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BARLEY: PRODUCTION, IMPROVEMENT, AND USES

STEVEN E. ULLRICH

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Barley

Production, Improvement, and Uses

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Edited by

Steven E. Ullrich

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Preface

Barley is a major world crop today and is one of the first agricultural domesticates. Yet it had been harvested and used long before the dawn of agriculture in the area known today as the Middle East. Barley is grown worldwide in many countries and regions with temperate climates in summer and in winter in some temperate and subtropical climates. Barley is highly variable in adaptation and utilization. These factors alone have caused barley to be the subject of much agronomic and end-use research. And largely because of its diploid and self-pollinated nature, barley has been the subject of much physiological and genetic research. Barley has been an important research model among plants long before the rise of *Arabidopsis* in this genre.

Because of the global status of barley, five books have been published in English dating back to 1978, some of which have summarized broad topics and others narrower topics of barley research and use. It has been some years since a broad compilation of barley research has been published. The aim of the book herein is to bring up-to-date reviews of the literature to reflect the broad nature of barley research on a global scale to a wide audience of students and professionals working in essentially all aspects of barley research, production, trade, and utilization.

Of course, literature is the foundation of a book such as this, and older classical publications are important, as are the latest findings in the various fields of knowledge. The authors of this book drew from nearly 3000 references from the world literature with more than a half (52%) published since 2000 and almost a third (30%) since 2005. The publication dates of the five books on barley mentioned above are 1978, 1985, 1992, 1993, and 2002.

Barley: Production, Improvement, and Uses contains 18 chapters, and after an introductory chapter on barley as a crop species, it covers the broad topics of the barley plant (three chapters); barley genetics, cytogenetics, germplasm, and breeding (six chapters); barley adaptation and cultural practices (four chapters); and barley utilization (four chapters). The chapters tend to include a historical perspective, current situations, and a look to the future. The chapters on breeding and cultural practices have four and five subchapters, respectively, to reflect the regionality of this global crop. Information from every continent, except Antarctica, is presented in this book.

A total of 51 expert renowned authors from 15 countries contributed to this book. The authors represent world class universities, federal and state or provincial government agencies and research centers or institutes, an international agriculture research center (International Center for Agricultural Research in the Dry Areas [ICARDA]), and the private industry. Yet there are many other internationally or nationally renowned "barley scientists" that could have contributed equally well in many of the topics covered in this book. Of course, there has to be a finite number of contributors to a book such as this.

It has truly been a pleasure to edit this book and to work with so many colleagues in barley from around the world. I am indebted to these many authors for their willingness and cooperation to contribute to this important book. I am also indebted to the editors, namely, Justin Jeffryes, Shelby Hayes Allen, and Susan Engelken, and Carrie Horn from Wiley-Blackwell, as well as Stephanie Sakson, editor and project manager from Best-set Premedia. Finally, I thank Mary Hope Ullrich, wife, teacher, book author, and artist, for her patience, understanding, professional advice, and the barley sketch on the title page.

Steven E. Ullrich

Barley

Production, Improvement, and Uses

Chapter 1
Significance, Adaptation, Production, and Trade of Barley

Steven E. Ullrich

SIGNIFICANCE OF BARLEY

Barley (*Hordeum vulgare* L.) is one of the most ancient crops, and it has played a role in the human development of agriculture, civilizations, and cultures and the sciences of agronomy, physiology, genetics, breeding, malting, and brewing. It is grown and/or used around the world. For many centuries, barley has fed livestock, poultry, people, and people's spirit. Barley was among the first domesticates playing an important role during the hundreds or thousands of years of human transition from a hunting and gathering to agrarian lifestyle in the "Fertile Crescent" of the Near East starting at least 10,000 years ago. The Fertile Crescent is considered the first of at least seven centers of agriculture origin in the world (Smith 1998). Barley, along with wheat (*Triticum* spp.), pea (*Pisum sativum* L.), lentil (*Lens culinaris* L.), goat (*Capra aegagrus hircus*), sheep (*Ovis aries*), and cow (*Bos taurus*), set the stage for the evolution of agriculture in the Near East, which eventually spread to North Africa, further east and north in Asia, and to Europe (Smith 1998). A concise history of the spread of barley cultivation is presented by Fischbeck (2002), and an update on the probable origin or origins of barley is presented in Chapter 2 of this book.

The prominence of barley can be seen from the interpretation of its genus name, *Hordeum*, which derives from the word by which Roman gladiators were known, "*hordearii,*" or "barley men," for eating barley to give them strength and stamina (Percival 1921). The English word "barn" derives from barley plus "aern" or barley house/building (*Webster's Dictionary*, various versions). Barley was presumably first used as human food, raw or roasted and in breads, porridges, and soups, but eventually evolved primarily into a feed, malting, brewing, and distilling grain. Barley's decrease in prominence as a food grain was due in part to the rise in prominence of wheat and rice. In recent times, 55%–60% of the barley crop has been used for feed, 30%–40% for malt, 2%–3% for food, and about 5% for seed.

Barley is best known around the world today as a feed grain and as the premier malting and brewing grain. Barley varieties are quite variable in feed quality, and barley is often compared to maize or corn (*Zea mays*) and wheat in feed quality. Considerable research and debate about the attributes of each have ensued throughout modern times. The presence of a fibrous hull on barley grain generally puts barley at a disadvantage, especially for use by nonruminants, mainly swine and poultry. However, the advantage of maize or wheat over barley is not clear-cut, and some studies have shown that barley can be of equal or greater quality compared with maize or wheat (Bowland 1974; Owens et al. 1995). Furthermore, hulless barley, due to removal of the hull with threshing, tends to be superior to hulled barley and more on par with maize and wheat (Joseph 1924; Mitchall et al. 1976; Bhatty et al. 1979). Given the adaptation of barley versus maize, barley is very important in areas where maize is not produced, especially where the climate is cool and/or dry, that is, in western

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North America, northern Europe, the Middle East, North Africa, and the Andean region of South America. Barley is utilized as forage as well as for grain. Details of barley feed use, characteristics, and value are given in Chapter 16 of this book.

When most people think of the composition of beer, they think of barley (but not necessarily malt). However, in much of Africa, sorghum (*Sorghum bicolor*), maize, and/or millet (various genera/species) beers abound and are part of cultural traditions. The history of alcoholic drinks including beer goes back thousands of years. The use of barley for beer likewise goes back thousands of years and dates from archeological evidence to at least 8000 years ago in the Middle East and in Egypt (Arnold 1911). The long history of brewing means that barley has long been selected for improvements in malting and brewing qualities. The traditions of using barley for brewing in the Middle East gradually migrated north into Europe where these traditions grew even stronger. Eventually, with the far reach of Europeans during the exploration and colonization of unknown and lesser known parts of the world (Americas, Africa, East Asia, and Australia), barley brewing traditions spread worldwide. Today, the sciences of malting and brewing are highly developed. Malting barley breeding is quite refined as well, with a host of barley and malt traits under consideration by industry. However, in spite of advanced technologies of analyses (e.g., near infrared) and breeding (marker-assisted selection), actual malting of grain and wet chemical analyses are still the principal procedures for analysis and selection. Major traits relate to the germination process and the physical and chemical composition of barley and malt including such things as kernel conformation, hull, carbohydrates, proteins, enzymes, and enzyme activity. Whereas the preponderance of malted barley is used for beer, some types are used for distilling (e.g., Scotch whisky and Irish whiskey) and for food applications. Details of traits and trait improvement can be found in Chapters 8 and 15 of this book.

Although barley utilization for food is relatively minor on a global basis today, throughout its history, barley has remained an important and major food source for some cultures principally in western and eastern Asia, as well as in the Himalayan nations and in northern and eastern Africa (Grando and Macpherson 2005; Newman and Newman 2006, 2008; Baik and Ullrich 2008). Furthermore, there has been a resurgence of interest and use of barley for food, primarily in the developed world due to an increasing emphasis on incorporating a diversity of whole grains in people's diets for health benefits. In addition, in 2006, the U.S. Food and Drug Administration issued an endorsement of the benefits of foods containing barley principally due to its soluble fiber content (β-glucans), which has been shown to lower blood cholesterol levels with implications for heart health. Barley also seems to lower blood glucose levels (glycemic index) with implications for those suffering from diabetes. See Grando and Macpherson (2005), Newman and Newman (2008), and Chapter 17 of this book for an expansion of barley food topics.

Barley has figured prominently on the frontiers of science in general, but especially in genetics. There is a long history of genetics research focused on trait inheritance and mapping in the conventional sense (Smith 1951; Nilan 1964; Barley Genetics Newsletter, 1971–2010), also on induced mutagenesis (Nilan 1981), and more recently on molecular and physical mapping and genetic analyses (e.g., Graner et al. 1991; Hayes et al. 1993; Kleinhofs et al. 1993; Yu et al. 2000; Kleinhofs and Han 2002; Caldwell et al. 2004; Close et al. 2004; Druka et al. 2006, 2008; Hayes and Szucs 2006; Kumlehn et al. 2006; Varshney et al. 2007; Xu and Jia 2007; Massman and Smith 2008; Potokina et al. 2008; Hamblin et al., 2010). Chapters 3–8 of this book focus on basic and applied molecular genetics and breeding advances in barley that are representative of the state of the science in crop species and plant species in general. As barley is one of the first domesticated crop species, much research has been done on the origins of barley and related small grains as well as phylogeny and systematics (see Chapter 2 of this book). Considerable research has been done with barley as a model in physiological and anatomical areas, especially of the grain (see Chapters 13 and 14).

ADAPTATION OF BARLEY

Barley has evolved to include several morphological and commercial forms, including winter, spring, two-row, six-row, awned, awnless, hooded, covered, naked, hulless, and malting, feed (grain and forage), and food types. Barley is arguably the most widely adapted cereal grain species with good drought, cold, and salt tolerance. It is generally produced in temperate (winter and/or spring planting) and semiarid subtropical (winter planting) climates. It does not tolerate highly humid warm climates. Grain production occurs at higher latitudes and altitudes and farther into deserts than any other cereal crop. For example, in the Nordic countries of Norway, Sweden, and Finland, six-row spring barley is grown further north (above 65°N lat.) than winter and spring two-row barley and spring wheat and oat. On the Altiplano of the Andean nations of Peru and Bolivia, barley is grown for grain at higher elevations (over 4500 m) than oat, wheat, and maize. In the North African country of Algeria, barley is grown further south toward the Sahara than the most drought-tolerant durum wheats (author, personal observation). Whereas barley can thrive and produce an acceptable crop at some of the earth's agricultural margins, it does very well under well-drained loam soils, at moderate rainfall (400–800 mm) or under irrigation, and at moderate temperature regimes (15–30°C). Barley production and reactions to biotic and nonbiotic stresses are detailed in Chapters 9–12 of this book. Below are a few rough illustrations of barley adaptation based on estimated yields from the United Nations Food and Agriculture Organization (FAO) database (FAO 2009). The estimated average barley yield in the world in 2006 was 2497 kg/ha, and in Western Europe, with a nearly ideal climate for barley with relatively high inputs of fertilizer and pesticides, it was 5956 kg/ha or 238% of the world average. Moving to warmer and drier Southern Europe, the average yield in 2006 was 2715 kg/ha. Moving to cooler and wetter Northern Europe, it was 4253, and in the Nordic countries mentioned above, the yield was 3550 kg/ha. The average yield across high-altitude Bolivia and Peru was 1045 kg/ha. In the North African countries bordering the Sahara desert, the average yield was 1168 kg/ha. Of course, a number of factors affect yield besides the adaptation effects of climate, soil, and biotic factors (+ and −), including the level of farmer inputs of cultivar, fertilizer, pesticides, and irrigation. All these things are reflected in the yield numbers above, especially lower inputs toward the margins of adaptation in southern and northern Europe, the Andean nations in South America, and in the Sahara desert bordering nations in North Africa. The wide adaptation of barley and production around the world have stimulated much study on the reactions of barley to abiotic and biotic stresses (see Chapters 10–12 of this book), and research and development of best management practices for barley production (see Chapter 9 of this book).

GLOBAL PRODUCTION OF BARLEY

How does barley figure into the whole scheme of crop agriculture around the world? Barley in recent years has been the fifth most-produced crop in the world and the fourth most-produced cereal on an approximate dry weight basis (Table 1.1). The three major food cereal grains, in order of production, are maize, rice (*Oryza sativa*), and wheat, which lead annual world crop production with 2000–2007 averages of ~600+ M t (millions of metric tons) each by a wide margin. Soybean (*Glycine max*), barley, sugarcane (*Saccharum* spp.), potato (*Solanum tuberosum*), and sorghum follow (196, 140, 93, 61, 58 M t, respectively). There have been some major shifts in production rank of the top crops over the last 20 years. Twenty years ago, the production rank from the top was wheat, maize, rice, barley, soybean, sugarcane, sorghum, and potato. The average rank so far in the twenty-first century is maize, rice, wheat, soybean, barley, sugarcane, potato, and sorghum. The shifts have involved greater surges in maize, rice, and soybean relative to the other crops (FAO 2009). In the mid-1980s, barley production was nearly twice that of soybean (160 vs. 88 M t). Table 1.2 complements Table 1.1 by reporting area and yield averages for the top six grain crops in the

Table 1.1 Global production estimates for 8 years of the top eight crops expressed "as is," in millions of metric tons (Mt), except dry weight (dw) for sugarcane and potato

Year/Crop	2000	2001	2002	2003	2004	2005	2006	2007	**Average**
Maize	593	616	604	641	727	713	695	785	**672**
Rice (paddy)	599	598	569	584	607	632	635	650	**609**
Wheat	586	590	575	560	633	629	606	607	**598**
Soybean	161	178	182	188	206	215	222	216	**196**
Barley	133	144	137	142	154	141	139	136	**140**
Sugarcane (dw)	88	88	93	95	93	91	98	102	**93**
Potato (dw)	63	59	60	60	63	61	60	61	**61**
Sorghum	56	60	54	59	58	59	56	65	**58**

Source: Food and Agriculture Organization of the United Nations (http://faostat.fao.org/site/567/default.aspx).

Table 1.2 Global grain production estimates for 8 years: first row in area harvested in millions of hectares (Mha), second row and grain yield in metric tons per hectare (t/ha)

Year/Crop	2000	2001	2002	2003	2004	2005	2006	2007	**Average**
Wheat	215	215	214	208	217	221	216	217	**215**
	2.7	2.7	2.7	2.7	2.9	2.8	2.8	2.8	**2.8**
Rice (paddy)	154	152	148	148	150	154	154	157	**152**
	3.9	3.9	3.9	3.9	4.0	4.1	4.1	4.2	**4.1**
Maize	140	139	138	142	146	145	144	158	**144**
	4.2	4.4	4.4	4.5	5.0	4.9	4.8	5.0	**4.7**
Soybean	74	77	79	83	91	93	93	95	**85**
	2.2	2.3	2.3	2.3	2.3	2.3	2.4	2.3	**2.3**
Barley	55	56	55	58	58	56	56	57	**56**
	2.4	2.6	2.5	2.5	2.7	2.5	2.5	2.4	2.5
Sorghum	41	44	41	45	41	44	42	44	**43**
	1.4	1.4	1.3	1.3	1.4	1.3	1.4	1.5	1.4

Source: Food and Agriculture Organization of the United Nations (http://faostat.fao.org/site/567/default.aspx).

world. One can get a good impression of differences in biology, adaptation, and production conditions from the combination of data in these two tables, especially relative yield data. For example, maize and rice are mostly produced under much less water stress than wheat and barley. This is reflected in total production versus total area grown and in global 8-year average yield estimates of 4.7 and 4.1 versus 2.8 and 2.5 t/ha for maize and rice versus wheat and barley, respectively. The fact that maize outyields rice (and many other crops) is at least partially reflective of the more efficient C4 versus C3 photosynthetic system of maize versus rice. The gap may be less than expected under similar production conditions because globally, maize typically experiences more drought than rice. Patterns over time in the overall production of these crops in Table 1.1 are reflected in the patterns in hectarage and yield in Table 1.2. Most area and yield data have been relatively stable in the twenty-first century so far. Exceptions are the trends of increasing yield of maize and increasing hectarage of soybean. Barley production, area, and yield data have been relatively stable this century, but barley has decreased by about 12% in overall production in the past 20 years.

The adaptation of barley described above can be seen in the regional distribution of barley production over the globe (Table 1.3). Thirty-eight countries in Europe (including the Russian Federation and Ukraine) produced 83 Mt of barley in 2007, which is more than 60% of the world's barley production. The 27 countries of the European Union (EU) produced over 40% of world production in 2007 and for the 5-year

Table 1.3 World distribution of barley production based on estimates in millions of metric tons (Mt), 2007

Region	No. of Countries	Production
World	**100**	**136**
Europe	**38**	**83**
European Union	27	59
Asia	**34**	**22**
West Asia	16	10
South Asia	7	5
East Asia	5	4
Central Asia	5	3
North America	**2**	**17**
Australia/New Zealand	**2**	**6**
Africa	**16**	**5**
North Africa	6	3
East Africa	6	2
South America	**8**	**3**

Source: Food and Agriculture Organization of the United Nations (http://faostat.fao.org/site/567/default.aspx).

Table 1.4 Barley production estimates by country—5-year averages (2003–2007)

Rank	Country	Production (M t)	Harvested (M ha)	Yield (t/ha)
World		**140.8**	**57**	**2.6**
1.	Russian Federation	16.7	9.4	2.2
2.	Canada	12.0	4.0	3.7
3.	Germany[a]	11.5	2.0	7.0
4.	France[a]	10.1	1.7	7.4
5.	Spain[a]	9.4	3.2	3.2
6.	Turkey	8.5	3.6	3.0
7.	Ukraine	8.3	4.3	2.5
8.	Australia	7.2	4.5	2.1
9.	United Kingdom[a]	5.5	1.0	7.1
10.	United States	5.2	1.6	4.2
11.	Poland[a]	3.6	1.1	3.7
12.	Denmark[a]	3.4	0.7	6.2
13.	China	3.4	0.8	4.7
14.	Iran	2.9	1.6	2.2
15.	Czech Republic[a]	2.1	0.5	4.1
16.	Kazakhstan	2.0		
17.	Finland[a]	1.9		
18.	Belarus	1.8		
19.	Morocco	1.8		
20.	Sweden[a]	1.5		
21.	Ethiopia	1.3		
22.	Algeria	1.3		
23.	India	1.3		
24.	Italy[a]	1.2		
25.	Ireland[a]	1.2		
26.	Argentina	1.2		
27.	Hungary[a]	1.1		

[a]European Union member countries.
Source: Food and Agriculture Organization of the United Nations (http://faostat.fao.org/site/567/default.aspx).

average of 2003–2007 (FAO 2009). Approximately 16% of the world's barley in 2007 was produced in 34 countries in Asia with 45% of Asian barley produced in 16 western Asian countries. North America (Canada and the United States) grew 12.5% of global production, followed by Australia/New Zealand (4.4%), 16 countries (mostly North and East) in Africa (~4%), and eight countries in South America (2%).

Barley production (5 year means, 2003–2007) by country is detailed in Table 1.4. These data again generally illustrate the adaptation of barley and complement the data in Table 1.3. The top 10 countries in descending order are the Russian Federation, Canada, Germany, France, Spain, Turkey, Ukraine, Australia, the United Kingdom, and the United States, and they produced approximately 67% of the world's barley over this 5-year period (94.4/140.8 M t). Seven of the top 10 countries are considered in Europe including the Russian Federation, Turkey, and Ukraine. Of these seven countries, four are currently (2009) in the EU. Of the 27 countries listed in Table 1.4, 15 are in Europe and 12 of these are currently in the EU with Turkey's membership pending. Although European countries dominate global barley production, each continent and subcontinent listed in Table 1.3 is represented by at least one country in Table 1.4. Country yield averages range from about 2 t/ha (Russian Federation, Australia, and Iran) to ≥7 t/ha (Germany, France, and the United Kingdom; Table 1.4). The country yield averages, hectarage, and total production reflect relative growing conditions (mainly precipitation) and management technology (mainly soil fertility and pest management). For example, the most influential factors in the countries with the lowest yields are climate for Australia and climate and management technology for the Russian Federation and Iran. At the high end of the yield spectrum, Germany, France, and the United Kingdom all have a favorable climate and a high level of management technology.

GLOBAL TRADE OF BARLEY

Most but not all barley-producing countries utilize the bulk of their production domestically. There is considerable trade of barley and barley products. FAO estimates of global trade of barley and barley products are summarized in Table 1.5, where import and export figures are presented and compared for the years 2000 and 2005. Over 20 M t of barley grain have typically been exported and imported annually this century, globally generating about US$3 billion per year. The estimated amount, value, and price of barley grain exports and imports rose between 2000 and 2005 by about 10%, 32%, and 22%, respectively. Barley malt trade has amounted to an estimated 5–6 M t/year with modest increases between 2000 and 2005 (exports: 5.5–6.2 M t [13%]; imports: 5.2–5.7 M t [10%]). The value of malt exports and imports rose from an average of about US$1.35 billion in 2000 to about US$2.0 billion in 2005 (48%). The increase in the value of malt outpaced the increase in the quantity of malt traded during these years. The quantity and value of beer exports and imports grew very dramatically between these two years. The quantity of beer exported and imported rose from 6.2 to 9.8 M t and from 6.3 to 9.4 M t or by 49% and 59%, respectively. The value of beer exports rose from US$4.8 billion to US$8.2 billion (71%) between 2000 and 2005, and the value of beer imports rose from US$5.4 billion to US$7.9 billion (46%). Furthermore, the increase in the value of beer outpaced the increase in the value of malt exported between 2000 and 2005, but import value gains were about equal.

Table 1.5 World trade estimates of barley and barley products

	2000		2005	
Year	Exports	Imports	Exports	Imports
Barley grain				
Quantity (M t)	23.8	22.3	25.8	23.4
Value (G US$)	2.7	2.8	3.6	3.7
Price (US$/t)	114	125	139	152
Malt				
Quantity (M t)	5.5	5.2	5.2	5.7
Value (G US$)	1.3	1.4	2.0	2.0
Beer				
Quantity (M t)	6.2	6.3	9.8	9.1
Value (G US$)	4.8	5.4	8.2	7.9
Malt extract				
Quantity (K t)	65	120	192	125
Value (M US$)	69	116	192	147
Pearled barley				
Quantity (K t)	15	32	69	29
Value (M US$)	4.0	13.5	11.6	6.3
Barley flour/grits				
Quantity (K t)	7	30	11	15
Value (M US$)	1.4	5.4	3.7	3.2

G, billions; M, millions; K, thousands; t, metric tons.
Source: Food and Agriculture Organization of the United Nations (http://faostat.fao.org/site/535/default.aspx#ancor).

Other value-added barley products traded are malt extract, pearled barley, and barley flour and grits. Compared to trade of barley grain, malt, and beer, the trade of these commodities is relatively minor. Generally, dramatic increases occurred from 2000 to 2005 in the export and import quantity and value of these commodities, except for import numbers for pearled barley and barley flour and grits, which actually went down, and in some cases dramatically. Given that all the data presented by the FAO are estimates from various sources with variable accuracies, export and import numbers for the various commodities in Table 1.5 agree fairly well. However, there are major discrepancies, and therefore, inaccuracies in the numbers for malt extract, pearled barley, and barley flour/grits.

Barley malt production data by country is difficult to obtain. Perhaps the reason is that maltsters guard production information for business purposes. Malt production capacity and production information is scattered across various Web sites. According to "Worldmalt Statistics" (http://www.coceral.com/), world malt production capacity is estimated at about 22 M t/year with actual annual production ranging from 18 to 22 M t in recent years. The EU countries typically produce 8–9 M t/year, about 42% of the world total. Typically, the top five EU malt producers in descending order are Germany, the United Kingdom, France, each with over a million metric tons, followed by Belgium (~700,000 t), and Spain and/or the Czech Republic (~500,000

t each). Furthermore, this Web site indicates that of the approximate 20 Mt of malt used per year globally, about 94% is used for beer, 4% for distillation, and 2% for food. Other estimates of annual malt production/capacity include a 5-year (2002–2006) average production of approximately 1.8 Mt in the United States (calculated from net import/export data from the United States Department of Agriculture [USDA] Foreign Agriculture Service [http://www.fas.usda.gov/ustrade/] and malt used in breweries from the U.S. Department of Treasury Alcohol and Tobacco Tax and Trade Bureau [http://www.ttb.gov/beer/beer-stats.shtml]), approximately 1.2 Mt capacity for Canada (http://www.wheatgrowers.ca/), about 700,000 t production for Australia (http://www.barleyaustralia.com/), and 4+ Mt capacity for China (Bormann 2007). China is capable of producing more malt than any other country in the world based on the data presented above. The surging economy in China in almost all sectors including the agricultural sector is well-known, so it is not surprising to see China's large capacity to produce malt. According to Bormann (2007), beer consumption has risen dramatically, driving an increasing demand for malt. In 1989, per capita beer consumption in China was ~5 L; in 1999, it was ~15 L, and the projection for 2009 is for over 25 L. In 1990, the demand for malt by Chinese brewers was 0.8 Mt, and in 2000, it was 2.7 Mt. By 2004, 200 maltsters had developed the capacity to produce 4.3 Mt of malt. Whereas China annually has imported about 2 Mt of barley in recent years, some of which is malting barley, it imports very little higher-cost malt, only about 3000–4000 t/year (FAO 2009). China, which has roughly the same amount of crop land and 4.4 times the population of the United States, has surged to the forefront in the production of a number of agricultural commodities. It is the number 1 producer, globally, of rice, wheat, potato, sweet potato (*Ipomoea batatas*), groundnut (*Arachis hypogaea*), cotton (*Gossypium* spp.), rapeseed (*Brassica* spp.), squash (*Cucurbita* spp.), peach (*Prunus persica*), apple (*Pyrus malus*), tobacco (*Nicotiana tabacum*), and cabbage (*Brassica oleracea/B. chinensis*). China is number 2 in maize, number 3 in banana (*Musa* spp.), and number 4 in soybean production in the world (FAO 2009). China, the world's most populous nation with 1.4 billion people estimated in 2008, is an agricultural giant.

A breakdown of leading exporting and importing countries of barley and malt by quantity traded is presented in Table 1.6. Data are presented for the years 2000 and 2005 to illustrate the dynamics of the trade. The EU dominates the export trade of both barley and malt. Three of the top five exporters of barley and malt in 2000 were EU countries (barley: Germany, France, the United Kingdom; malt: France, Belgium, Germany), and in 2005, France and Germany were among the top five barley exporters, and France, Belgium, and Germany were among the top five exporters of malt. Overall, EU countries exported 66% and 50% of the barley in 2000 and 2005, respectively, and 67% and 69% of the malt (FAO 2009). Australia and Canada, large countries with relatively small populations and high production, were among the top five barley and malt exporters in both years.

There tends to be greater diversification among the top barley and malt importing countries (Table 1.6). The consistently largest barley importer by far is Saudi Arabia, taking about 25% of the trade. Crop production is a relatively small component of Saudi Arabia's agriculture compared with animal production, hence, the emphasis on importing feed stocks like barley. China, Japan, and Belgium were among the top five importing countries in both 2000 and 2005. Japan, Brazil, the Russian Federation, and Venezuela were among the largest malt importers in both 2000 and 2005. Although the EU dominates the export market, it is a prominent importer of barley and malt as well, importing 25%–30% of the barley and about 25% of the malt traded in the world (FAO 2009).

Most of the world's people on every continent associate barley malt with beer. However, in rural areas in Africa, sorghum, maize, and millet beers are very important in local cultures. Unless otherwise indicated, the term beer in this section refers to beer made from malted barley. The average annual estimated global production of

Table 1.6 Leading barley, malt, and beer exporting and importing countries based on quantity estimates (millions of metric tons [Mt])

2000						2005					
Barley		Malt		Beer		Barley		Malt		Beer	
Exporters											
World	**23.8**	**World**	**5.5**	**World**	**6.24**	**World**	**25.8**	**World**	**6.2**	**World**	**9.84**
Germany	6.2	France	1.1	Mexico	1.05	France	5.4	France	1.2	Mexico	1.62
France	4.8	Belgium	0.60	The Netherlands	0.80	Australia	3.9	Belgium	1.1	The Netherlands	1.48
Australia	3.0	Germany	0.55	Germany	0.79	Ukraine	3.5	Canada	0.55	Germany	1.42
Canada	1.8	Canada	0.50	Belgium	0.43	Germany	2.9	Australia	0.49	Belgium	0.87
United Kingdom	1.6	Australia	0.47	Canada	0.39	Canada	2.0	Germany	0.42	Ireland	0.40
Importers											
World	**22.3**	**World**	**5.2**	**World**	**6.32**	**World**	**23.4**	**World**	**5.7**	**World**	**9.08**
Saudi Arabia	5.4	Japan	0.74	United States	2.35	Saudi Arabia	6.0	Brazil	0.65	United States	2.98
China	2.1	Brazil	0.64	United Kingdom	0.44	China	2.3	Japan	0.52	United Kingdom	0.75
Japan	1.7	Russian Federation	0.56	Italy	0.42	Spain	1.6	Belgium	0.48	Italy	0.53
Belgium	1.2	Germany	0.31	France	0.37	Belgium	1.4	Russian Federation	0.35	France	0.48
Iran	1.0	Venezuela	0.26	Germany	0.32	Japan	1.4	Venezuela	0.29	Germany	0.37

Source: Food and Agriculture Organization of the United Nations (http://faostat.fao.org/site/535/default.aspx#ancor).

barley malt beer over the 3-year period of 2005–2007 was 165 Mt. During this period, production increased rather sharply (11.5%) from 156 Mt in 2005 to 165 Mt in 2006, and to 174 Mt in 2007 (Table 1.7). Beer production in 2007 was 128% of the beer production in 2000 (136 vs. 174 Mt). By continent, in 2007, Europe produced the most beer, an estimated 57 Mt (EU with 40 Mt) followed by Asia at 53 Mt, North America at 35 Mt, South America at 18 Mt, Africa at 8 Mt, and Australia and New Zealand at 2 Mt (FAO 2009).

With 1.4 billion people, it is not surprising that China is now the world's largest beer producer with an estimated 40 Mt in 2007 (Table 1.7). The United States is a distant number 2 with an approximate production of 23 Mt, and the Russian Federation is a distant number 3 with approximately 11.5 Mt. Germany, Brazil, and Mexico closely follow at numbers 4, 5, and 6, respectively. The top 10 is rounded out with the United Kingdom, Japan, Spain, and Poland. The dynamics in beer production over the 3-year period of 2005–2007 are also depicted in Table 1.7. Among the top 10 producers, China is rapidly increasing its beer production, while the Russian Federation, Brazil, Mexico, and Poland have been slowly increasing production, and the United States, Germany, the United Kingdom, Japan, and Spain have been rather static in production.

Seven of the top 10 beer exporting and importing countries based on quantity of trade in 2000 and 2005 were EU countries. The EU countries as a whole exported approximately 60%, and they imported about 40%, of the beer traded in the world both of these years (FAO 2009). The top five exporting and importing countries in 2000 and 2005 are depicted in Table 1.6 along with the quantities exported and imported. Mexico has consistently been the leading beer exporting country in the world. Besides the European countries listed, Canada ranked fifth in 2000 and sixth in 2005 (FAO 2009). The United States has consistently ranked first in the importation of beer in the world and by a very wide margin versus the second-ranked United Kingdom. As noted above, global beer production has risen sharply (2007 was 128% of 2000) since the beginning of the twenty-first century. The trade of beer has risen even more sharply with export quantity in 2006 175% of export quantity in 2000, and import quantity in 2006 165% of that in 2000 (FAO 2009).

In contrast to the 165 Mt of beer brewed with barley malt in the world in 2006, there was an estimated 6.9, 2.5, and 1.5 Mt of beer brewed with malt of sorghum, maize, and millet, respectively (Table 1.7). Almost all of nonbarley malt beer is brewed in Africa. Canada (for maize beer) is the only country outside Africa among the

Table 1.7 Leading beer-producing countries based on estimates of quantity produced (millions of metric tons [Mt])

	2005	2006	2007
Barley beer			
World	**156**	**165**	**174**
China	31.7	35.9	40.0
United States	23.1	23.2	23.5
Russian Federation	9.1	10.0	11.5
Germany	9.5	9.9	9.7
Brazil	9.0	9.4	9.6
Mexico	7.3	7.8	8.1
United Kingdom	5.6	5.4	5.5
Japan	3.8	3.8	3.9
Spain	3.1	3.4	3.4
Poland	3.0	3.3	3.6
Sorghum beer			
World		**6.9**	
Tanzania		1.92	
Uganda		0.82	
Nigeria		0.79	
Burkina Faso		0.64	
Congo		0.59	
South Africa		0.56	
Cameroon		0.42	
Ghana		0.34	
Maize beer			
World		**2.5**	
South Africa		0.90	
Uganda		0.62	
Canada		0.52	
Congo		0.14	
Zambia		0.14	
Millet beer			
World		**1.5**	
Uganda		0.34	
Tanzania		0.33	
Ethiopia		0.21	

Source: Food and Agriculture Organization of the United Nations (http://faostat.fao.org/site/535/default.aspx#ancor).

leading producers of these beers. The leading countries are spread throughout sub-Saharan Africa. From the author's own experience living in Malawi in southern Africa, *msese*, a maize beer, brewed in 55-gal oil drums, was available in rural villages, and *chibuku*, another maize beer, commercially produced and packaged in paperboard cartons, was available in stores. Both types of beer are opaque with much sediment, even chunks, typically filtered out of msese, but not chibuku. The chibuku brand was "Shake Shake," probably to admonish the consumer to mix the contents thoroughly to get the full benefit (nutritional?) of the beer. Because considerable amounts of beer are brewed locally noncommercially in villages, the FAO production data are probably substantially underestimates. Nevertheless, these beers are produced on a much reduced scale compared with barley malt beer and are important mostly on a local or regional basis.

CONCLUDING REMARKS

This introductory chapter sets the stage for understanding the importance of barley as a major global crop. The following chapters will expand on some of the topics briefly discussed here. Barley has played a major role from the era of hunting and gathering, through the transition to agriculture, up to the present era. Barley was one of the first plants domesticated in the first agricultural region of the world, and it has maintained its prominence in the world for over 10,000 years in agriculture; human and animal food, feed, and nutrition; alcoholic beverage production and consumption; and in the continuing development of the biological sciences. Barley has played an important role in plant genetics and breeding, plant physiology, agronomy, cereal chemistry, human and animal nutrition, plant pathology, and entomology. Barley, as an experimental organism, has contributed to the development of scientific knowledge, and science has contributed to the improvement of barley as a crop. Barley, as the fifth most-produced crop in the world today, involves massive amounts of resources and people working in production agriculture; commodity transportation and trade; processing and end use product manufacture, transportation, marketing, and consumption, as well as, research and development for the improved production and use of the crop.

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Chapter 2
Barley Origin and Related Species

Roland von Bothmer and Takao Komatsuda

INTRODUCTION

The barley genus *Hordeum* was formerly considered a nonhomogeneous group with basically a number of unrelated but morphologically rather similar entities, thus not comprising a natural phylogenetic group. Today, there are new, powerful techniques available that have substantially increased our knowledge on evolutionary age, migration patterns, and differentiation of new species groups. Recent research has convincingly showed that *Hordeum* is indeed a monophyletic group with a common origin. All species, even cultivated barley, are thus related, some of them more distantly so. *Hordeum* is evidently an ancient genus, splitting from the wheat species some 13 million years ago (mya).

The stories of domestication of our cultivated plants are fascinating, combining biological and agricultural knowledge with archaeology and other sciences. Our view on the evolutionary history of domesticated barley and its relatives has been significantly altered in recent years. The former theory of a single domestication event of barley is now abandoned in favor of a view of at least two events, separated in time and place. The current research on the domestication process is largely directed toward understanding the mechanisms and pathways at the molecular level of the successive changes in the gene systems regulating traits responsible for major domestication syndromes.

Barley: Production, Improvement, and Uses. Edited by Steven E. Ullrich

Knowledge of the potential for utilization of the wild species has increased. The ancestral form of barley, *Hordeum vulgare* subsp. *spontaneum* (wild barley), despite belonging to the primary gene pool of barley, was earlier considered as an exotic germplasm and, as such, of limited value as a gene source in practical breeding programs. This view of wild barley has changed considerably. Today, the potential for practical utilization is highlighted for base broadening as well as for incorporation of individual, interesting genes regulating agronomic traits. Wild barley has also developed into a model organism in biological research on genetic diversity, differentiation in populations, disease resistance, and other important mechanisms.

Other wild species have lately become more in focus for utilitarian purposes. The secondary gene pool, *Hordeum bulbosum* (bulbous barley), has gained a renewed interest. Thirty years ago, it attracted attention for production of doubled haploids in wheat and barley, but now it has attained importance as a gene source particularly for incorporation of disease resistance. The South American *Hordeum chilense* is the target for creation of an entirely new crop, tritordeum, as a result of intergeneric hybridization with wheat. It has a number of promising characters, such as improved baking quality, stress tolerance, and disease resistance. Other species show potential for stress tolerance, for example, *Hordeum marinum* (sea barley) with a particularly high salt tolerance.

Some of the wild, perennial *Hordeum* species are important as components in natural pastures used for grazing in South America and in central

Asia. Attempts are being made to develop pastures for grazing in stressful areas, for example, in the Middle East, by introducing drought-tolerant annual species with a rapid development, such as *Hordeum murinum* (wall barley) and wild barley.

Some species have negative effects on the environment by successful, opportunistic behavior. They occur as serious weeds, such as *Hordeum jubatum* (squirreltail barley), in many parts of the world. Other species may cause severe problems in cultivation by acting as hosts for pests and diseases from which a pathogen may spread to the cereal crops. Irrespective of the fact that wild species may have a positive or a negative influence on agriculture or of being a real or potential gene source for crop improvement, it is important to increase knowledge of their biological systems, gene contents, and ecological preferences.

This chapter presents a review of the genus *Hordeum* and its species. It aims to give a comprehensive survey of the present knowledge of biological diversity and agricultural potential. Descriptions of the species together with outlooks on agricultural potentials are reviewed and, in particular, the current views on evolution and phylogeny are elucidated. The domestication process is mirrored by description of changes in the major gene systems.

THE TRIBE TRITICEAE

Barley belongs to one of the economically most important plant groups in the world, the Triticeae, which is a tribus (tribe) in the grass family, Poaceae. It comprises the major temperate cereals: wheat (*Triticum*, several species), rye (*Secale cereale*), barley (*H. vulgare*), and the "man-made" crop rye wheat or triticale (*Triticosecale*). There are other species of value as forage grasses, such as the crested wheatgrasses (*Agropyron cristatum* and related species) and Russian wildrye (*Psathyrostachys juncea*). Some species have gained attention, for example, as sand binders in eroded areas, such as lyme grass on Iceland (*Leymus arenarius*). Many Triticeae species are noxious weeds, such as quack grass (*Elymus repens*, formerly *Agropyron* or *Elytrigia repens*), mouse barley (*H. murinum* subsp. *leporinum*), and foxtail barley (*H. jubatum*).

All species in Triticeae have spikes (sessile florets); the basic chromosome number is $x = 7$, and they have large chromosomes reflecting large genomes. The tribe has a worldwide distribution occurring on all continents in most cold and warm temperate areas, and it even has some subtropical representatives.

The diversity centers of the tribe, defined as number of species, are in remote areas, such as in the mountainous regions of central Asia, where little material for research has been available and new species are still to be found. Thus, taxonomy at the species level is far from satisfactorily solved. No comprehensive monograph or review of the whole tribe is available, and there is still a great uncertainty about the number of species, ranging from ca. 325 (Dewey 1984) to ca. 500 (Löve 1984). More recently, Barkworth et al. (2007) estimated the number to be between 400 and 500 species.

The circumscription of genera, likewise, are in disagreement. Löve (1982, 1984), by applying a strict concept for generic delimitation based on genomes, recognized 38 genera, whereas Stebbins (1956) argued that all species should be lumped into one genus due to a high degree of crossability. Neither of these views has been widely accepted. Today, an intermediate number of genera are accepted, but still there is no consensus about the generic delimitations (Kellogg 1989; Barkworth 1992; Watson and Dallwitz 1992; Yen et al. 2005; Barkworth and von Bothmer 2009).

Triticeae is a very good example of a successful, widespread plant group with large diversity and high versatility in biological characters. It represents very complex modes of speciation, including polyploidy, and a high degree of interspecific and intergeneric hybridization leading to a reticulate pattern of evolution. A typical example of this phenomenon is the widespread **I** genome in *Hordeum* (see below concerning genome designations). This genome is also occurring as one component in polyploid species of *Elymus* and *Hystrix*, in combination with different other genomes, such as **Y**, **St**, **P**, and **W** (Mason-Gamer 2008; Zhang et al. 2008). The large number of

species, the large diversity, and the reticulate pattern of relationships makes the whole tribe a model group for studies of evolution and phylogeny as well as an interesting gene source for cereal and forage grass breeding.

Even though the phylogeny and evolutionary pattern in Triticeae is complex, phylogenetic studies of the grass family indicate that the tribe is monophyletic and that it constitutes an evolutionary homogeneous group (Catalan et al. 1997; Seberg and Frederiksen 2001; Petersen and Seberg 2005). Estimations have shown that the Triticeae diverged from tribus Avenae (oats) ca. 25 mya and that wheat (*Triticum*) and barley (*Hordeum*) diverged around 13 mya (Gaut 2002).

Species of the grass family differ in basic chromosome numbers and show large variation in genome size. All members of the Triticeae have a basic chromosome number of $x = 7$ and have the largest genome size of all grasses, where *Psathyrostachys fragilis* has the largest size of all with 17.9 pg (cf. Gaut 2002). The genome size is mainly a function of repetitive DNA. The complements of low-copy genes are, however, similar in rice and barley, but there is a 12-fold difference in DNA content (Saghai-Maroof et al. 1996; Gaut 2002). There is no evidence that annual, self-pollinated species should have smaller genomes than perennial, cross-pollinating species in the Triticeae (Eilam et al. 2007). During the 77 million years of grass evolution, there have been dramatic genome changes, but the major chromosomal organization has been surprisingly stable with conserved linkage blocks and gene orders (Devos and Gale 2000; Qi et al. 2006; Stein 2007 for references; Stein et al. 2007; Cuadrado et al. 2008).

The closest related genera to *Hordeum* in Triticeae are *Psathyrastachys*, which is an Asiatic group of tufted perennials growing in harsh, dry steppe environments, and *Taeniatherum*, an annual genus originally of Mediterranean origin (Frederiksen and von Bothmer 1989). *Hordeum*, together with *Psathyrostachys*, constitute a monophyletic group clearly separated from the rest of the Triticeae (Hsiao et al. 1995; Seberg and Frederiksen 2001). The relationships to the monotypic genus *Hordelymus* with the single species *Hordelymus europaeus* is not clarified (Ellneskog-Staam et al. 2006; Petersen and Seberg 2008). Significantly, newer data have revealed that the barley genus, *Hordeum*, is monophyletic; thus, the former splitting of the species into different genera (primarily *Hordeum* and *Critesion*) has no phylogenetic basis (Kellogg 1989; Petersen and Seberg 1997; Seberg and Frederiksen 2001).

THE GENUS *HORDEUM*

Distinguishing *Hordeum* species from all other Triticeae species are a few distinct characteristics. The major trait is the typical *triplet*, which are three one-flowered (one seeded) spikelets at each rachis node. In the wild species the lateral spikelets are always stalked, whereas they are sessile in *H. vulgare* (both in cultivated barley and its wild progenitor). The lateral spikelets in the triplets may be fertile and seed setting as in six-rowed barley, whereas the laterals are sterile in two-rowed barley. Despite the basic features characterizing the genus *Hordeum*, the morphological and genetic variation within and between species is considerable. A prominent feature is the large plasticity in morphological traits found particularly in some of the annuals. Under unfavorable stress conditions caused by drought, heat, salinity, or flooding, the plants may be slender with a single, short culm with a minute spike, with a low but secured seed set. Under favorable conditions, the same genotype may be luxuriant with a height of 1 m, with several culms and large spikes and florets. A good example of the extreme plasticity is *H. murinum*.

Distribution

Hordeum has a wide distribution and occurs in most temperate areas in the world. Southwest Asia is probably the original area for the genus, and the first representatives of *Hordeum* have been estimated to date from ca. 12 mya (Blattner 2004, 2006; Jakob and Blattner 2006; see under phylogeny). One group of perennial species is today present in central Asia (*Hordeum roshevitzii*, *Hordeum bogdanii*, and *Hordeum brevisubulatum*).

From this original Asian distribution, migration to North America occurred ca. 4 mya and some perennial species developed here (*Hordeum brachyantherum* and *H. jubatum*). Probably as a result of long-distance migration by birds, *Hordeum* spread from North to South America, where further speciation took place, and today, South America is the diversity center of the genus, defined as containing the highest number of species (18), including both diploid and polyploids. Annuality was developed here (*Hordeum euclaston*), and a secondary dispersal took place from South to North America where the two diploid annuals *Hordeum pusillum* (widespread) and *Hordeum intercedens* (endemic to southwestern California) were developed (Blattner 2006). Later, the annual, allotetraploid *Hordeum depressum* was differentiated in the southwestern (present-day) United States. The single Central American species, *Hordeum guatemalense*, was established, also probably through bird migration from the perennial North American taxa. In the Mediterranean area, *H. murinum* is of ancient origin and separated from the Southwest Asian *H. vulgare* and *H. bulbosum* ca. 10 mya. The other Mediterranean annual *H. marinum* differentiated from the **I** genome group ca. 6 mya. Theses two, originally Mediterranean annual species, have become widespread as weeds during the last centuries in suitable, temperate areas of the world. Somewhat puzzling are the two remaining species: *Hordeum secalinum*, with a European and North African distribution, and *Hordeum capense*, native to South Africa. Both are distinct, tetraploid, perennial species, and both have the diploid cytotype of *H. marinum* as one of its ancestors. *H. capense* has probably differentiated from *H. secalinum* or from a common ancestor in South Africa.

Habitats

The habitat preferences for *Hordeum* comprise a wide range of ecological niches. The majority of the perennial species are confined to dry or, more often, wet pastures. Some species, such as *H. bogdanii* and *H. depressum*, grow in saline environments; *H. marinum*, in particular, can be classified as a halophyte tolerant to high salt concentrations (see under the species). Dry steppes, stony hillsides, and salt pans are preferred particularly by some of the species in South America, for example, *Hordeum patagonicum*, *Hordeum comosum*, and *Hordeum tetraploidum*. One extreme specialization is *H. patagonicum* subsp. *magellanicum*, which is mainly confined to sandy beaches on Tierra del Fuego and has adapted a dispersal mechanism specialized to this environment. The altitude range is large, from sea level where several species are found up to 4000–5000 m in the Himalayas (*H. brevisubulatum* subsp. *nevskianum* and subsp. *turkestanicum*) and in the Andes (*Hordeum muticum*, *H. comosum*, and *Hordeum halophilum*).

Life forms, reproduction, and dispersal

Hordeum shows a large variation in reproductive pattern and in life forms. Most species are long- or short-lived perennials. Annuals have developed independently from perennials in different parts of the world through adaptation to particular ecological requirements, such as halophytic communities for *H. marinum*, summer or winter annuality in *H. murinum*, and adaptation to the vernal pool habitat for *H. intercedens* in California. The two species *Hordeum flexuosum* (South America) and *Hordeum arizonicum* (North America) have a versatile life form. They occur usually as short-lived perennials (often biennials) or as annuals, dying off under unfavorable conditions. The European species *H. secalinum* is very slow growing and it has a shallow and poorly developed root system. It grows in slightly saline environments, needs occasional flooding, and is very sensitive to competition from more aggressive species. Most perennial species are bunch grasses, but some species have a distinct vegetative reproduction by subterranean runners (rhizomes). This is particularly typical for *H. brevisubulatum*. The swollen shoot base (corm), looking like a "bulb," is significant for *H. bulbosum* and contributes to vegetative reproduction.

The original sexual reproduction was a versatile system with a capacity for self-pollination as well as for cross-pollination, with partly open flowers and a not well-differentiated time lapse for pollen maturity and stigma receptivity. This

is also the most common sexual reproduction system among present-day *Hordeum* species. However, specialization has been developed independently in various groups. Some of the annual species, such as *H. murinum* and *H. intercedens*, are more or less obligate inbreeders with cleistogamous flowers, short stigmas, small anthers with few pollen grains, and simultaneous development of stamens and stigma. They show generally a very high seed set, irrespective of external conditions. The ancestor of cultivated barley (*H. vulgare* subsp. *spontaneum*), in spite of being an annual, has a comparatively versatile system with often rather open flowers and thus with capacity for cross-pollination. The domestication process has led to a decreased outbreeding, and modern cultivars of barley are considered to be almost exclusively inbreeding. For the plant breeding process, barley is estimated to generally have $\ll$1% outbreeding. More primitive forms, such as older landraces, have a higher rate of outbreeding than modern cultivars. On the other extreme, there has been an evolution toward complete outbreeding. Some species, for example, *H. secalinum* and *H. tetraploidum*, are self-compatible but are mostly open flowering, with a rich pollen production and long exserted stigmas. The most extreme forms are *H. bulbosum* and *H. brevisubulatum*, which are both more or less obligate outbreeders and well adapted for wind pollination. They have both developed self-incompatibility mechanisms, which for *H. bulbosum* is a two-locus genetic system (Lundqvist 1962).

The seed dispersal mechanism is markedly differentiatied. Most species have a brittle rachis (main axis of the spike); that is, the spike disarticulates at maturity at each rachis node. The dispersal unit is a triplet; thus, a central spikelet together with the two lateral ones united with one internode of the spike. Only the two distantly related taxa *H. vulgare* subsp. *vulgare* and *H. bogdanii* have a tough spike where only the mature, central, seed-bearing spikelet disarticulates, leaving the main spike axis intact. The wild ancestor to barley, subsp. *spontaneum*, has a very fragile spike. The tough rachis in the cultivated form is a domestication syndrome and an advantage for easy and secure harvest. The genetic background for brittle versus tough rachis is fairly simple (two genes, see under Domestication of Barley). The genetic background for the tough rachis in *H. bogdanii* has not been investigated.

Most species have a rather unspecialized seed dispersal mechanism, but particularly, two types of specializations are evident. *H. murinum* and *H. vulgare* subsp. *spontaneum* have both long and tough bristles on awns, glumes, and on the edge of the rachis. These hairs are unilaterally directed, which means that the triplet, despite the comparatively large and heavy seeds, very easily attach to, for example, the furs of animals. This is an obvious adaptation to a zoochorous dispersal mechanism (see under *H. murinum*). The other extreme are those species adapted to wind (anemochorous) dispersal. They have small, light seeds and long, slender awns and glumes, which are spreading out (often 90°) at maturity. Several triplets are often attached to each other, forming a light "flying apparatus," which is easily spread in the wind, sometimes for long distances. Typical representatives with wind dispersal are the species in section *Critesion*, for example, *Hordeum lechleri* in South America and *H. jubatum* in North America.

It is obvious that an inbreeding reproductive system, which guarantees a secure seed setting also under unfavorable conditions, together with rather unspecialized ecological requirements (preferably open, ephemeral habitats) and an effective dispersal mechanism (by wind or by animals), is an effective prerequisite for a weedy habit, like in *H. murinum* and *H. jubatum*.

Chromosomes and genomes

The basic chromosome number is, like in all other Triticeae species, $x = 7$, and in the genus, both diploids ($2n = 14$) and polyploids ($2n = 4x = 28$ and $2n = 6x = 42$) occur. The chromosomes are large and the karyotype is mainly symmetrical with 2–5 nuclear organizing regions (NORs). The C-banding as well as the fluorescent *in situ* hybridization (FISH)/genomic *in situ* hybridization (GISH) patterns are distinct, which partly are species specific and can be used for determination of evolutionary relationships and phylogeny (Linde-Laursen et al. 1986a,b, 1989, 1995; Linde-

Laursen and von Bothmer 1989; de Bustos et al. 1996; Taketa et al. 1999, 2000, 2001, 2005). One particularly deviating species is the autopolyploid *H. brevisubulatum* complex, which shows a large cytological differentiation, from populations with minute, intercalary C-bands (subsp. *brevisubulatum*) to populations with large, distal constitutive heterochromatin similar to that in rye (subsp. *violaceum*; Linde-Laursen et al. 1980). All these distinct forms are, however, fully interfertile and with full meiotic pairing in the hybrids (Landström et al. 1984).

The concept of genomes was explored by Kihara (1940) in wheat and related species, and this concept has been widely accepted and used in several plant groups. The original definition of a genome is the degree of chromosomal pairing in the meiosis of interspecific and intergeneric hybrids. A high meiotic pairing rate in hybrids indicates a closer relationship between the parental species than a lower pairing rate. The conclusions of relationship may, however, be hampered by an autosyndetic pairing of chromosomes from each parent or by a meiotic regulation of pairing, which can both promote and reduce the chiasma frequency. Attempts, particularly in the Triticeae, have been made to use genome relationship as a basis for taxonomic delimitation, thus that only species containing identical haplomes (or combination of haplomes) should be combined in a genus. In the most ultimate form, such a taxonomy was proposed by Löve (1982, 1984) and was partly adopted by Dewey (1984), resulting in a large number of new and monotypic genera in Triticeae. This system has not been widely accepted, and the genome concept has been criticized as a basis for drawing phylogenetic conclusions and for taxonomic delimitations (Kellogg 1989; Seberg and Petersen 1998; Petersen and Seberg 2003). Nevertheless the genome concept based on a wider range of scientific data than only chromosomal meiotic pairing has shown to be a valuable tool for practical purposes as well as for considerations of relationships.

In *Hordeum*, the genome concept based on the traditional criteria, that is, the meiotic pairing in interspecific hybrids, was studied in a detail in the 1970s and 1980s (cf. von Bothmer et al. 1989c, 1995a, and references therein). The genomic content or delimitations in haplomes based on pairing behavior in the meiosis of hybrids have been widely verified in later studies including *in situ* hybridization and various nuclear and plastid molecular studies (see under Evolution and Phylogeny in *Hordeum*). The assembled data suggest that four basic haplomes occur in *Hordeum*, namely, the **H** haplome in *H. vulgare* and *H. bulbosum*, the $\mathbf{X}_a$ haplome in *H. marinum*, the $\mathbf{X}_u$ haplome in the *H. murinum* complex, and the **I** haplome or modifications thereof in all other *Hordeum* species. There is a differentiation within the haplome groups, so, for example, the **H** haplomes of *H. vulgare* and *H. bulbosum* are not identical. Within the large **I** haplome group, there is a tendency for differentiation between major distribution areas (Eurasia vs. South and North America).

The designations of the different genomes in *Hordeum* have obtained an unfortunate ambiguity. Formerly, when little data were available, all species of *Hordeum*, thus also including *H. vulgare*, were presumed to contain the same genome/haplome, named **H**. When more data became available, it was evident that at least two major genomic groups occur, namely, *H. vulgare* and *H. bulbosum* on the one hand, and most other *Hordeum* species on the other. At that time, the separate and distinct genomes of *H. murinum* ($\mathbf{X}_u$) and *H. marinum* ($\mathbf{X}_a$) were also identified. During the 1980s, it was internationally adopted that the haplome of the wild species should be named "**H**" and that of *vulgare-bulbosum* should be called "**I**." However, a working group assigned by the International Barley Genetics Symposium (IBGS) developed a classification system for chromosomes and genomes of cultivated barley. One major suggestion of the group was that the chromosome numbering in barley should follow the homoeologous wheat chromosome numbering, thus partly changing the chromosome numbering in barley. The group suggested further that the *H. vulgare* haplome should be named **H** (Linde-Laursen et al. 1997), ignoring that the haplome of the wild species had been named **H** for a number of years. This proposal was adopted at the IBGS and since then, both the chromosome numbering system and the barley haplome designation follow the recommendation of the working group. This

has caused a considerable problem since scientists working with wild species have continued to use the designations in the opposite way, thus **I** for *H. vulgare* and *H. bulbosum* and **H** for the other wild species (apart from *H. marinum* $\mathbf{X}_a$ and *H. murinum* $\mathbf{X}_u$). How this problem will be resolved is presently not clarified. In this presentation, the recommendation from the IBGS working group (Linde-Laursen et al. 1997) is followed.

Evolution and phylogeny in *Hordeum*

Over the last 25 years, a number of new tools have been developed for studies of evolutionary pathways and phylogenetic patterns. For *Hordeum*, the new data have refined our view on relationships, phylogeny, routes of migration, and age of the various species or species groups. There is now a general and rather detailed picture of relationships, but depending on methods used and interpretation of data, details may differ between studies. In order to draw valid conclusions on general evolutionary or phylogenetic patterns, a complete set of species or at least a fair amount of accessions and species need to be included, but few investigations fulfill these requirements. Several studies include a restricted number of species or populations and can then at the most be of value for a certain area or for a limited group of species.

Terzi et al. (2001) found a clear separation of the three gene pools of barley by using 200 rapid amplified fragment polymorphism (RAPD) and sequence-tagged site (STS) markers. Their results indicate that *H. bulbosum* is more closely related to *H. murinum* than to *H. vulgare*. Also based on RAPD markers, Marillia and Scoles (1996) studied 39 *Hordeum* taxa, and their tree topology was consistent with systematic treatments based on morphology. However, *Hordeum erectifolium*, *H. jubatum*, and, to a lesser extent, *H. bulbosum* occupied positions different from previous classifications. Based on six repetitive DNA sequences of barley and including 46 taxa (31 species), Svitashev et al. (1994) found a good congruence to the structure with four genomes. However, they reported a unique position of *H. bulbosum* more different from *H. vulgare* than found, for example, by hybridization and meiotic pairing.

Komatsuda et al. (1999) studied the phylogeny on 26 diploid *Hordeum* taxa by means of a nucleotide sequence linked to the *vrs1* (six-rowed spike) locus on chromosome 2**H** in barley, regulating the spike row number. The **H** (*H. vulgare* and *H. bulbosum*) and $\mathbf{X}_u$ (*H. murinum*) genome species are distinctly separated. The **I** and $\mathbf{X}_a$ (*H. marinum*) genomes are monophyletic; $\mathbf{X}_a$ is a sister group to the **I** genome; and the **I** genome group is highly homogeneous. Clark et al. (2005) studied 29 *Hordeum* species (42 taxa) by means of PCR in three different products from the seed-specific class of the sucrose nonfermenting-1-related protein kinase gene family. The variants A and B (with one exception) were distinct for the **H** genome (*H. vulgare* and *H. bulbosum*), and the variant C was diagnostic for the **I** genome species with the exception for *H. roshevitzii*. No bands were obtained for any subspecies or cytotypes of *H. murinum* and *H. marinum*, which is thus consistent with the distinct genomes for these two species. Vershinin et al. (1994), in the study of genera in the tribe Triticeae, included 29 species of *Hordeum* (32 taxa) by studying a family of tandemly organized nuclear DNA sequences (*dpTa1*). The **I** genome species could be separated from the ones with **H**, $\mathbf{X}_a$, and $\mathbf{X}_u$ genomes.

A number of studies including plastid molecular data are not in full agreement with results from studies of nuclear DNA. Provan et al. (1999), using SSR primers, found chloroplast polymorphism in 24 species (28 taxa). *H. vulgare* is separated from all other *Hordeum* species (*H. bulbosum* was not included in the study). The other *Hordeum* accessions tended to be resolved in groups containing American and Asian species. A number of South American diploids shared a single cpSSR haplotype. The chloroplast DNA study by means of restriction site variation by Doebley et al. (1992) of 37 *Hordeum* taxa divided the genus into several distinct groups. These groups mainly correspond to the major genomic groups. Their data suggest that *H. chilense* is the ancestor of *Hordeum procerum* and that diploid *H. brachyantherum* may be the maternal parent of *H. depressum*.

The hitherto largest study of the chloroplast genome in *Hordeum* was reported by Jakob and Blattner (2006) with altogether 249 populations

(875 individuals) representing all 31 species of the genus. By sequencing the *trnL–trnF* region, they found 88 different haplotypes and a complicated, reticulate diversity pattern. No less than 18 chloroplast haplotypes occurred in a single species (*H. patagonicum*) and up to six species shared the same haplotype. Missing chloroplast haplotypes in the European/Mediterranean taxa indicate a large extinction of chloroplast lineages in this region. Survival of the more ancient lineages in East Asia and in North America showed a pattern markedly differing from that found by nuclear DNA estimations (Blattner 2004, 2006). It is evident that ancient haplotypes occur together with their descendants, and these ancient chloroplast types have survived for at least 4 million years. The long-term survival of chloroplast haplotypes in some geographic areas and phylogenetic groups and their extinction in others will considerably affect phylogenetic inference from chloroplasts back to 9–12 million years. These unpredictable extinctions and survivals and the utilization of restricted materials are probably the main reasons for the large incongruence between studies of nuclear and cytoplasmic molecular DNA and between different reports on the DNA variation in plasmids.

The most comprehensive studies on evolution, phylogeny, and migration routes have been made by Blattner and coworkers based on nuclear as well as plastid DNA data (Blattner 2004, 2006; Jakob and Blattner 2006; Jakob et al. 2004). Their studies of nuclear sequences in a large material are completely congruent with the four *Hordeum* genomes (**I**, **H**, $\mathbf{X}_a$, and $\mathbf{X}_u$). The ancient split in the Triticeae took place ca. 13 mya when the two main lineages were separated, that is, between the *Triticum* and the *Hordeum* species groups (Gaut 2002). *Hordeum* as a genus differentiated around 12 mya, and with the **H** genome group from Southwest Asia (*H. vulgare* and *H. bulbosum*) being the oldest. This corresponds well with the theory that Eurasia is the original area for the Triticeae (Hsiao et al. 1999). The oldest split, which probably is simultaneous with the origin of the genus, was between the **H** and the $\mathbf{X}_u$ genome (*H. murinum*). It seems well proven that *H. marinum* (genome $\mathbf{X}_a$) is a sister group to the remaining species in the genus (the **I** genome), thus, in contrast to many other studies, particularly of the chloroplast genome, showing the monophyletic genealogy of *H. marinum*. The splits between *H. vulgare* and *H. bulbosum* and between *H. marinum* and the **I** genome species are both estimated to be around 7 million years. The Old and New World species were separted 5 mya. An increased evolutionary radiation rate took place in the **I** genome group ca. 3 mya, which corresponds well with the differentiation of the New World species. The North American species *H. brachyantherum* is a sister group to all South American species, and colonization took place through migrating birds. In the South American diploid group, *H. chilense* seems to be the most original one, giving rise to two separate lineages, one in southernmost South America (e.g., *H. comosum* and *H. patagonicum*) and the other with a more northerly distribution (*H. muticum* and *Hordeum cordobense*). There have been two independent and more recent long dispersals back to North America from a South American diploid, namely, the two annuals *H. intercedens* and *H. pusillum*, ca. 1.2 mya.

It is significant that polyploids occur in all the four major clades (genomic groups), but apart from *H. bulbosum* and *H. brevisubulatum*, which are of autopolyploid origin, all other species are alloploids or at least segmental alloploids. Most polyploids can be traced to different combinations of original diploids (see under the species). *H. roshevitzii*, which is evidently closely related to several polyploids (Blattner 2004) has a key role here. Central America (*H. guatemalense*) was colonized from the North. *H. marinum* took part in the differentiation of the two tetraploids *H. secalinum* and *H. capense* around 0.4 mya (Blattner 2006).

Some groups are in a very active phase of speciation, which is particularly evident in the *H. patagonicum* group in southern South America. Adaptive radiation has resulted in a complex morphological and ecological differentiation. An even larger differentiation, also over a much larger area (from western Turkey to eastern China) is evident in the *H. brevisubulatum* complex with genetic, cytogenetic, and haplotype

diversity (Linde-Laursen et al. 1980; Landström et al. 1984; Jakob and Blattner 2006).

Gene pools and the potential for gene transfer to cultivated barley

Over the years, much effort has been invested in the evaluation of the potential use of wild relatives in barley improvement. Several of the wild species have proven to be of interest as gene sources since they contain traits of agricultural interest, such as disease resistance, stress tolerance, and various quality traits. Hybridization patterns and the success rate in conventional crosses are important for the possibility to use exotic material and wild germplasm in breeding. Interspecific hybridization and backcrosses have been performed with almost all wild species in combination with barley, and there is a good picture of the interspecfic crossability and some general conclusions can be drawn (von Bothmer et al. 1983; Finch and Bennett 1984). Cultivated barley functions poorly as the female parent in combinations with all ploidy levels of wild species with <10% seed set, lowest with diploids (0.9%) and highest with hexaploids. Barley functions considerably better as the male parent, yielding a seed set of 37%–45% in combinations with all ploidy levels. A prominent pattern is that combinations at the diploid level are difficult to germinate and to raise viable hybrids even though there has been a relatively high seed set. The emerging seedlings are weak and die early. A postzygotic sterility barrier seems to be in operation. The only viable plants reported at the diploid level (apart from *H. bulbosum* and *H. vulgare* subsp. *spontaneum*) are in the combination with diploid *H. marinum*, but here the resulting plants were haploids of *H. marinum* (2n = 7), caused by selective elimination of the barley chromosomes. The raising of hybrid plants after a seed set is fairly good with tetra- and hexaploid wild species. The polyploid species of section *Critesion* give a high seed set and plant establishment in combination with barley. The hexaploid *H. lechleri*, in particular, showed good compatibility. Backcrosses of primary hybrids to barley have, however, turned out to be difficult; only a single case has been successful (von Bothmer et al. 1988; von Bothmer and Linde-Laursen 1989). A complicating factor is that hybrids often are cytologically unstable, resulting in elimination and, more rarely, duplication of *H. vulgare* chromosomes (Linde-Laursen and von Bothmer 1988). Triple and complex interspecific hybridization is more successful than direct backcrosses, and one combination gave rise to a presumed apomictic offspring (von Bothmer et al. 1988, 1989a).

Hybridization between cultivated barley and its wild ancestor, *H. vulgare* subsp. *spontaneum*, shows no sterility barriers. Crosses result in high seed set, good germination, and establishment of hybrids, which indicates a close relationship between the two diploid subspecies (Brown et al. 1978; Giles and von Bothmer 1985; Asfaw and von Bothmer 1990). Backcrosses to either of the parents or raising F_2 generations show no sterility barriers, so the wild form is a good source as a donor of exotic germplasm in breeding. Due to the genetic drag of "wild traits," subsp. *spontaneum* has only to a rather limited extent so far been used in conventional breeding programs despite the fact that several interesting agronomic traits (particularly disease resistance) have been found in this wild form (see under the species).

There are comparatively weak crossing barriers between cultivated barley and *H. bulbosum* showing good seed set and plant establishment (Pickering and Johnston 2005, for references). The mechanism of selective elimination of the *H. bulbosum* chromosomes was found early and was developed as a method for production of doubled haploids in barley and wheat (Kasha and Kao 1970; Forster et al. 2007). In later years, there has been a focus on the use of *H. bulbosum* as a gene donor particularly for disease resistance traits in stable hybrids with barley (see under *H. bulbosum*).

Based on the success rate in crosses, the gene pool concept has been applied to the species in *Hordeum* (cf. von Bothmer et al. 1995a). To the *primary gene pool* of *H. vulgare* subsp. *vulgare* belong all the various forms of cultivated barley, such as landraces, genetic stocks, and all primitive or advanced breeding material, as well as the wild progenitor (subsp. *spontaneum*). There are no

sterility barriers, and gene exchange is easily performed (apart from the problem to use exotic, nonadapted material in a conventional breeding program). The *secondary gene pool* consists of the single species *H. bulbosum*, also with the **H** genome. Hybridization and backcrosses to barley are possible, but with a certain reduction in crossability and hybrid fertility. All other species, that is, the species with the $\mathbf{X}_u$ (*H. murinum*), $\mathbf{X}_a$ (*H. marinum*), and **I** genomes (all other *Hordeum* species), constitute the *tertiary gene pool* of barley, namely, all those crossing combinations, which are extremely difficult to achieve.

Systematics in *Hordeum*

Despite homogeneity in some gross morphological traits such as the characteristic triplets and setaceous or flattened, dorsally placed glumes, there is no widely accepted infrageneric classification congruent with morphological, cytogenetic, or molecular data. Several systems have been proposed over the years (see Petersen and Seberg 2003 for a review and references). The Russian taxonomist Nevski (1941) was a pioneer in studies of grass systematics, and particularly in *Hordeum* and *Elymus*. He presented a monograph for the entire *Hordeum* genus, including all at that time known species (he also described some new ones; see Table 2.1). He recognized five sections where *H. bulbosum* was placed in a separate section, section *Bulbohordeum*, and all annual species, thus including *H. vulgare*, in section *Hordeastrum*. The perennial South American species were referred to section *Anisolepis*, and the South and North American species with glumes diverging at maturity were referred to section *Critesion*. Nevski included the remaining species, including, among others, the North American *H. brachyantherum*, in section *Stenostachys*.

When the concept of genomes was presented, it was suggested that a classification should follow the genomic groups and that a genus should be monophyletic, comprising a single haplome or an identical combination of haplomes in polyploids. Löve (1982) and Dewey (1984) both proposed this basis for classification, and Löve described a number of new genera in the Triticeae. *Hordeum* was split into two genera, *Hordeum* sensu stricto, including cultivated barley and its wild ancestor. All other species were referred to the genus *Critesion*. In the light of later studies on genomes revealing four basic haplomes, this classification is neither congruent with genomic content nor with molecular or morphological data, hence cannot be used as a taxonomic basis. Petersen and Seberg (2003) described a new section, *Sibirica*, including *H. bogdanii* and *H. roshevizii*, based on a combination of two nuclear and two plastid molecular data sets. Blattner (2004) argued that this classification bears more phylogenetic and geographic information than earlier systems (cf. von Bothmer et al. 1995a) but disagrees partly with karyological and genomic data. It is further complicated by adding the polyploid species, which, in several cases, is the result of intersectional hybridization. Blattner (2006) argues that internal transcribed spacer (ITS) region data strongly support the view that the four genome groups represent monophyletic units, which then should result in four sections. According to these data, *H. murinum* ($\mathbf{X}_u$) and *H. marinum* ($\mathbf{X}_a$) should be placed in two separate sections, but since the *H. marinum* haplome is also found in some of the polyploids (*H. secalinum*, *H. capense*, and *H. brachyantherum*, 6x), it would not reflect a classification into monophyletic groups. Blattner argues that this large section would consist of three genetically and geographically distinct clades of diploids, viz., *H. marinum*, Old World **I** genome species, and New World **I** genome species. Until further data have been presented, the more conservative infrageneric classification is kept here (Table 2.1).

THE SPECIES IN *HORDEUM*

Altogether, the genus *Hordeum* contains 31 presently recognized species and a number of infraspecfic units. This presentation treats all taxa but does not aim to give an extensive morphological and taxonomic treatment of them. For more elaborate descriptions on taxonomy, synonymy and morphological variation is referred to the more specialized taxonomic treatments cited under the species and in a monographic summary made by

Table 2.1. Species in the genus *Hordeum* (chromosome numbers: 2x = 14; 4x = 28; 6x = 42; life form: a = annual; p = perennial; a/p = annual or perennial)

Species and author	Number of subspecies	Chromosome number	Life form	Distribution
Section *Vulgare*				
H. vulgare L.	2*	2x	a	Cultivated; the wild form (subsp. *spontaneum*) Greece to Afghanistan
H. bulbosum L.	—**	2x, 4x	p	Mediterranean, east to Afghanistan
H. murinum L.	3	2x, 4x, 6x	a	Worldwide as a weed, native to Central Europe, Mediterranean, east to Afghanistan
Section *Anisolepis*				
H. pusillum Nuttal	—	2x	a	Most of USA
H. intercedens Nevski	—	2x	a	S.W. USA, N.W. Mexico
H. euclaston Steudel	—	2x	a	Central Argentina, S. Brazil, Uruguay
H. flexuosum Steudel	—	2x	a/p	N.E. Argentina, Uruguay
H. muticum Presl	—	2x	p	N.W. Argentina, N.E. Chile, Bolivia, Peru, Ecuador, Colombia
H. chilense Roemer & Schultes	—	2x	p	Central Chile, W. Argentina
H. cordobense Bothmer et al.	—	2x	p	Central and N. Argentina
H. stenostachys Godron	—	2x	p	Central and N. Argentina, Uruguay, S. Brazil
Section *Critesion*				
H. pubiflorum Hooker f.	2***	2x	p	The Andes area in Argentina and Chile
H. comosum Presl	—	2x	p	The Andes area in Argentina and Chile
H. jubatum L.	—****	4x	p	Native to N. America, widespread as a weed
H. arizonicum Covas	—	6x	a/p	S. USA and N. Mexico
H. procerum Nevski	—	6x	p	Central Argentina
H. lechleri (Steudel) Schenck	—	6x	p	Argentina and Chile

Section *Stenostachys*				
H. marinum Hudson	2*****	2x	a	Widespread as a weed, native to W. and Central Europe, Mediterranean, east to Afghanistan,
H. secalinum Schreber	—	4x	p	W. Europe, parts of the Mediterranean
H. capense Thunberg	—	4x	p	South Africa
H. bogdanii Wilensky	—	2x	p	Central Asia
H. roshevitzii Bowden	—	2x	p	Central Asia
H. brevisubulatum (Trinius) Link	5	2x, 4x, 6x	p	W. Turkey to N.E. China
H. brachyantherum Nevski	2******	2x, 4x, 6x	p	Western N. America, E. Canada, Kamtchatka
H. depressum (Scribn. & Sm.) Rydb.	—	4x	a	S.W. USA
H. guatemalense Bothmer *et al.*	—	4x	p	Guatemala
H. erectifolium Bothmer *et al.*	—	2x	p	E. Argentina
H. tetraploidum Covas	—	4x	p	S. Argentina
H. fuegianum Bothmer *et al.*	—	4x	p	S. Argentina, S. Chile
H. parodii Covas	—	6x	P	Central and S. Argentina, S. Chile
H. patagonicum (Haumann) Covas	5	2x	P	S. Argentina, S. Chile

*A third subspecies (subsp. *agriocrithon*) is sometimes recognized;
**the two cytotypes are sometimes recognized as separate subspecies;
***the two subspecies are sometimes recognized as separate species;
****two subspecies are sometimes recognized in the USA;
*****two separate species are sometimes recognized;
******two separate species are sometimes recognized.

von Bothmer et al. (1995a). Here, a broader, comprehensive summary of various aspects of the wild species such as relationships, genetic diversity, agronomic potential, or ecological preferences are given. English vernacular names for the different species and subspecies (when available) follow Barkworth et al. (2007).

Section *Vulgare*

Section *Vulgare* includes the three species *H. vulgare*, *H. bulbosum*, and *H. murinum*.

H. vulgare

Cultivated barley and its wild ancestor, which is still abundant in Southwest Asia, constitute a species complex where the different components are closely related. The systematic delimitation has been widely discussed particularly concerning at which taxonomic rank the different forms (cultivated and wild) should be treated. There are two main options: either to treat them as separate species, *H. vulgare* and *H. spontaneum* (cf. Nevo 2006), or as two subspecies, *H. vulgare* subsp. *vulgare* and subsp. *spontaneum*. Due to conspicuous adaptive radiation, domestication, migration, and subsequent breeding, a large variation amplitude has been acquired in the cultivated form. Spontaneous crosses and backcrosses between the two taxa in areas where they meet and overlap have further added to a complex picture of relationship, gene exchange, and variation pattern. All forms included in the *H. vulgare* species complex are highly crossable, giving rise to fully fertile offspring (apart from sterile mutants), which strongly indicates that the major forms (cultivated vs. wild) most appropriately are treated as subspecies, which is also followed here (cf. Asfaw and von Bothmer 1990).

A six-rowed, brittle rachis form collected in Tibet was recognized as a separate species, *Hordeum agriocrithon*, and was also considered as the ancestor of six-rowed, cultivated barley (Åberg 1938, 1940). However, brittle rachis types of two- and six-rowed barley are not uncommon and occur in many areas such as Morocco, the Middle East, and western China. They represent weedy forms and segregation products and are not true wild forms. The weedy, six-rowed, brittle rachis form is sometimes distinguished as subsp. *agriocrithon*.

The true wild form, subsp. *spontaneum*, is two-rowed throughout and is distinguished from cultivated barley by a combination of characters: brittle rachis, leading to easy shattering of the ripe, or near ripe, triplet. The edges of rachis as well as glumes and rachilla have long, tough, forward-pointing bristles, which is an adaptation to animal dispersal. The wild form has shrunken seed, not plump seed, as in cultivated barley. It is usually slender and with a weaker straw than cultivated barley.

To assess the distribution area of the true wild form is not easy due to the overlap in morphological characters, introgression, and occurrence of brittle rachis types in landraces of cultivated barley (see further under Domestication of Barley). The assumed natural occurrence of subsp. *spontaneum* is from the eastern Aegean islands and (possibly) Egypt over Middle East through Iran and eastward to Afghanistan, western Pakistan, and Tajikistan.

The ecological preferences cover a wide range of habitats such as meadows or mesic steppes to semiarid and arid regions, sometimes even on salty soils. Subsp. *spontaneum* and some brittle rachis forms of subsp. *vulgare* are often weedy and associated with other crops.

The center of diversity for subsp. *spontaneum* is the Middle East, particularly Israel and Jordan. Wild barley has been subject to intense studies, and the ecological and physiological diversity in the Middle East area has been particularly explored.

Cultivated and wild barley are primarily inbreeders, but the wild form is usually more open flowering and has a somewhat higher outcrossing rate than subsp. *vulgare*. Usually, wild barley has more or less *cleistogamou*s flowers, but under stress conditions, it can be more open flowering (or anther exserted; Parzies et al. 2000, 2008). Open flowering, *chasmogamy*, in subsp. *spontaneum* is controlled by a single recessive gene, which is located on chromosome 1**H** (Kurauchi and Makino 1998). The normal outcrossing rate

has been determined to maximum ca. 1.7%–1.8% in several studies from Israel (Brown et al. 1978) and Jordan (Abdel-Ghani et al. 2004; Parzies et al. 2008). Occasional outcrossing rate up to 10% has been noted (Brown et al. 1978; Nevo 1992). Despite a very high inbreeding rate of around 98%, a high degree of diversity is maintained. This is obviously sufficient as the basis for rapid adaptation to varying environmental conditions. The high level of heterozygosity linkage disequilibrium (LD) was shown in a study of 18 nuclear genes in 25 accessions of subsp. *spontaneum*. Intralocus LD decay is similar to that in outcrossing species (Lin et al. 2002; Morrell et al. 2005). The viability of pollen in subsp. *vulgare* and in subsp. *spontaneum* is high even after 26 h at temperatures of up to 40°, which shows that both subspecies retain a sufficiently high viability to ensure successful cross-fertilization (Parzies et al. 2005).

Seed dormancy is a physiological trait separating domesticated and wild forms. In contrast to cultivated barley, subsp. *spontaneum* has a strong, developed dormancy and needs "after-ripening," usually in dry conditions at high temperatures (30–35°) in order to sprout, which is an adaptation to survival in the hot summers (Gutterman et al. 1996; Zhang et al. 2002). After-ripening is important to prevent the seed from germinating directly after maturation and after a late rain at the beginning of the dry summer (Gutterman and Gozlan 1998; Gozlan and Gutterman 1999). The seed dormancy is regulated by a multigene system, located to several of the **H** chromosomes (Vanhala and Stam 2006). There are differential patterns of germination in mesic and xeric ecotypes, where adaption to the drier habitats includes increased diversity of after-ripening, increased drought tolerance, and improved root length growth (Chen et al. 2004). There are large fluctuations of population growth rate due to the variability in annual rainfall. The importance of the seed bank differs widely among populations. Seed dormancy has no demographic effect on populations from a Mediterranean climate area, whereas it is of fundamental importance for drier habitats (Volis et al. 2004).

Wild barley has an allelopathic effect on the germination and growth of other plants. Hamidi et al. (2006) found that a high concentration of the shoot extract of subsp. *spontaneum* gives a significantly reduced germination of wheat seeds. Lower concentrations of the wild barley extract stimulate the growth of its own plants. Since subsp. *spontaneum* sometimes occurs as a weed, allelopathic effects could possibly be used for weed control. Soil previously cropped with black mustard (*Brassica nigra*) considerably reduced the growth of wild barley (Tawaha and Turk 2003).

Genetic diversity

The Middle East, primarily Israel and Jordan, is the diversity center for subsp. *spontaneum*, as demonstrated in morphological and adaptive traits, proteins, and various types of molecular markers, whereas there is less variation in more peripheral areas such as central Asia (Volis et al. 2001; Liu et al. 2002; Tanyolac 2002; Baek et al. 2003; Vanhala et al. 2004; Ozkan et al. 2005; Al-Saghir et al. 2007). Much of the variation is correlated to adaptive traits, such as ecological and geographic parameters. Genetic diversity is larger among than within populations, though there can also be large intrapopulation diversity (Rijn et al. 2000; Turpeinen et al. 2003; Vanhala et al. 2004). A strong geographic differentiation is prominent as revealed by nucleotide sequence diversity in several genes (Lin et al. 2001, 2002; Morrell et al. 2003). In several gene loci, western populations show a significantly higher diversity than in other areas. Wild barley has a mosaic variation pattern generated by evolutionary processes, such as "selective sweeps" mirrored in genes showing a high homogeneity, whereas other loci (such as *Adh3* and *G3pdh*) have retained strong geographic patterns despite a high degree of migration. A similar pattern was shown in the *Isa* gene, with a putative role in plant defense and with a multipurpose function, possibly also important for grain quality (Cronin et al. 2007). Selective sweeps seem to be more common in mesic than in xeric conditions, resulting in a diversifying selection in the dryer climate (Turpeinen et al. 2001; Cronin et al. 2007). There is a positive correlation between climate and genome size as

shown in subsp. *spontaneum* from ecologically diverse populations in Israel (Turpeinen et al. 1999).

The pattern of single nucleotide polymorphisms (SNPs) indicates a large heterogeneity, and the supposed low outcrossing rate is obviously no barrier for the occurrence of recombinant haplotypes. Generally, despite the postulated high degree of self-pollination, subsp. *spontaneum* has a surprisingly high heterogeneity/heterozygosity. This may be due to a high rate of migration or to the wild form perhaps being more open flowering and hence more outcrossing than expected.

Ecotype differentiation

H. vulgare subsp. *spontaneum* has been a model plant visualizing the relation between genetic diversity and physiological adaptational traits, habitat preferences, and geographic differentiation. Distinct intrapopulational diversification has even been shown, particularly in the famous "Evolution Valley" in Israel. At the Neve Yaar microsite, significant genetic divergence and diversity were found in four microniches (Huang et al. 2002). Shade and soil subpopulations show higher genetic diversity than others, and the lowest diversity occurs in "sun-rock" subpopulations. Diversifying natural selection seems to act upon regulatory regions of the genome, which results in adaptive divergence. Fixation at particular loci at a certain microniche suggests directional selection. Various stress-related characters, such as salt tolerance (Pakniyat et al. 1997, 2003), drought tolerance, and sensitivity (Chen et al. 2002; Volis et al. 2002a,b, 2004; Elberse et al. 2003; Suprunova et al. 2004) have been extensively studied. Drought tolerance is related to certain morphological and physiological traits such as strong seed dormancy, small seed size, and low seedling vigor (Volis et al. 2002a,b; Chen et al. 2004). Wild barley shows a high drought tolerance of seedlings (Zhang and Gutterman 2003). The repair efficiency of double-strand DNA is tightly correlated to heat tolerance. For various populations, the repair mechanism functions well at a certain temperature but not at others (Lupu et al. 2006).

Disease resistance

Powdery mildew caused by the fungus *Blumeria graminis* f. sp. *hordei* is a serious disease in many areas. Screening of subsp. *spontaneum* material from various areas, for example, Greece and Turkey, revealed a high degree of resistance (Zeybek et al. 1999; Zeybek and Yigit 2002). The genetic background of the resistance to powdery mildew consists of monofactorial dominant and recessive loci. The identity linkage with the *Mla* resistance locus is especially of interest. Previously known alleles, new alleles, and bifactorially inherited resistance with a broader resistance spectrum have been reported (Kintzios and Fischbeck 1996; Lehmann et al. 1998; Backes et al. 2003; Repkova et al. 2006; Dreiseitl et al. 2007).

Resistance to scald (causal agent *Rhynchosporium secalis*) and development of molecular markers is another prebreeding goal where wild barley is a rich source for resistance. Resistance loci are present in at least five of the seven **H** chromosomes, and several new genes have been detected as well as quantitative trait loci (QTL) for field resistance (Abbot et al. 1991, Genger et al. 2003, 2005). Resistance to leaf rust caused by *Puccinia hordei*, located on chromosomes 2**H** and 3**H**, was reported from subsp. *spontaneum*, and further loci are probably also present (Feuerstein et al. 1990; Kopahnke et al. 2004). Even more promising for the perspective of barley improvement are the studies of multiple disease resistance. Fetch et al. (2003) found a high degree of multiple resistance in Israeli material, and two accessions showed resistance to all six pathogens studied.

Hordatines are *Hordeum*-specific secondary metabolites with a strong antimicrobial activity. Wild barley from various habitats in Israel showed significant differences between the 50 accessions studied in the accumulation of hordatines in seedlings. This is obviously correlated with ecogeographic factors (Batchu et al. 2006).

Breeding/prebreeding

The potential for subsp. *spontaneum* to be more widely used in barley breeding programs has been explored, particularly the development of efficient marker systems. The high level of diversity and heterozygosity for disease resistance indicates

that wild barley is a rich and largely untapped source of unique resistance alleles for barley improvement. The major locus for photoperiodic response was studied in introgression lines (ILs) of subsp. *spontaneum* into two barley lines (von Korff et al. 2004a). These lines containing the *Ppd-H1* locus were significantly earlier than the elite parents. QTLs for water stress tolerance and powdery mildew resistance located on chromosome 4**H** and 6**H**, respectively, were reported by Ivandic et al. (2003). Subsp. *spontaneum* shows large variation in developmental traits related to heading date.

Populations from the Himalayan region had no vernalization requirement but were extremely sensitive to short photoperiods, whereas other materials showed a winter habit and a third group had spring or facultative growth habit (Karsai et al. 2004).

Wild barley is mainly a winter annual and it may be used to establish permanent pastures for grazing in certain areas (El-Shatnawi et al. 2004). Barley straw is commonly used as animal feed in many developing countries, and increase in nutritional value would have a large impact on animal production. Nutritional quality is a complex character and it is greatly affected by environmental conditions. Grando et al. (2005) developed a marker system to facilitate the improvement of straw quality in barley by introgression from subsp. *spontaneum*.

QTLs for yield and yield-determining traits were detected in wild barley (von Korff et al. 2004b). Recombinant chromosome substitution lines (RCSLs) were developed by Matus et al. (2003) and Hori et al. (2005) to introduce chromosome segments of wild barley into cultivated barley, including yield components, malting quality, and domestication traits. Despite their overall inferior phenotypes, these RCSLs contributed favorable alleles for agronomic traits and malting quality. In most cases, the introgression of exotic alleles resulted in loss of desirable phenotypes in the cultivated parent. Hori et al. (2005) used five qualitative and nine quantitative traits, and several QTL alleles showed agronomic positive effects. Li et al. (2006) found 10 QTLs with increased effects for ear length, spikelet number, and number of grains per spike in advanced backcross populations. By using grain yield and drought-tolerant RCSLs, it was possible to select a group of genotypes tolerant and others sensitive to drought (Inostroza et al. 2007).

Subsp. *spontaneum* is compatible in crosses with wheat, and a complete set of whole chromosome addition lines and five telosomic addition lines have been developed. The evaluation of these addition lines may reveal agronomically important genes for wheat improvement (Taketa and Takeda 2001).

Improved methodology for gene discovery, development of efficient marker technology, better understanding of gene regulation, and improved technology for breeding has considerably increased the efficiency in introgression of exotic germplasm into an advanced background. This has also been the case for a better/more efficient utilization of subsp. *spontaneum* in barley improvement programs (Nevo 2006; Feuillet et al. 2008).

H. bulbosum, bulbous barley

H. bulbosum is a perennial, tall (up to 1.3 m), and erect growing species, with a spike similar in appearance to *H. vulgare*. The leaves are broad and have large auricles at the base. It is a geophytic grass with a bulbous swelling (corm) at the base of the culm contributing to an efficient vegetative reproduction. The species has an endodormancy system of the meristem, which means that it ceases to grow during the summer and it survives the summer drought by dormancy of the corm (Volaire and Norton 2006). The habitat preferences show large ecological amplitudes ranging from wet pastures, drier hillsides, abandoned fields, and roadsides. The occurrence is most often scattered, but rarely the species is found in denser stands.

The species is almost exclusively outcrossing with typical syndromes for an outbreeder, such as large, exserted anthers at anthesis with a very rich pollen production (Johansen and von Bothmer 1994). There is a clear time difference between maturity of pollen and receptivity of stigma, usually several days, as well as a variation in

pollen viability (Parzies et al. 2005). *H. bulbosum*, like probably also *H. brevisubulatum*, has a two-locus self-incompatibility system (Lundqvist 1962). Pistils of *H. bulbosum* have a high level of expression of the cDNA sequence *Hbc8-2*, which may be associated with the self-incompatibility system (Gudu et al. 2002). The allogamous breeding system of *H. bulbosum* is reflected in the variation pattern with primarily a large intrapopulational variation (de Bustos et al. 1998; Albayrak and Gözükirmizi 1999; Okumus and Uzun 2007).

The general distribution of *H. bulbosum* is the Mediterranean basin and eastward to Afghanistan and Tajikistan (Jørgensen 1982; Baum and Bailey 1985a). Two chromosomal races occur: a diploid cytotype is distributed in the western part of the Mediterranean area and a tetraploid race in the east. The border between the two races is very strict in Central Greece and in the Cyrenaica area in Libya. In a distance of only a few kilometers, there is a transition from one cytotype to the other without overlap between the two races. Occasional occurrence of triploids has been reported elsewhere, such as in Spain (Otiz et al. 1985). The two chromosomal races have been described as different subspecies (subsp. *bulbosum* and subsp. *nodosum*; Baum and Bailey 1985a,b), but the morphological characteristics show very much overlap, and these taxa are not widely accepted (Jørgensen 1982).

As a more or less obligate outbreeder, *H. bulbosum* shows a high degree of heterozygosity and polymorphism in comparison with barley and wheat as shown in a number of studies with molecular markers (Albayrak and Gözükirmizi 1999; Jaffé et al. 2000; Salvo-Garrido et al. 2001; Gudu et al. 2002; Okumus and Uzun 2007).

As the single species, *H. bulbosum* constitutes the secondary gene pool of barley (von Bothmer et al. 1995a). The two species share the **H** genome, however, in modified forms. The separation between them is estimated to ca. 6 mya (Blattner 2006). Most studies concur that *H. bulbosum* and *H. vulgare* are comparatively closely related (Doebley et al. 1992; Pelger and von Bothmer 1992; Baum and Johnson 1996; Komatsuda et al. 1999; Terzi et al. 2001; Petersen and Seberg 2003; Blattner 2004; Clark et al. 2005; Jakob and Blattner 2006), whereas others point to a more distant relationship (Svitashev et al. 1994; El-Rabey et al. 2002).

The first attempts to hybridize *H. vulgare* with *H. bulbosum* resulted in plants with the morphology of barley, and they turned out to be haploids of barley. The *bulbosum* chromosomes are eliminated in the first division cycles after fertilization and before a full-grown embryo has been established (Gernand et al. 2006; Forster et al. 2007). The phenomenon of selective chromosome elimination was explored and established as a breeding method to produce haploids in barley as well as in wheat (cf. Arabi and Nabulsi 2002). The elimination method for doubled-haploid production is now replaced by more efficient methods, like microspore and anther cultures, and crosses with maize, which causes selective elimination of the maize chromosomes. The elimination process is genotype-dependent as well as environmentally dependent, and use of other parental combinations (particularly of *H. bulbosum*) resulted in stable hybrids.

Several traits of agronomic interest are found in *H. bulbosum*, which has spawned great interest in the species as a gene donor (Pickering and Johnston 2005). The interspecific hybridization of *H. bulbosum* × *H. vulgare* and the exploration of introgression of desirable traits to barley, in contrast to the earlier developed chromosome elimination system for doubled-haploid production, have been widely explored in recent years. The development of efficient techniques for haploid production, as well as for production of stable hybrids, is reviewed by Pickering and Johnston (2005). As a basis for introgression, stable hybrids particularly with triploids and, to some extent, tetraploids have become important (Gilpin et al. 1997; Zhang et al. 2001). Even in supposedly stable hybrids, selective elimination of *H. bulbosum* repeat units as well as of *H. vulgare* genomic DNA may occur as shown in the *Rrn* loci (Kumar and Subrahmanyam 1999). For detecting and characterizing introgression of *H. bulbosum* chromatin in barley, several techniques have been applied: molecular cytogenetics (Zhang et al. 1999; Pickering et al. 2000), PCR detection of retrotransponson-like segments (Johnston and

Pickering 2002), and restriction fragment length polymorphism (RFLP) (Gilpin et al. 1997). Linkage maps, which can give more detailed information about relationships between the two species as well as create a basis for the development of marker-mediated introgression, have been developed (Jaffé et al. 2000; Salvo-Garrido et al. 2001). The complex cytogenetic behavior, especially in meiosis in hybrids and haploids, has been widely studied (Zhang et al. 2002; Pickering et al. 2004, 2005, 2006a).

H. bulbosum as a gene source for disease resistance is of particular interest for breeding purposes (Pickering and Futrier 1993). Introgression of resistance from *H. bulbosum* to cultivated barley has successfully been demonstrated for soil-borne viruses in the resistance gene $Rym16^{Hb}$ (Ruge et al. 2003; Ruge-Wehling et al. 2006), scald (Singh et al. 2004; Pickering et al. 2006b), leaf rust and mosaic viruses (Walther et al. 2000), leaf rust and powdery mildew (Shtaya et al. 2007). Resistances to other diseases and insects, which have successfully been transferred to barley, are *Septoria pessrinii* (Toubia-Rahme et al. 2003), rice blast fungus (Kim et al. 2002), various rusts (Sadravi et al. 2007), Russian wheat aphid, and greenbug (Gianoli and Niemeyer 1998). Other traits of interest may be nutritive value for forage purposes (Arzani et al. 2006) and hordein composition (Yan et al. 2003a).

H. murinum

H. murinum, a winter or summer annual, is a polyploid complex with a pronounced self-pollination system mostly with cleistogamous flowers, small anthers, and stigma, which are mature and receptive at the same time. Under certain conditions, the flowers are more chasmogamous, contributing to an increased outbreeding (Savova Bianchi et al. 2002). The *H. murinum* complex is morphologically distinct: the central and lateral spikelets are large and inflated, resulting in thick, somewhat flattened spikes. Glumes, particularly of the central spikelet, are distinctly flattened with long, stiff hairs at the margins. The spike is extremely brittle at maturity, and the tough bristles at the margin of the spike axes and of the glumes and awns are effective mechanisms for zoochorous dispersal. The mature seeds easily adhere to the furs of animals or the shoes, socks, or clothes of humans. This dispersal mechanism is very efficient as was demonstrated by Manzano and Malo (2006), describing examples of long-distance dispersal by migrating ungulates, sheep in particular. The dispersal distance can be several hundred kilometers, thus showing rapid plant migration routes, of special importance for spreading of weeds.

The various forms and cytotypes of the *H. murinum* complex are sometimes treated as three separate species (Baum and Bailey 1984, 1989) or as two species, one of them with two subspecies (El-Rabey et al. 2002), but mostly as a single species and with three subspecies (Jacobsen and von Bothmer 1995; Barkworth et al. 2007), which is followed here:

1. *glaucum*, smooth barley, is a diploid spring/summer annual with a rapid development. It is a slender plant with short anthers (<0.6 mm); the lateral florets are of about the same length as the central one, and both the lateral and the central florets are stalked. The original distribution of subsp. *glaucum* is the warm southern parts of the Mediterranean region (North Africa) eastward to Afghanistan and western Pakistan in Southwest Asia.
2. *murinum*, wall barley or way barley, is a tetraploid winter annual; the central spikelet is sessile to subsessile (in subsp. *glaucum* and subsp. *leporinum*, it is clearly stalked); the lateral spikelets are longer than the centeral ones; the anthers are intermediate in length (0.8–1.4 mm). The original distribution area for subsp. *murinum* is the northern part of the Mediterranean and Western Europe.
3. *leporinum*, mouse barley, is a tall and robust plant (up to 110 cm under favorable conditions); the central spikelet is stalked and the lateral spikelets are considerably longer than the central one; the anthers are long (up to 3.2 mm). Subsp. *leporinum* occurs in two cytotypes, which are not possible to distinguish morphologically. The tetraploid has an original distribution in the Mediterranean area eastward to western Iran, and the

hexaploid cytotype occurs from western Turkey to Afghanistan.

The relationships and evolutionary pathways of the *H. murinum* complex are well documented, and most studies agree on the general pattern. The group has a quite unique genome $\mathbf{X_u}$, and the closest relatives are the **H** genome species, *H. vulgare* and *H. bulbosum* (cf. Doebley et al. 1992; Molnar et al. 1992; Komatsuda et al. 1999; Terzi et al. 2001; de Bustos et al. 2002; Blattner 2004; Clark et al. 2005). The split between the **I** and the $\mathbf{X_u}$ species is the most ancient in the genus *Hordeum* estimated to ca. 12 mya (Petersen and Seberg 2003; Blattner 2006; Pleines and Blattner 2008). The nature of the polyploidy has, however, not been quite resolved, but most studies indicate that alloploidy or segmental alloploidy prevails (Taketa et al. 2000).

Due to the effective reproduction with predominantly self-pollination leading to a high seed set, even under rather unfavorable conditions, combined with very efficient seed dispersal, all forms of the *H. murinum* complex have been established as a severe weed in most parts of the world. It is a tendency for the different subspecies/cytotypes to invade or colonize different areas on other continents. In Australia, the *H. murinum* complex was introduced before the 1840s, and it is now widespread and has adapted to various regions (Cocks et al. 1976). Subsp. *glaucum* has invaded warmer temperate areas in northern Australia; subsp. *leporinum* occurs mainly in the south and subsp. *murinum* only on Tasmania (Cocks et al. 1976). Similar distribution patterns are found both in South and North America (Jacobsen and von Bothmer 1995; Barkworth et al. 2007). The variation, distribution, and spread in different areas are widely studied, such as in New Zealand (Powell 1968), Spain (Ruíz-Fernández and Soler 2004), Iran (Sahebi et al. 2001; Sheidai and Rashid 2007), Poland (Mizianty 2006), and on the island of Juan Fernández, Chile (Baeza et al. 2002). The establishment and dispersal of exotic weeds, such as *H. murinum*, is promoted in Californian grasslands by the giant kangaroo rat, which is an endangered species (Schiffman 1994). From Greece and Spain, new subspecies have recently been described, interpreted as local lowland and montane endemics in these respective countries (Scholz 1999). The variation in the *H. murinum* complex is, however, much more complicated, and the recognition of local taxa seems not appropriate. The large polymorphism is evident in hordein variation, mainly congruent with the taxonomic delimitation into subspecies (Pelger and von Bothmer 1992; Amirouche and Misset 2003). There is a strong correlation between hordein variability, ploidy levels, and bioclimatic conditions, which was revealed in a study of Mediterranean, mainly North African, material (Amirouche and Misset 2003).

The weedy nature and effective dispersal unit of *H. murinum* can cause severe health problems in animals. The awns, with their tough bristles, migrate and may cause severe damage. A study of different dog races in Czech Republic showed that the awns/seeds localize to interdigital webs, external ear canals, and conjunctival sacs (Crha et al. 2003). The awns of *H. murinum* can even cause health problems in humans (Karagöz et al. 2006).

The aggressive, weedy nature of the *H. murinum* complex is well demonstrated, and much effort has been invested into studies of weed control. Introduced weeds have generally a superior competitive ability to the native species; for example, subsp. *leporinum* dominates at high nutrient levels and is a severe weed in wheat crops and other habitats in Australia (Groves et al. 2003; Florentine and Westbrooke 2005). Establishment of *H. murinum* is favored in permanent pastures by open spaces in the vegetation. By minimizing the gap size in permanent pastures, the management of the species can be improved as in saffron (autumn crocus) fields in parts of the Mediterranean (Makarian et al. 2007). Herbicide treatments have resulted in resistance to several of the most common herbicides, such as paraquat (Preston et al. 1991, 2005) and others (Matthews et al. 2000; Yu et al. 2007), and there is a risk of pollen-mediated gene flow of the herbicide resistance (Hidayat et al. 2006). Infection by the seed-borne fungal pathogen *Pyrenophora semeniperda* results in either the failure of infected seeds to germinate or the reduction of seedling vigor, since the

fungus seems to have a potential as a seed-borne biocontrol herbicide. A complex butenolide compound stimulates germination of several noxious weeds, including *H. murinum* subsp. *leporinum*, and thus has a potential as an agent for broad weed control and possibly for land restoration (Stevens et al. 2007).

A comparison of the two weedy annuals *H. murinum* and *H. marinum* shows a much higher degree of salt tolerance typical for a halophyte in the latter species, whereas *H. murinum* has a higher sensitivity, typical for wild glycophytes (Lombardi et al. 2000). *H. murinum* has a potential value for revegetation in areas degraded by landfill leacheates and shows a particular tolerance to higher zinc and iron concentrations (Adarve et al. 1998). It has also a certain potential as a pasture grass in semiarid rangelands in Australia (Bolger et al. 1999) and in the Mediterranean (Khair et al. 1999; El-Shatnawi et al. 2003a,b). Age and environmental conditions affect the nutritive value (El-Shatnawi and Al-Qurran 2003). The sowing rate has an effect on growth, forage, and seed yield but less so on protein content (El-Shatnawi et al. 2003a,b). Subsp. *leporinum* has high requirements for phosphorus (P) and nitrogen (N) for optimal development (Hill et al. 2006). As is typical for a winter annual, it shows a strategy with early release from seed dormancy after survival of a long, dry summer, which contributes to a quick growth and establishment of pastures.

H. murinum has some potential as a gene source for traits of agronomic interest. A large variability for resistance to barley scald within and among populations was reported in subsp. *glaucum* and subsp. *leporinum* from Australia, where the former was generally more resistant (Jarosz and Burdon 1996).

Apart from being an aggressive weed, *H. murinum* has other deleterious effects. Stripe rust caused by *Puccinia striiformis* f. sp. *tritici* has become an endemic disease on wheat in South Africa, and isolates of the rust fungus was found on *H. murinum* in the Eastern Cape (Boshoff et al. 2002). Increased resistance of pathogens in the wild species may play an important role for "oversummering" of the pathogens and is thus deleterious for cereal cultivation. Leaf scald and spot blotch (caused by *Bipolaris sorokiniana*) were reported on *H. murinum* from Turkey (Kavak 2003, 2005). The widespread resistance may create a particular selection pressure of the fungus favoring increased pathogenicity, which will complicate the breeding for resistance in barley. *H. murinum* subsp. *leporinum* is highly susceptible to the two root lesion nematodes *Pratylenchus neglectus* and *Pratylenchus thornei* in Australia, and may thus allow an increase and persistence of the nematode in cultivated soils (Vanstone and Russ 2001). This may compromise the use of resistant crops in rotation for managements of the two pest species. In Turkey, the Russian wheat aphid *Diuraphis noxia* causes damage on the wheat crop. After harvest, the aphid moves to *H. murinum* subsp. *glaucum* and may cause potential problems for successive wheat (and barley) cultivation (Elmali 1998).

The insect *Neoseiulus californicus* is a worldwide distributed phytoseiid predator, which has a significant potential in biological control programs for several crops. Raworth et al. (1994) reported that *H. murinum*, which produces pollen in early spring, can be utilized as an early food source for the predator, for example, in fruit orchards.

In crosses with *H. vulgare*, the *H. murinum* complex shows poor performance (von Bothmer et al. 1983; Savova Bianchi et al. 2002). The seed set is comparatively high with all ploidy levels (29%–55%), but only a single combination, with the hexaploid subsp. *leporinum*, resulted in two hybrid plants, which died before flowering. Also in crossing combinations with other *Hordeum* species, *H. murinum* has low crossability. Adult interspecific hybrids were mainly obtained in combinations with the tetraploid subsp. *murinum* (von Bothmer and Jacobsen 1986).

Section *Anisolepis*

This section comprises a group of eight American, mainly South American, exclusively diploid species, all containing the common **I** genome. They are annuals or perennials with narrow, linear spikes and brittle rachis. The glumes of

lateral spikes are dissimilar; the upper one is setaceous, and the lower one is flattened. Glumes of central spikelets are flattened. The rachis is often distinctly winged.

The three annuals, *H. pusillum*, *H. intercedens*, and *H. euclaston*, are closely related (von Bothmer et al. 1982; Blattner 2004, 2006), though the former two species are North American and the latter is confined to Central Argentina, Uruguay, and southern Brazil. There has probably been a long-distance dispersal by migratory birds from South to North America followed by isolation and further speciation (Blattner 2006). The three annual species are low-grown (luxuriant specimens occur under favorable conditions) and have a dense, stiff spike. The rachis internodes are "winged" and are distinctly broadest at the top.

H. intercedens, vernal barley or bobtail barley, is confined to a restricted area in southwestern California, adjacent islands, and the northwestern part of Baja California of Mexico. It occurs in alkaline vernal pools and along rivers and in occasionally flooded areas. The abundance on the Californian mainland has diminished markedly in recent years due to urbanization and loss of natural biotopes, but it is still fairly abundant where the native habitats are available. *H. intercedens* has a certain salt tolerance (Garthwaite et al. 2005). It is probably one of the ancestors of the allotetraploid species *H. depressum* (Baum and Bailey 1987; Salomon and von Bothmer 1998).

H. pusillum, little barley, is a common species all over the United States except in the southwestern part, where it is replaced by *H. intercedens*. There are occasional findings in southern Canada and in northern Mexico, and it is introduced as a weed in parts of Europe (however uncommon). It occurs often as a weed in many types of grasslands, shortgrass pastures, roadsides, and waste places, often on alkaline soil and marshlands (Todd et al. 2004; Ashworth et al. 2006). It has a good salt tolerance (Kindscher et al. 2004). Carbonized caryopses of *H. pusillum* are abundant in archaeological remains in eastern North America. It was obviously formerly gathered as a wild plant by early settlers, but there are no indications that the species has been under cultivation at that time (Weiss et al. 2006). There is a hypothesis that *H. pusillum* was even domesticated, but that has not been proven and the old, preserved, carbonized caryopsis do not differ in any significant way from present-day wild material (Hunter 1992; Weiss et al. 2006). The variation pattern is correlated to the geographic origin of the populations (Ottosson et al. 2002).

H. euclaston, native to Northeast Argentina, Uruguay, and Southeast Brazil, is though a distinct species, closely related to the two North American annuals. It shows a large diversity as shown in hordein polypeptides (Echart-Almeida and Cavalli-Molina 2000).

The perennial species of section *Anisolepis* are all native to South America. They are a distinct and easily recognized species. *H. muticum* occurs in Northwest Argentina, northeastern Chile, Bolivia, Peru, Colombia, and Ecuador, where it grows mainly in Andean pastures and grasslands in altitudes around 3000 m and above. It is characterized by purple/blue spikes and spikelets with very short awns.

H. cordobense, *Hordeum stenostachys*, and *H. flexuosum* occur in Central and North Argentina, and the latter two also in Uruguay and southern Brazil. All of them have typical flattened lower glumes. They occur often in scattered stands but are sometimes more abundant in pastures and riverbeds and in drier conditions such as roadsides. Even though two or all three of the species may be found together, interspecific hybrids have not been reported. *H. flexuosum* has a very weak root system and occurs occasionally as an annual even though it is primarily a perennial. The crude protein concentration of the short winter grass *H. stenostachys* is ca. 10%, and it is considered a valuable grass for grazing in parts of the Pampas in Argentina (Hidalgo et al. 1998; Rodriguez Palma et al. 1999; Cerqueira et al. 2004). *H. cordobense* shows resistance to common root rot and spot blotch in a complex cross with cultivated barley (Kutcher et al. 1994).

H. chilense is native to central Chile and adjacent provinces of Argentina. It is usually a stout plant with broad leaves (in comparison to related species); the long glumes are usually spreading in the fruiting stage. The species grows in mesic inland habitats such as pastures, shores of lakes,

and streams. In coastal areas in Chile, it grows in drier locations roadsides, gravel, and alluvial soils. The species shows a large variation in morphology (von Bothmer et al. 1980) and other traits such as storage proteins (Atienza et al. 2005; Alvarez et al. 2006). *H. chilense* contains the common **I** genome, but it constitutes probably the most ancient genome variant in South America. Nuclear and plasmid molecular results together with cytogenetic data indicate that *H. chilense* is the original genome from which most of the diploid and polyploid South American species were differentiated (Linde-Laursen et al. 1989; Doebley et al. 1992; Taketa et al. 2001; Blattner 2004; Jakob and Blattner 2006). The separation between the North and South American genomes and the subsequent migration through long-distance dispersal is postulated to have taken place ca. 4 mya (Blattner 2006).

H. chilense has proven to contain an exceptionally good crossability with wheat, which is outstanding among *Hordeum* species. Initial crosses between *H. chilense* × *Triticum aestivum* and subsequent production of amphiploids resulted in partly fertile octoploids. These were, however, rather unstable. The hexaploid of the cross *H. chilense* × *Triticum turgidum* subsp. *durum* gave a more stable hexaploid offspring. The hybrids and amphiploids between the different wheats and *H. chilense* have been further developed to the new, man-made crop *Tritordeum* (review in Martín et al. 1999). This newly created species has a good potential particularly for widening the genetic basis of bread-making quality by increasing the variability for endosperm storage proteins in bread and durum wheats.

Apart from their quality traits, *H. chilense* and *Tritordeum* are good gene sources for a number of other valuable characters (Hernández et al. 2001: Atienza et al. 2007a,b), such as resistance to diseases and insects (for review, see Martín et al. 1999 and Atienza et al. 2005) like powdery mildew (Rubiales and Carver 2000), rust (Rubiales and Niks 1996; Vaz Patto et al. 2001, 2003), greenbug and Russian wheat aphid (Castro et al. 1996, 1998), *Blumeria formae* (Prats et al. 2006), wax layer resistance to leaf rust fungus *P. hordei* (Vaz Patto and Niks 2001), common bunt (Rubiales and Martín 1999), and resistance to *Septoria tritici* (Rubiales et al. 2001).

Other traits of agronomic importance are bread-making quality (Ballesteros et al. 2003), free threshing habit (Atienza et al. 2007a), carotenoid content (Ballesteros et al. 2005; Atienza et al. 2008), salt and drought tolerance (Forster et al. 1990), grain yield components (Pinto et al. 2002), storage proteins (Pistón et al. 2007, 2008), and alloplasmic male sterility (Martín et al. 2008).

Section *Critesion*

Critesion is a group of six or seven species (depending on taxonomic delimitation) that are all perennial and native to South and North America. Two South American species are diploids, *H. comosum* and *H. pubiflorum*, whereas the others are polyploids. The species of section *Critesion* have small, often very reduced or rudimentary lateral spikelets. The glumes and awns are long and thin, often brightly colored in dark purple or pink. In the fruiting stage, the glumes and awns are bending outward (often in 90°), forming an efficient unit for wind dispersal of the small and light seeds. All species of section *Critesion* are dominantly inbreeding and possess variants of the basic **I** genome (in diploids) in different combinations (in polyploids).

H. jubatum, foxtail barley or squirreltail barley, has an original distribution in Central and Northern Mexico, United States, and southern Canada, but it is probably native also to easternmost Siberia. The species has spread as a noxious weed to many areas of the world. It grows naturally in pastures, prairies, around riverbeds, and (seasonal) lakes, most often in saline habitats. As a weed, it is found on roadsides and in ephemeral areas. It occurs from sea level up to 3000 m.

H. jubatum is a segmental allotetraploid. In nature, *H. jubatum* crosses readily with *H. brachyantherum*, which occurs in similar habitats. The hybrid was described as a separate species (*H. caespitosum*). It is semisterile and hybrid swarms may occur where the parents are abundant, especially in parts of the United States. Hybridization with *H. vulgare* produces sterile offspring, and some parental combinations result

in the elimination of single chromosomes or of the entire genome of cultivated barley (von Bothmer et al. 1991).

H. jubatum contains resistance to specific races of stem rust (*Puccinia graminis* f. sp. *tritici*) with changed virulence in Canada (Fetch 2003) and specific mating-type genes for leaf blotch (*Phaeosphaeria avenaria* f. sp. *triticea*) (Ueng et al. 2003; Malkus et al. 2005; Reszka et al. 2005). Both pathogens may be putative problems for cereal cultivation.

H. jubatum has shown promising results of biomass production and has also been the target for several ecological studies. Fall burning enhances the abundance of the species (McWilliams et al. 2007). For the treatment of the weedy nature of foxtail barley, tillage treatment has an effect on the soil seed bank, where a reduced tillage resulted in a higher seed density and thus an increased spreading of the weed (Conn 2006; Conn et al. 2006). The seed viability is comparatively short. While all foxtail barley seed loses viability after ca. 7 years, 80% of the viability has been lost already after 3.7 years (Conn 2006; Conn et al. 2006). The emergence of seedlings is significantly reduced with surfaced seeds by light exposure and fluctuating soil moisture owing to reduced osmotic potential (Boyd and van Acker 2003, 2004).

Since the species often grows in saline environments, the tolerance for salinity has been widely studied, including the possibility to establish salt-accumulating halophytes on brine-affected soils (e.g., Keiffer and Ungar 2001, 2002; Cramer 2003; Garthwaite et al. 2005). In Canada, string and net-patterned salt marshes are rare landscape elements where *H. jubatum* is an important element for salinity adaptation (Wang and Redman 1996; Timoney 2001). Restoration of wetland vegetation from former rangeland and saltland where *H. jubatum* is a prominent part is important (e.g., for migratory birds by sheet flow) (Ashworth et al. 2006).

H. arizonicum, Arizona barley, occurs in a very restricted area in southern Arizona and in a few locations in southeastern California and northern Mexico. It is probably a threatened species as its natural, saline habitats along ditches, water canals, and ponds are mostly changed to concrete-covered canals and irrigated fields. It has a shallow and weak root system, and the life form is mostly biennial, sometimes perennial, and even annual. *H. arizonicum* is an allohexaploid with presumably *H. jubatum* (4x) and *H. pusillum* (2x) as parental genome donors (Chakrabati et al. 1986; Baden and von Bothmer 1994; Blattner 2004).

H. procerum grows in pastures, salt marshes, and riverbeds up to 1200 m in Central Argentina. It is an allohexaploid, probably with *H. jubatum* as one of the parents and a South American diploid, either *H. muticum* or *H. cordobense*, as the other (Blattner 2004).

H. arizonicum and *H. procerum* cross rather easily with barley and yield sterile hybrids, often with a varying chromosome number (2n = 28–35), or, in some cases, also haploids through the elimination of the barley chromosomes (Subrahmanyam 1977, 1980; von Bothmer et al. 1983).

The two diploid species *H. comosum* and *H. pubiflorum* are both bound to the Andean area up to ca. 4000 m. Both species have their southernmost distribution in Tierra del Fuego, but the former species is restricted to Chile and Argentina (up to the province of Mendoza) and confined to dry hillsides and steppes often as solitary plants. *H. pubiflorum* extends northward, scattered to Bolivia and Peru, and grows in wet mountain pastures, in both fresh and saline habitats. It is subdivided into two subspecies, one in the extreme south (subsp. *pubiflorum*) and one in the rest of the distribution area (subsp. *breviaristatum*; Baden and von Bothmer 1994; Baden and Kristensen 1996). Both species are common and important components of the Andean pastures and, as such, are of great value for grazing (Defosse et al. 1990; Díaz Barradas et al. 2001; Utrilla et al. 2006). Survival of *H. comosum* seedlings in the high Andes is partly dependent on microclimatic modifications in stands close to native cushion plants (Acuña-Rodríguez et al. 2006; Cavieres et al. 2007). *H. comosum* contains endosymbiotic fungi of the genus *Neotyphodium*, which may be a source of insect resistance in cultivated barley and in other cereals (Wilson 2007). Hybridization between cultivated barley and *H. pubiflorum* and

H. comosum has, so far, been unsuccessful (von Bothmer et al. 1983).

H. lechleri is an allohexaploid South American species. It is very common and is often a dominant plant in river valleys and pastures in both saline and freshwater environments, as well as on roadsides and other disturbed habitats. It occurs from Tierra del Fuego to the province of Mendoza and on the Falkland Islands.

Two indole alkaloids with defense-related functions are found in several species in *Hordeum*. The genes for benzoxazinoid 2,4-dihydroxy-2H-1,4-benzoxazin-3(4H)-one (DIBOA) biosynthetic pathways have evolved independently and are unique for *H. lechleri* relative to other *Hordeum* species (Grün et al. 2005).

H. lechleri has attained a particular interest due to the rather outstanding crossability with *H. vulgare* among the **I** genome species (von Bothmer et al. 1983). The crossing efficiency was tested in a number of combinations with various barley cultivars and in different *H. lechleri* genotypes. They readily produce hybrids with barley as the paternal parent, but only the combination with cv. Bonus as female gave rise to a viable offspring (von Bothmer et al. 1995b). A single successful backcross of a primary hybrid with *H. lechleri* has been reported (von Bothmer and Linde-Laursen 1989). Crosses of *H. jubatum* and *H. lechleri* with cv. Gull showed a particularly high crossability. It is postulated that cv. Gull has a gene for crossability not present in the other cultivars tested (von Bothmer et al. 1989a). Many of the produced offspring plants in *H. lechleri* × *H. vulgare* show deviating chromosome numbers. True trihaploids (2n = 21) of *H. lechleri* occur after selective elimination of the *H. vulgare* chromosomes, plants with 2n = 22–27 representing a full set of three genomes of the *H. lechleri* parent, plus individual barley chromosomes or a combination of chromosomes (stable in each plant) and true hybrids with all seven barley chromosomes (Linde-Laursen and von Bothmer 1988; von Bothmer et al. 1999). In the aneuploid and euploid plants, the influence of individual barley chromosomes on the meiotic pairing has been assessed and the 4**H** chromosome shows a negative effect on the autosyndetic pairing (von Bothmer et al. 1999).

Section *Stenostachys*

Section *Stenostachys* comprises a number of annual and perennial, diploid and polyploid species, mainly with the **I** genome, but some species also with the $\mathbf{X}_a$ genome. It is distributed in Eurasia, in South Africa, and in North and South America. All species are rather slender plants with short awns and versatile reproductive and dispersal patterns.

H. marinum, Maritime Barley, Sea Barley

The *H. marinum* complex consists of three entities, which have been treated variously. They comprise one tetraploid and two diploids, usually treated as two separate subspecies, subsp. *marinum* (2x), sea barley, and subsp. *gussoneanum* (2x and 4x, which is kept in this presentation), Mediterranean barley or geniculate barley. Some authors have recognized two species (*H. marinum* and *Hordeum gussoneanum*; cf. Baum and Bailey 1984; Baum and Johnson 1998; Jakob et al. 2007). *H. marinum* sensu lato is an annual, slender plant with short, ovate, stiff spikes with tough awns and glumes. The breeding system is almost complete inbreeding (small and included anthers). The diploid subsp. *marinum* has a very broad, winged lower glume of the lateral spikelet, whereas subsp. *gussoneanum* (the diploid as well as the tetraploid cytotype) has setaceous lower glumes (von Bothmer et al. 1995a).

The whole complex has a natural distribution area in eastern and central Europe and the Mediterranean basin, Southwest Asia, and eastward to Afghanistan. Subsp. *marinum* is native to the western part of the Mediterranean area, extending eastward to Greece (scattered, probably secondary locations are found more to the east). The diploid subsp. *gussoneanum* is found in the Eastern Mediterranean to southwestern Asia, and the tetraploid occurs from Turkey to Afghanistan. Although it is expanding its distribution in most areas, it is declining in some of its native environments. The species was previously rather common along the Atlantic Coast in Europe but is now declining drastically and is a threatened species, for example, in The Netherlands (Jager and Weeda 2000).

The ecology is quite distinct. *H. marinum* is confined as a natural plant to saline meadows, riverbeds, and marshes along the sea coast and inland, and as a weed in pastures and waste grounds. It is common in highly saline environments to receive attention as a putative donor of genes for stress tolerance. The species has spread in many areas of the world and is particularly common in Australia, North America, and South Africa. As an unwanted introduction, *H. marinum* is a serious weed both within parts of its natural distribution area and in foreign environments, not least because of its high salt tolerance and deep rooted nature (Browning et al. 2006). It easily builds up a considerable seed bank from which aggressive populations are quickly established, for example, in Southwest Asia. To manage aggressive weediness, chisel plowing in combination with barley cropping generally reduces the weed seed bank, particularly of *H. marinum* (Ghosheh and Al-Hajaj 2005). *H. marinum* as a nonnative invader is very aggressive and suppresses survival of native flora in dense stands, as shown in Californian grasslands (Hoopes and Hall 2002). Various methods for the restoration of the native flora from invading species have been proposed, such as in a State Park in the San Joaquin Valley, California, where spring burning has shown to be effective (Solomeshch and Barbour 2006).

Earlier cytogenetic studies indicated that the tetraploid cytotype of subsp. *gussoneanum* is an autotetraploid with the basic genome from the diploid subspecies. However, based on restriction analysis and DNA sequences of the chloroplast translation elongation factor, Komatsuda et al. (2001) reported two different fragments of the tetraploid form, one of which showed that the diploid is the immediate donor of one genome, whereas the other fragment is modified. It was assumed to emanate from a now extinct form of the *H. marinum* complex. The other diploid, subsp. *marinum*, has a distinct pattern, not identical to any of the genomic variants of subsp. *gussoneanum*. Using chloroplast sequences, Jakob et al. (2007) showed a similar differentiation pattern and explained this with niche differentiation in Mediterranean Quaternary refugia. The distribution areas of the two cytotypes of subsp. *gussoneanum* overlap in central Turkey. After the origin of the tetraploid, somewhere in the Eastern Mediterranean, it underwent a pronounced ecological shift, which allowed it to colonize more mountainous inland habitats eastward to Afghanistan.

Both Komatsuda et al. (2001) and Jakob et al. (2007) found that subsp. *marinum* is subdivided into two forms: one variable form on the Iberian peninsula and one more uniform type from the rest of the distribution range.

Even though there is a distinct morphological difference between the two subspecies, they can easily be crossed and the offspring has an intermediate morphology and with full meiotic pairing and seed set (von Bothmer and Jacobsen 1986, von Bothmer et al. 1989b). Natural hybrids have not been found. The very distinct and deviating $\mathbf{X}_a$ genome in the *H. marinum* group makes it possible to trace the occurrence of this genome in other *Hordeum* species. The allopolyploid nature of *H. capense* and *H. secalinum* with one $\mathbf{X}_a$ genome has been shown by genomic *in situ* hybridization (Taketa et al. 1999). An example of ongoing speciation is the finding of a hexploid cytotype of *H. brachyantherum*. In the area where the diploid and tetraploid cytotypes of *H. brachyantherum* meet, particular attention was paid when surveying the region. The hexaploid cytotype was found as a single, small but stable population along a creek in California. The hexaploid nature was revealed afterward, and further studies by *in situ* hybridization (Taketa et al. 1999) showed that one of the genomes clearly emanates from *H. marinum*. The interspecific hybridization followed by a subsequent chromosome doubling resulted in this new cytotype, which, by strict definition, is a new, distinct species, however, not yet formally described. This speciation event has taken place quite recently, thus after the introduction of *H. marinum* as a weed to North America.

Due to its obvious salt tolerance as observed in native habitats, *H. marinum* has been the subject of a number of studies of its stress tolerance ability such as salt tolerance (Lombardi et al. 2000; Garthwaite et al. 2005, 2006; Colmer et al. 2006), oxygen deficiency tolerance (Malik et al. 2009),

and waterlogging tolerance (McDonald et al. 2001; Setter and Waters 2003).

H. marinum has attracted attention as a possible gene donor due to its particular crossability with wheat. By studying wild populations of *H. marinum* growing in the vicinity of wheat fields in Austria and England, Guadagnuolo et al. (2001a,b) showed that numerous wheat species-specific DNA markers were amplified in *H. marinum* indicating previous spontaneous hybridization. They conclude that introgression of wheat traits into this wild relative seems to be possible under natural conditions.

In crosses with cultivated barley some viable embryos and plants are obtained with diploid as well as tetraploid cytotypes of subsp. *gussoneanum*. However, in all cases reported, the barley chromosomes are eliminated, leaving haploid plants of *H. marinum* (von Bothmer et al. 1983; Jørgensen and von Bothmer 1988; Solntseva and Pendinen 1999).

In contrast to the poor cross compatibility with barley, *H. marinum* has a particular crossability to wheat, which has been widely explored. Alloplasmic recombinant wheat lines, addition and alloplasmic lines (Troubacheeva et al. 2005, 2008), backcrosses to wheat (Pershina et al. 2006), and the effect of rye chromosomes on polyembryony in the combination *H. marinum* × *T. aestivum* and construction of amphiploids for the transfer of increased salt tolerance to wheat (Islam et al. 2007) have been reported.

European and South African perennial species

H. secalinum and *H. capense* are closely related alloteraploids sharing one genome from *H. marinum* ($\mathbf{X}_a$) and one from the common **I** genome species, probably *H. brevisubulatum* or a common diploid ancestor (von Bothmer and Jacobsen 1980; Linde-Laursen et al. 1986b; Baum and Johnson 2003; Blattner 2004, 2006; Petersen and Seberg 2004; Jakob and Blattner 2006).

H. secalinum, false-rye barley or meadow barley, has a distribution from southernmost Sweden along the Atlantic Coast to the Mediterranean area where it occurs in suitable biotopes both in North Africa (very rare) and in Europe. *H. secalinum* was formerly abundant in inland localities in Europe (von Bothmer and Jacobsen 1980) but is now rare due to urbanization or the expansion of cultivation (Cronberg et al. 1997). It grows in loose tufts and with a poorly developed root system, which makes the species vulnerable to competition from more aggressive invaders. The lower leaf sheaths are densely hairy. The long anthers and rather open flowers indicate a certain amount of outbreeding. It grows in moist, saline habitats, mainly in coastal areas, such as seashore pastures, where it can be a prominent component and important for grazing for cattle and sheep (Cilev et al. 2003, Braghieri et al. 2007). In Tunisia, it extends to 2100 m. A comparatively high seed set and hybrid establishment was obtained in crosses with *H. vulgare* (von Bothmer et al. 1983).

H. capense is the only sub-Saharan species of the genus and is distributed in inland localities in South Africa and in Lesotho. It occurs mainly on fertile soil in pastures and wet places like river banks up to 2700 m. It is a rather robust plant with fibrous outer leaf sheaths growing in dense tufts that can withstand heavy grazing. The outer glume of the lateral spikelet is somewhat flattened, and glumes are spreading in the fruiting stage. The sexual reproduction is mainly inbreeding, and the species has a certain vegetative reproduction by means of subterranean lateral shoots. Hybrids with *H. secalinum* are sterile, and a single sterile hybrid with *H. vulgare* has been reported (von Bothmer et al. 1983). There have been various theories of the occurrence of a *Hordeum* species with such a large disjunction to related species, even that it could be a recent introduction of *H. secalinum*. However, it is evident from several studies that *H. capense* is quite a distinct and separate species and differentiated ca. 0.4 mya. It has probably emerged from a common ancestor to *H. secalinum* (von Bothmer and Jacobsen 1980; Linde-Laursen et al. 1986b; Baum and Johnson 2003; Blattner 2004; Petersen and Seberg 2004).

Asiatic perennials

In Asia, three rather specialized perennial species occur. They are abundant and in some areas important as forages in natural vegetation.

The diploid *H. roshevitzii* grows in loose tufts in moist habitats like pastures, shores of lakes, and streams, rarely at roadsides. It is a slender, delicate grass with often pink and dark violet spikes and rudimentary lateral spikelets and glumes, which are spreading in the fruiting stage. The distribution is central Asia, Russia, Kyrgyzstan, North China, and Mongolia (von Bothmer and Jacobsen 1980; Baum and Johnson 2002).

H. bogdanii is a distinct, tufted, and hairy diploid species with long awns. It is the single species in *Hordeum*, beside cultivated barley, having a tough rachis, such that the single florets break off at maturity. It has often fertile, lateral spikelets (hence being "six-rowed"). The reproductive system is versatile, mainly inbreeding, but outbreeding may occur. The seed set is often very good. The distribution is central Asia, from Iran and Turkmenistan to eastern China. *H. bogdanii* has reached a certain interest due to its habitat preferences. It grows at altitudes from 1000 to 3800 m, mainly in saline environments in pastures, at streams, ponds, and lakes, and rarely as a weed. The genetic diversity is large (Guo et al. 2002; Zheng et al. 2003), but crosses with cultivated barley have not yielded offspring (von Bothmer et al. 1983). *H. bogdanii* is very sensitive to radiation. It had a five times higher RAPD variability at the Semipalatinsk nuclear testing region in Kazakhstan than in materials from other areas (Turuspekov et al. 2002). The authors conclude that *H. bogdanii* could be a good indicator for mapping radiation pollution at nuclear test sites or after nuclear accidents. Clement et al. (1997) found that *H. bogdanii* and *H. brevisubulatum* subsp. *violaceum* may be infected by endophytic fungi, which seems to be correlated with a high level of resistance against aphids.

The third exclusively Asiatic species is *H. brevisubulatum*. It is a di- and polyploid species complex with intricate morphological, cytological, and genetic variation patterns. The complex has a wide distribution from western Turkey to easternmost parts of Russia and China. Five subspecies have been recognized, earlier treated as separate species (cf. von Bothmer and Jacobsen 1980; Baum and Johnson 2007), each with a distinct distribution and habitat preference. In areas where the subspecies meet and overlap, there are obvious intercrossings and transitional forms are abundant. Common in all taxa is the outcrossing habit, with long, exserted anthers and large, branched stigmas. It has a self-incompatibility system, probably similar to that in *H. bulbosum*. The seeds have a very short viability. All subspecies have a vegetative reproduction by rhizomes. *H. brevisubulatum* has a conspicuous cytological variation. The constitutive heterochromatin varies from very large, terminal segments, as in rye, in subsp. *violaceum* to almost no heterochromatin in subsp. *brevisubulatum* (Linde-Laursen et al. 1980, Linde-Laursen and von Bothmer 1984). All subspecies and chromosomal races are intercrossable and yield fully fertile hybrids (somewhat reduced in triploids) with full meiotic pairing indicating that the complex is of autoploid nature (Landström et al. 1984; von Bothmer and Jacobsen 1986). Almost all crossing combinations with cultivated barley and the various chromosomal races of *H. brevisubulatum* yielded subviable plants (von Bothmer et al. 1983).

The following subspecies, mainly growing in pastures and along streams, are recognized in *H. brevisubulatum*:

brevisubulatum: diploids and tetraploids; southeastern Siberia, Mongolia, and China; at altitudes of 1400–3000 m;

nevskianum: diploids and tetraploids; central Asia, viz., northeastern Afghanistan, western Siberia to western China; at altitudes of 1500–5000m;

turkestanicum: tetraploids and hexaploids; a restricted area in central and northeastern Afghanistan, northern Pakistan, Tajikistan, and Xinjiang in China; it prefers dry biotopes such as stony slopes at altitudes of 2000–4600 m;

violaceum: diploids and tetraploids; from western Turkey to western Iran; up to 3500 m; and

iranicum: tetraploids and hexaploids; confined to western Iran; up to 3500 m.

South American species

Five species in section *Stenostachys* are found in southern South America. They represent diploids

as well as polyploids and are all perennials, mainly growing in pastures along streams and shores of lakes and ponds. All have in common the **I** genome or variants of it.

H. erectifolium is a morphologically distinct diploid species rather recently described and known from a single location, in the western part of the Buenos Aires province in Argentina. It grows in halophytic vegetation near a salt lake (von Bothmer et al. 1985). It is densely tufted with erect basal leaves, laterally compressed spikes, and flattened lower glumes of lateral spikelets. The whole plant has a very dense wax cover, being glaucous in appearance.

The three polyploid, rather similar species, *H. tetraploidum* (4x), *Hordeum fuegianum* (4x), and *Hordeum parodii* (6x), are all native to the Andean area in Argentina and Chile (von Bothmer et al. 1986b). They are segmental alloploids with variants of the common **I** genome. *H. fuegianum* is restricted to Tierra del Fuego and the adjacent Magellanes region in southern Chile, occuring mainly along the seacoast. *H. tetraploidum* has a wide distribution from the Santa Cruz province in the south to Mendoza in Central Argentina at altitudes up to 1500 m. *H. parodii* is partly sympatric with *H. tetraploidum* but extends scattered in Central Argentina and in southernmost Chile and Tierra del Fuego (where it somewhat overlaps with *H. fuegianum*). All three species are fairly common and important for grazing in natural pastures. In crosses with cultivated barley as the male parent, the three species yielded a high seed set, but the plant establishment was poor (von Bothmer et al. 1983).

The most variable and intricate of the South American species is the perennial, diploid, inbreeding *H. patagonicum*, which is a species complex with high diversity (von Bothmer et al. 1986a; Blattner 2006; Jakob and Blattner 2006). All forms are low-grown and densely tufted with short spikes. The complex is restricted to southernmost Chile and Argentina up to Chubut province in Argentina and occupies many different habitats in the Patagonian area in the lowland. The main habitats are salt pans, various types of salty areas around lakes, and along rivers.

All forms are compatible in crosses and yield fertile offspring with high meiotic pairing. The cytogenetic differentiation includes translocations and inversions (von Bothmer et al. 1986a). There is much overlap between the taxa and many intermediate forms indicate frequent gene exchange. It is a group in a very active phase of speciation, probably mainly due to ecological differentiation (Blattner 2006; Jakob and Blattner 2006). *H. patagonicum* easily yields hybrids with most other *Hordeum* species but subviable hybrid plants with *H. vulgare* (von Bothmer et al 1983; von Bothmer and Jacobsen 1986).

The subspecies are the following:

patagonicum: very short culm (<12 cm) and spike; Chubut and Santa Cruz provinces;
setifolium: up to 40 cm; Chubut and Santa Cruz provinces; salt pans and disturbed areas (roadsides and salty and grazed slopes);
santacrucense: up to 20 cm; in southern Santa Cruz province in Argentina and to the strait of Magellanes in Chile; salty areas around lakes, often on open soil;
mustersii: up to 30 cm, spikelets densely hairy; only known from two locations in Santa Cruz province; salt pans and dry steppe; and
magellanicum: short runners, long and lax, nodding spikes; Tierra del Fuego and southernmost part of Santa Cruz province; apart from the inland locations, it occurs along the coast in sand dunes.

North and Central American species

Three species occur here. *H. guatemalense* is an endemic, tetraploid, perennial species occurring only in the mountain area Cuchumatanes of northern Guatemala and is known only from a few sites. It grows in marshy meadows in the alpine zone, 3000–3500 m. The spike is usually purple, and the awns and glumes are short. It is probably most closely related to the North American species (von Bothmer et al. 1985; Blattner 2006).

H. depressum, low barley, is an annual, inbreeding tetraploid species confined to a restricted area in western California and to a few scattered

populations in northern Oregon and Washington State. It is a low-grown and gracile plant. The lower glumes of the lateral spikelets are flattened at the base, and the glumes are bending outward at the fruiting stage. It occurs in saline areas and vernal pools, but may occur as a weed. It has a high seed set in crosses with cultivated barley and a comparatively high formation of hybrid plants (von Bothmer et al. 1983). The hybrids show a cytogenetic instability, mainly as aneuploids (Pendinen and Chernov 1995).

H. depressum is a segmental allotetraploid with the **I** genome and has evolved as a result of hybridization and subsequent polyploidization between the two diploids native to California, *H. intercedens* and *H. brachyantherum* subsp. *californicum*, probably with the latter as a female parent (Knutsson and von Bothmer 1993; Salomon and von Bothmer 1998; Blattner 2004).

H. brachyantherum, meadow barley or northern barley, is a species complex consisting of diploids and polyploids occurring in North America and in easternmost Asia. The tetraploid form (subsp. *brachyantherum*) has a wide distribution in western United States, adjacent Mexico, and Canada to Alaska, the Aleutian island chain over to Kamtchatka and the Kuril islands. It has an odd disjunction across the continent and is found also in a small area in the northern part of Newfoundland and adjacent Labrador. It occurs mostly in moist biotopes such as pastures and along streams on grassy slopes and in woodlands and alpine meadows up to 4000 m. In many places, it occurs together with *H. jubatum* and hybrid swarms are not uncommon. It is usually a robust, broadleaved, and glabrous plant with straight awns and glumes in the fruiting stage.

Subsp. *californicum*, California barley, is a diploid, slender, densely pubescent plant with narrow leaves. The glumes and awns are bending outward at the fruiting stage. It has a restricted distribution in central California mainly along the coast, but inland locations are not uncommon. Subsp. *californicum* grows in a wide array of habitats from dry and moist grassy pastures, pebble beds of streams, oak woodland, often in serpentine and on disturbed ground from sea level up to 2300m. In a few sites, subsp. *californicum* is found together with subsp. *brachyantherum*, but triploids have not been reported. Both diploids and tetraploids contain variants of the common *Hordeum* **I** genome. Analysis of hybrids between the two subspecies shows that the diploid has contributed with one genome in the tetraploid. The single hexaploid population of *H. brachyantherum* was the result of a spontaneous hybridization event of *H. brachyantherum*, 4x, and *H. marinum* (see under this species).

All forms of *H. brachyantherum* are prominent representatives of the native flora of California, but they are sensitive for competition from invasive, nonnative grass species (Kolb et al. 2002; Lulow 2006). Properties of biological and chemical restored perennial grasslands are studied for reestablishment of the native taxa, including both subspecies of *H. brachyantherum* (Bugg et al. 1997; Posthoff et al. 2005). Ingham and Wilson (1999) report on the native wetland species, among others, *H. brachyantherum*, for the colonization of vesicular-arbuscular mycorrhizal fungi (VAMF), which can possibly be of importance for the revegetation of native plant species. All cytotypes cross readily with *H. vulgare* as the female parent (von Bothmer et al. 1983).

DOMESTICATION OF BARLEY

Ancient farmers selected wild barley leading to domestication, on which humans are utilizing today (Purugganan and Fuller 2009). The first definite sign of barley cultivation has been recorded from the Middle East "arc" more than 10,000 BP (Zohary and Hopf 2000). To elucidate the time and place of barley domestication, studies of genes related to key steps in domestication can give valuable insights. During the process of domestication, barley has gradually accumulated traits that facilitated agricultural production. Selection may have been unconscious, that is, as a result of environmental selection, or conscious, as a result of deliberate choice by humans (von Bothmer et al. 2003).

A monophyletic origin with a single domestication in the Israel-Jordan region was suggested mainly by analysis of genome-wide amplified

fragment length polymorphism (AFLP) (Badr et al. 2000; Salamini et al. 2002). However, a simulation study showed that crops with multiple origins are more likely to produce monophyletic clades than do crops with a single origin in the study of genome-wide multilocus marker systems (Allaby et al. 2008). Two phylogenetic trees constructed based on AFLPs of whole genomes and AFLP loci linked to the nonbrittle rachis gene were in good contrast, where the latter showed a clear separation of western and eastern barley cultivars (Komatsuda et al. 2004). Two domestications of barley were indicated by evidence on nonbrittle rachis (Takahashi 1955; Azhaguvel and Komatsuda 2007). Genetic discontinuity existed between the Fertile Crescent and central Asia, and the second domestication was suggested to be within central Asia 1500–3000 km farther east of the Fertile Crescent (Morrell and Clegg 2007), indicating multiple origins of barley. Genetic structure diverged geographically in wild barley (Morrell et al. 2003; Kilian et al. 2006) and in cultivated barley (Morrell and Clegg 2007; Saisho and Purugganan 2007). Recent genetic and archaeology studies detected multiple domestication origins for each species in the Fertile Crescent (Zeder 2008; Brown et al. 2009).

Selections for nonbrittle rachis, six-rowed spike, and naked caryopsis are three key traits involved in barley domestication. Migration of barley to regions outside its place of origin was accelerated through mutations and recombinations to develop reduced vernalization requirement and photoperiod insensitivity (Salamini et al. 2002).

Nonbrittle rachis

Seed shattering is a character of natural adaptation in wild plants (Fuller et al. 2009; Purugganan and Fuller 2009). The brittleness of the rachis promotes seed dispersal, together with the rough awn, which becomes attached to animals for effective dispersal and for entering into soil (Elbaum et al. 2007). Nonbrittle rachis is the most important trait for domestication and results in efficient harvest without loss of grains. Spikes of the nonbrittle mutant remain longer on the plant in the field after maturation. The earliest archeological clue for nonbrittle barley comes from Tell Abu Hureyra in Syria from 9500 BP (Hillman et al. 1989). In wild barley as well as all the wild species of *Hordeum*, spikes disarticulate immediately above each rachis node to form typical wedge-shaped spikelets (von Bothmer et al. 1995a). Disarticulation scars in wild barley are smooth, which helps in seed dispersal, whereas in cultivated barley, threshing produces rough dehiscence scars on grains detached from rachis segments (Tanno and Willcox 2006). Anatomically, the rachis nodes are clearly constricted in brittle spikes but are not constricted in nonbrittle spikes (Ubisch 1915).

Nonbrittle rachis of cultivated barley is inherited by a monogenic recessive gene (Ubisch 1915; Schiemann 1921; Johnson and Åberg 1943). The *Btr1-Btr2* (double dominant) genotype of wild barley strongly constricts the rachis node, whereas one recessive allele *btr1Btr2* or *Btr1btr2* does not result in the constriction of the rachis node. The *btr1* and *btr2* loci are tightly linked and located on the short arm of chromosome 3**H** (Takahashi and Hayashi 1964; Komatsuda et al. 2004). The recessive nature of nonbrittle rachis suggests a loss of function-type mutation in *Btr1* and *Btr2*. The *btr1* gene has been fine mapped and is now delimited to a region less than 1 cM (Azhaguvel et al. 2006).

Allelic variation occurs in *Btr1* and *Btr2*. Alleles of *Btr1.a* and *Btr2.k* complementarily produce brittle rachis in the presence of the dominant D gene (chromosome 7**H**) (Komatsuda and Mano 2002; Komatsuda et al. 2004), whereas *Btr1.h* and *Btr2.h* of wild barley do not need the D factor to produce brittle rachis (Senthil and Komatsuda 2005). In addition to *Btr1* and *Btr2*, two QTLs for brittle rachis were detected on chromosomes 5**H** and 7**H** (the D gene) (Komatsuda et al. 2004). Cultivated barley consists of two geographic types, western and eastern (Takahashi 1955; Komatsuda et al. 2004). The diphyletic origin of barley is assumed to have evolved via independent mutations from previously diverged wild barley (Azhaguvel and Komatsuda 2007). Spike shattering is another form of yield loss caused by

weak rachis under the different gene controls (Kandemir et al. 2000).

Triticeae species may share orthologous genes for brittle rachis because they are located on homeologous group 3 chromosomes (Watanabe and Ikebata 2000). In wheat, *Br-A2* and *Br-A3* that produce wedge-type diaspores were mapped on chromosomes 3**A** and 3**B** of *T. turgidum*, and the two genes and barley *Btr1* and *Btr2* were suggested to be homologous based on the comparative mapping (Kandemir et al. 2004; Nalam et al. 2006). *Br-A1* of *Triticum timopheevii* was mapped on the short arm of chromosome 3**A** (Li and Gill 2006), which also produces wedge-type diaspores. *Br-S1* of *Aegilops speltoides* was mapped on the short arm of chromosome 3**S**. *Br-S1* produces *ligustica*-type disarticulation (wedge), which is dominant over *speltoides*-type disarticulation (whole spike shattering). The wheat *Br-A1* and *Br-S1* and barley *Btr1* and *Btr2* were suggested not to be orthologous (Li and Gill 2006), but the evidence was rather weak.

Br2 was mapped on the long arm of chromosome 3**D** of *Aegilops tauschii*, which produces barrel-type diaspores. Based on map position, *Br2* was unlikely an ortholog of the brittle rachis genes of barley. Rice shattering gene *qSH1* encoding a BEL1-type homeobox gene is located on the long arm of rice chromosome 1 (Konishi et al. 2006), which is syntenous with barley chromosome 3**H** (Devos 2005). *JuBel2*, the barley ortholog of *qSH1* (Li and Gill 2006), was mapped on the long arm of barley chromosome 3**H** (Castiglioni et al. 1998; Muller et al. 2001). As *JuBel2* and *btr1/btr2* are located in different arms of barley chromosome 3H, *JuBel2* does not correspond to barley *btr1/btr2*. Therefore, the *btr1* and *btr2* genes remain to be cloned.

Six-rowed spike

Hordeum species possess three spikelets at each rachis node and the spike architecture is unique among the Triticeae (von Bothmer and Jacobsen 1985). The two-rowed type is the exclusive phenotype in wild barley, suggesting that the two-rowed spike is the ancestral form. Six-rowed spontaneous mutants are obviously not preferred for survival in the wild, and they are eliminated naturally and rapidly from wild barley populations (Zohary 1964). The central spikelet is fertile and goes on to develop into a grain in wild and cultivated barley. The two lateral spikelets are sterile in the two-rowed type (wild and cultivated barley), and are fertile in the six-rowed type (only cultivated barley). The fertile lateral spikelets that develop into grain appear during barley domestication. In wild barley, the three spikelets form a light, arrowhead-like dispersal unit that both facilitates seed dispersal by animals and aids seed burial. The numerous upward-oriented barbs on the lemma and awn are also part of the dispersal and self-planting mechanisms. Reduced awn barbing seems important in its utilization as animal feed, which is found in two-rowed cultivars (von Bothmer et al. 1995a).

The archeological remains of six-rowed barley appeared very early in the aceramic Neolithic beds in Tell Abu Hureyra from 8800 BP onward (Helbaek 1959). In that site, remains of two-rowed barley are dated at about 9000 BP with sporadic six-rowed elements among the two-rowed materials (Helbaek 1969). This is a sign that the six-rowed character in barley was derived from two-rowed barley during domestication. The appearance of six-rowed spike was probably a result of conscious selections during barley domestication (Harlan and de Wet 1973). Six-rowed barley produces three times as many seeds per spike as two-rowed barley and is thus a change of dramatic agronomic importance. Yield component compensation kicks in to balance seed number/spike with seed weight and tiller number. Six-rows tiller less and have lighter seeds on average. Therefore, it is not automatic that six-rowed spikes give a huge yield increase versus two-rowed spiked barley. In the past, six-rowed cultivated barley was assumed to be derived from *H. agriocrithon*, which is a six-rowed form of brittle rachis found in the early 1930s in western China (Åberg 1938). Alleles of these materials will be sequenced soon.

Six-rowed spike is controlled by a recessive gene, *vrs1*, on the long arm of chromosome 2**H**. The *Vrs1* gene encodes a member of the homeodomain-leucine zipper (HD-ZIP) I class of

transcription factors (Komatsuda et al. 2007). This gene is expressed only in lateral spikelets at the immature stage, which is the reason why this gene only affects the development of lateral spikelets. The dominant nature of *Vrs1* in two-rowed barley suggests the *Vrs1* protein is a repressor that regulates the development of lateral spikelets. The result agrees with a theory that most domestication genes involve changes to transcription factors (Doebley 2006). The *Vrs1* gene is located in a gene-poor region, and recombination is highly suppressed around the *vrs1* locus (Pourkheirandish et al. 2007). Comparison of the barley *vrs1* BAC contig sequence and rice genome sequence revealed that the rice ortholog of *vrs1* (*Oshox14*) is located in rice chromosome 7 instead of the collinear region in rice chromosome 4. The *Vrs1* gene originated by a duplication of the ancestral homeobox gene *HvHox2*, and *Vrs1* may be specific in the genus *Hordeum* but may not exist in other genera in the Triticeae (Sakuma, et al. 2010).

Wild barleys and two-rowed barley have the dominant allele *Vrs1*. The hypothesis that six-rowed barley was derived from two-rowed barley was based on archaeological studies (Helbaek 1959), and it was primarily supported by genetic studies showing that there were more than 90 mutant lines induced for the *vrs1* locus from two-rowed barley (Lundqvist et al. 1997). Isolation of the *Vrs1* gene revealed point mutations on the gene to produce six-rowed barley (Komatsuda et al. 2007). Sequence analysis of the *vrs1* gene of six-rowed cultivars revealed three independent origins for six-rowed barley. Two of them (alleles *vrs1.a2* and *vrs1.a3*) were derived from their immediate ancestor, two-rowed cultivated barley (alleles *Vrs1.b2* and *Vrs1.b3*), by single nucleotide mutations causing a frame shift and nonsynonymous substitution, respectively. The *vrs1.a1* allele represents a single nucleotide mutation causing a frame shift. The origin of *vrs1.a1*, which is the most widespread allele and probably the first allele of six-rowed barley, was not found among two-rowed cultivars tested so far (Komatsuda et al. 2007). It remains to be studied whether this six-rowed allele was derived from extinct, two-rowed cultivated barley or it is directly derived from wild barley. More extended surveys of a worldwide huge germplasm or analysis of ancient DNA in archaeobotany may answer this question (Schlumbaum et al. 2008).

Most of six-rowed cultivars have an awn length of lateral spikelets of nearly the same size as the central spikelets. The *vrs1.c* allele forms awnlike appendages on the lemma of lateral spikelets. *Lks1* forms six-rowed, awnless spikes, and it is suggested to be nonallelic but linked with *vrs1* (Lundqvist et al. 1997). Six-rowed barley with *vrs1.c* and *Lks1* genes does not show a causable mutation on the *Vrs1* gene (Saisho et al. 2009). Allele variation of spike morphology also occurs in two-rowed barley; *Vrs1.p* with pointed-tip lateral lemma, *Vrs1.b* with a round tip of the lateral lemma, and *Vrs1.t* with extremely rudimentary lateral spikelets. These alleles show a variation at the DNA level, but whether they are a functional polymorphism remains unclear (Saisho et al. 2009).

I series (*I*, I^h, and *i*) are a natural allele variation located in the short arm of chromosome 4**H**, and many six-rowed cultivars have the dominant *I* or the more pronounced I^h allele promoting the development of anthers and seed set in lateral spikelets (Leonard 1942; Woodward 1947). It remains to be determined whether the wild ancestor of domesticated barley has a dominant allele for *I* or I^h or the wild ancestor had recessive *i* and a dominant mutation created *I* and I^h. *Six-rowed spike 5* (*v5*) and *intermedium spike c* (*int-c*) are induced mutant lines (Fukuyama et al. 1982; Lundqvist and Lundqvist 1988). These lines are allelic (Lundqvist 1991) and are located on the short arm of chromosome 4**H** (Lundqvist et al. 1997). Recessive *int-c* allows partial fertility in lateral spikelets and produces an *intermedium* spike. This gene was detected in more than 20 induced mutant lines (Lundqvist and Lundqvist 1988). Since both *int-c* and *vrs1* in recessive state promote the development of lateral spikelets, their dominat alleles *Int-c* and *Vrs1* may encode repressors of lateral development. In contrast, *I* and I^h genes in the dominant state promote development of lateral spikelets. The *I* series and *int-c* are postulated to be allelic (Lundqvist et al. 1997), but the direction of their dominance is opposite

to each other and allelism is not clear. *Six-rowed spike 2* (*vrs2*), *3* (*vrs3*), and *4* (*vrs4*) are also recessive genes located on the long arms of chromosomes 5**H**, 1**H**, and 3**H**, respectively (Lundqvist et al. 1997). These recessive genes are detected only in induced mutant lines but not in cultivars. They probably enhance the development of lateral spikelets to various degrees depending on their position in the spike.

Naked caryopsis

Uncommonly in cereals, barley cultivars have caryopses with adhering hulls at maturity (Taketa et al. 2008). However, a few cultivars have grains that thresh free, known as naked barley. All wild barleys have hulled grains, indicating that hulled grain is an ancestral type in barley as well as in the genus *Hordeum*. The remains of naked kernels have been found about 8000 BP in Ali Kosh, indicating naked barley occurred early in the domestication of barley (Helbaek 1969). Hulled barley has caryopses with the husk cemented to the grain, while naked barley has easily separable husks upon threshing. The naked caryopsis of barley allowed its direct link to dietary use. Naked barley is distributed worldwide, but there is a higher preference for naked barleys in East Asian countries such as China, Korea, and Japan, and it is especially common in Tibet and the northern parts of Nepal, India, and Pakistan (von Bothmer et al. 2003). In these areas, barley is used as a major component of the human diet and naked barley is preferred. In the southwestern part of Japan, a special semidwarf type called "uzu," earlier covering about 80% of the whole barley acreage in Japan, is now used mainly in the naked form (Takahashi 1951). The smaller kernel of the uzu type is preferred to cook together with rice in Japan due to the similar sizes of the two cereals. Although the frequency is low in Western countries, naked barley was grown in Anatolia and in Northern Europe in ancient times (Helbaek 1969). A single recessive gene, *nud*, is located on the long arm of chromosome 7**H**. The *Nud* gene encodes an ethylene response factor (ERF) family transcription factor, and this gene was located in a 17 kb region, which was deleted in all naked barley. The *Nud* gene controls the formation of a lipid layer between the pericarp epidermis and the hull. Naked barley has a single origin (Taketa et al. 2008).

Reduced seed dormancy

The temporary reduced metabolic rate of viable seed results in the inability to germinate under favorable environment. Dormancy allows seed survival and dispersal to wide areas, although deep seed dormancy is a problem in cultivation and it is economically undesirable in malting. Therefore, a moderate level of seed dormancy is considered as an appropriate condition for barley cultivars. Stringent selection against seed dormancy leads to the genotype susceptible to preharvest sprouting (Prada et al. 2004). A clear geographic pattern of seed dormancy was shown by the analysis of 4365 cultivated and 177 wild barley (subsp. *spontaneum*) accessions derived from the world (Takeda and Hori 2007). Cultivars from East Asia, Turkey, and North Africa showed high dormancy (low germination percentage). Cultivars with an extremely high level of dormancy were introduced in the Far East to escape preharvest sprouting caused by heavy rain at the ripening time in this area. Uniquely, more than 70% of cultivars in Ethiopia showed germination higher than 80% immediately after the ripening. Presumably, barley is cultivated continuously throughout the whole year in this country.

The seed dormancy of barley is largely due to the presence of hulls that prevent the availability of oxygen (O_2) to the embryo. In addition, abscisic acid (ABA) and gibberellins (GAs) interact with O_2 to regulate seed dormancy. The median O_2 requirement for germination of dormant seeds was 400-fold greater than for excised embryos, indicating that the tissues enclosing the embryo markedly limit O_2 penetration (Bradford et al. 2008). An ABA hydroxylase gene is expressed to a much higher level in nondormant seeds than in dormant seeds (Millar et al. 2006). This gene is expressed in the coleorhizae, indicating the enzyme activity confined to this particular organ.

Seed dormancy is affected by several genes, environmental factors, and by gene × environment

interactions. *SD1* is a major QTL detected in all the eight mapping populations analyzed by Hori et al. (2007), where 30%–50% of variance for seed dormancy was explained by *SD1*. *SD1* was mapped in the centromeric region of chromosome **5H** (Han et al. 1996; Prada et al. 2004; Zhang et al. 2005). The location of *SD1* was delimited to a 4.4-cM region by near-isogenic lines (Han et al. 1999), and a fine map for the cloning of *SD1* was constructed by Sato et al. (2009). *SD1* is epistatic to *SD2* at early-ripening stages, but they seem to act additively at later ripening stages (Romagosa et al. 1999). *SD2* controls a moderate level of seed dormancy, which makes it a promising candidate gene for utilization in barley breeding (Gao et al. 2003). *SD2* was mapped at the distal end of the long arm of chromosome **5H** (Han et al., 1996; Hori et al. 2007). *SD2* was fine-mapped in an interval of 0.8 cM by Gao et al. (2003). A gene coding for GA20 oxidase was identified as a candidate gene of *SD2* using barley–rice synteny, but its gene identity remains to be proven (Li et al. 2004).

Reduced vernalization requirement

Vernalization is the exposure of plants to low temperatures to induce transition from a vegetative to a reproductive state. The vernalization requirements of winter species prevent flowering during the winter for the protection of floral organs against cold. Both winter and spring barley can be cultivated in midlatitudinal regions according to climate conditions, and development of barley with a reduced vernalization requirement expanded barley cultivation to areas where spring sowing is necessary (von Bothmer et al. 2003). Wild barley and the first domesticated barleys were likely with a *Sgh1sgh2sgh3* genotype state for the vernalization requirement. The three genes are located on chromosomes **4H**, **5H**, and **7H**, respectively (Takahashi and Yasuda 1956, Yan et al. 2006). Mutation in one of the three genes was sufficient to provide a reduced vernalization requirement. Multiple evolutionary pathways were proposed and the multiple origin of *Sgh2* implies very complex stories (Yasuda 1969; Yan et al. 2004).

Barley *Sgh1* (*VRN-H2*) is a wheat *VRN2* ortholog, both dominant for vernalization requirement. Wheat *VRN2* encodes zinc finger and CCT (CONSTANS, CONSTANS-LIKE, TOC1) domain (called *ZCCT1*). The *ZCCT1* gene, as does *CONSTANS*, inhibits the transition of plants from the vegetative to the reproductive stage (Yan et al. 2004). *VRN2* is a trans-acting repressor of *VRN1*; therefore, wheat *VRN1* and *VRN2* have opposite transcription profiles (Yan et al. 2004, 2006). Transcription of the *VRN2* gene is gradually downregulated by vernalization. In barley, Southern blot analysis of barley genomic DNA using wheat *ZCCT1* as a probe in 85 barley cultivars showed the presence of this gene in 23 winter barley lines and the deletion of this gene in 62 spring barley lines (Yan et al. 2004, Dubcovsky et al. 2005). The physical deletion of the *VRN-H2* results in the loss of a repressor of the winter *vrn-H1* allele, yielding the pseudo-spring facultative phenotype (Zitzewitz et al. 2005). There are three linked *ZCCT* genes designated *ZCCT-Ha*, *ZCCT-Hb*, and *ZCCT-Hc*. The *ZCCT* genes were absent in all the facultative and spring varieties, indicating *ZCCT* genes are candidate genes for *VRN-H2* in barley (Zitzewitz et al. 2005). Single origin was suggested for the *ZCCT* deletion found in *vrn-H2* varieties collected from China, Japan, Korea, Nepal, Southwest Asia, Turkey, Ethiopia, Europe, and America, but independent point mutations and deletions of the *ZCCT* genes were found in *Triticum monococcum* (Dubcovsky et al. 2005).

Barley *Sgh2* (*Vrn-H1*) and wheat *VRN1* are orthologous, dominant for reduced vernalization requirment (spring habit). *VRN1* is the wheat *APETALA1* gene of the MADS-box family (Murai et al. 2003; Yan et al. 2003b). Promoter mutations in *VRN-A* and *VRN-A^{m}1* were sufficient to determine the dominant spring growth habit (Yan et al. 2003b; Fu et al. 2005), but no promoter polymorphism, which likely correlates spring and winter growth habit, was found in barley except in a special case (Zitzewitz et al. 2005). Large deletions (2.8–5.2 kb) within the first intron of barley *VRN-H1* and wheat *VRN-1* orthologs were always associated with spring growth habit in barley and wheat (Fu et al. 2005; Zitzewitz et al. 2005). The first 0.44-kb minimal

region was the most critical motif shared in Triticeae species with a vernalization requirement. The variable deletion patterns in the first intron suggested evolution of the *VRN-H1* alleles by independent mutations in the genes followed by recombination and gene conversion in European barley (Cockram et al. 2007).

Barley *Sgh3* (*VRN-H3*) and wheat *VRN3* are orthologs, dominant for reduced vernalization requirement. These genes are orthologs of *Flowering Locus T* (*FT*) of rice and *Arabidopsis* related to flowering time (Yan et al. 2006). The dominant allele with a high level of transcripts of this gene induced early flowering in barley and wheat. *VRN2* is downregulated by vernalization and *VRN2* negatively regulates *VRN1* and *VRN3* (Yan et al. 2006).

Photoperiod insensitivity

Plants respond to periodic change in relative day length (Turner et al. 2005). Flowering time is highly variable under the control by photoperiodic response genes in barley (Laurie 1997). *Ppd-H1* is the major determinant of long-day response in barley. *Ppd-H1* is located on the short arm of chromosome 2**H** (Laurie et al. 1995; Karsai et al. 1997; Decousset et al. 2000). Since *Ppd-H1* encodes a pseudo-response regulator (PRR), it is different from rice major photoperiod response genes, *Hd1* and *Hd3a*, but likely orthologous to rice *Hd2* (Yano et al. 2000; Murakami et al. 2005; Turner et al. 2005). Wild barley requires long-day condition for flowering and has a dominant allele at the *Ppd-H1* locus. Spring barleys lack the wild allele, and *Ppd-H1* plants head about 20 days earlier than *ppd-H1* plants under long-day conditions (16 h of light) (Turner et al. 2005). Spring barley acquired a long-day insensitive mutation to allow extended vegetative growth under long days at high latitudes. This obviously favored expansion of the barley production area into higher latitudes. Significant pleiotropic effects of the *Ppd-H1* locus under long days on plant height, plant yield, tiller yield, spike length, and grain number per tiller were detected (Laurie et al. 1994; Sameri et al. 2006).

An association study revealed a new causative SNP, SNP48 (Jones et al. 2008), which has a stronger association with phenotype than SNP79 proposed formerly (Turner et al. 2005). Latitude-dependent geographic distribution of barley in Europe was shown, and the origin of a photoperiod nonresponsive European landrace was suggested to be wild barley in Iran (Jones et al. 2008).

Ppd-H2 is the second major photoperiod response gene, which was mapped on the long arm of the chromosome 1**H**. Explicit differences in flowering time under short days (10 h) were observed, but *Ppd-H2* has little effect under long days (13–16 h) (Laurie et al. 1995; Szucs et al. 2006). *HvFT3* was considered as a candidate for *Ppd-H2* because the two are closely linked on chromosome 1**H** (Faure et al. 2007; Kikuchi et al. 2009). Five *FT*-like genes were found in barley: *HvFT1*, *HvFT2*, *HvFT3*, and *HvFT4* and were shown to be highly homologous to rice *OsFTL2* (the *Hd3a*), *OsFTL1*, *OsFTL10*, and OsFTL12, respectively. These relationships were supported by comparative mapping between barley and rice (Faure et al. 2007). No rice candidate of *HvFT5* ortholog was found. Genes and mutants at more than 14 other loci have been associated with earliness in barley (Lundqvist et al. 1997). These genes in various combinations permit plant breeders to adapt barley for production in many parts of the world.

Photoperiod response is interactive with vernalization response in determining flowering time (Trevaskis et al. 2006; Karsai et al. 2008). Low temperature and long-day response pathways are integrated to induce *VRN3* (*HvFT1*) through the induction of *VRN2* (Hemming et al. 2008).

Cleistogamy

Although cleistogamy would not be a domestication trait, its relevance on the reproduction system and gene flow is interesting. Barley is predominantly self-pollinated, even though much of the pollen is released only after the anthers have been exerted; this is because the stigmas become receptive prior to anther exertion and are able to capture sufficient self-pollen not to require fertilization by wind-borne nonself pollen. The

exertion of the anthers is pronounced in some wild barleys so that their rate of outcrossing is higher than that of cultivated barley (Abdel-Ghani et al. 2004). The palea and lemma remain tightly closed throughout anthesis in some barley cultivars (Lord 1981). The cleistogamous flowers force plants with this habit to be almost entirely autogamous. Cleistogamy also provides a means of escape from cereal head blight infection and minimizes pollen-mediated gene flow. The size of the lodicule in the cleistogamous flower is typically smaller than that in the noncleistogamous type (Honda et al. 2005). Cleistogamy in barley is under the control of a single recessive gene (Ceccarelli 1978), and the *cleistogamy 1* locus (*cly1*) was mapped to the long arm of chromosome 2**H** (Turuspekov et al. 2004; Turuspekov et al. 2009). *Cly1* has been isolated by positional cloning (Nair et al. 2010).

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Chapter 3
Barley Genome Organization, Mapping, and Synteny

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INTRODUCTION AND HISTORY

The high diversity of barley (*Hordeum* subsp. *vulgare* or *spontaneum*) phenotypes, true diploid nature, ease of hybridization, and cultivation, has made barley a favorite genetic organism since the rediscovery of Mendel's laws. Later, it was discovered that barley was easily mutated, resulting in an even larger diversity of phenotypes enhancing genetic studies, which naturally led to chromosome analyses and mapping. Barley is a true diploid ($2n = 2x = 14$), self-fertile, and has large chromosomes (6–8 μm). Barley (cultivated *vulgare* and wild *spontaneum*) has seven pairs of distinct chromosomes originally designated by Arabic numbers 1–7 (Nilan 1964; Ramage 1985). In spite of priority in naming, the barley community has agreed to redesignate the chromosomes according to their homoeologous relationships with chromosomes of other *Triticeae* species (Linde-Laursen 1997). Thus, barley chromosomes 1, 2, 3, 4, 5, 6, and 7 are now officially designated 7H, 2H, 3H, 4H, 1H, 6H, and 5H, respectively. The homoeology conclusion is well supported by morphological, biochemical, and molecular studies and the ability of the barley chromosomes to substitute for the equivalent wheat chromosomes in substitution lines (reviewed in Shepherd and Islam 1992). In this report, we will add the original barley chromosome designations in parentheses in order to avoid confusion with the older genetic and cytological literature.

The five chromosomes without satellites are designated 1H(5), 2H, 3H, 4H, and 7H(1). The two chromosomes with satellites are designated 5H(7) and 6H. Chromosome 6H has the larger satellite and is shorter than chromosome 5H(7), which has the smaller satellite.

Distinctive Giemsa C- and N-banding pattern identifies each of the seven barley chromosomes (reviewed in Ramage 1985; Linde-Laursen and Jensen 1992). Based on the few reports of barley pachytene chromosome analyses (Sarvella et al. 1958; Singh and Tsuchiya 1975), the relative lengths and arm ratios agree reasonably well with those published for mitotic metaphase chromosomes.

Barley genetic mapping from the first part of the twentieth century was summarized by Luther Smith (1951 and references therein). Briefly, seven barley linkage groups named I–VII were identified and associated genes were listed. Large numbers of chromosome translocations were being identified at the time and were summarized (Burnham and Hagberg 1956). Translocations turned out to be useful in associating the seven linkage groups with specific chromosomes (Burnham and Hagberg 1956). The literature is summarized in Nilan (1964). Briefly, chromosome 7H(1) encompassed linkage groups III and VII, 2(H) I, 3H VI, 4H IV, 1H(5) II, 6H not previously identified, and 5H(7) V. Besides translocations, trisomics also were used to associate genes with chromosomes (Tsuchiya 1960) and to confirm the translocation assignments. Other chromosome mutations were discovered and

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studied in the mid-twentieth century by many noted cytogeneticists of that time (reviewed in Nilan 1964).

Isolation of mutants and mapping of genes continued at a rapid pace until it became necessary to coordinate the effort. Publication of the *Barley Genetics Newsletter* (BGN) was discussed during the First International Barley Genetics Symposium held at Wageningen, The Netherlands, on August 26–31, 1963 and was implemented with Volume 1 in 1971. All current and back issues of the BGN are available on the Internet at http://wheat.pw.usda.gov/ggpages/bgn/. The BGN Volume 1 shows the current barley linkage map of that time with 113 naked eye polymorphism (NEP) (morphological and disease resistance) loci. This represented a great improvement over the maps presented by Smith (1951), which contained 32 loci. The cover of BGN 32, 2002 shows the NEP loci integrated into a single map by Jerry Franckowiak. This map includes 280 loci, but the exact positions of the loci are approximations in many cases since the different NEP markers occur in different lines and are mapped in different segregating populations.

The molecular age arrived with the publication of a rudimentary chromosome 6H map using restriction fragment length polymorphism (RFLP) markers to map the NADH-specific nitrate reductase gene (Kleinhofs et al. 1988). Polymerase chain reaction (PCR)-generated markers were soon introduced with the publication of a partial barley genome map (Shin et al. 1990). Complete molecular barley genome maps followed in rapid succession (Graner et al. 1991; Heun et al. 1991; Kleinhofs et al. 1993). These were primarily RFLP marker maps made with the progeny from a single cross. Once the basic maps were established, centromeres and telomeres were mapped. Due to the availability of ditelosomic addition lines, centromeres can be easily approximated (Kleinhofs et al. 1993; Kunzel et al. 2000). Telomeres presented a more difficult problem, but most of the arms were capped with markers by cloning telomere-associated sequences and by mapping them by a combination of RFLP and PCR methods (Kilian and Kleinhofs 1992; Kilian et al. 1999). Consensus maps were made by merging data from several individual maps (Langridge et al. 1995; Qi et al. 1996), and a "bin" map was developed to facilitate integration of maps (Kleinhofs and Graner 2001).

The RFLP technology is very reliable, and RFLP-based markers constitute the backbone of barley genome maps. However, RFLP technology is also slow and expensive. Therefore, new faster and cheaper (per data point) technologies were being developed such as random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP). The RAPD technology was not very reliable, and except for a few RAPD markers on some established maps, it did not contribute significantly to barley genome mapping. It is however, a technology that can be applied to identify closely linked markers to specific genes and it is useful for that purpose. The AFLP technology was considerably better, and several whole-genome maps were produced (Waugh et al. 1997b; Qi et al. 1998). The problem with the AFLP technology was that the markers were mostly dominant and could not be easily transferred to crosses with different parents. Nevertheless, it was useful for preparing maps rapidly and for finding closely linked markers for map-based cloning (Hinze et al. 1991; Mano et al. 2001). A similar PCR technology, labeled sequence-specific amplified polymorphism (S-SAP), anchored one primer in retrotransposon conserved sequence and was used to expand existing maps (Waugh et al. 1997a; Rodriguez et al. 2006). The S-SAP technology had some of the same problems as AFLP.

A rapidly emerging PCR technology based on simple sequence repeats (SSRs) has become favored by plant breeders for molecular marker-assisted selection (MMAS) and by geneticists for map building. This is due to their codominant nature, ease of use, high information content, abundance in barley genomes, potential for automation, and ready transferability among diverse crosses (Saghai Maroof et al. 1994; Becker and Heun 1995; Liu and Sommerville 1996; Struss and Plieske 1998; Varshney et al. 2004). Comprehensive SSR maps (Ramsay et al. 2000; Li et al. 2003) and an integrated consensus map merging SSR, RFLP, and AFLP markers (Karakousis et al. 2003) were

developed. A consensus map, containing 775 SSR loci and integrating six commonly used mapping populations, has been published (Varshney et al. 2007). This highly integrated map merged data from the Steptoe × Morex, Igri × Franka, Oregon Wolfe barley (OWB) recessive × OWB dominant, Lina × Canada Park, L94 × Vada, and SusPtrit × Vada populations. It is highly useful for scientists looking for specific SSR markers in defined regions of the genome because it is very dense and fully integrated with the RFLP markers used in earlier maps.

Integrated consensus maps were also developed using multiple mapping populations and multiple-type markers combining RFLP, SSR, and single nucleotide polymorphism (SNP) techniques to produce an integrated transcript map of the barley genome with 1032 expressed sequence tags (ESTs) (Stein et al. 2007). This map, providing a genetic resolution of approximately 1 cM, provides an excellent resource for targeted marker saturation, candidate gene identification, map-based gene cloning, and structural genomics.

A map integrating SSR and diversity arrays technology (DArT) markers was developed for a doubled-haploid population derived from the cross of cv. Barque-73 × *H. vulgare* spp. *spontaneum* accession CPI71284-48 (Hearnden et al. 2007). Due to the wide cross between *H. vulgare* spp. *vulgare* and *H. vulgare* spp. *spontaneum*, a very high level of polymorphism was observed allowing the mapping of SSR markers for which genomic assignments were not previously available.

SSR markers continue to be highly useful for breeders and geneticists; however, newer technologies with much higher throughputs have been developed, which are altering the barley mapping landscape. These, including DArTs and SNPs, will be discussed in a separate section below.

DARTS AND SNPS—HIGH-THROUGHPUT GENOTYPING SYSTEMS

DArT is a whole-genome profiling technology based on DNA/DNA hybridization (primarily on a microarray platform) using complexity reduced representation of genomes (Jaccoud et al. 2001). DArT assays a large number of SNP and InDel polymorphisms at many thousands (usually 10,000–50,000) of restriction enzyme (RE) sites distributed throughout the genome. Polymorphism detection is based on measuring the abundance of individual fragments in genome representations prepared by RE digestion and adapter ligation (Kilian et al. 2005). While there are many other possible implementations, the most common format of DArT makes use of the methylation filtration effect of *Pst*I RE to enrich representations for hypomethylated, low-copy genome regions, which tend to contain actively expressed genes (Wenzl et al. 2004; Kilian et al. 2005). In this format, the probes on DArT arrays are very similar to genomic probes used in RFLP, and therefore, DArT can be described as a highly parallel, reverse RFLP. However, the scoring of markers is not strictly based on the restriction fragment length but rather on the presence (abundance) of a fragment in genomic representation. DArT markers are usually assayed in a dominant fashion; the underlying signal intensities of hybridized genomic representations (targets) are highly quantitative (described in detail below) and allow the identification of heterozygotes by applying a more statistically sophisticated classification algorithm (Diversity Arrays Technology Pty Ltd, Yarralumla, ACT, Australia, unpublished data).

While the initial proof of concept for DArT was developed using the rice (*Oryza sativa*) genome (Jaccoud et al. 2001), the first comprehensive genotyping DArT array was reported for barley (Wenzl et al. 2004). This first paper reported technology development/optimization and its deployment to genetic mapping and diversity analysis. This was also a first report on creating a medium-density genetic map of any crop species based on detecting many hundreds of markers in a single assay. A map of nearly 1000 markers for the Steptoe × Morex population was reported, with a fairly uniform marker distribution among the linkage groups and along each chromosome (Wenzl et al. 2004).

Following the initial paper describing DArT performance in barley, the technology has gained an appreciable level of application through a

service provided by Triticarte Pty Ltd in Canberra, Australia. The most recent DArT array, containing over 3000 polymorphic markers, was developed by screening 45,312 unique genomic clones, most of which were obtained using PstI/BstNI methyl–filtration complexity reduction. In the last 4 years, over 20,000 barley samples were processed using DArT arrays for whole-genome profiling. A large proportion of these samples were from mapping populations. The utility of DArT arrays in barley has been enhanced by construction of a barley consensus map (Wenzl et al. 2006). This map was constructed using the seven doubled haploid (DH) populations Barque-73 × CPI71284-48, Clipper × Sahara, Dayton × Zhepi2, Igri × Atlas68a, Steptoe × Morex, TX9425 × Franklin, and Yerong × Franklin, and the three recombinant inbred (RI) populations Foster × CI4196 F8-9, Frederickson × Stander, and Patty × Tallon. The map contained 2935 markers (2085 DArT and 850 other markers) and spanned 1161 cM. The 2935 loci of the whole data set were distributed into 1629 bins (unique loci) with an average interbin distance of 0.71 ± 1.01 cM (median = 0.32 cM). This resolution was only moderately greater than the resolution obtained with DArT loci alone (1.03 ± 1.59 cM; median = 0.40 cM). DArT markers also exhibited a moderate preference toward more distal (telomeric) regions in some of the chromosomes (1H, 2H, 3H, 6H, and 7H). Chromosome 5H had a more even distribution of loci than the other chromosomes.

In order to further increase the resolution power of DArT-based genome profiling, a new set of markers was derived from *H. vulgare* subsp. *spontaneum* accessions and was mapped in four additional populations, leading to the establishment of new integrated barley map with 3542 markers including over 600 new DArT markers (Alsop et al. 2010). In each of the four mapping populations reported in this study (two involving cultivated × wild cross and two cultivated × cultivated), over 550 DArT markers (average of 573.3 per map) were mapped confirming improved coverage compared to the earlier generation array, which allowed mapping just 442 markers in a cultivated × wild barley map (Hearnden et al. 2007).

High genome coverage and good performance across a wide range of germplasm including wild barley accessions prompted interest in deploying DArT in a number of association studies. DArT markers were evaluated for 318 Wild Barley Diversity Collection (WBDC) accessions from the Fertile Crescent, Central Asia, North Africa, and the Caucasus region for resistance to leaf rust, stem rust, and/or stripe rust (Steffenson et al. 2007). Association mapping for stem rust resistance applied in the WBDC detected significant marker associations. Two significant marker associations were detected for resistance to the wheat stem rust race MCCF in the same bin as the *rpg4/Rpg5* complex on chromosome 7(5H). The presence of a major stem rust resistance gene in this bin on chromosome 7(5H) was validated in a biparental population (WBDC accession Damon × cv. Harrington) mapped with DArT markers (Steffenson et al. 2007).

Another study reported a whole-genome scan with 1130 DArT markers of a population of 192 genotypes representing landraces and old and contemporary cultivars from key regions around the Mediterranean basin and the rest of Europe (Comadran et al. 2008). Significant associations of yield under drought stress with barley genomic regions were detected by combining DArT marker data with yield data in mixed model analyses. Quantitative trait loci (QTLs) with good consistency across environments were detected on barley chromosomes 3H, 4H, 5H, and 7H. These studies heralded an era of whole-genome association studies in barley (Waugh et al. 2009).

A sequence analysis of approximately 2500 DArT markers from a *Pst*I representation of barley suggested that approximately 50% of the markers were derived from actively expressed sequences (unpublished data). This is consistent with the method of complexity reduction and the process of marker discovery providing an effective filtering system for removal of repetitive sequences among DArT markers. Recently, when an additional 1400 DArT markers were sequenced (many of them derived from *H. vulgare* subsp. *spontaneum* accessions), a very similar frequency of protein-coding sequences was obtained, which established that the current array (2008) contains

over 2000 unique sequences (unpublished data).

The quantitative nature of DArT assays was demonstrated in a bulked segregant analysis (BSA) of the previously well-characterized Steptoe × Morex mapping population using the DArT platform (Wenzl et al. 2007). In a reconstruction experiment, the allele frequency difference between the bulks constructed based on a leaf pubescence phenotype was tightly correlated with the measured hybridization difference ($r = 0.96$) (Wenzl et al. 2007). The hybridization intensities, therefore, are proportional to the abundance of alleles in DNA pools. This linear relationship between array signal and allele frequency allows not only reliable detection of linked markers but also approximate estimation of the genetic distance between markers and a gene. Quantitative BSA based on DArT has been successfully applied for rapid identification of molecular markers linked to phenotypes, especially disease resistance genes (unpublished data). In addition, the quantitative nature of DArT assay enables delivery of seed purity testing with a higher level of resolution compared with gel-based technologies.

SNPs are, by far, the most abundant molecular markers identified in all genomes studied and barley is not an exception (Brookes 1999). SNP discovery relies on the availability of sequencing data, and with the advent of improved and cheaper sequencing techniques, it is becoming ever more accessible. If sequencing data are not readily accessible, techniques for SNP discovery include prescreening for polymorphism using single-strand conformation polymorphism (SSCP) (Andersen et al. 2003) or EcoTILLING (Comai et al. 2004). In barley, SNP discovery has been achieved by searching assemblies of ESTs (Kota et al. 2003), sequencing selected sets of unigenes in several barley accessions (Rostoks et al. 2005b), or exploiting the Affymetrix Barley1 GeneChip (Close et al. 2004) to identify single feature polymorphisms (Rostoks et al. 2005a). An SNP-based integrated barley linkage map was developed by sequencing 1338 barley unigenes selected for association with various abiotic stresses in eight barley genotypes (Rostoks et al. 2005b). The SNP frequency observed in this study was 1/200 bp, which is similar to that observed by others using different sets of germplasm and across different loci (Kota et al. 2001; Kanazin et al. 2002; Bundock et al. 2003; Bundock and Henry 2004). The map integrated SSR, RFLP, and SNP loci for a total of 1237 and a map distance of 1211 cM. Barley genome map lengths of 1173–1387 cM have been reported previously (Kleinhofs et al. 1993; Ramsay et al. 2000; Costa et al. 2001). The aforementioned map was recently complemented by a less comprehensive consensus map comprising 216 additional SNP markers derived from ESTs (Kota et al. 2008). Transcript-derived markers were combined with SNP markers to add 1596 gene loci to the Steptoe × Morex map (Potokina et al. 2008).

The development of the Illumina GoldenGate SNP assay ushered in a new era of SNP mapping. In barley, 4596 SNPs have been arranged in three pilot GoldenGate assays, two of which, barley oligonucleotide pool assays 1 and 2 (BOPA1 and BOPA2), were used to develop a consensus genetic linkage map composed of 2943 SNPs from Steptoe × Morex, OWB, Haruna Nijo × OHU602, and Morex × Barke doubled-haploid mapping populations (Close et al. 2009). The SNP-derived genetic map is available online (http://www.harvest-web.org) or through HarvEST:Barley for Windows (http://harvest.ucr.edu/ or http://www.harvest-web.org).

Other maps exploiting the Illumina GoldenGate SNP mapping tools and combining with other molecular markers, particularly ESTs, are published or are nearing publication (Aghnoum et al., 2010; Close et al., 2009; Sato and Takeda 2009; Sato et al. 2009; Szucs et al. 2009; N. Stein, pers. comm.; Table 3.1).

MERGING MOLECULAR AND TRADITIONAL MAPS

While barley genome molecular mapping has undergone a revolution and more and more sophisticated maps are being developed, integrating the traditional NEP maps with the molecular

Table 3.1 Summary of recent and extensive barley molecular marker genetic maps[a]

Reference[b]	Marker[c]	Loci	cM	Population[d]
Rostoks et al. (2005b)	SNP	1237	1211	LH, OWB,SM
Wenzl et al. (2006)	DArT	2935	1161	Multiple
Varshney et al. (2007)	SSR	775	1068	Multiple
Marcel et al. (2007)	Multiple	3258	1081	Multiple
Stein et al. (2007)	EST	1255	1118	SM, OWB, IF
Hearnden et al. (2007)	SSR and DArT	1000	1100	Barque-73 × CPI71284-48
Potokina et al. (2008)	TDM	1596	1010	SM
Szucs et al. (2009)	Multiple	2383	?	OWB
Sato et al. (2009)	EST	2890	?	HNH602
Sato and Takeda (2009)	SNP	2890	1187	HNH602
Close et al. (2009)	SNP	2943	1099	Multiple
Aghnoum et al. (2010)	Multiple	6990	1093	Multiple
N. Stein (pers. comm.)	Multiple	1794	1068	MB

[a]Only relatively recent maps and with multiple marker loci are included.
[b]References are listed in the chapter except N. Stein (pers. comm.).
[c]The marker type listed is the most predominant type mapped. Multiple—indicates that many different marker types were integrated in the map. TDM, transcript-derived markers.
[d]Abbreviations used for populations: LH, Lina × HS92 (Canada Park); OWB, Oregon wolf barley recessive × dominant; SM, Steptoe × Morex; IF, Igri × Franka; HNH602, Haruna Nijo × H602; MB, Morex × Barke; multiple—indicates more than three populations were used.

maps has had relatively little progress. There have been a few, relatively large-scale efforts (Kudrna et al. 1996; Castiglioni et al. 1998; Costa et al. 2001; Pozzi et al. 2003).

Kudrna et al. (1996) used BSA to position 33 morphological genes on the Steptoe × Morex bin map. Integrating molecular marker maps with phenotypic trait maps has been updated every year in the BGN since 1997 (http://wheat.pw.usda.gov/ggpages/bgn/). Mapping of the OWB dominant × recessive population resulted in placing the following 14 morphological genes on a well-developed molecular map: *wax*, *nud*, *lks2*, *Vrs1*, *Zeo*, *wst*, *alm*, *Pub*, *Kap*, *Hsh*, *Blp*, *rob*, *raw1*, and *srh* (Costa et al. 2001). This map has been updated with additional molecular markers (http://barleyworld.org/oregonwolfe.php). These markers provide a backbone upon which other morphological and molecular markers can be integrated. The genes *Wax*, *Nud*, *Vrs1*, *Zeo*, and *Kap* have been cloned, and thus, gene-specific markers are available (http://wheat.pw.usda.gov/ggpages/bgn/).

An interesting AFLP-based procedure for efficient mapping of mutations was developed (Castiglioni et al. 1998). Large numbers of mutant genes were set up for potential mapping; however, only one mutant gene, *branched-5* (*brc-5*), was mapped as a proof of principle. The mutant *brc-5* is located on chromosome 2H, most likely, bin 6 between the RFLP markers CDO665 and BCD351B and between the closely linked AFLP markers E3636-2 and E4338-2. The same procedure was used to map the *calcaroides* genes *cal.a*, *b*, *C*, *d*, and *23* and *leafy lemma lel1* and *lel2* to chromosomes 2H, 5H(7), 5H(7), 3H, 5H(7), 1H(5), and 5H(7), respectively (Pozzi et al. 2000). These have been incorporated into the morphologic/molecular maps as much as possible, but integration may not be too precise due to the preponderance of AFLP markers, which are poorly integrated with other maps. The Castiglioni et al. (1998) RFLP-AFLP map was further improved by adding additional AFLP markers and was used to map more or less precisely a large number of genes including *adp*, *als*, *aur-a1* (*lig*), *aur-a2* (*lig*), *br1* (*brh1*), *br2* (*brh2*), *bra-d7* (*trd*), *cul3* (*cul4*), *cul5* (*cul4*), *cul15* (*cul4*), *cul16* (*cul4*), *den6* (*mnd6*), *den8* (*mnd6*), *dub-1*, *hex-v3* (*vrs1*), *hex-v4* (*vrs1*), *int-c5* (*int-c*), *K* (*Kap*),

li (*lig*), *lig-a2* (*lig*), *lk2* (*lks2*), *lk5* (*lks5*), *sld1*, *sld4*, *tr* (*trp*), *trd*, *unc*^{k}, *uc2* (*cul2*), and *uz* (*uzu*) (Pozzi et al. 2000). Several of these are alleles of the same gene, and integration of these markers is facilitated, because they have also been mapped by different approaches and in different populations.

The genes of the subunits of barley magnesium chelatase and their mutants have been analyzed (Hansson et al. 1998). It was shown that the mutations in semidominant chlorina mutants *clo-125*, *-157*, and *-161* are in the 42-kDa subunit of the magnesium chelatase gene. Furthermore, these mutations are allelic to the recessive mutation *xantha-h* (not the same as *xanh* now *xnt8* described under BGS140). The *xantha-h* mutation has been mapped to the long arm of chromosome 7H(1) and is almost certainly allelic to *Xnt1* based on map location and phenotype. The 140-kDa subunit is encoded by *Xantha-f* and maps to the short arm of chromosome 2H, and the 70-kDa magnesium chelatase subunit is encoded by *Xantha-g* and has not been mapped.

There are a few NEP genes that have been cloned, and additional NEP genes have been mapped with good precision but have not yet been cloned (http://wheat.pw.usda.gov/ggpages/bgn/). There are other genes, which have been mapped with molecular markers but not in reference to NEP genes found on the classical map. These genes, however, can be positioned with fair accuracy with reference to the other NEP genes. These analyses resulted in the development of an integrated NEP/molecular marker map that places the NEP genes, which can be positioned with reasonable accuracy in reference to well-established molecular markers composed mostly of RFLP and SSR markers (http://wheat.pw.usda.gov/ggpages/bgn/). The overall NEP/molecular marker map is still probably fairly inaccurate because NEP genes have been insufficiently mapped in relation to established molecular markers or in relation to other NEP genes.

A currently active project to map 480 NEP genes with respect to the SNP markers will dramatically change the NEP/molecular marker maps (Druka et al. 2010). This project attempts to localize the NEP genes with respect to the SNP maps in a population of mutants that were backcrossed to cv. Bowman by Jerry Franckowiak. After numerous backcrosses, one can reasonably expect that the genomic background would become homogenized to be like Bowman except for the linkage drag resulting from selection for the observable mutant trait. Thus, markers that are identical with the parent cultivar where the mutant was induced and are different from the Bowman marker at the same locus should be closely linked to the trait and will allow the estimation of the size of the donor fragment in the Near Isogenic Line. Mapping of the closely linked markers that have been dragged along with the mutant trait will place the mutant in the approximately correct location. Since the project at the Scottish Crop Research Institute is on a large scale, numerous NEP genes will be positioned on the molecular maps and will facilitate incorporating additional markers in the map (Druka et al. 2010).

SYNTENY WITH OTHER SPECIES

Rice

Development of molecular markers such as RFLPs, which can be transferred between related species, led to the establishment of syntenic relationships among several plant and animal species. The degree of synteny observed varies greatly; however, in plants, good marker order conservation has been observed among members of the *Triticeae* tribe (Devos et al. 1993). Gross synteny has been observed between rice and maize (*Zea mays*), wheat (*Triticum* spp.), and barley (Ahn and Tanksley 1993; Kurata et al. 1994; Saghai Maroof et al. 1996). Since barley and wheat are closely related and have been shown to exhibit a high degree of synteny, the synteny between rice and wheat is also relevant to barley (Devos et al. 1993; Dubcovsky et al. 1996). This observation is relevant since extensive analyses of rice–wheat synteny have been conducted (Van Deynze et al. 1995). These early analyses with their limited resolution led to the identification of regions of

the rice genome that appeared to be syntenic or even colinear with the barley genome. Therefore, it was anticipated that mapping and sequencing information of the small rice genome (ca. 400 Mb) (Arumuganathan and Earle 1991) could be used to identify probes and to saturate genomic regions in large genome organisms (ca. 4900 Mb) such as barley (Arumuganathan and Earle 1991), leading to eventual cloning of genes (Kilian et al. 1995). Comparison of high-density EST maps of barley to the rice genomic sequence revealed that about 50% of the barley genes are retained in an orthologous position (Stein et al. 2007). The ability to exploit rice information for saturation mapping in barley has been demonstrated on numerous occasions. However, high-resolution mapping often also identified regions within the genomes where colinearity of gene order broke down (Dunford et al. 1995; Kilian et al. 1995, 1997; Han et al. 1998, 1999; Smilde et al. 2001; Brunner et al. 2003; Collins et al. 2003; Yan et al. 2003a; Caldwell et al. 2004; Stein et al. 2005; Turner et al. 2005; Komatsuda et al. 2007). Direct cloning of barley genes based on rice orthologs and their map position has been successful in relatively few cases such as the *ror2* gene (Collins et al. 2003), *rym4/5* (Kanyuka et al. 2005; Stein et al. 2005), *Ppd-H1* (Turner et al. 2005), and the wheat *Vrn1* gene (Yan et al. 2003b), which was relevant to the cloning of the homologous barley gene (von Zitzewitz et al. 2005). However, in other cases, the orthologs were located in nonsyntenous positions or were absent, or at least not entirely recognizable. Thus, for example, the barley stem rust genes *Rpg1*, *Rpg5*, and *rpg4* were not found in the syntenous rice locations (Brueggeman et al. 2002, 2008). Similarly, the *Vrs1* gene (Pourkheirandish et al. 2007), the *Sh2* gene (Li and Gill 2002), and the *Ph1* gene (Griffiths et al. 2006) were not located in syntenous rice positions. The *Nud* gene homolog was found on the syntenous chromosome but was identified only after the barley gene was cloned (Taketa et al. 2008). In all of these cases, however, barley–rice synteny played an important part in identifying closely linked markers that helped to develop high-resolution maps and eventual cloning of the genes.

Brachypodium

While rice has been a good model system for the *Triticeae*, it is a tropical grass that diverged from barley and wheat about 50 million years ago (Paterson et al. 2004) and has its limitations. A better model grass, *Brachypodium*, has recently emerged. There are two species of *Brachypodium* that are diploid and have been investigated, that is, annual *Brachypodium distachyon* and the perennial species *Brachypodium sylvaticum*. *Brachypodium* is a small-stature temperate grass with a small genome of ca. 350 Mb (Huo et al. 2008), with self-fertility, with rapid generation time, and with simple growth requirements (Draper et al. 2001). Another advantage of *Brachypodium* is that it is readily transformable (Vogel et al. 2006a; Vogel and Hill 2008; for a recent review, see Garvin et al. 2008).

Phylogenic analysis based on sequenced ESTs and bacterial artificial chromosome (BAC) end sequences confirmed a close relationship between *Brachypodium* and barley and wheat, and a more distant relationship with rice, maize, and sorghum (Vogel et al. 2006b; Huo et al. 2008). Microcolinearity between *Brachypodium* and *Triticeae* species has been further confirmed in recent publications (Bossolini et al. 2007; Faris et al. 2008; Huo et al. 2008). Detailed analysis of the *Lr34/Yr18* leaf and stripe (yellow) rust resistance in wheat and comparison with *Brachypodium* and rice showed that the gene order was conserved in *Brachypodium* but not in rice (Spielmeyer et al. 2008). However, the region containing the *Lr34/Yr18* locus did not seem to be present in either rice or *Brachypodium*. This is a situation that has been observed with other disease resistance genes in comparisons between barley and rice (Brueggeman et al. 2002, 2008; Scherrer et al. 2005) and in line with the hypothesis that rapid reorganization takes place at disease resistance loci as proposed much earlier by Leister et al. (1998). We have observed that some regions of the barley genome are highly colinear with *Brachypodium*, while others, particularly around the disease resistance loci, are less so (Drader and Kleinhofs 2010). In summary, it seems that the availability of the *Brachypodium*

sequence together with rice will provide an excellent model for the large genome grass family crops such as barley.

Molecular marker maps, synteny studies, and sequencing have also provided significant insight into the evolution of the barley genome (Rostoks et al. 2002; Wei et al. 2002). Based on conserved marker colinearity in grass genomes, comparison of the corresponding molecular marker maps and sequences has substantiated previous assumptions that the ancestral cereal genome comprised five chromosomes only (Bolot et al. 2009). Recently, it was shown that a genome duplication that occurred some 50 million years ago is still traceable in wheat and barley through the presence of seven duplicated chromosome segments corresponding to the segmental genome duplications present in rice (Salse et al. 2008; Thiel et al. 2009).

PHYSICAL MAPPING OF THE BARLEY GENOME

In the absence of a complete genomic sequence of an organism, physical maps are required to relate data obtained from the genetic mapping of phenotypic traits to the level of tangible genes. In general terms, a physical map is an ordered set of DNA markers or DNA fragments among which the distances are expressed in physical distance units, such as "genome equivalent" (GE) or "length fraction" (LF) of chromosomes. While these estimates are still relatively crude and depend on a level of cytogenetic resolution and accuracy, the least biased measurement of a given chromosome fragment is in base pairs. The following paragraphs describe the major technologies applied to physically mapping the barley genome along with their potentials and limitations.

In situ hybridization

In the wake of the development of hybridization-based DNA markers, *in situ* techniques were developed for the direct visualization of the position of a given marker on a chromosome. Because of the limited sensitivity of the probe detection system, visualization of single-copy sequences by *in situ* hybridization is a critical issue. As a result, the majority of efforts were focussed on multi-copy genes encoding ribosomal DNA or storage proteins, for example (Lehfer et al. 1993; Leitch and Heslop-Harrison 1993; Pedersen and Linde-Laursen 1995; Pedersen et al. 1995). Only few efforts to map single-copy sequences were successful, resulting in less than a dozen single- or low-copy genes assigned to a chromosomal position by this procedure (Pedersen and Linde-Laursen 1995; Butnaru et al. 1998). Using ultrasensitive fluorescence *in situ* hybridization (FISH) by indirect tyramid signal amplification (TSA), 14 cDNA probes were assigned to a physical location on one or more chromosomes (Stephens et al. 2004). However, TSA often results in very high background fluorescence because plant cells contain endogenous biotin that also cross-interacts with this signal amplification procedure. It remains to be seen whether directly labeled *in situ* hybridization probes will improve the visualization of small DNA targets in barley, as has been demonstrated for maize (Kato et al. 2006).

The use of complete BAC inserts allowed for the incorporation of sufficient label into hybridization probes. After masking the repetitive sequences present on BACs, the remaining single- and low-copy sequences yield sufficient fluorescent signal to facilitate a systematic *in situ* anchoring of the corresponding sequences. Using this approach in combination with the analysis of pachytene chromosomes, a physical map of rice chromosome 10 was constructed (Cheng et al. 2001). Applying FISH to 18 BAC clones, each harboring at least one genetically mapped marker, allowed for integration with the genetic map and provided evidence that the telomeres of the long and short arm were well tagged on the genetic map. Moreover, precise estimates were obtained of the ratio of genetic versus physical map distances. In the same study, *in situ* hybridization on artificially stretched interphase chromosome (fiber-FISH) analysis of closely spaced markers yielded a resolution of up to 40 ± 2 kb. In a similar way, FISH analysis of barley BAC clones was successfully established (Lapitan et al. 1997).

However, as the length extension of pachytene chromosomes is not significantly different from samples isolated from mitotic prophase chromosomes, *in situ* mapping in barley remains restricted to metaphase chromosome preparations. Based on these factors, the maximal resolution was estimated to be in the order of 10 Mb (Pedersen and Linde-Laursen 1995). An artificial increase of physical *in situ* hybridization resolution has been achieved by using super-stretched flow-sorted barley chromosomes as targets. Spatial resolution of neighboring loci was improved down to 70 kbp as compared with 5–10 Mbp after FISH on mitotic chromosomes (Valarik et al. 2004).

Fiber-FISH has been successfully applied to resolve the topology of the binding sites for the centromere-specific histone (CENH3) and gametocidal chromosome (GC)-rich centromeric satellite sequences within barley centromeres (Houben et al. 2007). However, given an average length of a barley chromosome arm of ca. 380 Mb, and keeping in mind that hybridization of at least three probes is required to estimate their physical distance and to define their orientation on the chromosome, the resolution of fiber-FISH seems too high for the construction of whole-genome physical maps. Hence, use of this technology will remain restricted to address specific issues in target areas comprising only a few hundred kilobases in size. In conclusion, *in situ* hybridization-based methods lack the throughput or accuracy that is required for the systematic construction of physical maps. However, on a case-by-case basis, they can provide valuable information to prove the consistency of physical maps developed by alternative procedures, to resolve the genetic make up of restricted chromosome regions, or to determine gap sizes on BAC contig maps (see below).

Physical mapping using genetic stocks

Being an amphiploid species, wheat can tolerate the presence of additional chromosomes in the nucleus. When present in a diploid state, these are meiotically stable. This feature has been successfully exploited for the development of wheat–barley addition lines. Altogether, this resource comprises all but one disomic addition of a complete barley chromosome (additions of 1H leads to sterility) and all chromosome arms except for 1HL (Islam et al. 1981). These disomic and ditelosomic additions have proved to be very useful in locating genes controlling proteins, isozymes, and molecular markers to individual barley chromosomes and chromosome arms (for references, see Islam and Shepherd 2000). While the assignment of a marker to an entire chromosome or chromosome arm is inadequate for physical map construction, it still allows rapid assignment of large quantities of markers. Using this approach, Nasuda et al. (2005) allocated 701 ESTs to a corresponding chromosome arm.

In an alternative approach, Barley1 GeneChip containing about 23,000 genes was employed for rapid parsing of more than 1250 genes to their corresponding chromosome arms. The assignments were performed by comparing the hybridization signal obtained from wheat and wheat–barley addition lines and by inferring that genes exclusively or predominantly expressed in a given addition line must be located on the corresponding barley chromosome (Cho et al. 2006; Bilgic et al. 2007). Similarly, isolation of individual barley chromosomes and chromosome arms by flow sorting allows multiparallel chromosomal assignment of markers. Using DNA from sorted chromosome 1H as a hybridization probe to interrogate an Illumina bead array representing 1536 genes, 40 hitherto unmapped genes could be assigned to this chromosome (Simkova et al. 2008). The extension of this approach to chromosome arms that were isolated from the wheat–barley addition lines facilitated the localization of the centromere positions in a high-density transcript map of barley at hitherto unprecedented precision (T.J. Close, pers. comm.).

Translocation mapping

Another approach to obtain information on the location of DNA markers exploits information that is available regarding the position of translocation breakpoints or the physical size of chromosome deletions. The corresponding positions on a chromosome are given as relative fragment (RF)

length (ratio of the length of the intact chromosome to the deleted or translocated segment) or milliGeNome (mGN) units (1 mGN = 1/1000 mitotic metaphase genome length) (Jensen and Linde-Laursen 1992; quoted by Kunzel et al. 2000).

The systematic analysis of translocation stocks only was possible after PCR-based marker assays became available, as these require only minute amounts of template DNA. Chromosomes carrying distinct translocations were collected by microisolation from mitotic cells and were subsequently used for localization of sequence tagged site (STS) markers by PCR (Sorokin et al. 1994). Based on PCR analysis of a set of barley chromosomes, which covered a total of 240 physically mapped translocation breakpoints, a physical map comprising 301 PCR markers that had previously been genetically mapped was constructed (Kunzel et al. 2000). The results confirmed, at a then unresolved resolution, that recombination rates are not evenly distributed along the barley chromosomes: only 4.9% of the genome showed high recombination frequencies (≤1 Mb/cM); 18% showed medium recombination frequencies (1–4.4 Mb/cM) approximating the expected average recombination frequency of 4.4 Mb/cM (total genome size [~5.500 Mb] divided by genetic map length [1.200 cM]). The vast remainder of the genome (77.1%) is characterized by low recombination rates (4.5–298 Mb/cM). Interestingly, the highly recombinogenic portion of the genome harbored 47.3 % of the mapped markers. As most of the markers used were derived from genes, the study provided evidence that (i) genes are not evenly distributed along the chromosomes and (ii) recombination is greatly increased in the gene space, which is located toward the distal portions of the chromosome arms. While reduced recombination in centromeric and pericentromeric regions is a common phenomenon seen in most plant genomes analyzed to date, the large extent of suppression observed in wheat and barley seems to be unique and may reflect a common signature in the evolution of *Triticeae* genomes.

Triticeae genomes are extraordinarily rich in repetitive sequences. More than 80% of the barley genome is composed of repetitive DNA, which in turn is mostly composed of retroelements (for a review, see Schulman and Kalendar 2005). The increasing availability of sequencing data sheds light on the genome dynamics, which is the result of massive insertions of transposons, for example, through copy and paste multiplication of retroelements, which, on the other hand, are counterbalanced by the loss of DNA sequences through unequal crossing-over, gene conversion, or sequential deletion (Fig. 3.1).

Apart from polyploidization, the delicate balance of gain and loss of repetitive elements is the major evolutionary force influencing genome size. In this context, a comparative sequence analysis of *copia* elements from *Arabidopsis*, rice and *Triticeae* suggests that their half-life, that is, the estimated time span until half of the full-length elements are at least partially removed from the genome, follows the order *Arabidopsis* < rice < *Triticeae* (Wicker and Keller 2007). Hence, the slower elimination of retrotransposons may account for the large genome sizes observed for *Triticeae* species. A sequence analysis of 30 kb of putative centromeric sequence revealed the presence of three intact copies of *cereba* elements including their 1-kb long terminal repeats (LTRs)

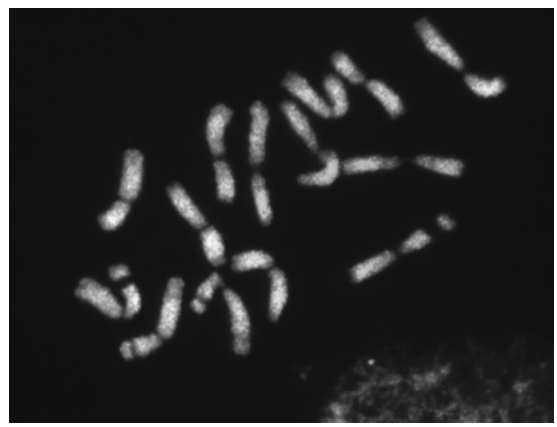

Fig. 3.1. Metaphase cell of barley cv. Morex after fluorescence *in situ* hybridizations with a probe specific for the *Gypsy*-type transposable element *BAGY2*. Hybridization signals (in yellow) are distributed throughout all chromosomes except the subtelomeric and pericentromeric regions (Wicker et al. 2009). For color detail, please see color plate section.

(Hudakova et al. 2001). The fact that the inspected sequence did not contain any internally deleted retrotransposons or solo LTRs lends strength to the hypothesis that regions that are suppressed in recombination tend to accumulate repetitive elements.

Deletion mapping

In addition to intact barley chromosomes or chromosome arms, the alloploid wheat genome is also able to accommodate deleted chromosomes. The corresponding genetic stocks were developed by first introgressing a so-called GC 2C from *Aegilops cylindrica* into the genome of common wheat (*Triticum aestivum*). Wheat lines carrying disomic additions of this chromosome undergo stable meiosis. Once the GC is present only in a hemizygous state (e.g., after backcrossing to wild-type bread wheat), those gametes that during meiosis have lost the GC contain deleted and translocated chromosomes (Endo 1988; Endo and Gill 1996). While the molecular mechanism of this process is not yet fully understood, it has been successfully exploited to develop a comprehensive set of deletion lines covering all three wheat genomes, which have been extensively used for the physical mapping of RFLP markers (Hohmann et al. 1995) and for the assignment of 7104 ESTs to 119 defined chromosomal regions (chromosome bins) (Qi et al. 2004). More recently, 154 additional markers were mapped at increased physical resolution into 159 bins. During this process, rare inconsistencies with previously reported marker orders were observed, which seem due to the presence of small secondary and tertiary deletions present in mapping lines (Ishikawa et al. 2009).

The GC system was also shown to work for barley. By crossing a hexaploid wheat line carrying a disomic addition of a GC to a disomic wheat barley addition line, the hybrid will be hemizygous for both the GC and the barley chromosome. When this hybrid is backcrossed to the original wheat–barley addition line, those progeny lines that lack the corresponding GC may contain translocated or deleted barley chromosomes that are stably inherited (Shi and Endo 1997, 1999). Following this approach, deletion-based libraries for barley chromosomes 7H(1) and 5H(7) have been constructed (Shi and Endo 2000; Serizawa et al. 2001; Ashida et al. 2007). The authors performed physical mapping of 17 AFLP and 28 STS markers derived from chromosome 7H(1) in a panel of 22 deletion/translocation lines. A similar approach was used to assign 97 EST markers to 20 bins on chromosome 5H(7) (Ashida et al. 2007).

The above-mentioned deletion lines form a stable and easily accessible resource to place large numbers of genetic markers without relying on the detection of polymorphism between parental lines. Like in meiotic mapping, where the number of crossover events determines the genetic resolution, the resolution in physical mapping depends on the number of distinct deletion/translocation events under study. In the case of chromosome 5H(7), 20 bins were defined by the available deletion lines (Ashida et al. 2007). Given an estimated physical size of 800 Mb, the average bin size on chromosome 5H(7) amounts to $800/20 = 40$ Mb. While this resolution is sufficient to develop a crude picture on gene distribution or synteny, it falls short of developing a physical order of closely linked genes or markers. Hence, attempts are underway to increase the physical resolution by increasing population size. In this regard, 90 deletion/translocation lines were analyzed to refine the resolution of the above-mentioned 7H(1) map. Cytogenetic analysis of the distribution of breakpoints revealed a high incidence (28%) in the centromeric region, providing a first hint that heterochromatic regions, which hardly show meiotic recombination, could be resolved by physical mapping (Masoudi-Nejad et al. 2005). Still, about 800 evenly distributed bins per chromosome would be required to increase the resolution to about 1 Mb/bin. This level is expected to be required to efficiently anchor and order nonoverlapping BAC contigs to the physical map.

Radiation hybrid (RH) mapping

The physical placement of markers at an even higher resolution can be achieved by the analysis of populations composed of RHs. These are

generated by lethally irradiating cells from a donor species (e.g., human) to induce random chromosomal breaks. The corresponding fragments are subsequently rescued as intergenomic translocations by fusion with a suitable recipient cell line from a different species (e.g., mouse or hamster). A panel of about 90 such lines has been successfully used for the construction of a high-resolution (100 kb) physical map of the human genome comprising about 42,000 STS markers (for references, see Riera-Lizarazu et al. 2008). The corresponding nuclei still contained 10%–50% of the human genome mostly as fragments translocated into rodent chromosomes. Based on the facts that (i) individual hybrid cells no longer harbor the full genome of the human donor cell and that (ii) the donor genome has been fragmented by irradiation, markers can be mapped based on the coretention frequency of marker pairs. The closer the loci are physically linked, the higher is the coretention frequency in the panel (Barrett 1992).

Up to now, RH mapping has only been of minor importance in the physical mapping of plant genomes. The generation of RH lines requires the availability of efficient protocols for the generation of viable protoplasts and the corresponding regeneration protocols to develop callus or suspension cultures for the maintenance of RH cell lines. As to barley, RHs were successfully generated by electrofusion of X-ray-treated protoplasts of cv. Golden Promise with recipient protoplasts from *Nicotiana tabacum*. The barley protoplasts contained the *bar* gene conferring tolerance to bialaphos as a selectable marker (Wardrop et al. 2002). The RH cell lines had retained barley DNA at an average frequency of about 25%. The limited amount of DNA available from the cell lines only allowed for the analysis of 35 SSR markers across a panel of 40 distinct RH lines. By the application of a whole-genome amplification protocol, the DNA yield of the panel could be increased by a factor of 20 (Wardrop et al. 2004). In combination with an increase in the number of RHs in the panel, this might pave the way for the construction of physical maps ordering several thousand markers at high physical resolution.

A more sustainable resource of RH lines has been developed for maize. Here, oat (*Avena sativa*) plants carrying a single addition of maize chromosome 9 have been subjected to γ-irradiation. After selfing, progeny plants that carried between 1 and 10 (average 3) rearranged maize chromosomes (intergenomic translocations, deletions, or a combination of both) were selected. In theory, a panel consisting of 100 RH plants would yield a genetic resolution of 600 kb (Riera-Lizarazu et al. 2000). In the meantime, a complete set of oat–maize addition lines has been produced and provides the basis for the development of further RH panels, which can be stably propagated through seeds (Kynast et al. 2004). A similar approach was employed in wheat (Hossain et al. 2004). By γ-irradiation of an alloplasmic (*Aegilops longissima*) *Triticum durum* line containing chromosome 1D of *Triticum tauschii* in a hemizygous state, an RH panel was developed, which allowed physical localization of the *scs*^{ae} gene (located on 1D) regulating the cytonuclear compatibility between *T. durum* and *Ae. longissima*. With an average physical resolution that amounts to an unprecedented 200 kb/bin, this RH panel represents a promising resource for future studies. These examples illustrate that genetically well-buffered alloploid plants such as wheat or oats may provide an excellent substrate for the development of sustainable, seed-based RH panels. Hence, the available stocks of wheat–barley addition lines should be well suited to investigate the development of the corresponding resources for barley.

Development of a BAC contig map

The above-described approaches aim at ordering DNA markers as landmarks for the estimation of physical distances between selected DNA fragments (loci). The availability of sufficient numbers of DNA markers as well as the efforts and resources required for their detection poses a serious limitation to the construction of whole-genome physical maps. As an alternative to the analytical ordering of markers, sequential ordering of large insert clones aims at reconstituting chromosomes or parts thereof. A minimal set of

overlapping clones that together provide complete coverage across a genomic region (minimum tiling path) provides the interface to link up genetic maps along with their phenotypic information to the DNA sequence that can be readily obtained for individual clones. Clearly, the best physical map will be the DNA sequence itself. However, despite the rapid progress in DNA sequencing technology (Shendure and Ji 2008), it is still not clear whether whole-genome sequencing approaches will provide sufficient information to produce a linear set of sequences in a complex crop plant genome, if these are not linked to a robust physical map consisting of overlapping DNA fragments cloned into BACs (Shizuya et al. 1992).

Small regional BAC contigs ranging up to about 500 kb have been constructed to isolate genes by map-based cloning (Table 3.2). This cumbersome approach of gene isolation relies on a "chromosome walking" strategy, which works well in small eukaryotic genomes (Bender et al. 1983) but quickly reaches its limits in complex genomes owing to the presence of excess repetitive DNA and chromosome regions that display suppressed recombination.

In recent years, the availability of automatic sequencing technologies has ushered in the development of generic physical mapping approaches that rely on (i) the digestion of BAC DNA with restriction enzymes (REs), (ii) the enzymatic labeling of the overhanging DNA ends of the resulting DNA fragments with fluorescent dideoxynucleotides, and (iii) the electrophoretic separation of the labeled fragments by automatic DNA sequencers. There is a series of technical variants of this approach, which mainly differ in the kind and number of REs used, the way DNA is labeled and subsequently analyzed by electrophoresis (for a review, see Meyers et al. 2004). Upon capturing the fragments by image analysis, the fingerprints of any two BAC clones are compared using "fingerprint contig" (FPC) software (Soderlund et al. 1997) (http://www.agcol.arizona.edu/software/fpc/) and parsed into singletons or contigs depending on the number of shared bands.

The feasibility of physical map construction in a *Triticeae* genome was recently shown for *T. aestivum* chromosome 3B, representing the largest chromosome in this species (995 Mb). Here, a chromosome-specific BAC library (Safar et al. 2004) was fingerprinted to about sevenfold coverage yielding 1036 contigs with an average size of 783 kb and covering 82% of the chromosome. Individual contigs were ordered by anchoring to the genetic map and by using a panel of 184 RH lines of chromosome 3B, resulting in an average resolution of about 260 kb/bin (Paux et al. 2008).

The development of a whole-genome physical map of the barley genome forms a constituent effort of the International Barley Sequencing Consortium (IBSC; http://www.barleygenome.

Table 3.2 Barley BAC contigs > 200 kb that were generated during map-based cloning experiments (National Center for Biotechnology GenBank, db nucleotide status 4/2009)

Chromosome	Contig Length (kb)	Genes	GenBank Accession	Library	Reference
5H	200	*rpg4, Rpg5*	EU812563	Morex	Brueggeman et al. (2008)
7H	244	*nud*	AP009567	Morex	Taketa et al. (2008)
7H	232	*Rh2*	AY853252	Morex	Hanemann et al. (2009)
3H	212	*Rph7*	AF521177	Morex	Brunner et al. (2003)
3H	440	*rym4*	AY661558	Morex	Stein et al. (2005)
3H	300[a]	*Rph7*	AY642926	Cebada Capa	Scherrer et al. (2005)
5H	300	*Ha*	AY643842–AY643844	Morex	Caldwell et al. (2004)
1H	261	*Mla*	AF427791	Morex	Wei et al. (2002)
7H	330	*Rpg1*	AF509748–AF509765	Morex	Brueggeman et al. (2002)
2H	518	*Vrs1*	EF067844	Morex	Komatsuda et al. (2007)

[a]Contig 300 kb of which 184 kb is sequenced.

org). This consortium was established in 2006 by researchers from eight founding institutions located in Australia, Finland, Germany, Japan, the United Kingdom, and the United States with the objective of generating a high-quality reference genome sequence (Schulte et al. 2009). Against this backdrop, the construction of a physical BAC contig map was initiated by the identification of a subset of about 70,000 gene-containing BAC clones from the original Morex BAC library (Yu et al. 2000; Madishetty et al. 2007). The corresponding clones were further characterized by high content information fingerprinting (HCIF) (Luo et al. 2003). Subsequently, a generic effort to construct a whole-genome physical map by HCIF was initiated, aiming at fingerprinting 14 GE (Schulte et al. 2009).

In order to improve the genomic sampling of the original Morex BAC library, four additional BAC libraries of this cultivar were constructed. The complete set now comprises five BAC libraries that have been cloned with three different REs (2x *Hin*dIII, *Bam*HI, *Mbo*I) and one library of randomly sheared DNA, whose average insert sizes range from 92 to 147 kb and which altogether represent 26.9 GE. The critical influence of the average insert length of the fingerprinted BAC clones, the genome coverage of the libraries, and the influence of the alignment parameter (*T*) on the expected number of BAC contigs is illustrated in Table 3.3. A simulation revealed that by having an average BAC insert size of 125 kb, fingerprinting to 15-fold coverage and requiring an overlap of 70% to merge two clones into a contig (=70% of the fingerprinted bands need to be identical), one can expect about 7000 contigs with an average size of 800 kb. Hence, even after deep fingerprinting of the barley genome, a single coherent contig for each chromosome cannot be expected.

Currently, 340,000 high-quality fingerprints have been generated, that is, eightfold redundancy has been achieved. Of these 287,000 clones fall into 25,000 contigs, which, on average, comprise 11.5 BAC clones. At least a 12× genome coverage is expected to be completed by the end of 2009 (N. Stein and D. Schulte, pers. comm.). It will be a challenging task to order the individual contigs along the chromosomes and to connect them to the genetic map. Anchoring of the BAC contigs to the genetic map will require the systematic assignment of genetic markers to BAC contigs and their subsequent mapping in populations that provide sufficient genetic resolution to separate a maximum of contigs into different bins (i.e., >7000 bins).

High-density genetic maps based on DArT and SNP markers are being explored in the barley physical mapping project of the IBSC. The strategy is based on anchoring markers to fingerprinted BAC clones using methods previously described in wheat (Paux et al. 2008). The approach utilizes hybridization of fluorescent-labeled representations derived from three-dimensional (3D) pools

Table 3.3 Modeling of the influence of several key parameters (genome coverage, average BAC insert size, *T*, which represents the overlap between BAC clones) on the outcome of whole-genome BAC fingerprinting in barley. Results were obtained by simulations of a generalization of the models of Lander and Waterman (1988) and Wendl and Waterston (2002). Simulations are based on a genome size of 5 Gbp (J. Keilwagen and I. Grosse, unpublished results)

		10× coverage			15× coverage		
		T = 0.6	*T* = 0.7	*T* = 0.8	*T* = 0.6	*T* = 0.7	*T* = 0.8
av insert size = 100 kb	Number of contigs	9160	24,800	67,700	1860	8330	37,340
	av contig length (Mb)	0.6	0.26	0.14	2.7	0.66	0.21
av insert size = 120 kb	Number of contigs	7630	20,700	56,400	1550	6940	31,120
	av contig length (Mb)	0.72	0.31	0.17	3.3	0.79	0.25
av insert size = 150 kb	Number of contigs	6100	16,590	45,110	1240	5550	24,890
	av contig length (Mb)	0.89	0.39	0.22	4.1	0.99	0.31

of BAC clones to molecular marker arrays. Preliminary experiments with DArT markers showed that very efficient anchoring was observed with superpools containing seven plates of 384 BAC clones. With this complexity of superpools (corresponding to approximately 0.07 barley GE), it required hybridizing several hundred DArT arrays to anchor approximately 2000 DArT markers to >1 GE of BAC clones from cv. Morex (N. Stein, pers. comm.). The available mapping populations are of insufficient resolution. Therefore, anchoring and ordering of contigs will require the development of novel resources such as high-resolution panels of RH and/or deletion/translocation lines. It has been demonstrated that linkage disequilibrium (LD) mapping can be applied for accurate placement of markers on a map (Rostoks et al. 2006). Therefore, natural barley populations, exhibiting a low level of LD, could serve as a future resource to locate contigs at high genetic resolution. In addition, the implementation of the increasing body of information about synteny between barley, rice, *Brachypodium*, and other grasses will greatly assist map integration.

CONCLUSIONS

Despite the large size and complexity of the barley genome, there has been tremendous progress in molecular mapping. Major factors driving this development were the following: (i) generation of a comprehensive EST resource that allowed systematic development of gene-based markers, (ii) development of a microarray containing 23,000 barley genes, and (iii) development of a large array of DArT markers. Comprehensive consensus maps provide a seminal resource to select markers for specific chromosomal regions and to exploit the information available for other grass species by comparative mapping. Compared to the first generation of RFLP markers, the application of high-throughput genotyping procedures for SNP and DArT markers increased the speed of data generation by several orders of magnitude. Hence, proper curation of the large amounts of molecular marker data will become a major issue.

Similar to genetic mapping, there has been a long tradition of physical mapping in barley, and a wealth of resources has been developed along the way. In conjunction with next-generation sequencing technologies, the construction of a physical BAC contig map will lead toward the whole barley genome sequence. While the BAC contig map is still a *studium nascendi*, the availability of a steadily increasing number of BAC contigs will be of great benefit for the map-based isolation of genes underlying qualitative and quantitative traits. Notwithstanding the obstacles posed by genome size, repetitive DNA, and the lack of recombination in proximal chromosome regions, which need to be surmounted on the way toward a coherent BAC contig map, the prospects of a genomic sequence will continue to motivate geneticists, breeders, and evolutionary biologists to take up the challenge and to develop this seminal resource.

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Chapter 4
Genome Analysis: The State of Knowledge of Barley Genes

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INTRODUCTION

Barley (*Hordeum vulgare* L.) is well adapted to short growing seasons and exhibits high tolerance to a range of abiotic stresses (e.g., cold, drought, alkali, and salinity). Thus, barley cultivation is well suited to all temperate regions of the world and ranks fourth in terms of total cereal production. The primary uses of barley grain are for animal feed and in the malting and brewing industry, where approximately two-thirds and one-third of the annual barley crop are used, respectively. Recently, barley has been promoted as a human food due to a number of potential health benefits (Baik and Ullrich 2008). These benefits include high levels of β-glucans, tocopherols and tocotrienols, and total and soluble dietary fiber (Fastnaught 2001). Because of such recognized human health-promoting properties, the worldwide acreage of barley may increase.

Due to the global importance of barley to agriculture, geneticists have a long history of studying the role and function of barley genes. The primary goal in these studies has been to develop an understanding of processes that underlie agronomic phenotypes (e.g., yield, heading date, and height), pest resistance, abiotic stress tolerance, and malting quality traits. Barley is a self-pollinating member of the grass family with a diploid genome comprising seven chromosomes (2n = 2x = 14). The estimated barley genome size is 5.3 billion nucleotides (Arumuganathan and Earle 1991) with over 80% of the genome composed of repetitive elements. For many decades, barley geneticists collected and characterized mutant phenotypes and developed genetic maps. The large genome size has slowed efforts to connect genes to phenotypes. With the advent of genomics tools and technologies, genes controlling important agronomic, quality, and development traits can be isolated and studied on a larger scale and more efficiently. In this chapter, we review the structural and functional genomic resources and databases that have been developed, and genes that control important traits that have been isolated and characterized.

STRUCTURAL GENOMICS TOOLS

Historically, isolation of barley genes has been difficult due to the large genome size and the lack of genomics tools. Beginning in the 1990s, an explosion in development of barley genomics tools enabled the isolation of a number of key genes underlying important traits. In this section, we briefly summarize the current genomics tools.

Expressed sequence tags (ESTs)

ESTs are generally 300- to 600-nucleotide long sequences derived from cDNA clones. ESTs provide a way to identify a large number of gene sequences from expressed genes. cDNA libraries are made from mRNA and thus they represent the expressed genes in the tissue that the mRNA was isolated from. Since 2000, several large barley

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EST projects generated significant numbers of sequences from expressed genes (e.g., Zhang et al. 2004). The total number of barley ESTs in GenBank (http://www.ncbi.nlm.nih.gov/) as of January 2009 is 539,689. These EST data were largely contributed by research groups in five countries including: the United States, Germany (IPK Gaterslaben), Japan (Okayama University), United Kingdom (Scottish Crop Research Institute), and Finland (University of Helsinki). The cDNA libraries used for the EST projects were derived from multiple developmental stages and tissue types and from abiotic and biotic treatments. In addition, eight different genotypes were used for cDNA library development. Thus, the barley community has a large collection of gene sequences that represent the expressed genes in many tissues, treatments, and genotypes.

Full-length cDNAs (FLcDNAs)

FLcDNAs are derived from entire mRNA molecules, including the coding sequence and 5′- and 3′-untranslated sequences (UTRs). There are several procedures for developing FLcDNA-rich cDNA libraries. The biotinylated cap trapper method is popular as it provides a high proportion of complete cDNAs (Carninci et al. 1996, 1997). FLcDNAs are useful for mapping on the genome sequence, gene prediction, and estimation of open reading frames. Sequences of FLcDNA also provide a primary structure for the protein they encode and can be used to develop a database for peptide mass fingerprinting to identify the expressed protein derived from the gene. The first comprehensive set of barley FLcDNA sequences was generated by Sato et al. (2009b). The mRNA was isolated from 15 different organs and treatments, followed by pooling to develop an FLcDNA library using the CAP trapper method (Carninci et al. 1998). A total of 5006 clones of the target goal of 25,000 were sequenced and deposited in GenBank, as well as in a separate database at http://www.shigen.nig.ac.jp/barley/. Sixty percent of the clones were confirmed to have complete coding sequences from comparison with rice amino acid and UniProt sequences. The high homology of FLcDNAs to mapped barley ESTs (27%) enabled anchoring of 151–233 FLcDNAs on each of the seven barley chromosomes. In the future, this will be a powerful resource for annotating the genome sequence.

Bacterial artificial chromosome (BAC) libraries

Large-insert genomic libraries are important resources for physical mapping, map-based gene cloning, and genome sequencing. The BAC vector system (Shizuya et al. 1992) has become an invaluable tool because of its ability to maintain stably large DNA fragments and its ease of handling. Seven BAC libraries have been constructed, six using the North American malting barley cv. Morex (Lapitan et al. 1997; Yu et al. 2000; Schulte et al. 2009) and one using the Japanese malting variety "Haruna Nijo" (Saisho et al. 2007). The five Morex BAC libraries cover over 25 genome equivalents (Schulte et al. 2009). The Haruna Nijo library consists of 294,912 clones with an average insert size of 115.2 kb and a genome coverage of about 6.6 genome equivalents (Saisho et al. 2007). Several genomics resource centers have been established that maintain and distribute large-insert genomic libraries. Clemson University Genomics Institute (CUGI; https://www.genome.clemson.edu/) is responsible for the distribution of the Morex BAC library developed by Yu and colleagues (2000) (coded as HV_MBa). The Haruna Nijo BAC library (coded as HNB) is available from the National Bioresource Project (http://www.nbrp.jp).

Genetic maps

Historically, barley geneticists spent decades developing high-quality genetic maps, composed of mutants, using classical three-point linkage tests (Franckowiak et al. 1997). However, with the development of molecular markers, genetic maps with hundreds to thousands of markers have been constructed. Several barley populations were first mapped with restriction fragment length polymorphisms (RFLPs) (e.g., Graner et al. 1991; Heun et al. 1991; Kleinhofs et al. 1993). Due to their transferability across species, RFLPs were essential for the early synteny studies that

identified evolutionary patterns and genome similarities among cereal crop species (Moore et al. 1995; Devos and Gale 1997; Devos 2005). Subsequently, amplified fragment length polymorphism (AFLP)-based markers were widely used for higher-density map development (Waugh et al. 1997; Hori et al. 2003; Takahashi et al. 2006). However, AFLP markers have problems with laboratory transferability and consistency. Sequence-based polymerase chain reaction (PCR) markers—that is, sequence tagged sites (STSs) (e.g., Mano et al. 1999) and simple sequence repeats (SSRs) (e.g., Ramsay et al. 2000; Varshney et al. 2006)—proved to be more reliable and informative. Stein et al. (2007) developed a consensus barley map with 1032 EST-based loci assayed using a combination of marker assays. Sato et al. (2009a) developed a high-resolution barley EST map with 2890 loci using a single mapping population. RFLP, STS, and SSR markers continue to serve as useful anchor loci for maps.

Single nucleotide polymorphisms (SNPs) have recently been adopted as the marker system of choice for many organisms including barley. EST-SNPs are derived from gene sequences and are amenable to high-throughput genotyping. Rostoks et al. (2005b) described the construction of an SNP-based linkage map. However, the major breakthrough in recent years in marker system and genetic linkage map development in barley was the adaptation of very cost-efficient, high-throughput, and high-quality genotyping assays based on the GoldenGate BeadArray technology (Illumina Inc., San Diego, CA). The GoldenGate technology is based on an allele-specific detection of SNPs. SNP assays for barley were developed primarily through the collaborative effort of an National Science Foundation (NSF)-funded grant to Timothy Close (University of California, Riverside) and two large association mapping projects: the Barley Coordinated Agricultural Project (CAP) in the United States (http://barleycap.cfans.umn.edu/), and Association Genetics of UK Elite Barley (AGOUEB) in the United Kingdom (http://www.agoueb.org/). A consensus genetic linkage map containing over 2943 gene-based SNP markers was developed (Close et al. 2009; http://harvest.ucr.edu/).

Two other high-throughput parallel marker systems are diversity arrays technology (DArT) and single-feature polymorphism (SFP). Over 2000 DArT markers have been developed and mapped on the barley genome (Wenzl et al. 2004, 2006). More than 4000 SFPs were mapped on the barley genome (Luo et al. 2007). Taken together, the barley genome has considerable marker density for molecular breeding, positional cloning, and integration of the genetic map to BAC-based physical maps. For a more complete description of genetic maps, see Chapter 3 in this book.

Physical mapping and genome sequencing

Physically mapping genes to chromosomes can be conducted using a variety of methodologies. Künzel et al. (2000) microdissected barley translocations and used PCR of mapped RFLP markers to identify the position of 240 translocation breakpoints. This work provided the opportunity to integrate physical and genetic distances and resulted in the identification of regions of high and low recombination. Wheat–barley chromosome addition lines can also be used for physical mapping genes to chromosomes. These stocks contain the full complement of wheat chromosomes and a single barley chromosome (e.g., Islam et al. 1981). Nasuda et al. (2005) assigned nonredundant barley 3′ ESTs to the seven barley chromosomes and their arms by PCR analysis using a set of wheat–barley chromosome and chromosome arm addition lines (Islam et al. 1981; Islam 1983; Islam and Shepherd 1990, 2000). These authors conducted PCR with the primers from ESTs using the DNA templates of euploid wheat (*Triticum aestivum* cv. Chinese Spring) and barley (*H. vulgare* cv. Betzes) to identify ESTs that were amplified only on the barley DNA template. They assigned 701 ESTs to single barley chromosomes and chromosome arms: 75 to 1H, 127 to 2H, 119 to 3H, 93 to 4H, 108 to 5H, 82 to 6H, and 97 to 7H.

The development of a BAC-based physical map for eventual genome sequencing is a large-scale worldwide effort. An NSF grant to Timothy Close (University of California, Riverside)

provided the funding to initiate this effort. Madishetty et al. (2007) developed a high-throughput approach to use overgo probes to identify gene-containing BAC clones. Over 10,000 overgo probes derived from EST sequences were used to identify 83,381 gene-containing clones from the Morex BAC library. Fingerprinting of these clones resulted in contigs that comprise roughly two-thirds of all barley genes (http://phymap.ucdavis.edu/barley/). Recently, four new Morex BAC libraries have been prepared that cover an estimated 20 haploid genome equivalents (Schulte et al. 2009). From these libraries, approximately 550,000 clones (covering approximately 14 genome equivalents) will be fingerprinted and assembled in contigs (Schulte et al. 2009). To complement these efforts, BAC-end sequencing will be conducted on 350,000 BACs (http://www.public.iastate.edu/~imagefpc/IBSC%20Webpage/IBSC%20Template-home.html). In addition, there are efforts to integrate the resulting BAC contigs with the SNP and DArT-based genetic maps. Thus, in the near future, there will be a robust BAC-based physical map integrated with the genetic map.

The International Barley Sequencing Consortium (IBSC; http://www.public.iastate.edu/~imagefpc/IBSC%20Webpage/IBSC%20Template-home.html) has developed a white paper for barley genome sequencing and provides coordination and communication for barley genome physical mapping and sequencing efforts. The overall goal of the IBSC is to develop a "gold standard" genome sequence. There are two general approaches to genome sequencing: identifying a minimum tilling path of genetically mapped BAC clones and sequencing these clones (BAC-by-BAC strategy), and whole genome shotgun sequencing. The large size of the barley genome and the high percentage of repetitive elements would seem to preclude the whole-genome shotgun approach. However, next-generation sequencing technologies (e.g., Shendure and Ji 2008) may eventually have the potential to overcome these constraints. Recent work with these technologies shows that genes can be identified within barley BAC clones and genome sequence (Wicker et al. 2006, 2008). Sequencing the barley genome will likely employ next-generation sequencing technologies and some combination of the BAC-by-BAC sequencing strategy coupled with whole-genome shotgun sequencing.

EXPRESSION PROFILING

One of the milestones of the international collaborative effort in generating ESTs from barley was the public release of the 22K Barley1 GeneChip by Affymetrix (http://www.affymetrix.com/). The chip design was based on approximately 350,000 ESTs from a range of tissues and genotypes representing 21,439 barley genes (Close et al. 2004). Since its release, the Barley1 GeneChip has become a platform of choice for most of the published large-scale mRNA profiling experiments. In the next sections, we describe the Affymetrix GeneChip technology, several published experiments that represent the range of the GeneChip uses for barley, as well as outline the Agilent spotted array technology as an alternative to the Affymetrix GeneChip.

Affymetrix GeneChip arrays

The basic building blocks of the Affymetrix GeneChip microarrays are 25-base long oligodeoxynucleotides (probes) that are synthesized at specific locations (features) on a coated quartz surface by photolithography (http://www.affymetrix.com/; Barone et al. 2001). Over a million features per microarray are usually available for the probe synthesis, allowing multiple (typically 22) probes per gene. Eleven probes are perfectly matched (PM) to the mRNA sequence of the representative gene (often called the "exemplar"). The remaining 11 mismatch (MM) probes have a noncomplementary middle base designed to detect and eliminate any false or contaminating signal within the gene expression measurement. The assumption is that the MM probe will hybridize to nonspecific sequences (background) as effectively as the PM probe, thus allowing spurious signals to be quantified and subtracted. This logic is incorporated into the statistical algorithms

that estimate a single expression value using all 22 probes from the probe set. Alternative statistical algorithms, where the MM signal is not used for the calculation of relative expression values, have been developed and are available (Hochreiter et al. 2006; Millenaar et al. 2006). For Affymetrix GeneChips, the labeling process and signal generation involve synthesis and labeling of cRNA with biotin, followed by a two-step signal enhancement procedure. GeneChip hybridizations are based on a single sample per single chip, which is different from the classical, two-dye mRNA profiling experiment designs where two RNA samples, each labeled with different colors, are hybridized to a single microarray.

Microarrays—examples of chip use

The concept behind mRNA profiling is that the mRNA abundance of genes measured across a range of different conditions or "treatments" can provide clues about gene function, or mRNA profiling can provide biological insights if known genes are considered. Microarrays measure mRNA abundance for thousands of genes simultaneously. Within this section, we describe the various uses of the Barley1 GeneChip.

Reference study

One of the first experiments using the Barley1 GeneChip was designed to provide a reference gene expression data set for barley researchers. The mRNA abundance in 15 tissues derived from 21,439 genes was measured in the barley cv. Morex (Druka et al. 2006). The experiment was planned, performed, and financially supported by barley researchers from all around the globe, including groups from the United Kingdom, United States, Germany, Finland, Japan, and Australia. The data set has been subsequently extensively used to advance many different projects.

Responses to abiotic and biotic stress

Barley is a salt-tolerant crop species and is therefore important in salinity-affected arid and semi-arid regions of the world. mRNA profiling experiments have reported reprogramming of the transcriptome during salinity, drought, and low-temperature stress. In one such experiment using salt stressed and unstressed seedlings, genes associated with jasmonic acid signaling were identified (Walia et al. 2007). A similar experiment was conducted to identify barley genes involved in responses to low temperature and drought (Tommasini et al. 2008). In these experiments, over 80% of the responsive genes were similar to *Arabidopsis* genes, suggesting conserved molecular mechanisms underlying these responses.

A number of mRNA profiling experiments have examined barley interactions with fungal pathogens that cause stem rust (*Puccinia graminis* f. sp. *tritici*), powdery mildew (*Blumeria graminis* f. sp. *hordei*), and fusarium head blight (*Fusarium graminearum*) (Caldo et al. 2004, 2006; Boddu et al. 2006, 2007; Zhang et al. 2008). One such experiment used a transgenic barley line carrying the *Rpg1* stem rust resistance gene (Zhang et al. 2008). The dominant barley stem rust resistance gene *Rpg1* confers resistance to many but not all pathotypes of the stem rust fungus *P. graminis* f. sp. *tritici* (*Pgt*). Transformation of *Rpg1* into the susceptible cv. Golden Promise rendered the transgenic plants resistant to *Pgt* pathotype MCC but not to *Pgt* pathotype QCC. The objective was to identify genes that were induced/repressed during the early stages of pathogen infection to elucidate the molecular mechanisms and the role of *Rpg1* in defense. All pairwise combinations of cv. Golden Promise and *Rpg1* transgenic line G02-448F-3R, and two *Pgt* pathotypes (MCC and QCC), were used for mRNA profiling. The subsequent analysis identified differentially expressed genes that were activated during the early infection process, before the hypersensitive response or fungal growth inhibition occurred. These results provided a list of candidate signaling components, which can be analyzed for function in *Rpg1*-mediated disease resistance.

Chip-based cloning

By comparing mRNA abundances of mutant and wild-type barley, it is possible to identify genes that have been "knocked out" in the mutant

during mutagenesis. These genes are candidate genes for the associated phenotypic change, or at least, they can help to identify the physical locus where the mutation (deletion) has occurred. Additionally, mRNA profiling can identify other genes that have mRNA levels affected by the mutation, thus enabling infererences of regulatory networks. An example of the application of this methodology has been demonstrated using the barley–stem rust pathosystem (Zhang et al. 2006; Druka et al. 2008b). Fast neutron mutagenesis usually generates subchromosomal deletions. A fast neutron-induced deletion mutant, showing susceptibility to stem rust pathotype *Pgt*-MCC, was identified in barley cv. Morex, which carries the stem rust resistance gene *Rpg1*. Genetic analyses and *Rpg1* mRNA and protein expression level analyses showed that the mutation was a suppressor of *Rpg1* and was designated *Rpr1* (required for *P. graminis* resistance). mRNA profiling identified a set of genes that were downregulated in the mutant compared to the wild type. Genetic analyses identified three genes that were deleted in the *rpr1* mutant, and each of these genes cosegregated with the *rpr1*-mediated susceptible phenotype. The loss of resistance was presumed to be due to a mutation in one or more of these genes or in other genes within the deletion, which may not be present on the Barley1 GeneChip (Zhang et al. 2006). Two other differentially expressed genes from this data set mapped to a different chromosome, coinciding with a stem rust resistance quantitative trait locus (QTL) (Druka et al. 2008b). These are prime candidates for a regulatory pathway underlying the barley–stem rust interaction.

Physical mapping

GeneChips can also be used to physically map genes to chromosomes and chromosome arms. Wheat–barley disomic and ditelosomic chromosome addition lines have been used as genetic tools for a range of applications since their development in the 1980s (Islam et al. 1981; Islam 1983; Islam and Shepherd 1990). The Barley1 GeneChip was used for comparative transcript analysis of the barley cv. Betzes, the wheat cv. Chinese Spring, and Chinese Spring–Betzes chromosome and ditelosomic addition lines to physically map barley genes to their respective chromosomes and chromosome arm locations, respectively (Cho et al. 2006; Bilgic et al. 2007). For example, those transcripts that were detected in Betzes and a ditelosomic addition line but not in Chinese Spring were placed on the chromosome arm carried by the ditelosomic addition line. This resulted in mapping 1257 barley genes to chromosome arms 1HS, 2HS, 2HL, 3HS, 3HL, 4HS, 4HL, 5HS, 5HL, 7HS, and 7HL (Bilgic et al. 2007).

SFP mapping

Binding of the short, 25-nucleotide long oligodeoxyribonucleotides (basic building block of the GeneChip) to their target sequences can be affected by SNPs and indels. Such small differences represent some of the forms of naturally occurring sequence variation. This has spawned considerable interest in using GeneChips for genotyping and mapping (Borevitz et al. 2003; Rostoks et al. 2005a; Luo et al. 2007). Over 4000 separate SFPs that accurately predicted the SNP genotype of >98% of the doubled-haploid (DH) lines of the 150-line Steptoe × Morex population have been reported for barley. They were highly enriched for features containing sequence polymorphisms, but the majority of SFPs (approximately 64%) did not exibit sequence polymorphism (Luo et al. 2007). This finding reflects the design of the Barley1 GeneChip; probe selection was based on EST alignments that comprised multiple barley genotypes. In most cases, probes were designed to the conserved part of the alignment. This can explain the relatively poor SFP-based SNP prediction but raises questions about what is the cause of the thousands of SFPs that have no DNA polymorphisms in the probe binding region. The most obvious explanations are alternative splicing or alternative polyadenylation, which both can directly affect probe binding. The distance from the 3′-end of the gene, base composition, and the sequence, as well as closely located polymorphisms can indirectly affect the binding to the probe.

Genetical genomics

In 1994, J. Damerval and colleagues described a method to "dissect the genetic architecture of quantitative variation of numerous gene products simultaneously." They proposed that "such methodology might help to understand the architecture of regulatory networks and the possible adaptive or phenotypic significance of polymorphism in the genes involved" (citations from Damerval et al. 1994). About a decade later, this approach was named "genetical genomics" (Jansen and Nap 2001). Originally, Damerval's experiments exploited variation in protein abundance as a molecular phenotype, but today, with the development of large-scale mRNA profiling platforms, expression-based approaches are used extensively. Advancements in high-throughput genotyping and genome sequencing were the key factors in developing this approach (for reviews, see Schadt et al. 2005; Rockman and Kruglyak 2006; Druka et al. 2010).

The basis of the genetical genomics approach is the comparison of mRNA abundance between different genotypes of the same species. Such comparisons revealed that hundreds or even thousands of genes are differentially expressed in a genotype-dependent manner. Classical genetical genomics experiments involve examining the expression profiles of individuals from the experimental mapping population that was derived from a cross between two distinct genotypes (parents). The resulting mRNA abundance measurements in each of the progeny are then considered a "phenotype" or "surrogate phenotype." Such phenotypes can be mapped using the well-established statistical tools of quantitative genetic analysis. Thus, the mRNA abundance (also often referred to as gene expression level) can be analyzed as a trait, no different from any other conventional (higher-order) traits (e.g., grain yield) in terms of QTL detection (Fig. 4.1.). The major difference from the classical higher-order traits is that available genomics tools allow obtaining mRNA abundance values for tens of thousands of genes in a highly parallel fashion. However, there is a profound difference between interpreting mRNA abundance and higher-order-trait QTL. For higher-order-trait QTLs, the underlying genes are either unknown or hypotheses are tested about the genes that have been inferred from other experiments. For the mRNA abundance trait, the sequence of the gene is known. Therefore, the first question when addressing transcript abundance QTL (also referred to as expression quantitative trait locus [eQTL]) is whether the gene's location coincides with its transcript abundance QTL. A positive answer means that the factors regulating mRNA abundance or the gene expression are in the proximity of the gene (called *cis*-regulation). On the other hand, if the eQTL location is different from the physical location of the gene, *trans*-regulation of the gene is inferred. For unsequenced species like barley, where a majority of genes are not mapped, the identification of the *cis*-eQTLs for thousands of genes has a significant added value by enabling construction of high-density genetic maps. Figure 4.1 shows how eQTL mapping can complement classical genetic approaches to infer genotype–phenotype linkages. Once the linkage is established, a line of inference can be further elaborated by exploiting the concept of *cis*- and *trans*-regulation.

In barley, a genetical genomics experiment was reported using a population of 150 recombinant DH lines and the Barley1 GeneChip (Potokina et al. 2008). This population was developed by crossing the high-yielding feed barley cv. Steptoe with the malting quality cv. Morex. The population has been genotyped primarily using RFLP markers, consecutively leading to the development of one of the first barley gene molecular maps (Kleinhofs et al. 1993). The population has also been extensively phenotyped, mostly for malting- and yield-related traits, and underlying loci (QTLs) were identified (e.g., Hayes et al. 1993; Han et al. 1995; Kandemir et al. 2000; Clancy et al. 2003; Ullrich et al. 2008). Thus, mRNA abundance data from the genetical genomics experiment can also be analyzed in the context of the legacy data. The following points summarize the results of the genetical genomics experiment:

1. About 16,000 barley genes identified 23,738 significant eQTLs that affected the expres-

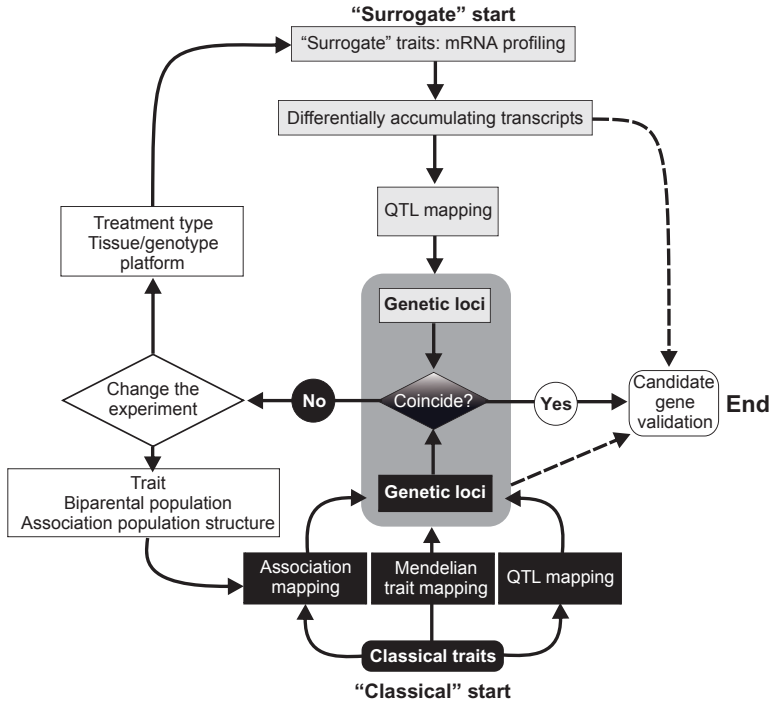

Fig. 4.1. Flowchart showing the exploitation of the concept of "surrogate" phenotypes to infer candidate genes for the higher-order trait. Parallel, high-throughput mRNA profiling using classical dose–response and/or time course experiments is usually aimed at the identification of reactive genes. Reactive genes are useful to determine metabolic or developmental pathways associated with the biological process. However, such experiments often yield large numbers of genes offering too many choices for validation. The number of candidate genes can be reduced by selecting those that have mRNA abundance QTLs coinciding with the higher-order-trait QTL. In case no coinciding QTLs are found, one has to consider redesigning either the mRNA profiling experiment or the higher-order-trait QTL mapping experiment. The use of mRNA profiling is limited to the traits that are determined or result in mRNA abundance variation. However, many traits may not necessarily be associated with mRNA abundance variation. For example, protein abundance or enzymatic activity also needs to be considered as an alternative "surrogate" phenotype for the higher-order trait.

sion of 12,987 genes. Over a third of these genes with expression variation had only one identified eQTL. The number of QTLs linked to the expression of the rest of the genes varied from two to six. Both *cis*- and *trans*-effects can be observed in a large proportion of the quantitatively controlled transcripts. In this population, more than half of the quantitatively controlled transcripts appear to be primarily regulated by *cis*-eQTLs (Potokina et al. 2008).

2. The potential of using these mRNA abundance QTL traits as surrogates for the identification of candidate genes underlying higher-order traits was explored on a well-studied interaction between barley and the wheat stem rust fungus *Pgt*. In this case, the transcript abundance QTL complements or even replaces more traditional gene isolation methods (Druka et al. 2008b).
3. By aligning previously generated SNP-based genetic linkage maps with transcript abundance QTLs and rice physical maps, an integrated barley gene map consisting of over 6000 markers was generated (A. Druka and R. Waugh, unpublished data).

Agilent microarray: alternative to the Affymetrix GeneChip

The Barley1 GeneChip has proven to be an efficient platform. However, the technology is relatively expensive and currently, many more novel ESTs are available, but they are not represented on the Barley1 GeneChip. Therefore, customized microarrays can provide a cheaper, more flexible alternative platform to the GeneChip. Agilent (http://www.chem.agilent.com/) manufactures off-the-shelf and custom arrays in several formats for many commonly studied species. Agilent's patented Ink Jet *in situ* Synthesis (IJISS) builds oligonucleotide sequences (60 mers), or probes, which represent genes of interest, directly onto the surface of modified glass slides. This produces very high-quality arrays with extremely flexible formatting depending upon the number of genes to be studied, with up to 240,000 gene sequences represented on a single slide. Currently, two 44K custom Agilent microarrays have been designed for barley (Druka et al. 2010). The 44K microarrays include at least twice as many genes as the Barley1 GeneChip.

FUNCTIONAL GENOMICS

Assigning gene function to sequences requires a functional test showing that a specific gene(s) is responsible for the phenotype. The tools to conduct functional genomics in barley have been slow to develop for a variety of reasons, including lack of an endogenous active transposon system, large genome size, and low-throughput transformation. In the last few years, a variety of functional genomics tools have been developed to study gene function. There are two general approaches to determine gene function, notably, through forward or reverse genetics.

Reverse genetics

Reverse genetics starts with the gene sequence and uses genetics tools to identify gene function. As described above, the international barley community has generated a large collection of ESTs and has used the Barley1 GeneChip to monitor transcript accumulation in many developmental stages, tissue types, and abiotic and biotic stresses. ESTs and results from Barley1 GeneChip experiments are rich resources for developing hypotheses for gene function or for verifying the gene underlying a mutant phenotype. To test these hypotheses, functional tests are required. A variety of reverse genetics tools have been developed to study gene function.

Repressing and overexpressing genes in transgenic plants

Testing genes in transgenic plants is a common functional genetics approach. Barley transformation techniques were developed in the 1990s (e.g., Wan and Lemaux 1994; Tingay et al. 1997). For a complete description of the status of barley transformation, see Chapter 6. Although barley transformation is possible, the throughput is low and only a few genes have been characterized in transgenic plants. As described above, one example is the introduction of the *Rpg1* (stem rust resistance) gene in susceptible barley (*rpg1*) resulted in stem rust resistant transgenic plants (Horvath et al. 2003). Another example is described below for the *Hv-elF4E* gene, which provides resistance to the *barley yellow mosaic virus* (BYMV) complex. Transformation of a resistant genotype with the Hv-elF4E gene from a susceptible genotype resulted in plants that were susceptible to *barley mild mosaic virus* (Stein et al. 2005). These examples indicate that testing genes in transgenic barley is a useful functional genomics tool.

Downregulation of genes in plants has been greatly improved through the development of RNA interference (RNAi). This approach is frequently used to downregulate genes in many plants (e.g., Chuang and Meyerowitz 2000). Double-stranded RNA interference (dsRNAi) functions through a homology-dependent post-transcriptional gene silencing pathway. Thus, RNAi is an ideal tool to downregulate genes that are family members and thus to overcome gene redundancy. Although RNAi should work well in barley, the inefficiency of barley transformation has precluded the use of this technology in stable transgenic barley. To date, these authors do not

know of any published work using RNAi to downregulate barley genes in stable transgenic barley.

Targeting-induced local lesions in genomes (TILLING)

TILLING was first developed for *Arabidopsis* (McCallum et al. 2000a,b). The goal of TILLING is to identify alleles of a gene where the gene sequence is known but the function is not. Once identified, plants carrying such alleles are used for detailed phenotypic characterization leading to inferences of gene function. TILLING relies on mutagenizing a large population of seed of a single genotype, isolating DNA from tissue from pooled plants, PCR amplification of a target gene from pooled DNA, and subsequent detection of mutations in the gene of interest. The following is a general approach to developing and characterizing a TILLING population. M_0 seeds from a single genotype are treated with a mutagen and are planted to generate M_1 plants. To ensure each mutation is derived from independent mutagenic events, one seed from each M_1 plant is harvested for the M_2 generation. M_2 plants are sampled and pooled for DNA isolation and are harvested for M_3 seeds. DNA from the pooled M_2 plants serves as the template for mutation screening. Mutation detection in the target gene involves digesting a heteroduplex DNA structure and visualizing the digestion product. There are multiple methodologies for detecting heteroduplex structures; many of them involve using the *Cel*I enzyme to digest the heteroduplex DNA and electrophoresis to detect the digested fragments. The M_3 seed serves as the resource for forward genetics and for confirming mutants detected in M_2 plants. The goal is to obtain an allelic series of mutations in the target gene that exhibit a range of phenotypes from loss of function to partial function. The final association of the mutant phenotypes with allelic differences in the target gene requires multiple alleles.

Two TILLING populations have been developed with two types of mutagens in barley. Caldwell et al. (2004) used ethyl methanesulfonate (EMS) to develop a population in cv. Optic. These authors demonstrated the utility of the population by identifying mutations in the *Hordoindoline-a* and the *Floral Organ Regulator-1* genes. Talame et al. (2008) used sodium azide (NaN_3) to develop a population in cv. Morex. Novel alleles were detected in four genes including *HvCO1*, *Rpg1*, *eIF4E*, and *NR*. Both the Optic and Morex populations are available for screening for mutations in target genes and for identifying novel mutant phenotypes.

TILLING has multiple advantages. Once the population is established and the pooled DNAs are isolated, screening for mutations in specific genes is high throughput. Many amplicons can be examined quickly and for a relatively low cost. In addition, in contrast to insertional mutagenesis, which generally results in loss-of-function mutations, TILLING results in an allelic series with a range of phenotypes from loss of function to partial function. This range of phenotypes may be directly applicable in breeding programs or in precise gene function research. Moreover, TILLING does not require transformation, and thus special containment facilities are not required.

Virus-induced gene silencing (VIGS)

VIGS was developed to rapidly and transiently test gene function. VIGS provides the opportunity to quickly test gene function before developing stable transgenic plants. The VIGS system functions when plants are infected with a virus carrying a plant gene with homology to a host gene. Viral infection and replication induces post-transcriptional gene silencing of any gene that exhibits homology to the gene carried in the virus. All homologous RNA sequences including the viral RNA are targeted for degradation (Ruiz et al. 1998). In barley, the VIGS system has been developed using the *barley stripe mosaic virus* (BSMV) (Holzberg et al. 2002). BSMV is a tripartite positive-sense RNA virus in the Hordeivirus family. Holzberg et al. (2002) showed that VIGS could be used to downregulate the phytoene desaturase (PDS) gene. PDS is required for carotenoid biosyntheis, which protects chorophyll from degradation. Plants inoculated with a

VIGS vector carrying the PDS gene exhibited bleaching, indicating that it is possible to down-regulate genes transiently in barley.

BSMV-VIGS has been used to test the function of *Rar1*, *Sgt1*, and *heat shock protein 90* (*Hsp90*) in *Mla13*-mediated powdery mildew resistance (Hein et al. 2005). As described below, *Rar1*, *Sgt1*, and *Hsp90* are required for *Mla*-mediated resistance to powdery mildew. These authors found that silencing *Rar1*, *Sgt1*, or *Hsp90* during infection by an *Avr13* powdery mildew isolate resulted in a susceptible phenotype. These results confirm previous results that *Rar1* and *Sgt1* are required for *Mla13*-mediated resistance and demonstrate that *Hsp90* is also required. In addition, these results confirm that the BSMV-VIGS system can be used to examine gene function in barley.

Transient-induced gene silencing (TIGS)

A rapid specialized TIGS assay has been developed to study the barley–powdery mildew interaction (Douchkov et al. 2005). The causal pathogen of powdery mildew is *B. graminis* f. sp. *hordei* (*Bgh*), which is a biotrophic pathogen that infects epidermal cells in a cell autonomous fashion. The basic idea of TIGS is to express RNAi hairpin constructs carrying the gene to be tested in epidermal cells and then to study the *Bgh* infection process in those cells. Thus, the RNAi constructs result in the down-regulation of the target gene through posttranscriptional gene silencing in the epidermal cells, and the impact of the gene on powdery mildew infection can be studied (Douchkov et al. 2005). Image analysis have been improved and, coupled with the ability to rapidly create constructs using the Gateway™ technology, approximately 300 genes can be analyzed per month (Wise et al. 2009). To date, many genes have been tested using this system (Wise et al. 2009).

Forward genetics

The main tools of forward genetics are mutants. Barley geneticists have spent decades collecting and characterizing mutants. Thus, the barley community has a large collection of mutants to begin forward genetic approaches. In addition, recently additional genomics tools have been developed to complement the array of mutants and to accelerate forward genetics.

Mutants

Barley has been extensively used for mutation research and for subsequent mutant collection and characterization. The diploid nature of barley makes it amenable to mutant screens and characterization. Historically, barley geneticists have collected spontaneous and artificially induced mutants. These mutants have been systematically maintained in geneticist and breeder collections, at the National Small Grain Collection, Aberdeen, ID, and at the Nordic Genetic Resource Center, Alnarp, Sweden (Lundqvist and Franckowiak 2003). To enhance the utility and accessibility of the mutants, over 400 mutant alleles have been introgressed into cv. Bowman (Franckowiak et al. 1997). Information on the Bowman introgression lines is available at *Barley Genetics Newsletter*, Volume 26 (http://wheat.pw.usda.gov/ggpages/bgn/26/bgn26tc.html). These mutants provide a rich resource for functional studies and gene cloning.

Multiple mutagens have been used to induce mutations in barley. X-rays were the first treatments used to generate mutants in barley (Stadler 1928). Neutrons and chemical mutagens have also been used. Neutrons often cause large chromosomal deletions accompanied by male and female sterility. Chemical mutagens such as EMS and sodium azide have been used intensively due to the effectiveness of inducing point mutations. A summary of the mutagens used to induce mutations in barley can be found in Lundqvist and Franckowiak (2003).

Several different methods for assigning gene symbols have been described and used (Smith 1951; Nilan 1964; Lundqvist et al. 1996). Locus names and gene symbols of barley mutants were proposed in the *Barley Genetics Newsletter* and were approved by the International Barley Genetic Symposium (Franckowiak et al. 1997).

The current nomenclature system uses three descriptive letters for each locus. A recessive mutation uses all lowercase letters; otherwise, the first letter is a capital letter. Locus designations are followed by a number describing a particular locus, and a small letter is used to define a particular allele. For example, *Lnt1* is the abbreviation for the *low number of tillers1* locus and *lnt1.a* is a specific allele. Some of the representative phenotypes underlying several barley reproductive and vegetative development traits are shown in Figs. 4.2 and 4.3.

Activator (Ac)/dissociator (Ds) transposon tagging

The maize *Activator* transposase (*Ac*TPase) gene is an autonomous transposon that directs the transposition of itself and the nonautonomous element *Ds*. *Ac*/*Ds* transposons have been used for transposon tagging genes in maize and in heterologous plants such as tomato (Jones et al. 1994), *Arabidopsis* (Springer et al. 1995), tobacco (Whitham et al. 1994), and petunia (Chuck et al. 1993). Koprek et al. (2000) pioneered the development of transgenic barley plants carrying the maize *Ac*/*Ds* transposons for use in transposon tagging genes. Transgenic barley that carried the maize *Ac* element and independent lines carrying the maize *Ds* element were developed. Crosses of the *Ac*-containing lines with the *Ds*-containing lines resulted in the transposition of the *Ds* element. In F_2 plants, approximately 47% of the *Ds* elements had transposed. Examination of F_3 plants revealed that 75% of the *Ds* transposition events were linked to the original *Ds* genomic locations, and 25% were not linked to the original Ds genomic locations. Multiple lines carrying the *Ds* element have been developed with the elements positioned throughout the barley genome. Nineteen and 100 independent *Ds* insertion sites have been mapped in two laboratories (Cooper et al. 2004; Zhao et al. 2006). These insertion sites can be used as launching points for future mutagenesis. Singh et al. (2006) showed the transposition of *Ds* elements over multiple generations and showed that the element transposed primarily into genic regions. This study demonstrated the ability of the *Ac*/*Ds* transposon system to conduct saturation mutagenesis. After the mutants are identified, the *Ds* element can be stabilized by segregation and selection of plants without the *Ac* element. Isolating the gene sequence underlying the mutant phenotype is straightforward as the *Ds* sequence can be used as a probe on genomic libraries prepared from the plants carrying the *Ds* element in the gene of interest. Alternatively, there are a variety of PCR-based approaches (e.g., thermal asymmetric interlace [TAIL]-PCR) to amplify sequences that flank the *Ds* insertion site. Although this system is useful for activating *Ds* elements, the number of lines carrying independent *Ds* elements is low; thus, this is primarily a forward genetic tool.

Activation tagging

Developing transgenic plants with a construct containing a strong promoter is referred to as activation tagging. The rationale is that plants exhibiting a phenotype carry the transgenic promoter ectopically expressing a random gene that is located in close proximity to the transgenic promoter insertion site. Ayliffe et al. (2007) generated transgenic barley carrying a *Ds* transposable element driven by two maize ubiquitin promoters. The rationale is that through *Ac* activity, *Ds* will transpose in the vicinity of a gene and the highly active ubiquitin promoters will drive the expression of the gene. A majority of the transposed *Ds* elements drove expression of genes flanking the *Ds* element. These lines are important for identifying dominant overexpression mutations and for overcoming the effect of gene redundancy on the recovery of loss-of-function mutations.

GENOMICS INFORMATION AND DATABASES

The barley community has a diverse array of databases that house information pertaining to barley genetics and genomics, which can be easily accessed on the Internet. Here, we present a list of these databases and the information that each contains (Table 4.1).

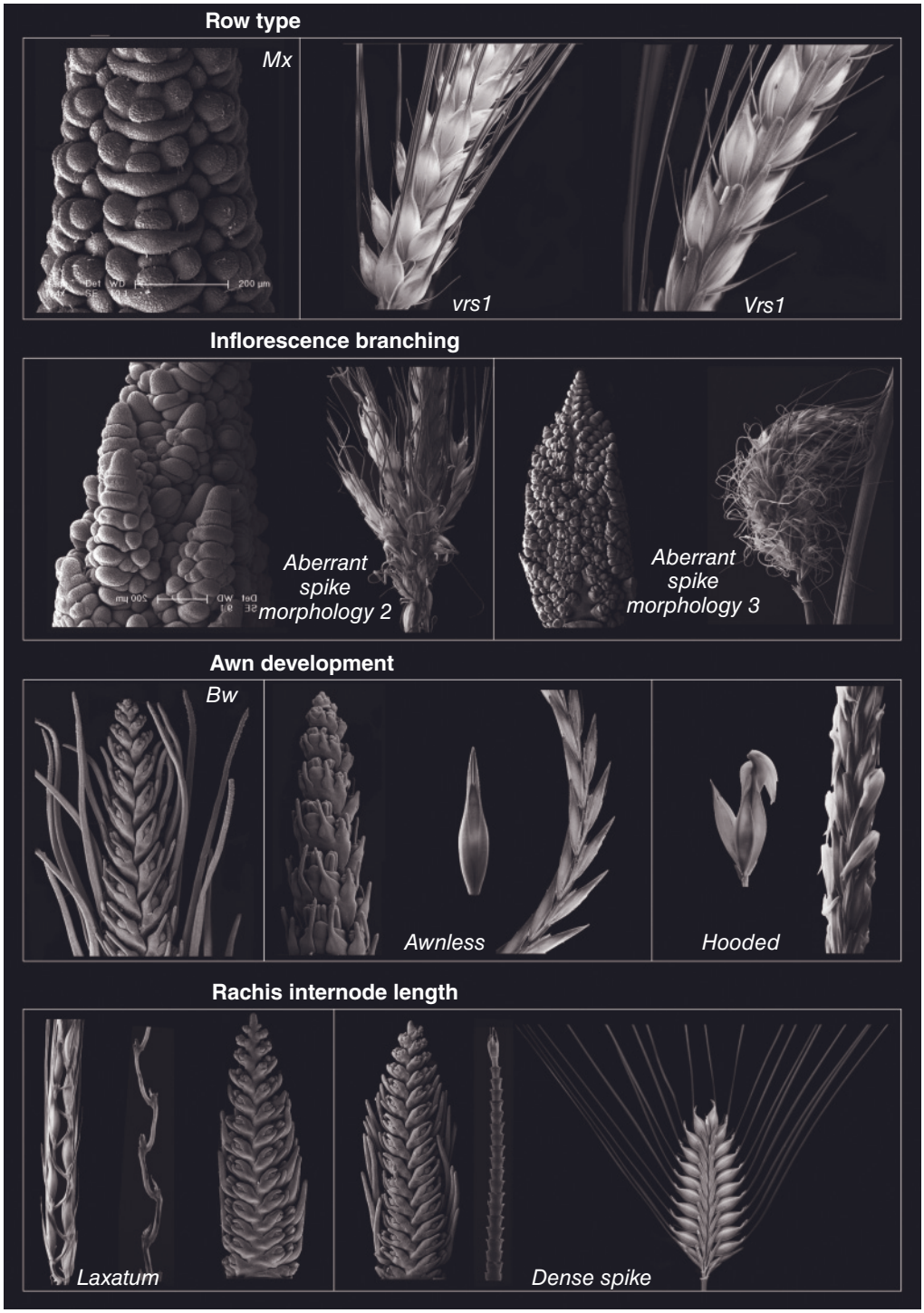

Fig. 4.2. Phenotypes of barley inflorescences representing row type, inflorescence branching, awn, and rachis development traits. The *vrs1* and *Vrs1* alleles represent six-row and two-row inflorescence types, respectively. The *aberrant spike morphology 2* and *aberrant spike morphology 3* mutants represent examples of excessive inflorescence branching mutants. The *awnless* and *Hooded* mutants represent mutants that exhibit altered awn development. The *laxatum* and *dense spike mutants* represent mutants exhibiting different rachis internode lengths. The rachis with spikelets removed is also shown for both mutants. Scanning electron microscopy images of developing barley inflorescences dissected from cv. Morex (Mx) and cv. Bowman (Bw) and the mutants are also shown. For color details, please see color plate section.

Fig. 4.3. Phenotypes associated with barley vegetative development representing leaf color, tillering, and ligular region development traits. For color details, please see color plate section.

Table 4.1 Relevant public databases that harbor barley genomics-related information and are accessible online

Database	URL	Reference
NCBI	http://www.ncbi.nlm.nih.gov/	U.S. government maintained site
ArrayExpress	http://www.ebi.ac.uk/microarray-as/ae/	Parkinson et al. (2007)
BarleyBase	http://barleybase.org/	Shen et al. (2005) Wise et al. (2007)
GrainGenes	http://www.graingenes.org/	O'Sullivan (2007)
HarvEST	http://www.harvest-web.org/	S. Wanamaker and T.J. Close (unpublished data)
GeneNetwork	http://www.genenetwork.org/	Druka et al. (2008a) Wang et al. (2003)
CR-EST	http://pgrc.ipk-gatersleben.de/cr-est/	Not available
MapMan	http://mapman.gabipd.org/web/guest	Thimm et al. (2004)
Barley Database	http://www.shigen.nig.ac.jp/barley/	Not available
The Gene Index Project	http://compbio.dfci.harvard.edu/tgi/	Not available
Barley SNP Database	http://bioinf.scri.ac.uk/barley_snpdb/	Rostoks et al. (2005b)
Barley Genetic Stocks AceDB Database	http://ace.untamo.net/	Not available
Barley Mutant Database	http://germinate.scri.ac.uk/barley/mutants/index.php?option=com_wrapper&Itemid=35	Caldwell et al. (2004)
TILLMore	http://www.distagenomics.unibo.it/TILLMore/	Talame et al. (2008)
The Hordeum Toolbox	http://www.hordeumtoolbox.org/	Not available
List of germplasm databases	http://www.bioversityinternational.org/index.php?id=168	Not available

The National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/) was established in 1988 as a division of the National Library of Medicine (NLM) at the National Institutes of Health (NIH). As a recognized resource for molecular biology information, NCBI aims to develop new information technologies to aid understanding of fundamental molecular and genetic processes through creating automated systems for storing and analyzing molecular biology, biochemistry, and genetics-related data sets. NCBI also contributes to developing and promoting the standards for databases, data deposition and exchange, and biological nomenclature. The two major repositories of sequence data and mRNA profiling data sets are GenBank (GB) and Gene Expression Omnibus (GEO), respectively. Both are linked to PubMed, a database for scientific publications. PubMed is a service of the U.S. NLM that includes over 17 million citations from MEDLINE and other life science journals for biomedical articles dating back to the 1950s. PubMed includes links to full text articles and other related resources. Currently (as of January 2009), the word "barley" in the NCBI databases is associated with 9957 literature citations and abstracts (PubMed), 539,689 EST records, and 23,106 UniGene entries (gene-oriented clusters of transcript sequences). UniGene is an organized view of the transcriptome in the species collected and provided by NCBI (http://www.ncbi.nlm.nih.gov/sites/entrez?db=unigene). Each UniGene entry is a consensus nucleotide sequence derived from the same transcription locus (gene or expressed pseudogene), together with information on protein similarities, gene expression, cDNA clone reagents, and genomic location. NCBI also hosts a number of barley genetic linkage maps at http://www.ncbi.nlm.nih.gov/projects/mapview/map_search.cgi?taxid=4513.

GEO (http://www.ncbi.nlm.nih.gov/geo) at the NCBI has emerged as the leading, fully public repository for gene expression data. The database has web-based interfaces, applications, and graphics for efficient exploration, visualization, and interpretation of hundreds of microarray studies. Data can be examined from both experiment-centric and gene-centric perspectives using user-friendly tools that do not require specialized

expertise in microarray analysis or time-consuming downloads of massive data sets. Currently, there are 25 barley experiments deposited in GEO.

ArrayExpress(http://www.ebi.ac.uk/microarray-as/ae/) is another public resource for microarray data (Parkinson et al. 2007). ArrayExpress serves as an archive, providing access to microarray data, related publications, and a knowledge base of gene expression profiles. Data in the ArrayExpress repository can be queried by various parameters such as species, authors, or words used in the experiment description. The ArrayExpress resource also includes the Expression Profiler (EP) suite of tools for microarray data mining, analysis, and visualization, and MIAMExpress—an online data submission tool. As of August 2008, there are 20 barley experiments representing 754 assays in the ArrayExpress database.

Microarray Retriever (http://www.lgtc.nl/MaRe/) is a tool developed to enable meta-analysis studies, in which expression data from GEO and ArrayExpress can be combined. The tool allows access to the two repositories simultaneously. Complex queries can be conducted to search the repositories and retrieve microarray data from published articles and to download data in one structured archive.

BarleyBase (http://barleybase.org/) (Shen et al. 2005) and its successor, PLEXdb (http://plexdb.org/), are public resources for large-scale gene expression analysis for plants and plant pathogens. BarleyBase hosts microarray data sets for a range of species including *Arabidopsis*, barley, citrus, cotton, fusarium, grape, maize, medicago, poplar, rice, soybean, sugarcane, tomato, and wheat.

The database of choice for legacy and classical genetics data is GrainGenes (http://www.graingenes.org/) (O'Sullivan 2007). It hosts information such as genetic maps, genes, alleles, genetic markers, phenotypic data, QTL studies, experimental protocols, and publications about Triticeae species (wheat [*T. aestivum*], barley [*H. vulgare*], rye [*Secale cereale*], and their wild relatives) and oat (*Avena sativa*) and its wild relatives. The database can be queried by text searches, browsing, Boolean queries, MySQL commands, or by using premade queries created by the curators. GrainGenes also serves as an informative site for researchers and a means to communicate project aims and outcomes, and as a forum for discussion.

The Hordeum Toolbox (THT; http://www.hordeumtoolbox.org/), developed by the Barley CAP (http://www.barleycap.org/), contains pedigree, SNP genotype, and phenotype data from advanced breeding lines from 10 U.S. barley breeding programs. THT is housed within the GrainGenes database structure. The user can select any combination of data types and breeding lines for download. In addition, THT also houses SNP maps and provides linkage (via BarleyBase) of the probe sets on the Barley1 GeneChip to SNP markers.

One of the most popular tools among barley molecular geneticists has become HarvEST (http://harvest.ucr.edu/). HarvEST is principally an EST database-viewing software that emphasizes gene function and is oriented to comparative genomics and the design of oligonucleotides, in support of activities such as microarray content design, functional annotation, and physical and genetic mapping. The "HarvEST:Barley" component of HarvEST has additional functions to support comparative genome mapping. HarvEST was developed at the University of California, Riverside by Timothy Close, Steve Wanamaker, Mikeal Roose, and Matthew Lyon. HarvEST includes an ACE file viewer that allows the user to examine the sequence alignment and to readily determine where individual sequences reliably deviate from a consensus sequence. The sequences were assembled using CAP3 software (Huang and Madan 1999) and were used for the development of the Barley1 GeneChip microarray produced by Affymetrix (Close et al. 2004), for the development of overgo oligos to select BAC clones in gene rich regions (Zheng et al. 2006), and as a source of SNPs for Illumina oligonucleotide pool assays (Rostoks et al. 2006b). Integrated maps of approximately 3000 SNP markers from four barley mapping populations, along with a rice synteny viewer, are displayed on the site. A subset of these mapped SNPs is integrated with the minimum tiling path of

BAC clones. HarvEST also contains hyperlinks to other sequence databases and facilitates connection to NCBI for live Basic Local Alignment Search Tool (BLAST) searches of the public dbEST database.

One of the recently adapted tools for the analysis of barley genetic data is GeneNetwork (http://www.genenetwork.org/) (Druka et al. 2008a). GeneNetwork is an online analytical environment that enables the user to test genetic hypotheses about how component traits, such as mRNA abundance, may interact to condition more complex biological phenotypes (higher-order traits). Associations between transcript abundance, phenotypic traits, and genotype can be established by either using correlation or genetic linkage mapping functions.

The Barley DB database (http://www.shigen.nig.ac.jp/barley/) includes information on barley germplasm and barley genome resources at Okayama University. The database contains 5006 FLcDNAs and 134,928 entries of ESTs. The barley germplasm collection at Okayama University and their information are also available online.

The Crop EST database (CR-EST) is a publicly available online resource providing access to sequence, classification, clustering, and annotation data of crop EST projects at the IPK Gatersleben (http://pgrc.ipk-gatersleben.de/cr-est/). The main part of the database is occupied by barley EST data. A web application on this site allows BLAST searches against CR-EST ESTs and to query and retrieve data from Gene Ontology and metabolic pathway annotations as well as sequence similarities from stored results of BLASTX searches against the protein databases. CR-EST also features interactive JAVA-based tools, such as open reading frame visualization and explorative analysis of gene ontology mappings to ESTs.

MapMan (http://mapman.gabipd.org/web/guest) is a user-driven tool that displays gene expression data onto diagrams of metabolic pathways or other processes. MapMan was developed as one of the Genomanalyse im biologischen System Pflanze (GABI) projects funded by the Federal Ministry of Education and Research in Germany (Thimm et al. 2004). In barley, MapMan has been used to investigate the transcriptome relationships between seed maturation and germination (Sreenivasulu et al. 2008b).

The Gene Index Project (http://compbio.dfci.harvard.edu/tgi/) uses available EST and gene sequences, along with the reference genomes wherever available, to provide an inventory of likely genes and their variants and to annotate these with information regarding the functional roles played by these genes and their products. The latest HvGI release 10.0 on June 3, 2008 contains 41,206 tentative consensus (TC) sequences.

There are several databases describing barley mutant genetic stocks. The classical database is the Barley Genetic Stocks AceDB Database (http://ace.untamo.net/). It holds information on genetic stocks mostly described in the *Barley Genetics Newsletter* (http://wheat.pw.usda.gov/ggpages/bgn/). Two other databases house the forward genetic component (mutant phenotypes are displayed) of two TILLING projects: Barley Mutant Database (http://germinate.scri.ac.uk/cgi-bin/mutantsdatabase/index.pl) (Caldwell et al. 2004) and TILLMore (http://www.distagenomics.unibo.it/TILLMore/) (Talame et al. 2008).

Additionally, there is an abundance of Web sites that have various barley-related information made available for browsing or download. A comprehensive overview of these resources has been reported by Sreenivasulu et al. (2008a).

GENE CLONING AND CHARACTERIZATION

In the past few years, many of the tools and information described above have been used to isolate barley genes that control agronomic traits, provide pest resistance, and underlie interesting mutants. Even with the advent of genomics tools, the large size of the barley genome and the lack of genome sequence have precluded large-scale positional cloning of important barley genes. Here, we present examples of some of the genes underlying important or interesting phenotypes that have been isolated and characterized.

Genes controlling developmental phenotypes

The first gene in barley to be isolated that controls a developmental phenotype was *Hooded* (Müller et al. 1995) (Fig. 4.2). Barley florets consist of the abaxial (dorsal) lemma and adaxial (ventral) palea surrounding two lodicules, three stamens, and the pistil. The awn develops from the lemma. A dominant mutation at the barley *Hooded* gene results in the development of an inverted floret on the awn. *Hooded* was cloned from a cDNA library using the homeobox region from the maize homeotic gene *Knotted1* (*Kn1*) as a probe (Müller et al. 1995). The *Hooded* phenotype is due to a 305-bp duplication in intron 4, which results in the ectopic expression of the gene in the lemma–awn interface leading to the formation of the extra floret primordium. Dominant mutations in the maize *Kn1* gene result in ectopic expression of *Kn1* in the leaf, a blade-to-sheath tissue transformation and disruption of the ligular region (Smith et al. 1992). Interestingly, ectopic expression of the maize *kn1* gene in transgenic barley exhibited a barley *Hooded* phenotype (Williams-Carrier et al. 1997). These results indicate that ectopic expression of homeobox genes in barley and maize exhibits different phenotypes.

Barley spikes are composed of spikelet triplets (one central and two lateral) that are arranged alternatively at each rachis node. In cultivated barley, the central spikelet is fertile, whereas the two lateral spikelets can be fertile (six-row) or sterile (two-row). Lateral spikelet fertility is primarily controlled by the *vrs1* locus. Dominant alleles (*Vrs1*) at the *vrs1* locus result in two-rowed spikes, whereas recessive loss-of-function alleles (*vrs1*) result in six-rowed spikes (Fig. 4.2). Wild barley exhibits a two-rowed spike type, and thus mutations at the *vrs1* locus resulted in six-rowed barleys. The discovery of six-rowed spike phenotypes likely resulted in increased yield. The *Vrs1* gene was isolated via a map-based cloning approach and was shown to encode a homeodomain-leucine zipper I transcription factor (Komatsuda et al. 2007). Sequence analysis of six- and two-rowed cultivars and wild accessions showed that two-rowed barley is the progenitor to six-rowed barley, and six-rowed barley likely arose multiple times. Finally, RNA *in situ* hybridizations in two-rowed barley showed that the *vrs1* gene is specifically expressed in the lateral, but not central, spikelet primordia, thus further strengthening the hypothesis of its role in the suppression of lateral spikelet development (Komatsuda et al. 2007).

Barley is predominantly a self-pollinated plant due to cleistogamy. Cleistogamy is the result of the lemma and palea remaining closed during pollen release. Large and small lodicules are associated with noncleistogamy (i.e., chasmogamy) and cleistogamy, respectively (Honda et al. 2005). The cleistogamous state is recessive to the non-cleistogamous state and is under control of the *Cly1* gene. Nair and colleagues (2010) isolated the *Cly1* gene via a map-based cloning approach. *Cly1* encodes an ortholog of the *Arabidopsis AP2* transcription factor and contains two *AP2* domains and a putative microRNA172-targeting site. Associations between two independent synonymous nucleotide substitutions within the microRNA172-targeting site and cleistogamy were detected. In the noncleistogamous background, microRNA172-directed cleavage of *Cly1* was detected. Thus, Nair and coauthors (2010) proposed that microRNA172 directs the down-regulation of the *Cly1* allele, resulting in lodicule development and the noncleistogamous state. The nucleotide changes in the microRNA172-targeting site in the *cly1* allele prevent down-regulation of *cly1*, resulting in the inability of the lodicules to develop and the cleistogamous state.

Barley can be generally classified into winter and spring growth habit. Winter types require vernalization for flowering, whereas spring types do not require vernalization for flowering. Three genes (*VRN-H1*, *VRN-H2*, and *VRN-H3*) have been identified through homology-based approaches that control spring/winter growth habit. *VRN-H1* located on chromosome 5H encodes a MADS box transcription factor (Trevaskis et al. 2003), , which is a conserved sequence motif found in a family of transcription factors essential for the correct timing of flowering in plants. It is not usually defined. It is standard term in gene terminology. *VRN-H2* located on chromosome 4H encodes a zinc finger protein *CCT* (CONSTANS, CONSTANS-like, and

TOC) domain transcription factor (*ZCCT*) (Yan et al. 2004). *VRN-H3* encodes a gene product similar to that encoded by the *Arabidopsis Flowering Locus T* (*FT*) gene (Yan et al. 2006). One model that describes the interactions of these vernalization genes under different vernalization and day length conditions is that *VRN-H2* negatively regulates the *VRN-H1* and *VRN-H3* genes, thus suppressing flowering. Both vernalization and short-day conditions repress the expression of *VRN-H2*, resulting in the expression of *VRN-H3* and *VRN-H1* and, subsequently, flowering. In addition, *VRN-H3* is upregulated by long days (LDs) and induces the expression of *VRN-H1*, also leading to flowering. While *VRN-H1* is increased, it downregulates *VRN-H2*, thus exhibiting a feedback loop. Further work is required to determine if the protein products are interacting directly or indirectly and to identify the other interacting genes in this pathway.

Photoperiod, or day length, is used by plants to regulate flowering. In barley, the *Ppd-H1* locus is a major regulator of the response to LDs and flowering. The recessive *ppd-H1* allele exhibits reduced response to LDs, whereas the dominant *Ppd-H1* allele responds to LDs. The barley *Ppd-H1* gene was isolated through a map-based cloning strategy and colinearity between barley, rice, and *Brachypodium* (Turner et al. 2005). These authors showed that *Ppd-H1* encodes a pseudo-response regulator and regulates the circadian expression of photoperiod pathway genes. It also appears that the photoperiod and vernalization pathways are interconnected through the action of downstream photoperiod pathway genes on VRN-H3 (Cockram et al. 2007). Sequencing of *Ppd-H1* in landraces and wild barleys showed a relationship between genotype and geographic location (Cockram et al. 2007). Alleles predicting a nonresponsive phenotype were predominantly found in central and northern Europe consistent with the long growing season. Alleles predicting a responsive phenotype were found exclusively in wild barleys in southern Europe, Southwest Asia, and the Mediterranean basin, consistent with the LD flowering nature of wild barleys. These results show the impact of the *Ppd-H1* gene in a given geographic location on barley production.

Covered/naked caryopsis is controlled by a single locus Nud on the long arm of chromosome 7H. The *Nud* gene has been isolated via positional cloning and has been shown to encode an ethylene response factor (ERF) family transcription factor protein (Taketa et al. 2008). Confirmation of the gene was shown by the fact that all 100 naked barleys tested contained a 17-kb deletion that encompassed the ERF gene. Additionally, two X-ray-induced nud *alleles* contained DNA lesions in the ERF transcription factor gene. The observation that all naked barleys examined contain a 17-kb deletion is evidence for the monphyletic origin of naked barley. *Nud* gene expression is strictly localized to the testa and is proposed to function to regulate the composition of lipids on the surface of the pericarp epidermis (Taketa et al. 2008).

Two genes that underlie the *uzu* and *slender* mutant phenotypes have been characterized and isolated. The recessive *uzu* mutant exhibits a dwarf phenotype, accumulates a higher level of brassinosteroids, and a significantly reduced response to brassinosteriod application than wild-type plants. The *Uzu* gene was isolated and appears to encode a brassinosteroid receptor (Chono et al. 2003). Dominant mutations at the *Slender1* (*Sln1*) locus are gibberellic acid (GA) insensitive, resulting in a dwarf phenotype. In contrast, recessive mutations at the *Sln1* locus have constitutive GA responses and result in spindly plants. The *Sln1* gene was isolated and shown to be related to the *Arabidopsis GAI/RGA* and the wheat *Rht* and maize D8 genes (Peng et al. 1999; Chandler et al. 2002). These results indicate that *Sln1* is a GA response modulator.

Plant lesion mimic or necrotic mutants are a broad category of morphological mutants that display a wide range of disease lesion phenotypes in the absence of a pathogen. The mutants play a key role in the identification of the genes underlying the programmed cell death (PCD) pathway. PCD is associated with senescence and plays a key role in plant–pathogen interactions. Two genes, represented by *nec1* and *necS1* mutants, have been isolated, which are causal to disease lesion mimic or necrotic phenotype (Fig. 4.3). *Nec1* encodes a cyclic nucleotide-gated ion channel 4 homolog of the *Arabidopsis HLM1* gene (Rostoks et al. 2006a),

and *NecS1* appears to encode a cation/proton-exchanging protein (Zhang et al. 2009).

Genes controlling physiological phenotypes

Boron toxicity is an agronomic problem for barley and limits production worldwide (Sutton et al. 2007). The major QTL on chromosome 4H and several other minor QTLs underlying boron tolerance were identified in a population derived from the cross of the boron-tolerant Algerian landrace Sahara 3771 by the boron-sensitive genotype Clipper (Jefferies et al. 1999). Map-based cloning of the boron tolerance gene (*Bot1*) underlying the chromosome 4H QTL was conducted utilizing 6720 meiotic events and rice–barley synteny to identify markers and to define the region carrying the *Bot1* gene. A barley EST exhibiting homology to an *Arabidopsis* BOR1 efflux transporter was found to cosegregate with the *Bot1* gene. Compared with a boron-sensitive genotype Clipper, the tolerant genotype Sahara 3771 carries about four times more copies of the *Bot1* gene and exhibits an increase in transcript levels. The Sahara *Bot1* allele provides increased boron tolerance in yeast compared with the Clipper *Bot1* allele. The proposed role of the *Bot1* gene product is to limit the amount of boron entering the root and to increase boron removal from the leaves.

Aluminum toxicity is a problem for barley production in acid soils (Minella and Sorrells 1992). Barley genotypes that are resistant to high levels of aluminum exhibit the ability to tranport citrate from the roots into the soil, effectively detoxifying aluminum. A QTL for aluminum resistance was identified on chromosome 4H in a population derived from the resistant parent "Murasakimochi" and the susceptible parent Morex (Ma et al. 2004). This QTL was associated with aluminum-activated citrate secretion from the roots into the soil. Map-based cloning combined with GeneChip analysis was used to isolate the gene underlying the QTL (Furukawa et al. 2007). The gene, referred to as *HvAACT1*, encodes a multidrug and toxic compound extrusion (MATE) family member. Functional characterization of this transporter in *Xenopus* oocytes indicates that it transports citrate (Furukawa et al. 2007).

Two other genes that impact physiological traits have been isolated and characterized, including nitrate reductase (Cheng et al. 1986) and an *ent*-kaurenoic acid oxidase (Helliwell et al. 2001). The nitrate reductase gene encodes an enzyme that catalyzes the reduction of nitrate to nitrite in the nitrogen-reducing pathway. Numerous nitrate reductase-deficient mutants have been identified and characterized (e.g., Warner et al. 1977). The *Grd5* gene encodes *ent*-kaurenoic acid oxidase and catalyzes three steps in the gibberellin biosynthetic pathway. Gibberellin-responsive *grd5* mutants have been identified, each resulting in the accumulation of *ent*-kaurenoic acid in the grain and exhibiting a dwarf phenotype (Helliwell et al. 2001).

Genes controlling resistance to pathogens

Barley stem rust disease is caused by the stem rust fungus *Pgt*. Several genes confer resistance to stem rust, including the dominant alleles at the *Rpg1* and *Rpg5* loci and the recessive allele at the *Rpg4* locus. All three loci were cloned using a map-based cloning approach (Brueggeman et al. 2002, 2008). The *Rpg1* gene encodes a receptor kinase-like protein with two tandem protein kinase domains (Brueggeman et al. 2002). Nirmala et al. (2006) showed that the RPG1 protein was localized mainly in the cytosol, plasma membrane, and intracellular membranes. During infection by avirulent pathotypes, RPG1 protein levels are reduced to undetectable levels via the proteasome degradation pathway (Nirmala et al. 2007). However, the amount of RPG1 protein did not change during infection by virulent pathotypes. These results indicate that RPG1 degradation plays an important signaling role in the barley–*Pgt* resistance interaction. Recently, the *Rpg5* gene was isolated and shown to encode a novel three-domain protein composed of a nucleotide binding site, a leucine-rich repeat, and a serine threonine protein kinase (Brueggeman et al. 2008). Preliminary evidence suggests that *Rpg4* encodes an actin-depolymerizing factor (Brueggeman

et al. 2008). Future work is required to confirm the identity of the *Rpg4* gene.

Recessive mutations at the *Mlo* locus confer non-race-specific resistance to all known powdery mildew (*B. graminis* f. sp. *hordei*) isolates (Jørgensen 1992). *Mlo* was the first barley gene cloned using a map-based cloning approach (Buschges et al. 1997). *Mlo* encodes a 60-kDa protein with six membrane-spanning helices. A series of naturally occurring and artificially induced alleles have been identified to study the fine structure of the *Mlo* locus. The naturally occurring *mlo-11*-resistant allele contains a wild-type coding sequence, and a tandem repeat of the 5′ regulatory region and a portion of the coding sequence. This genomic configuration results in aberrant transcripts and the reduction of wild-type and mutant transcript and protein accumulation (Piffanelli et al. 2004). Thus, *mlo-11*-derived resistance is conferred by disruption of transcription via a *cis*-mediated alteration in the promoter region.

Genes that play a role in *mlo*-based resistance have been identified. Loss-of-function mutations in the *Ror1* and *Ror2* genes in a recessive *mlo* background result in plants susceptible to powdery mildew, indicating that *Ror1* and *Ror2* are required for *mlo*-specified resistance (Freialdenhoven et al. 1996). *Ror2* has been cloned and it encodes a syntaxin (Collins et al. 2003); however, the identity of *Ror1* is unknown.

The *Mla* resistance locus confers gene-for-gene resistance to powdery mildew isolates and contains more than 30 alleles in the resistance gene region. Wei et al. (1999) positionally cloned the *Mla* locus and sequenced a 240-kb portion of this region. Three gene families encoding nucleotide binding site–leucine-rich repeats (NBS-LRR) were identified. The *Mla1* and *Mla6* alleles have been identified as coiled-coil NBS-LRR proteins that confer resistance to the *AvrMla1* and *AvrMla6* powdery mildew-containing strains, respectively (Halterman et al. 2001; Zhou et al. 2001). To date, the relationship between each *Mla* allele and resistance has not been elucidated (Caldo et al. 2004).

Several other genes including *Rar1*, *Sgt1*, and *Hsp90* are essential for powdery mildew resistance governed by *Mla* genes (Hein et al. 2005). *Rar1* was isolated via a positional cloning strategy and was shown to encode a zinc-binding protein (Shirasu et al. 1999). *Rar1* is essential for a subset of *Mla* loci to specific races of mildew, especially required for *Mla6*, *Mla12* and *Mla13* (Halterman et al. 2003; Hein et al. 2005). *Rar1* may control steady-state levels of MLA protein. There was a strong reduction of both *Mla1* and *Mla6* steady-state levels in plants carrying the *rar1* mutant allele (Bieri et al. 2004). The *Sgt1* locus encodes a protein that functions in ubiquitin-dependent cell cycle control (Shen et al. 2003). *Mla6* was significantly compromised upon transient *Sgt1* single-cell silencing (Shen et al. 2003). The cytosolic *Hsp90* gene encodes a tetratricopeptide repeat motif and a domain with similarity to the cochaperone p23, and it interacts with the RAR1 and SGT1 proteins. VIGS experiments showed that *Hsp90* is required for *Mla13* resistance (Hein et al. 2005).

Recessive alleles of *Rym4/5* confer resistance to the BYMV complex. Resistance to *barley mild mosaic virus* and BYMV is conferred by the recessive *rym4* allele, whereas resistance to the BYMV 2 is conferred by the recessive *rym5* allele. The *rym4/5* resistance locus was fine mapped to chromosome 3HL (Pellio et al. 2005). Map-based and homology-based cloning of the *rym4/5* locus was conducted, and the gene was shown to encode a *eukaryotic translation initiation factor 4E* (*Hv-elF4E*) (Kanyuka et al. 2005; Stein et al. 2005). Sequence analysis of *Hv-elF4E* in plants carrying the *rym4* and *rym5* alleles showed that these carried distinct SNP alleles that conferred different resistance specificities. Transformation of a resistant genotype with the *Hv-elF4E* gene from a susceptible genotype resulted in plants that were susceptible to *barley mild mosaic virus*. Subsequent analysis showed that recessive allelic variation at *Hv-elF4E* confers different resistance specificities.

FUTURE DIRECTIONS

Historically, functional characterization of barley genes has progressed slowly due to the large genome size, lack of genomics tools, and the

recalcitrance of barley transformation. Recent improvements in structural and functional genomics tools, the development of databases, and improved barley transformation efficiency have increased the ability to isolate genes and to characterize gene function. These tools are now just beginning to be used in the barley research community, and the speed by which geneticists can isolate and test the functions of a gene is increasing rapidly. Although the barley genome has not been sequenced, efforts have been initiated under the auspices of the IBSC (http://barleygenome.org/). The genome sequence will provide another tool to more rapidly clone genes known by phenotype and will increase the number of markers for mapping QTL and marker-assisted selection.

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Chapter 5
Cytogenetics and Molecular Cytogenetics of Barley: A Model Cereal Crop with a Large Genome

Waheeb K. Heneen

INTRODUCTION

Barley (*Hordeum vulgare* L. ssp. *vulgare*) is an important cereal crop that ranks fourth after maize (*Zea mays*), wheat (*Triticum* spp.), and rice (*Oryza sativa*) based on world production. Its uses span over feed, beer, and food production. The genome of barley cv. Betzes has been recommended as a reference genome in the Triticeae (Linde-Laursen et al. 1997). The advantages of the choice of barley are its diploid nature, the acquired knowledge of its cytology, genetics and genomics, and the availability of abundant barley genetic stocks. Barley has the lowest chromosome number (2n = 14) compared with wheat (2n = 42), rice (2n = 24), and maize (2n = 20). The genome size (1C) of barley (4873–5096 Mbp) is less than that of wheat (15,966 Mbp) and larger than that of maize (2292–3313 Mbp) and rice (401–466 Mbp) (Arumuganathan and Earle 1991; Bennett and Leitch 2005). As in most other plants, more than 75% of the DNA is repetitive sequences. The DNA sequencing program of barley is in progress (http://barleygenome.org/), and a variety of morphological and molecular DNA markers have been assigned to its seven chromosomes in genetic linkage maps (Kleinhofs et al. 1993; Stein et al. 2007; Varshney et al. 2007; Sreenivasulu et al. 2008). The development of wheat–barley disomic and ditelosomic addition lines harboring different barley chromosomes or chromosome arms (Islam and Shepherd 1990) provided suitable material for mapping purposes. Of further relevance to the choice of barley is access to rich collections of genetic and chromosome structural mutant lines, the gained knowledge about genes that control important functions, and the colinearity and synteny of barley to wheat, rye, and rice (Lundqvist et al. 1996; Cho et al. 2006; Varshney et al. 2006; Sreenivasulu et al. 2008). Taken together, these various features support the choice of barley, with its relatively large genome, as a model cereal crop for molecular mapping, molecular cytogenetic studies, and development of an integrated cytogenetic map (Harper 2000).

The aim of this chapter is to briefly review the earlier cytogenetic and current molecular work done on barley complemented by current molecular cytogenetic and novel microscopic approaches and findings. Obviously, advances in molecular genetics and in cytological techniques and microscopical analysis are behind the progress of our knowledge in molecular cytogenetics. The recent discoveries in this field might give us some hints as to future prospects.

CYTOGENETICS

Karyotype

Staining with aceto-orcein, aceto-carmine, or Feulgen, and the application of the squash technique provided the first adequate mitotic

Barley: Production, Improvement, and Uses. Edited by Steven E. Ullrich

chromosome spreads that were suitable for karyotype analysis. Karyotype and idiogram configurations were mainly based on chromosome size, position of the centromere, and occurrence of distinct secondary "nucleolar" constrictions and eventual weak tertiary constrictions. Less contraction of mitotic prometaphase or late prophase chromosomes, as well as meiotic pachytene chromosomes provided further morphological details due to differential chromatin condensation and appearance of chromomeric patterns along the chromosomes. The accuracy of chromosome identification and characterization was speeded up by the visualization of additional morphological, structural, and functional criteria, following the introduction of chromosome banding techniques. Examples of these are the C-, N-, and Q-banding for differential staining of constitutive heterochromatin (Fig. 5.1) and the use of $AgNO_3$ for staining of active ribosomal DNA (rDNA) sites (see Linde-Laursen et al. 1997). Studies on DNA synthesis and late DNA replication also provided some kind of a banding pattern that coincided with the distribution of heterochromatin (Uozu et al. 1997). A new fluorescence chromosome banding technique, using 4′,6-diamidino-2-phenylindole (DAPI), followed by image three-dimensional reconstruction and deconvolution, was applied on barley and gave rise to a banding pattern similar to that of G-banding (Liu et al. 2004). In another fluorochrome banding technique for the visualization of guanine-cytosine (GC)-rich DNA regions, barley chromosomes were stained with propidium iodide and DAPI, resulting in a banding pattern similar to that induced by chromomycin A_3 (She et al. 2006). The designations 1H–7H, internationally recommended for the barley chromosomes, are based on the homoeologous relationships to wheat and the Triticeae system (Linde-Laursen et al. 1997). Barley cv. Betzes was chosen as the reference genome for the Triticeae (Linde-Laursen et al. 1997).

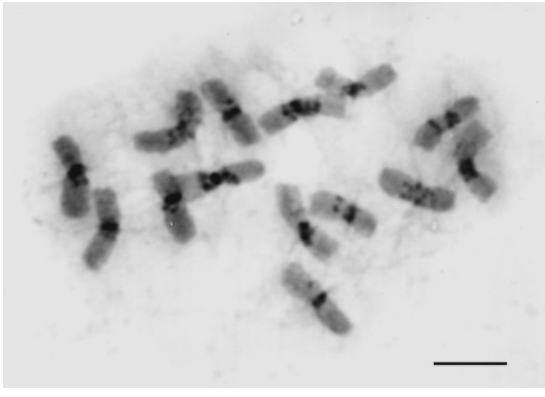

Fig. 5.1. Giemsa C-banding of somatic metaphase chromosomes of barley. Bar = 10 µm.

Gene mutations and reconstructed karyotypes

The use of mutagenic agents for the induction of gene mutations and structural chromosome changes has provided a wealth of mutant lines (Hagberg 1986; Marthe and Künzel 1994; Gecheff 1996; Lundqvist et al. 1996; Sreenivasulu et al. 2008). These lines are useful as a genetic resource in breeding and for gene mapping. Of special interest from a cytological point of view are the lines with reconstructed karyotypes, resulting from translocations, inversions, and duplications. The application of C-banding was instrumental in defining the chromosomes involved and the breakpoints in many of the reconstructed karyotypes. A nonrandom distribution of breakpoints was observed, being higher in heterochromatin in the first posttreatment mitosis and in the gene-rich regions of euchromatin in viable offspring (Künzel et al. 2001). The effects of these gross chromosome structural changes on gene expression, mitotic and meiotic cell division, fertility and phenotype are among some further aspects of study in such materials.

Interspecific and intergeneric hybrids and addition lines

Interspecific and intergeneric hybrids between barley and other Triticeae species have been quite informative as to the homoeology, elimination, and territories of chromosomes, also as to haploid production (see Linde-Laursen et al. 1997) and nucleolar dominance (see below). The wheat–barley addition lines developed carrying different

barley chromosomes or chromosome arms (Islam and Shepherd 1990) are a useful means for assigning genes to specific chromosomes or chromosome arms and for studying homoeologous chromosome pairing, also for monitoring of gene introgression, expression, and regulation.

Ribosomal genes and nucleolar dominance

The size and number of repeats of the ribosomal genes in barley diploids, trisomics, and translocation and duplication lines have been determined (Subrahmanyam and Azad 1978; Subrahmanyam et al. 1994). In diploid barley, higher numbers of rDNA cistrons occurred in 5H than in 6H chromosomes. The interrelationships between the number of gene repeats in rDNA, the size of $AgNO_3$ bands at nucleolar organizer regions (NORs) in metaphase chromosomes denoting sites and relative amounts of active repeats, and the number and size of nucleoli, in normal and reconstructed karyotypes, have been the subject of many studies (see Linde-Laursen et al. 1997). The phenomenon of nucleolar dominance/suppression in hybrids and in reconstructed complements, on inter-and intrachromosomal levels, has been repeatedly documented and discussed (see Linde-Laursen et al. 1997; Pikaard 2000).

MOLECULAR GENETICS

The lack of a fully sequenced barley genome (http://barleygenome.org/) is compensated by the available genetic maps based on morphological traits and various molecular markers (see Sreenivasulu et al. 2008). Due to the copious occurrence of repetitive DNA in Triticeae species, sequencing of expressed sequence tags (ESTs), obtained through cDNA sequences, has been the best approach for gene identification on the DNA level. The most common molecular markers that have been developed from ESTs are simple sequence repeats (SSRs). EST-SSRs have provided a rich collection of molecular linkage maps in barley (Stein et al. 2007; Varshney et al. 2007; Sreenivasulu et al. 2008). The EST-based single nucleotide polymorphism (SNP) markers are also expected to be a valuable tool, being the most polymorphic (Stein et al. 2007). A number of bacterial artificial chromosome (BAC) libraries, with inserts in the range 20–270 kbp covering up to 6.3 genome equivalents, have been developed in barley (Lapitan et al. 1997; Yu et al. 2000; Isidore et al. 2005). The possibility to detect contigs was verified by defining a contig of sex BAC clones spanning 230 kbp at the *Rph7* locus on the short arm of chromosome 3H (Isidore et al. 2005). One way to interrelate molecular and cytological markers, or genetic and physical distances, is tagging of translocation breakpoints of microdissected translocation chromosomes by restriction fragment length polymorphism (RFLP)-based molecular probes (Sorokin et al. 1994; Künzel et al. 2000). For RNA- and transcript-based analysis, the Affymetrix Barley1 GeneChip probe array, including 22,792 probe sets, representing at least 21,439 barley genes, was the first chip developed for a large-genome plant (Close et al. 2004). This array has been used for mapping barley genes to specific chromosome arms taking advantage of the wheat–barley disomic and ditelosomic chromosome addition lines (Cho et al. 2006; Bilgic et al. 2007). The array has also been used for monitoring regulatory or encoding defense products in response to biotic or abiotic stress in barley (Collinge et al. 2008).Common to genetic linkage maps with many localized genes is the finding that these are unevenly distributed in the genome that contained gene-rich and gene-poor regions. This is in concordance with the frequent occurrence of single- and low-copy sequences used for RFLP analysis in distal chromosome regions (Laurie et al. 1993).

MOLECULAR CYTOGENETICS

Fluorescence *in situ* hybridization (FISH) and genomic *in situ* hybridization (GISH)

The use of FISH and GISH has noticeably contributed to the revival of cytology and the start of the chromosome painting era (Schubert et al. 2001). FISH provides several new landmarks, thus facilitating chromosome characterization

and physical mapping of specific DNA sequences. The hybridization of genetically mapped sequences onto chromosomes is a direct way to integrate genetic and cytological maps. Bacterial plasmids with inserted short DNA sequences, as well as BACs and yeast artificial chromosomes (YACs), with inserted relatively larger DNA sequences, act as probes. The probes can be specific for single- or low-copy genes, tandem or dispersed repetitive sequences, or relatively large chromosome segments. Various detection systems that yield different colors are used for visualizing the labeling patterns in a fluorescence microscope. In the case of GISH, the DNA of a whole genome is labeled and used as a probe to detect genomes, chromosomes, or chromosome segments present in a different genomic background. Differentiation here is based on differences in dispersed DNA repeats. Applications of FISH on barley, using plasmid probes, comprised the localization of tandem repetitive sequences such as 18S-5.8S-25S and 5S rRNA genes (Fig. 5.2), di- and trinucleotide EST-SSRs, the pHcKB6 sequence, and the Afa family sequence; dispersed repetitive sequences, transposable elements, or retroelements such as the BIS-1 sequence; and single- or low-copy sequences such as transgenes and genes for beta-hordein, *Hor 1*, *Hor 2*, *alpha-amylase 2*, and a peroxidase gene (see Linde-Laursen et al. 1997; Pedersen et al. 1997; Tsujimoto et al. 1997; Brown et al. 1999; Nasuda et al. 2005a; Cuadrado and Jouve 2007; Cuadrado et al. 2008). Labeling with EST-SSRs yielded nonrandom patterns of FISH signals, providing a banding pattern that contributed to the characterization of the barley chromosomes (Pedersen et al. 1996; Cuadrado and Jouve 2007; Cuadrado et al. 2008). For the detection of signals from RFLP clones, ultrasensitive FISH techniques were applied in an effort to integrate genetic linkage and physical maps (Stephens et al. 2004). FISH has also been useful in a study on micronuclei formation following induced chromosome aberrations in barley (Juchimiuk et al. 2007). BAC probes with barley DNA inserts were utilized for the localization of C-hordein genes (Lapitan et al. 1997). Flow sorting of barley chromosomes, with or without the application of FISH, enables

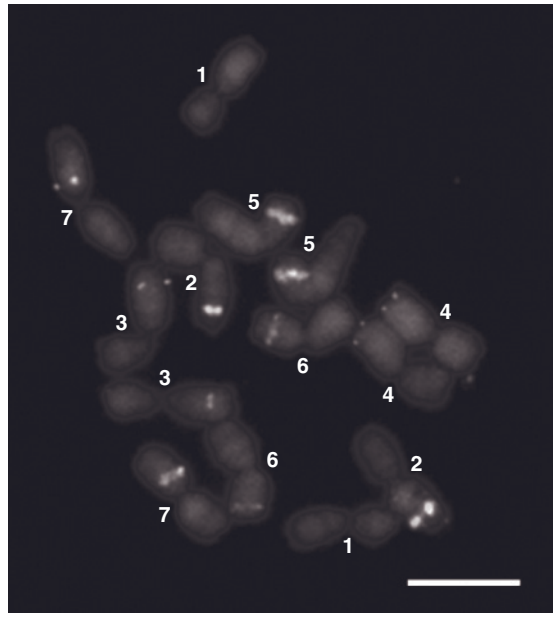

Fig. 5.2. DAPI-stained somatic metaphase chromosomes of barley cv. Bonus, showing signals after FISH of 25S (green) and 5S (red) rDNA probes, labeled with digoxigenin-11-dUTP and immunodetected with FITC-conjugated antidigoxigenin antibody and with tetramethyl-rhodamine-5-dUTP, respectively. Numerals designate chromosomes 1H–7H (Linde-Laursen et al. 1997; Brown et al. 1999). Bar = 10 µm (courtesy of Łukasz Kubica and Robert Hasterok, Department of Plant Anatomy and Cytology, University of Silesia, Katowice, Poland). For color details, please see color plate section.

marking and isolation of normal or reconstructed chromosomes, and telocentrics representing chromosome arms, for further use (Lysák et al. 1999; Ma et al. 2005; Suchánková et al. 2006; Doležel et al. 2007). In addition, FISH has been useful in studies on the molecular organization and function of the centromere in structurally changed chromosomes of barley (Nasuda et al. 2005b). Worth mentioning in this context is that the primed *in situ* labeling (PRINS) as well as cycling-primed *in situ* labeling (C-PRINS) methods of localization of DNA sequences on chromosomes have been successfully tested on barley (Kubaláková et al. 2001). Chromosome segments or whole chromosomes of barley were detectable in a wheat background also by using GISH (Mukai and Gill 1991; Schwarzacher et al. 1992).

Nucleolar dominance, DNA methylation, histone modification, and epigenetics

FISH advantageously complemented C-/N-banding and $AgNO_3$ staining, in studies on rDNA, NOR, and nucleoli in diploid barley and in lines with inversion or translocation chromosomes (Georgiev et al. 2001; Kitanova and Georgiev 2005). The phenomenon of nucleolar dominance, encountered in reconstructed barley karyotypes and in hybrids and addition lines, has been thoroughly discussed in search of interpretations (Pikaard 2000; Viegas et al. 2002). DNA methylation and histone acetylation have been inferred to be interrelated with regulation of gene activity (Chen and Pikaard 1997). DNA methylation patterns along the chromosomes of normal and reconstructed barley have been mapped and compared with the distribution of constitutive heterochromatin and high-gene density regions (Ruffini Castiglione et al. 2008). That suppressed rRNA genes can be derepressed by inhibitors of cytosine methylation and by histone deacetylation indicates that gene silencing is controlled at the chromatin level (see Pikaard 2000). Accordingly, the phenomenon has been denoted under the realm of epigenetics. Mutual influence of DNA methylation, histone modifications (acetylation, methylation, phosphorylation, etc.), and noncoding small RNA interference (RNAi) is a factor that determines chromatin topography, which in turn acts as a "chromatin code," "histone code," or "epigenetic code" (Lusser 2002; Turner 2002;. Pontes et al. 2003; Loidl 2004; Tariq and Paszkowski 2004).

Interpecific and intergeneric hybrids and addition lines

The use of suitable probes and FISH enabled the differentiation of all chromosome arms and the determination of homoeological relationships and differences in pairing patterns and introgressions in *H. vulgare* × *Hordeum bulbosum* hybrids and their progenies (Pickering et al. 2004, 2005). The process of chromosome elimination in these hybrids has also been highlighted (Gernand et al. 2006).

Meiosis

Diakinesis is a suitable meiotic stage for studies on chromosome homologous and homoeologous pairing. This stage is also suitable for studies on number, size, and sites of associations of nucleoli with chromosomes (Fig. 5.3a) when access to analyzable pachytene stages is not at hand. Labeling of diakinesis chromosomes after the application of FISH with 25S and 5S rDNA probes made chromosome identification possible (Fig. 5.3b).

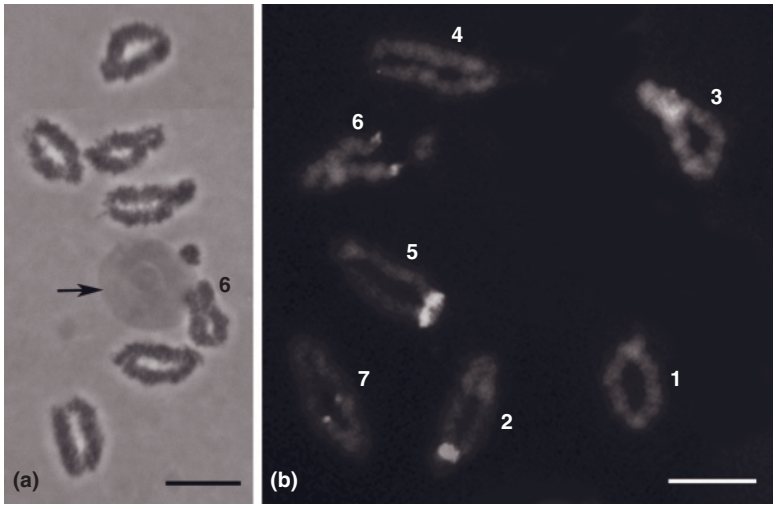

Fig. 5.3. Diakinesis bivalents of barley cv. Bonus. (a) Stained by Snow's carmine. A large nucleolus (arrow) associated with the secondary constriction of chromosome 6H. (b) FISH and chromosome numbering as in Fig. 5.2. Bar = 10 µm. Fig. 5.3b (courtesy of Łukasz Kubica and Robert Hasterok, Department of Plant Anatomy and Cytology, University of Silesia, Katowice, Poland). For color details, please see color plate section.

MICROSCOPY

The low number and large size of barley chromosomes favor their choice as a suitable material for studies on plant chromosome structure. Thus, scanning electron microscopy (SEM) and atomic force microscopy (AFM), as well as scanning near-field optical microscopy/atomic force microscopy (SNOM/AFM) for simultaneous topographic and fluorescent imaging of high-density GC-rich DNA following YOYO-1 staining, have been attempted for elucidating the internal structure of barley chromosomes (Yoshino et al. 2002 and references therein). The combination of immunocytochemistry with SEM for the localization of phosphorylated histone H3 at serine 10 and the features observed at centromeric and pericentromeric regions contribute to the further understanding of chromosome structure–function interrelationships (Schroeder-Reiter et al. 2003).

PROSPECTS

FISH mapping and chromosome painting

Parallel with the progress in the barley genome sequencing program (http://barleygenome.org/) and when all genome sequences become available, efforts are needed to provide a complete and defined contiguous large-insert genomic library for physical gene mapping, genomic structural analysis, map-based cloning and chromosome painting. The available BAC libraries (Lapitan et al. 1997; Yu et al. 2000; Isidore et al. 2005) are welcomed efforts in this direction. When using cosmids or BACs with large inserts containing single-copy and repetitive sequences, it has been shown that competitive *in situ* suppression (CISS) hybridization was necessary to suppress signals generated by the repetitive DNA sequences for the detection of single-copy genes (Sadder et al. 2000). This approach as well as multicolor FISH might favorably be applicable on barley. The use of chromosome-specific contigs for labeling *Arabidopsis* chromosomes has successfully resulted in painting the different chromosomes with different colors (Lysák and Lexer 2006). Comparative FISH analysis using SSRs and BAC contigs and application of comparative chromosome painting, in barley and its close relatives, would be informative as to the possible role of these sequences in chromosome structure, function, and evolution. DNA sequence colinearity between barley and rice (Pourkheirandish et al. 2007) and application of comparative chromosome painting between these two species would be given priority, considering the availability of the complete rice genomic sequence (IRGSP 2005). The potential of combining FISH with chromosome flow sorting should also be taken advantage of. To be remembered as well is that barley cv. Betzes has been recommended as a reference genome for Triticeae (Linde-Laursen et al. 1997).

Nucleolar dominance

The mechanisms that control gene transcription through changes in chromatin topography comprise DNA cytosine methylation, covalent modifications of histones (methylation, acetylation, phosphorylation, ubiquitination, etc.), and the RNAi pathway implying the production of small interfering RNAs (siRNAs), which can be involved in degrading target transcripts and in triggering DNA and histone methylation. Collectively, these mechanisms are epigenetic phenomena and are exciting current research areas (Neves et al. 2005; Henderson and Jacobsen 2007; Matzke et al. 2007; Zhang 2008). Continued efforts are needed to reveal these interactions and to find out if further details of chromatin structural modifications can be visualized cytologically (Harper 2000; Bowler et al. 2004). The use of the DNA methyltransferase inhibitor 5-azacytidine in combination with $AgNO_3$ staining and FISH with an rDNA probe (e.g., Caperta et al. 2007) could be informative in studies on nucleolar dominance.

Meiosis

Of interest would be the analysis of number, size, and chromosomal association sites of nucleoli during the pachytene–diakinesis stages, in

comparison with these features during mitosis. FISH localization of rDNAs on pachytene–diakinesis chromosomes in normal and reconstructed chromosomes would also be informative. Further, new efforts are needed to optimize cytological techniques for the analysis of the pachytene stage in barley. For future DNA and gene localization purposes using FISH, it is desirable to have relatively less-condensed well-spread long pachytene chromosomes as a substrate, for a better mapping resolution. The use of haploids (Sadasivaiah and Kasha 1971) for this purpose would further simplify chromosome painting. That designed autonomous minichromosomes were mitotically and meiotically maintained in maize (Carlson et al. 2007) makes it worthwhile to develop such minichromosomes in barley, for transformation purposes.

Microscopy

Optimizing the combination of SEM and cytochemistry (Yoshino et al. 2002; Schroeder-Reiter et al. 2003, 2006) permitted better structural preservation, labeling efficiency, and signal localization. This would further elucidate structure–function interrelationships and facilitate locating single-copy, low-copy, and repetitive DNA sequences to high-resolution chromosome substructures. Such an approach for the analysis of active and inactive rDNA in dominant and suppressed NORs of normal and reconstructed nucleolar chromosomes is compelling. Novel microscopical approaches as well, such as three-dimensional structured illumination microscopy (Carlton 2008), are worthy of trial for revealing further structural and functional details of mitotic and meiotic chromosomes.

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Chapter 6
Application of Molecular Genetics and Transformation to Barley Improvement

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CURRENT STATUS OF BARLEY IMPROVEMENT

Barley is a diploid inbreeder and its improvement has generally been achieved through the use of pedigree selection and variations upon it. Barley breeding program development has occurred throughout the twentieth century so that all countries where barley is an important crop have at least some breeding effort. Estimates of the rate of yield progress due to breeding generally show improvement has occurred at rates of around 1% per annum (Thomas 2003; Ordon et al. 2005). Studies identifying changes in other characters are much fewer, but, in some regions of the world, development of shorter-strawed cultivars, especially using dwarfing genes such as *sdw1*, has reduced crop height and hence susceptibility to lodging. Where malting quality is a major objective, clear genetic improvements in malt extract potential and general processing characters have also occurred (Grausgruber et al. 2002; Macaulay et al. 2004). Such improvement can also be observed in data produced from official trials in the United Kingdom (http://www.hgca.com/) and also in comparative trials of new malting cultivars around Europe (http://www.europeanbreweryconvention.org/PDF/2009/EBC - B&MC - 2 Year Report 2007-2008.pdf).

While there is little evidence that the rate of progress is declining, breeding programs have dramatically increased in size, with larger programs working with total F2 populations of over 1 million per year. With doubled haploidy being used increasingly in many programs and the costs of yield trials increasing, breeders need to identify better crossing and selection strategies to make their programs cost-effective. Molecular biology offers a range of techniques that can be deployed, either directly or indirectly, to develop more cost-effective breeding programs. In this chapter, we will consider the examples of marker-assisted selection (MAS)/marker-assisted breeding (MAB) and genetic transformation. While both techniques have the potential to be used directly in the improvement of crop species, genetic transformation is currently used as a means of hypothesis testing, especially in the identification of candidate genes.

MOLECULAR MARKERS IN BARLEY

Members of the international barley research community were early adopters of molecular marker technology and produced some of the first comprehensive whole-genome linkage maps (Graner et al. 1991; Heun et al. 1991; Kleinhofs et al. 1993) and quantitative trait locus (QTL) analyses in small grain cereals (Hayes et al. 1993; Thomas et al. 1995). Much of the impetus for these efforts came from plant breeders who were keen to improve selection efficiency for quantitative traits. In the intervening 15 years, map resolution and speed of genotyping have progressively improved (see Chapter 3) and no longer limit the generation and integration of mapping data. QTL reports have proliferated (see Chapter 4) and MAS has been the topic of several important

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review papers (Xu and Crouch 2008 and references cited therein). These authors describe four broad scenarios where plant breeders are likely to use MAS:

1. when phenotypes show complex inheritance,
2. when phenotypes show developmental or environmental specificity,
3. to accelerate the advance of specific allele combinations, and
4. to pyramid target alleles.

As costs of breeding programs increase, the demand for more efficient methods increases and the exciting prospects for MAS lie at the core of integrated European and U.S. association genetics projects, which have a strong plant breeder involvement. The Association Genetics of UK Elite Barley project (AGOUEB; http://www.agoueb.org/) utilizes the extensive phenotypic database associated with the U.K. official testing system where data have been collected on over 80 characters for over 500 spring and winter barley lines over the past 15 years. By integrating these data with genotypes produced from some 3000 mapped single nucleotide polymorphisms (SNPs) using Illumina oligo pooled arrays (OPAs), markers of relevance to U.K. breeders should be identified. The U.S. component, the Barley Coordinated Agricultural Project (CAP; http://www.barleycap.org/), involves 29 scientists at 19 research institutions and is funded by the United States Department of Agriculture/Cooperative State Research, Education, and Extension Service. Over the next 4 years, the same SNPs as used in AGOUEB will be assayed on ~4000 barley cultivars and experimental lines submitted by 10 breeding programs. Approximately 40 traits will be measured on the same germplasm. This very large data set will be stored in the GERMINATE database, accessed through The Hordeum Toolbox web portal, and analyzed using QTL Miner software developed specifically for this application. In Germany, GABI-GENOBAR also utilizes the same Illumina OPAs, and we are aware of other worldwide genotyping initiatives that will also do so, thus facilitating the integration and interpretation of projects from around the world.

APPLICATIONS OF MOLECULAR MARKERS

Studies of gene pool variation

Many studies have examined the comparative diversity in the elite and less-adapted gene pools. *Hordeum vulgare* ssp. *spontaneum*, the wild progenitor of cultivated barley, has a gene pool far more diverse than cultivated barley, highlighting a domestication bottleneck (Russell et al. 2000). While this suggests a comparative lack of diversity in the adapted gene pool, there is evidence that the levels of diversity in old and current breeding germplasm are more similar (Malysheva-Otto et al. 2007); that is, breeding has not yet further eroded the genetic basis of the barley gene pool to any great extent. Further evidence that there was still a considerable amount of variation that could be exploited within the adapted gene pool was provided by a survey of five spring barley cultivars that were on the U.K. Recommended List in 2007. Just using 35 simple sequence repeat (SSR) markers revealed that it was possible to make over 1×10^{11} different allelic combinations from the five cultivars (Rae et al. 2007). The challenge is to identify how many of these differences represented functional polymorphisms and thus potential for genetic improvement.

The barley crop is divided by some major developmental differences. In some regions, the crop requires a period of cold (winter) chilling before it will flower; this effect is principally controlled by allelic variation at the *Vrn-H1*, *Vrn-H2*, and *Vrn-H3* loci (Takahashi and Yasuda 1971). The vernalization response in cultivated barley is mainly due to an epistatic interaction between *Vrn-H1* and *Vrn-H2*, a model that has been validated (Szucs et al. 2007) by studies of the molecular variation at the two loci. Early flowering, with or without fulfillment of the vernalization requirement in the winter and spring crops, respectively, can be achieved through deploying alleles at various major gene loci affecting flowering time, such as *Ppd-H1* affecting photoperiodic response (Turner et al. 2005). Furthermore, a mutation in a homeodomain-leucine zipper I-class homeobox

gene at the *vrs1* locus causes lateral floret fertility giving rise to six-row barley (Komatsuda et al. 2007). Breeders generally tend to work within these well-defined gene pools; while they do make crosses between lines from different pools, they generally prefer to work within the same pool. This has led to some of the differences in diversity that have been observed between two- and six-row barley (Russell et al. 1997). Using a relatively small sample of SSR markers, Yahiaoui et al. (2008) were able to reveal subpopulations within Spanish six-row landraces, most likely associated with ecogeographic features. As the saturation of molecular maps increases, it is likely that more differentiation within some of the major gene pools will be detected.

As higher-resolution genetic maps, coupled with knowledge about location of genes, becomes available, barley breeders can make more informed choices about the crosses that they would wish to make within their target germplasm groupings. In addition, this type of knowledge will also enable barley breeders to make greater, but more targeted, use of the gene pools that they are not working with, thus providing extra germplasm to maintain breeding progress.

QTL detection

"Biparental" populations

The current state of QTL discovery in barley has been thoroughly covered in Chapter 4. For the purposes of this chapter, we emphasize that much of the current QTL literature is based upon specially constructed segregating populations, either as progenies of various types, principally doubled-haploid lines (DHLs) and recombinant inbred lines (RILs), or as derivations from a backcrossing program, mainly advanced backcross QTL studies or segmental substitution lines. While many of these studies have increased the pool of barley knowledge, few have led to markers for key characters. There are some notable exceptions to this; for example, Collins et al. (2003) identified QTLs for malting quality characters that are being used in breeding programs (Langridge 2005).

Studies of dormancy have revealed that loci detected originally in the Steptoe × Morex population (Romagosa et al. 1999a) have frequently been detected in other populations. This is best illustrated by the SD1 locus centromeric on 5H, which has also been found in a number of populations, including several involving relatively unadapted germplasm (Hori et al. 2007). Despite this, the main problem of most QTL studies carried out so far is that the results are really only relevant to the special cross in which they are detected. QTLs detected in one genetic background have been observed to have different effects in another in validation studies (Meyer et al. 2004). Furthermore, the size of the effects detected can vary even within samples of the same germplasm. Reciprocal crosses were studied for the association of variation at the rDNA locus on barley chromosome 5H, and the amount of genetic variation for grain hardness associated with the locus varied fourfold between the Blenheim × E224/3 cross and its reciprocal (Powell et al. 1992). There are, however, several potential explanations for such variation. In this case, the sample sizes for the reciprocal crosses were small and thus, the size of the effect will be very sensitive to random differences in the amount of recombination between the reciprocal crosses. Nevertheless, similar phenomena have been observed in resampling the Steptoe × Morex population, where a second sample has shown variation in the significance of QTLs for yield (Romagosa et al. 1999b) and malt quality (Han et al. 1997).

Association genetics approaches

Approaches that rely upon creating special segregating populations to identify QTLs are limited in that they are generally only especially suited for those characters that differ widely between the parents. Association genetics approaches offer an attractive alternative because of the following:

1. You can survey a large panel of appropriate germplasm at the same time, and thus the average effects detected will apply over a range of genetic backgrounds.
2. Often, studies can be based upon contemporary germplasm for which extensive phenotypic data may already exist, such as the AGOUEB project (http://www.agoueb.

org/). Failing this, the effort in generating *de novo* phenotypic information can be derived from current breeding programs, such as the U.S. Barley CAP (http://www.barleycap.org/).

3. A representative sample of germplasm will encapsulate a number of lines that contrast for most phenotypes of interest.
4. They have the potential for a higher resolution of the genetic interval containing a QTL.

The drawback with such approaches is that one requires comprehensive genome coverage as QTL detection depends upon both the size of the effect being detected and the extent of linkage disequilibrium in the area, which is dependent upon both the genetic distance between marker loci and the number of cycles of recombination (Mackay and Powell 2007), that is, the opportunity for linked alleles at loci to disassociate. If linkage disequilibrium decays rapidly, then the chances of detecting markers linked to a QTL of small effect diminish rapidly the greater the distance between the marker and the QTL position. If the QTL effect is relatively large, then the effect will persist over a greater distance. Despite these restrictions, a medium-density amplified fragment length polymorphism (AFLP) marker coverage of one mapped marker approximately every 10 cM was successfully used to identify QTLs for yield (Kraakman et al. 2004) and other characters (Kraakman et al. 2006). More recently, a higher marker coverage provided by Diversity Arrays Technology (DArT) markers (Wenzl et al. 2004, 2006) was also used to identify yield QTL across a broad range of germplasm grown in diverse Mediterranean environments (Comadran et al. 2008). The development of a high-density SNP genotyping platform for barley promises to develop this approach even further as just one Illumina OPA offers 1536 SNP markers. If all are polymorphic across the association panel, that results in a genome coverage of one marker for <1 cM. Several detailed studies in barley have shown that linkage disequilbrium persists on average between 1 and 3 cM, so the levels of marker density now available in barley are more than enough for association genetics studies.

Assuming that marker coverage is adequate, significant marker character associations can be due to underlying population substructure differences rather than differences associated with a causal gene. There are a number of approaches that can be deployed to account for the underlying population substructure and these have been reviewed by Mackay and Powell (2007). The structured association approach, implemented by the software STRUCTURE (Pritchard et al. 2000), has been frequently used in association genetics studies of crop plants reported so far. STRUCTURE classifies genotypes into one of k groups so the grouping factor can be utilized as a means of correcting for structure effects. Studies of germplasm of various levels of diversity highlight that there is often considerable amounts of admixture between different groupings, so a better way of correcting may be to utilize the probabilities of belonging to one of the k groups as a matrix in detecting associations. The effects of accounting for the underlying population structure using either estimates of grouping or kinship have been discussed by Yu et al. (2006), who have demonstrated that the level of false-positive detection is markedly reduced by including an estimate of kinship in mixed models to detect marker character associations in maize. This approach is implemented in the software TASSEL. In summary, there are few genome-wide studies in barley that have identified suitable methods to account for population substructure, but, at the very least, accounting for kinship relationships should be carried out.

It remains to be seen how effective association genetics approaches will be in detecting QTLs of value compared to other approaches such as doubled haploid lines (DHLs) or substitution lines. A major drawback is that association genetics approaches are not effective for alleles of low frequency. For example, cv. Optic has been the most widely grown spring barley in the United Kingdom over the past 10 years and also features in the pedigrees of a number of other advanced breeding lines. Genotyping of all lines that have completed at least 2 years of official trials in the United Kingdom reveals that there are a number of alleles with frequencies of less than 5% at some

loci, and most current U.K. Recommended List cultivars possess at least one rare allele, with Optic possessing five. While this might not be a problem in providing a general characterization of a germplasm pool, it may well be that one reason an allele is rare is that it has recently been introgressed into the elite gene pool, but, despite being associated with a selective advantage, it has yet to become relatively frequent in the elite gene pool. In this scenario, the novel allele would be classified as minor and ignored in the analysis. For the plant breeder, it is essential to identify such beneficial novel alleles as soon as possible and alternative approaches should be identified. In such cases, it may be beneficial to generate several small populations utilizing the novel allele(s) and to conduct a multipopulation mapping approach to determine whether or not the alleles are associated with any beneficial phenotypes. More simply, it would be possible to conduct a form of reverse bulk segregant analysis at the novel loci, that is, to classify sets of random lines from crosses according to the novel and alternative alleles and to test for phenotypic differences.

Table 6.1 Questionnaire on usage of markers in barley breeding

1	Organization:	
2	Contact:	
3	Breeding target countries/region:	
4	Do you use markers in barley variety development?	Yes/No
5	Do you use markers to introgress "exotic" germplasm?	Yes/No
If you answered yes to question 4 and/or question 5, please answer questions 6–10		
6	Do you use markers to compare possible parents in crossing?	Yes/No
7	Do you use markers to select for disease/pest resistance?	Yes/No
8	Do you use markers to select for a quality character?	Yes/No
9	Do you use markers to select for a yield character?	Yes/No
10	Do you use markers to select for an agronomic character?	Yes/No
What prevents or limits the current use of markers in your program?		
11	Lack of resources for implementation?	Yes/No
12	QTLs not detected in right background?	Yes/No
13	Detected QTL effects too small?	Yes/No
14	Lack of useful targets?	Yes/No
15	Phenotypic selection is successful enough?	Yes/No
16	Any other comments?	

MAS/MAB in barley

Estimate number of breeding programs worldwide and those using MAS

In order to estimate MAS usage in the international barley research community, we conducted an informal survey via email. The questionnaire is presented in Table 6.1. Over 50 questionnaires were sent out, and of the 37 organizations who responded, 17 were from Europe; 12 were from North America; 5 were from South America; and 1 each was from North Africa, Australia, and East Asia.

In Figs. 6.1–6.3, we summarize responses to the three categories of questions and highlight key trends. For the purposes of this summary, we included North Africa with the "Europe" regional group. Australia, East Asia, and South America were grouped as "elsewhere." In Fig. 6.1, it is apparent that breeders in North America are using MAS at a lower rate than their colleagues in the rest of the world. The highest adopters are in Europe, and they are using MAS for cultivar development and introgression, with high frequencies of use in both categories. The dynamic and progressive programs grouped as "elsewhere" are using MAS at equal rates for cultivar development and allele introgression. For those using MAS, the highest use by all three groups is for disease traits (Fig. 6.2). Quality and parent building were important uses in Europe and in North America. Figure 6.3 may explain the low rate of MAS usage in North America—many researchers in this region reported that resource constraints prevented them from using MAS and that phenotypic selection is effective. MAS users in Europe also reported that resource constraints limited their use of MAS. However, they rated biological factors—genetic background and size of QTL effects—as equally important limiting factors.

It is inappropriate to infer too much from this informal survey. The high MAS adoption rates

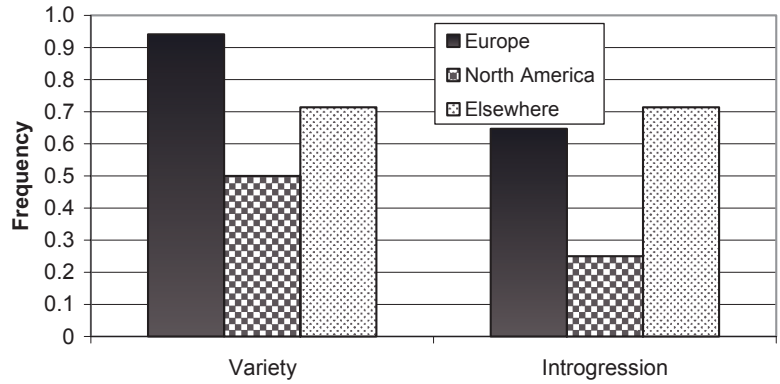

Fig. 6.1. Frequencies of usage of barley markers in breeding with purely elite germplasm and wider introgression strategies.

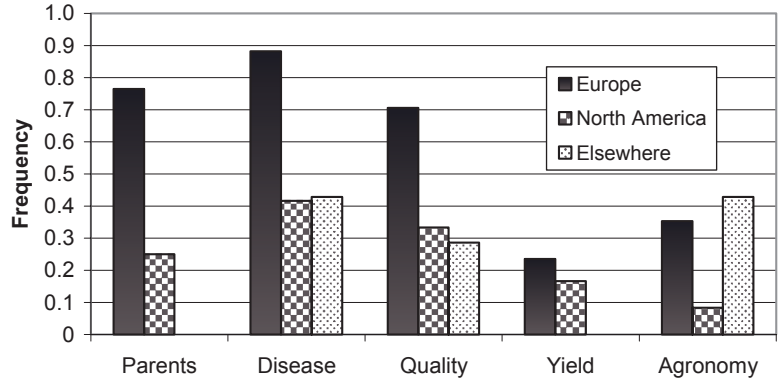

Fig. 6.2. Frequencies of usage of markers in breeding for different target categories.

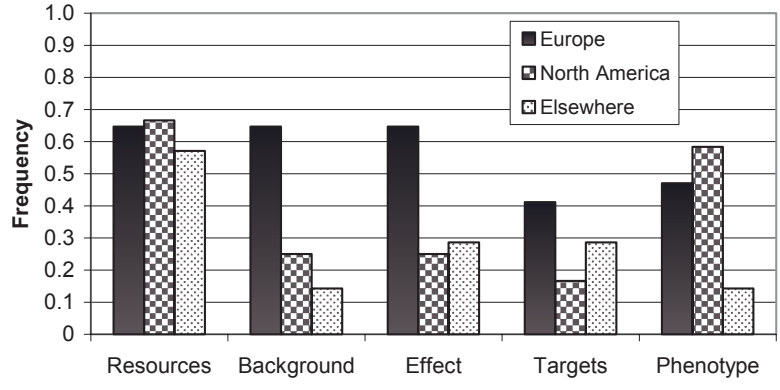

Fig. 6.3. Frequencies of reasons cited that restricted the use of markers in barley breeding.

in Europe may be driven in part by the preponderance of private sector barley breeding in this region and the importance of barley yellow mosaic virus (BaYMV) resistance (see section on Transformation below). Private sector barley breeding is generally carried out by companies whose main focus is on other more profitable crops, and thus the investment in the resources for MAS can be spread over them. This informal survey may overestimate MAS usage in all regions, since three important questions were not included in the survey and remain to be answered:

1. "What percentage of the germplasm in breeding programs was developed by MAS?"
2. "How many cultivars have been released using MAS?"
3. "What new knowledge and/or hypotheses did you generate in the process of your MAS endeavors?"

It is, however, arguable that all recent U.K. winter barley recommended cultivars with either the *rym4* or *rym5* resistance alleles to BaYMV are the products of MAS. Nearly all European breeders working with these resistance alleles use MAS to facilitate selection. It should, however, be emphasized that the marker is generally used either to select parents for crossing or among advanced breeding lines in the final stages of selection to either confirm resistance or choose resistant lines for submission to official trials.

Single gene marker uses

In barley, the use of molecular markers, to date, has had the most impact on a number of single gene characters, the majority of which can be classified as disease resistance factors. A reliable and cost-effective phenotypic screen is essential to score such characters, and while that may exist for many common fungal pathogens, phenotypic screening for resistance to insects and other diseases may not be so cost-efficient or reliable. In such cases, use of markers as a surrogate to select for a resistant phenotype is an attractive alternative. Resistance to BaYMV and barley mild mosaic virus (BaMMV) is arguably the most well-worked example of the use of markers in small grain cereals. Initial work identified an isozyme marker (Konishi et al. 1989) that could be used to select for resistance at the *rmm/rym4/rym5* locus on chromosome 3H. This was followed by the identification of restriction fragment length polymorphism (RFLP) (Graner and Bauer 1993), random amplified polymorphic DNA (RAPD) (Weyen et al. 1996), and SSR (Graner et al. 1999) markers that could be used to select for resistance. In fact, the Bmac29 SSR marker has been used by most European cereal breeders whose targets include the German and French winter barley markets. The SSR marker is a linked marker, located approximately 1 cM from the resistance gene (Graner et al. 1999), but the association with the resistance phenotype has proved remarkably durable, probably due to linkage drag in the region surrounding the resistance gene. More recently, the resistance gene has been identified (Stein et al. 2005) together with the functional polymorphism (Kanyuka et al. 2005), opening up the possibility of a "perfect" marker.

Another major benefit of using markers to select for a resistance phenotype is that it is relatively easy to build a resistance gene pyramid, that is, to incorporate several different resistance genes into a genotype in order to buffer against the possible breakdown of any one of them. Again, resistance to BaYMV and BaMMV provides a good example of how markers can be deployed to accumulate several different resistance gene loci in a cost-effective manner (Werner et al. 2005).

Considering barley malting quality, an experiment involving *Bmy1* and diastatic power conducted by the Oregon Barley Project is an example of the potential use of MAS for quality and a useful case study related to the questionnaire above. Diastatic power, a measurement of the total combined activity of all starch-degrading enzymes during the brewing process, is a key malting quality parameter with a defined genetic basis. Many studies show β-amylase is the principal enzyme whose activity is significantly correlated with diastatic power (Sun and Henson 1991; Evans et al. 2005). The *Bmy1* gene, located on chromosome 4H, encodes the endosperm-specific β-amylase that is reported to be most important for malting (Kreis et al. 1987). Variation in β-amylase activity and thermostability, which would be useful for barley cultivar improvement, has been described at the DNA and protein levels. Chiapparino et al. (2006) provided an excellent review of this work and the converging nomenclature relating allelic variation at the DNA and protein isoform levels. At the DNA level, differences in β-amylase activity among genotypes have been correlated with sequence variations in introns (Erkkila 1999; Coventry et al. 2003) and exons of *Bmy1* (Clark et al. 2003; Chiapparino et al. 2006). Based on extensive reports of associations of specific sequence polymorphisms with

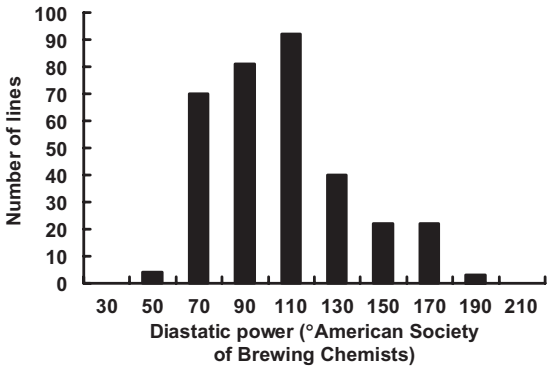

Fig. 6.4. Distribution of diastatic power values (n = 334) in an array of experimental barley germplasm described in Table 6.2.

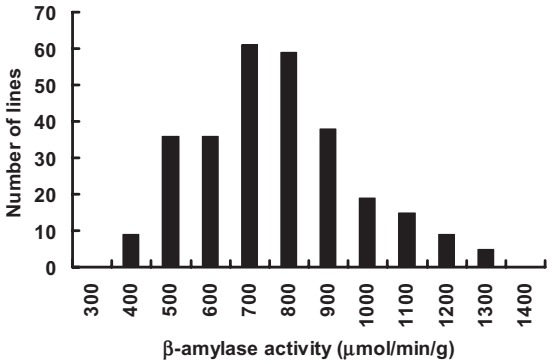

Fig. 6.5. Distribution of β-amylase activity values in an array of experimental barley germplasm (n = 287).

malting versus feed descriptors in germplasm arrays (Chiapparino et al. 2006; Malysheva-Otto and Roder 2006; Ovesna et al. 2006), it was hypothesized that the abundant phenotypic variation for diastatic power and β-amylase activity in Oregon breeding germplasm (Figs. 6.4 and 6.5) was due to allelic variation in *Bmy1*. Optimism prevailed that specific sequence variations in a set of germplasm would explain the variation in diastatic power and β-amylase activity and that these could serve as perfect markers for future MAS efforts in this germplasm base.

The test germplasm array for this experiment consisted of 151 lines (all F_5 or later generation) representing winter, facultative, and spring growth habits evaluated in an array of environments (Table 6.2). Because the 151 lines were derived from crosses involving only nine different parents, it was efficient to obtain full gene sequences (~3840–3850 bp) of *Bmy1* in the nine parental accessions and to infer allele sequence in the 151 progenies. As shown in Tables 6.3 and 6.4, we found that eight of the parental accessions were monomorphic at the intron III and coding region positions reported as determinants of diastatic power and/or enzyme activity in the literature. Strider is a unique haplotype in terms of both intron III and coding region variants. Interestingly, despite the fact that Strider was a parent of all progeny in the winter/facultative germplasm array, the Strider *Bmy1* allele was present in only 3/54 progeny. This low frequency may be attributable to (indirect) phenotypic selection against the Strider allele based on malting quality assessment during germplasm advance. This same phenotypic selection was effective in coupling the high yield potential of Strider with suitable diastatic power, meaning that Strider is no longer a necessary parent for winter/facultative malting cultivar development. Thus, at this point in time, there is no need to develop and apply a MAS assay against the Strider *Bmy1* allele. If this information had been available when the germplasm was first developed, we could have effectively selected against the Strider *Bmy1* allele in early generations of the winter and facultative material and presumably have saved effort and expense in the phenotypic evaluation of the germplasm that was ultimately discarded. However, the fact remains that even with the Strider allele at low frequency in the winter and facultative germplasm and absence in the spring germplasm, there is a tremendous amount of phenotypic variation for diastatic power and β-amylase activity.

These findings shift MAS for diastatic power in this germplasm array from the simplicity of a perfect marker in known functional regions of *Bmy1* to the complexity of unknown genetic factors. These may be as simple as functional polymorphisms in as yet uncharacterized upstream and/or downstream regulatory sequences or as complex as transcription factors encoded by other genes, or gene families. Progenies with pedigrees tracing to selections from the 54 winter and

Table 6.2 Summary of the breeding program trials, and the germplasm therein, used for characterization of diastatic power and β-amylase activity

Breeding Trial and Number of Lines in Trial	Growth Habit	Parental Genotypes	Location/Year	Traits Measured
ORELT—54	Winter and facultative	Strider, Kold, 88ab536, Orca	COR-2004	DP, BAA
			ABID-2004	DP, BAA
			COR-2005	DP
			ABID-2005	DP
			PID-2005	DP, BAA
			FID-2005	DP, BAA
			POR-2005	
SOTE—37	Spring	Stander, Orca, Tango, Excel	TCA-2003	DP
STUC—60	Spring	Stander, UC960, UC958	TCA-2004	DP, BAA

ABID, Aberdeen, Idaho; COR, Corvallis, Oregon; FID, Filer, Idaho; PID, Parma, Idaho; POR, Pendleton, Oregon; TCA, Tulelake, California; BAA, β-amylase activity; DP, diastatic power.

Table 6.3 *Bmy1*INDELIII polymorphisms for eight barley genotypes (in boldface) compared to representative *Bmy1* haplotype standards. The nine genotypes were parents of the germplasm arrays, listed in Table 6.2, which were phenotyped for diastatic power and β-amylase activity (Figs. 6.4 and 6.5)

		A1310/T1311	G1883/A1904
Genotypes	GenBank Accession No.	127 bp	21 bp
Strider	EU589327	–	–
Excel	EF175466	+	+
Kold	EF175466	+	+
Orca	EF175466	+	+
Stander	EF175466	+	+
Tango	EF175466	+	+
UC958	EF175466	+	+
UC960	EF175466	+	+
88Ab536	AB306504	+	+

Table 6.4 Polymorphic *Bmy1* coding region SNPs for barley parental genotypes, listed in Table 6.2, which were phenotyped for diastatic power and β-amylase activity (Figs. 6.4–6.5). The positions of the nucleotide substitutions are per Chiapparino et al. (2006)

	343	495	1357	1462	1552
Genotypes	(R115C)	(D165E)	(A453T)	(V488I)	(G518R)
Strider	T (C)	G(E)	A(T)	A(I)	A(R)
Excel	C (R)	C(D)	G(A)	G(V)	G(G)
Kold	C (R)	C(D)	G(A)	G(V)	G(G)
Orca	C (R)	C(D)	G(A)	G(V)	G(G)
Stander	C (R)	C(D)	G(A)	G(V)	G(G)
Tango	C (R)	C(D)	G(A)	G(V)	G(G)
UC958	C (R)	C(D)	G(A)	G(V)	G(G)
UC960	C (R)	C(D)	G(A)	G(V)	G(G)
88Ab536	C (R)	C(D)	G(A)	G(V)	G(G)

facultative lines in the germplasm array are in the U.S. Barley CAP project, providing an opportunity to discover the basis of the abundant variation in diastatic power and β-amylase in this germplasm via association mapping.

This experiment underscores key issues highlighted in the MAS survey described earlier in this chapter:

1. The experiment was designed to lay the groundwork for MAS for a quality trait.
2. If the *Bmy1*-based markers had been informative, they would have been valuable for allele introgression and cultivar development.
3. *Bmy1* polymorphisms reported in the literature are not suitable targets for MAS in this germplasm.

An important and challenging outcome of this project relates to the third question that we neglected to include in the survey: "what new knowledge and/or hypotheses did you generate in the process of your MAS endeavors?" These data provide an impetus to develop a complete understanding of *Bmy1* regulation. Finally, these data should not be interpreted to mean that MAS for specific *Bmy1* alleles is ineffective. In other germplasm, such selection has been effective (Coventry et al. 2003). MAS transfer of the Haruna Nijo Bmy1 allele to Strider, for example, might lead to levels of diastatic power and enzyme activity equal to, or higher than, those already present in the winter/facultative germplasm base. However, such a "wide" cross, in which nearly every character contrasts (e.g., growth habit, cold tolerance, disease resistance, and inflorescence type), would require considerable effort and allocation of MAS resources.

Multigene phenotypes

One of the most cited uses of MAS is pyramiding multiple genes affecting the same trait (Xu and Crouch 2008). The challenges and opportunities are even greater when dealing with genes conferring quantitative disease resistance. Barley stripe rust (BSR), incited by *Puccinia striiformis* Westend. f. sp. *hordei*, is a serious disease of barley in many parts of the world. Multiple BSR resistance QTLs have been mapped (Chen et al. 1994; Hayes et al. 1996; Toojinda et al. 2000; Castro et al. 2003a; Rossi et al. 2006), and their effects validated via introgression of QTL alleles into susceptible cultivars via MAS (Toojinda et al. 1998; Castro et al. 2002, 2003a,b; Richardson et al. 2006). A qualitative resistance gene on chromosome 7H was also mapped (Castro et al. 2003a). These qualitative and quantitative resistance sources have conferred resistance to the spectrum of BSR races encountered in Mexico and in the United States for over 20 years (Rossi et al. 2006).

A BSR resistance QTL allele transfer experiment was recently undertaken using cv. Kurtford as the susceptible recurrent parent and iBISON 95-2 as the resistance donor parent. iBISON 95-2 is a near-isogenic line developed using MAS for resistance QTL alleles on chromosomes 1H, 4H, and 5H (Richardson et al. 2006). iBISON 95-2 is a spring two-row awned genotype, whereas Kurtford is a spring six-row hooded cultivar, developed by Westbred LLC (http://www.westbred.com/). Kurtford is used for forage (hay, haylage, or green chop) in California, where stripe rust is endemic. Stripe rust susceptibility is the major defect of this cultivar.

The MAS backcrossing scheme used to achieve the transfer of the three resistance QTL alleles to Kurtford is shown in Fig. 6.6. The practical goal of the project was to develop six-row, hooded, stripe rust-resistant germplasm with commercial potential. To achieve this goal, we used a mix of phenotypic and genotypic selection at four morphological trait loci and three QTLs. We used phenotypic selection for six-row and hooded during germplasm development. Six-row is determined by recessive alleles at *Vrs1* (2H) and dominant alleles at *Int-c* (4H). Hooded is determined by dominant alleles at *Kap* (4H) and *Lks2* (7H). The genes determining *Vrs1* and *Kap* have been cloned: *Vrs1* = *HvHox1* (Komatsuda et al. 2007) and *Kap* = *HvKnox3* (Williams-Carrier et al. 1997). Perfect markers based on functional polymorphisms are therefore available for these two genes, and markers tightly linked to *Int-c* and

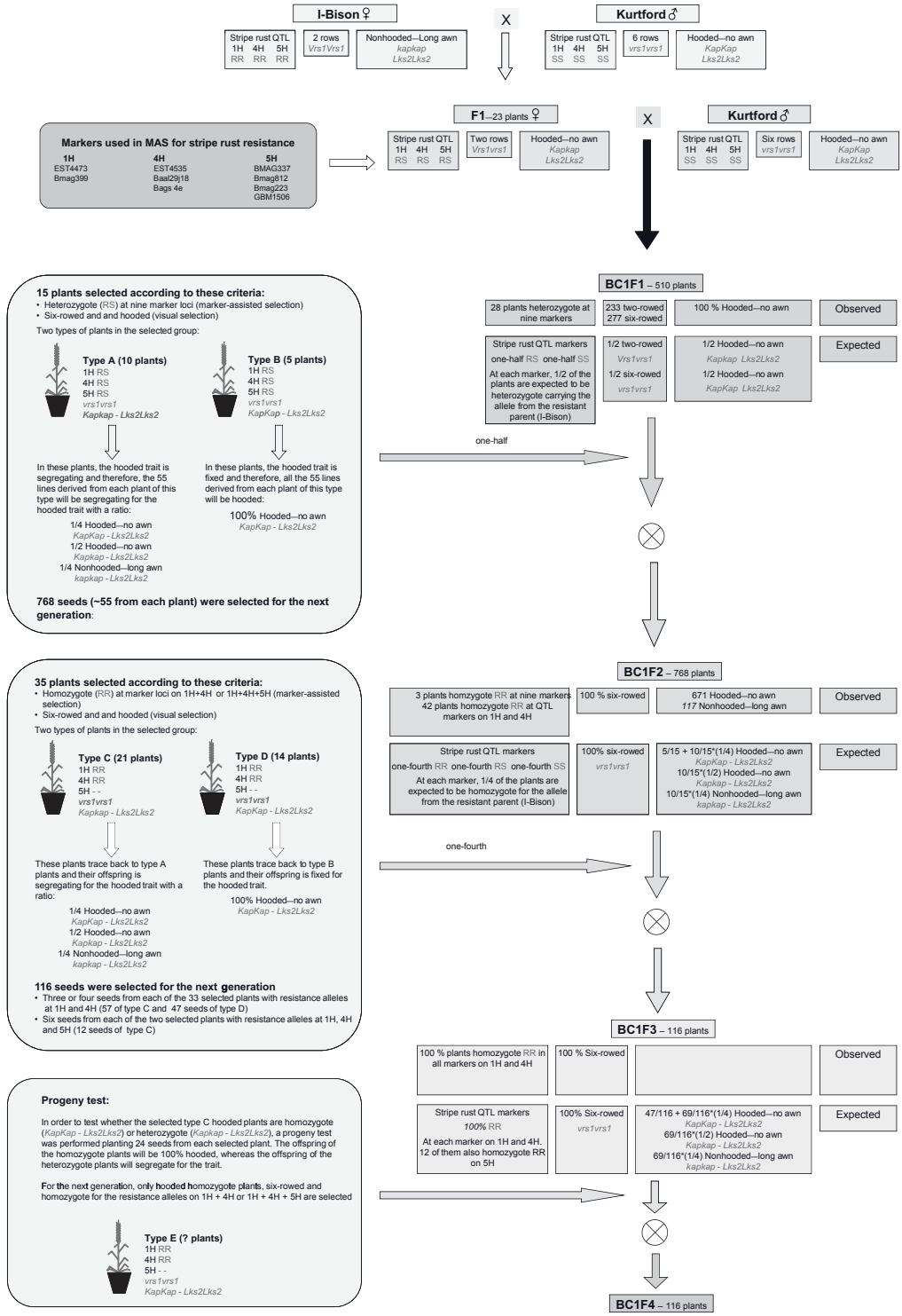

Fig. 6.6. Strategy for introgressing stripe rust resistance into Kurtford. For color details, please see color plate section.

Lks2 are also available (http://barleyworld.org/oregonwolfe.php). However, phenotypic selection for six-row and hooded was chosen due to cost considerations and the absolute reliability of scoring these morphological traits based on phenotype. MAS was used for the BSR resistance QTL alleles on 1H, 4H, and 5H because of the challenges inherent in scoring quantitative resistance (Vales et al. 2005). The tools developed for BSR-MAS are available at http://barleyworld.org/osubreeding/striperustmapping.php.

From planting the BC_1 generation in the greenhouse to planting BC_1F_4 lines in the field would have taken approximately 15 months, 200 m^2 of greenhouse space, and 13,000 genotypic data points. The field tests required for phenotypic validation and selection are in progress and involve 106 MAS target lines plus 16 controls. Fifty-six MAS target lines are homozygous for target alleles at the four morphological trait loci and two of the QTLs (1H and 4H). Nine lines are homozygous for BSR resistance QTL alleles on chromosomes 1H, 4H, and 5H and target *vrs1* and *Int-c* alleles. These lines are all hooded, but some are still segregating at *Kap* and/or *Lks2*. Forty-one lines are homozygous for BSR resistance QTL alleles on 1H and 4H and target *vrs1* and *Int-c* alleles. Again, these lines are all hooded, but some are still segregating at *Kap* and/or *Lks2*. No background selection was used in this project due to cost considerations. The expense could not be justified since there are no QTLs reported for forage quality and quantity. Therefore, the only suitable targets in Kurtford were the aforementioned morphological traits: six-row and hooded.

This experiment underscores key issues highlighted in the MAS survey described earlier in this chapter:

1. It was an example of MAS applied for the purposes of simultaneous exotic allele introgression and cultivar development.
2. MAS was used for improving disease resistance.
3. Available resources precluded the use of larger population sizes and MAS for target alleles at the four loci determining spike type.
4. A lack of suitable targets precluded MAS for forage quality and quantity.

Hybrid barley

While there have been numerous reports of heterosis for a range of characters in barley (e.g., Thomas and Powell 1990; Gupta and Singh 1999), hybrid barley has made little impact on world barley production. Systems such as balanced tertiary trisomics (Ramage 1965) and cytoplasmic male sterility (Ahokas 1980) have been proposed for F_1 hybrid barley production, but, until recently, there had been no development of commercial F_1 hybrid barley. Recently, the breeding company Syngenta Seeds Ltd. has produced several F_1 hybrid winter barley cultivars such as Colossus, Boost, and Bronx, which have been placed on the U.K. Recommended List (http://www.hgca.com/) since 2004. The hybrids have been produced using a system based upon cytoplasmic male sterility. In such a system, the ability to manipulate the genetic background associated with both the male sterile cytoplasm and the pollinator line containing the restorer gene greatly aids the development of new hybrids. A major male restorer gene has been located on chromosome 6H (Matsui et al. 2001) and markers can be utilized to develop different pollinator lines. Molecular markers can also be used to produce cytoplasmic male sterile lines that will give the maximum heterozygosity with one or more pollinator lines and thus attempt to maximize the heterotic gain.

TRANSFORMATION

Successful transformation of barley was first reported in the early 1990s using DNA uptake, particle bombardment, and electroporation. By the late 1990s, the first reports of *Agrobacterium tumefaciens*-mediated barley transformation were published. Since then, substantial efforts to improve methods and to insert genes of interest into barley have been made with varied success (for reviews, see Dahleen et al. 2001; Dahleen and Manoharan 2007; Goedeke et al. 2007; Ganeshan

et al. 2008; Lemaux et al. 2008; Harwood and Smedley 2009; Harwood et al. 2009). Currently, no commercial transgenic barley cultivars are available because of government and industry policies and the sociological controversy of issues such as containment of transgenes and potential health effects. But transformation is a valuable research tool to determine gene effects and interactions.

Techniques

Routine transformation of barley for breeding and genetic studies requires several components. The method must be efficient so many lines with independent transgene insertions can be generated in a reasonable amount of time. The time in tissue culture must be limited to reduce or eliminate somaclonal variation. The method should be genotype independent so the transgenes can be inserted into the cultivar of choice rather than necessity. And there should be a simple way to remove the selectable marker genes so the transgenic line only contains the DNA for the gene of interest. Major steps have been made toward these goals as described below.

Particle bombardment

The first production of fertile transformed barley was reported in 1994 using particle bombardment to introduce new genes into immature embryos, derived callus, and microspores (Jahne et al. 1994; Ritala et al. 1994; Wan and Lemaux 1994). These and other early studies inserted genes for selectable markers to optimize components of the bombardment process, including distance between the macrocarrier and the barley sample, particle size, and gas pressure. An optimized method for barley transformation using the PDS 1000/He gene gun (Bio-Rad, Hercules, CA) was developed (Lemaux et al. 1996), which is still used by many laboratories with minor modifications of the bombardment technique. Most transformation efficiencies with particle bombardment are below 3%. These early efforts used cultivars with high regeneration potential, such as Golden Promise and Igri, because of the lack of success with cultivars that have breeding value. Experiments with the high regenerating cultivars allowed examination of variables affecting transformation efficiency and improvements in methods. As success became more widespread, efforts to identify high-regeneration, locally adapted modern cultivars helped produce transgenic plants in lines that would be more useful for breeding. Alterations of media components have led to improvements in regeneration up to 15-fold in adapted North American cultivars and 10-fold in Golden Promise (Dahleen and Bregitzer 2002). These improvements allowed the successful transformation by particle bombardment of the two-row malting barley cv. Conlon (Manoharan and Dahleen 2002). Additional improvements in tissue culture methods or explant selection (Chang et al. 2003) continue to expand the number of cultivars amenable to transformation.

Agrobacterium

A. tumefaciens-mediated transformation of barley was first reported by Tingay et al. (1997) through the use of a supervirulent *Agrobacterium* strain. The tissue culture protocol was similar to that used for particle bombardment of immature embryos. Targeting cv. Golden Promise resulted in an average 4.2% transformation efficiency over multiple experiments, with 1–10 transgene copies per T_0 plant. Several studies have since evaluated multiple variables to determine their effect on barley transformation efficiency in immature embryos with generally higher transformation efficiencies in Golden Promise (Matthews et al. 2001; Trifonova et al. 2001; Murray et al. 2004; Shrawat et al. 2007; Hensel et al. 2008). Wounding by sonication or bombardment, alteration of media components, and inclusion of acetosyringone had mixed effects on transformation efficiency. Several *Agrobacterium* strains (AGL0, AGL1, and LBA4404) were efficient in transferring DNA into barley. A simplified transformation method was recently published (Bartlett et al. 2008) using improved tissue culture methods and media. Their transformation efficiency was 25% over six experiments, and almost half of the

260 independently transformed lines had single-copy insertions.

Wang et al. (2001) transformed the elite Australian cv. Schooner using *Agrobacterium* by modifying media and transformation procedures. Murray et al. (2004) transformed three Australian cultivars using visual selection of callus expressing the green fluorescent protein (GFP) gene, although efficiencies were much lower than in Golden Promise. Hensel et al. (2008) used their method improvements to test three winter barley and six spring barley genotypes. Results were varied, with two winter and one spring genotypes producing no transgenic plants. The transformation efficiencies of the other genotypes were substantially lower than those of Golden Promise, but several genotypes produced transgenic plants at reasonable levels for routine use. Southern blot analysis indicated about half of the lines contained a single-copy integration, and most lines showed Mendelian inheritance and gene expression in their progeny. This move beyond model cv. Golden Promise has made it possible to transform high-quality commercial barley cultivars that are useful in breeding programs.

There has been similar interest in transforming barley pollen using *Agrobacterium*-mediated methods to potentially produce large numbers of homozygous transgenic lines. Kumlehn et al. (2006) examined the effects of a number of variables on the success of pollen transformation in winter barley cv. "Igri." The best combination of variables gave 2.2 fertile plants per spike, comparing well with immature embryo transformation frequencies. Between 31.4% and 68.6% of the T_0 plants contained single copies of the transgenes, depending on the *Agrobacterium* strain used.

Another potential target explant for *Agrobacterium* is *in vitro* cultured ovules. Holme et al. (2006) used Golden Promise to develop a method to culture recently pollinated ovules with *Agrobacteria*. With selection, they recovered 3.1 transgenic plants per 100 ovules cultured with 37% of the plants showing single-copy insertion. They then used the method to test four additional cultivars that do not regenerate well from callus culture systems (Holme et al. 2008). Transformed plants containing one or two transgene copies were obtained from all four cultivars, indicating this method may be genotype independent, in contrast to methods requiring callus formation before regeneration. The short time frame makes this an attractive method to produce plants with little or no somaclonal variation, and they even had a transformation efficiency of 0.8% without selection (Holme et al. 2006).

Agrobacterium-mediated and particle bombardment of Golden Promise immature embryos were compared by Travella et al. (2005). *Agrobacterium*-mediated methods of Tingay et al. (1997) were modified to include an osmotic treatment of the immature embryos before cocultivation and transfer of callus surviving selection to an intermediate medium containing benzylaminopurine (BAP) and increased copper concentrations before regeneration. The modified method had twice the transformation efficiency (2%) compared to particle bombardment (1%) with fewer copies of the transgene integrated into the genome and little to no transgene silencing. Particle bombardment produced some lines with more than eight copies of the transgene and frequent transgene silencing in the progeny. It would be interesting to see a comparison using some of the more recent improvements in both methods.

Applications

While initial efforts to transform barley concentrated on improving the efficiency of methods using selectable antibiotic or herbicide resistance genes or markers for β-glucuronidase, GFP, or luciferase, efforts using genes of practical interest quickly followed. Traits targeted for alteration have included nutritional and malting quality, disease resistance, tolerance to various soil minerals, and production of pharmaceutical compounds. Gene silencing by RNA interference and transformation of candidate genes are being used to better understand gene function. The following sections will briefly describe some of the transgenic research being conducted to increase disease resistance and the malting quality of barley.

Disease resistance

In one of the first experiments, barley was transformed with the coat protein (CP) gene from barley yellow dwarf virus (BYDV) pathovar P-PAV (Wan and Lemaux 1994). Eight of these plants from six transgenic lines had moderate to high levels of resistance to P-PAV that correlated with the presence of the CP gene (McGrath et al. 1997). Later, Wang et al. (2000) transformed Golden Promise with a transgene designed to produce hairpin (hp) RNA containing BYDV-PAV sequences, using *Agrobacterium*-mediated methods. They tested 25 independent barley lines for their reaction to BYDV-PAV and found nine lines that showed extreme resistance to the virus. Further investigation of the progeny of two independent transgenic lines showed a single transgene inheritance that consistently correlated with protection against BYDV-PAV.

Many compounds produced in plants have been implicated in disease resistance, including the stilbene-type phytoalexin resveratrol. The stilbene synthase gene from grape (*Vitis vinifera* L.) with its native promoter and an enhancer was inserted into Igri using particle bombardment of microspores (Leckband and Lorz 1998). Transfer of this gene in tobacco, rice, and other species enhanced disease resistance. The stilbene synthase gene was inherited and expressed in T_1 and T_2 progeny. Wounding by rubbing leaves with sand induced the gene, giving maximal expression approximately 8 h later, as measured by RNA levels. Pathogen inoculation also induced the transgene. Inoculation with *Botrytis cinerea* indicated that the stilbene synthase transgene enhanced resistance in barley.

Fungal genes also have been used to transform barley for disease resistance. The Tri101 gene from *Fusarium sporotrichioides* that acetylates trichothecene mycotoxins was inserted into cv. Conlon by particle bombardments. The goal of transformation with this gene was to reduce toxins that interfere with plant protein synthesis, to reduce fusarium head blight (Manoharan et al. 2006). Recent information on the characterization of Tri101 proteins from *F. sporotrichioides* and *F. graminearum* indicated that FsTri101 had low specificity for deoxynivalenol, while FgTri101 had high specificity for this trichothecene toxin (Garvey et al. 2008). The use of FsTri101 might explain the lack of an effect on deoxynivalenol and fusarium head blight in the field.

Another compound involved in disease resistance is lipoxygenase (LOX), which Weber (2002) found to be involved in cell death associated with the hypersensitive response and gene regulation during pathogenesis. To investigate this association, Sharma et al. (2006) transformed cv. Salome by particle bombardment to overexpress barley *LOX2*. High expression levels from using a constitutive promoter were detrimental to plant growth and increased senescence. Plant death after successful regeneration from tissue culture and poor transmission of the transgene to progeny indirectly supports the role of LOX in programmed cell death.

Transformation has been valuable in elucidating mechanisms of barley interactions with the powdery mildew fungus *Blumeria graminis*. Schultheiss et al. (2005) transformed barley with *RACB*, which produces a small monomeric GTPase, and showed the gene was involved in susceptibility to *B. graminis*. The interaction between *MLA1* and *MLA6* resistance genes with *RAR1* was examined by Bieri et al. (2004) using transgenic lines. Transformation allowed development of specific gene combinations to look at protein abundance and interactions in the various powdery mildew resistance gene pathways. Transformation with the gene for synthesis of BAX inhibitor-1 (Huckelhoven et al. 2003; Eichmann et al. 2004) provided materials to examine the role of programmed cell death in *mlo*-mediated penetration resistance to powdery mildew.

Another use for barley transformation has been to verify candidate resistance genes. *Agrobacterium*-mediated transformation of susceptible Golden Promise with the *Rpg1* gene for resistance to the stem rust fungus (*Puccinia graminis* f. sp. *tritici*) was used to confirm that the isolated sequence was the resistance gene (Horvath et al. 2003). A single copy of the resistance gene expressed in Golden Promise provided more resistance than found in the source of the transgene, Morex. In

contrast, barley transformation with the maize Rp1-D gene for resistance to common leaf rust (*Puccinia sorghi*) did not provide resistance to the stem or the leaf rust (Ayliffe et al. 2004) because of aberrant RNA processing in barley resulting in truncated transcripts that contained the nucleotide binding site but lacked the leucine-rich repeat of the maize gene.

A candidate gene, for BaYMV and BaMMV resistance (Stein et al. 2005) was confirmed using barley transformation. A eukaryotic translation initiation factor (Hv-eIF4E) was proposed as a candidate for the recessive *rym4* BaYMV and BaMMV resistance genes in barley. When the Hv-eIF4E sequences from a susceptible cultivar were transformed into a resistant line containing *rym4*, the transgenic lines showed dominant susceptibility to BaMMV. These results helped determine that response to BaMMV infection is controlled by variants of Hv-eIF4E in monocots.

Malting quality parameters

A major target of transformation has been amylolytic enzymes involved in the malting of barley. These enzymes are active in the germinating grain and hydrolyze starch into fermentable sugars. The enzymes are inactivated during the increased temperatures of mashing and kilning. Increasing the activity or thermal tolerance of these enzymes should increase the amounts of substrate for fermentation.

Modified amylases were the focus of two studies. Kihara et al. (2000) substituted seven amino acids in the barley β-amylase enzyme to increase thermostability by as much as 11.6°C. Igri lines stably expressed the gene after transformation by polyethylene glycol (PEG) treatment of protoplasts and had higher β-amylase activity at 65°C compared to wild-type Igri. An alkalophilic α-amylase from *Bacillus* was identified by Tull et al. (2003) and was used to transform Golden Promise by particle bombardment. In transgenic plants expressing this gene, α-amylase activity increased 30%–100% above native α-amylase levels. Adding a signal sequence for the secretion of the enzyme from aleurone cells helped retain high levels of activity during micromashing.

Glucanases also have been targeted because β-glucans can interfere with wort filtration. Horvath et al. (2001) tested Golden Promise lines expressing a protein-engineered (1,3-1,4) β-glucanase developed by particle bombardment (Jensen et al. 1996). Micromalting showed some transgenic lines had moderate to high levels of the heat-stable β-glucanase throughout germination and even after kiln drying. A thermotolerant endo-1,4-β-glucanase (cellulase) from *Trichoderma reesei* was inserted into Golden Promise and cv. Kymppi using particle bombardment (Nuutila et al. 1999). The enzyme retained activity after 2 h at 65°C. Xue et al. (2003) used a hybrid cellulase with both cellulase and glucanase activity to transform Golden Promise by *Agrobacterium* methods. High levels of expression were maintained in the progeny of transgenic lines, even through postharvest storage. Production of these transgenic starch and β-glucan-modifying enzymes in barley could increase malt house and brewery yields by improving efficiencies, thereby lowering production costs.

FUTURE PROSPECTS OF MOLECULAR TECHNOLOGIES AND TRANSFORMATION

As can be seen from the above, molecular technologies have, apart from some specific components, not really impacted barley improvement for complex characters such as yield and malting quality. From our survey, and from comments from various other breeders, many feel that the effects detected in elite germplasm for yield and malting quality are small and do not account for much of the phenotypic variation. Given that yield and malting quality are characters with low heritabilities, it is perhaps not surprising that the detected effects are small. The question is whether or not it is cost-efficient to use MAS to select among progenies for small effects. It may well be a better use of the information to use markers to select parents that carry the beneficial alleles and then to use phenotypic selection to screen among the progenies in the normal manner. The availability of a low-cost genome-wide marker assay

could also be applied effectively to seed stock production. The time from crossing to submission for official trials is 4 years in some barley breeding programs, which means that seed stocks are generated from relatively early-generation single plants. Residual heterozygosity in the originating plant could cause some problems in seed stock production, which can be partially alleviated by growing out more families, but this increases the resources needed for the multiplication phase. An alternative strategy would be to utilize genome-wide profiling with markers to identify mother plants with the same or similar homozygous genotypes upon which to base the seed stock. As the chromosomal regions of the characters used to test for distinctness, uniformity, and stability as part of the official trial process become apparent, markers could be targeted to these specific regions.

It is arguable that QTL information alone is insufficient to be utilized in identifying markers of value in the selection for complex characters. For instance, crossing two good malting quality parents does not automatically mean that the progeny will also be of good malting quality. Where the information is given, published results frequently demonstrate that the mean of a random inbred population is significantly less than the midparental value. This is indicative of the type of epistatic interactions that might result from a complex of component characters that are dependent upon each other. Crossing and segregation can all too easily disrupt the fine balance in expression of the individual components, and applying MAS to any one unknown component without knowledge of the consequences up- or downstream of the target is not likely to produce any greater genetic gain than normal phenotypic selection. The detailed whole-genome profiles that current marker technologies such as DArT and Illumina OPAs offer, coupled with the information that can be gathered from expression profiling (Chapter 4), can be utilized to identify discrete genomic regions that affect specific aspects of a complex character and how these interact to provide a "good" phenotype.

The benefit of the whole-genome profiles produced from technologies such as DArT and Illumina OPAs is that one can generate a detailed view of the variation being manipulated in elite gene pools. Just surveying the current U.K. Recommended Spring Barleys with 1536 SNPs from barley OPA1 reveals that two cultivars, Doyen and Waggon, produced by the same breeding company (Syngenta Seeds), differ at over 350 loci. Thus, it would be impossible for the worldwide breeding effort to evaluate the number of different potential combinations that can be made from a cross between just these two cultivars. Even assuming that just 10% of the polymorphisms between the two cultivars detected by the SNP markers on barley OPA1 are functional, there are still over 3×10^{10} potential inbreds. While distal localization of chiasmata in barley has been known for a long time (Linde-Laursen 1982), detailed whole-genome profiling is now revealing just how few recombination events in centromeric regions have occurred in elite barley germplasms. For many chromosomes, there are just two major centromeric haplotypes.

DArT and barley OPAs provide sufficient genome coverage to provide markers that are linked sufficiently strongly to phenotypic characters that they can be deployed in MAS strategies. Markers linked within 2 cM of a target trait gene/QTL can be used effectively, but a breeder must accept that there will be a certain frequency of false positives. In addition, such a strategy requires knowledge of the parental genotypes and phenotypes for it to be implemented effectively. A "perfect" marker, that is, one where the polymorphism is in the causal gene, is more desirable. However, it requires identification of a candidate gene, either through chromosome walking or bioinformatic approaches or a combination of the two and then validation through genetic transformation. This obviously is resource intensive and cannot be conducted for many targets, although eQTLs may help in the identification of some candidate genes.

While markers such as those provided by the barley OPA are very useful for surveying variation based upon functional genes in the adapted barley gene pool, it remains to be seen how effective they will be in describing the variation in less-adapted material, particularly wild barley *Hordeum vulgare* ssp. *spontaneum*, where there are

many more haplotypes per gene than in cultivated barley (Caldwell et al. 2004). This therefore, limits the value of relying on just one SNP, even if it was a perfect marker, in mining germplasm collections for alternative alleles of a gene affecting a target character.

Although release of commercial transgenic barley has yet to happen, transformation has the potential to improve adaptation and yields through biotic and abiotic resistances, and quality through manipulation of enzymes and components in the grain. In the mean time, transformation techniques are a valuable tool to help elucidate the functions and interactions of genes in metabolic pathways. Continued improvements in methods will increase transformation efficiencies in a wide variety of genotypes and will allow use of these technologies for further barley improvement.

In summary, molecular biology has provided a key tool for barley improvement, namely, molecular markers for use in MAS and MAB. This tool is also valuable in fine mapping approaches to identify potential candidate genes affecting a character, and genetic transformation can then be deployed to validate candidates. Molecular markers have also highlighted the amount of variation that still exists in the elite barley gene pool, which is surprisingly high considering the long history of crossing and selection that has been applied to a diploid inbreeder. The future challenge for molecular biology in barley is to understand the control of chiasma formation and thereby to develop strategies to generate more recombination in the large centromeric linkage blocks and to facilitate the generation of more variations.

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Chapter 7
Barley Germplasm Conservation and Resources

Harold E. Bockelman and Jan Valkoun

INTRODUCTION

A primary objective of germplasm resource programs is to assure continued availability of genetically diverse germplasms with the characteristics required for developing stable, productive, and high-quality cultivars. To meet this objective, it is important that germplasm resource programs include collection and evaluation of accessions in addition to the basic function of maintenance. Development and maintenance of barley germplasm collections, identification of germplasm with characteristics required for cultivar development, and introduction and maintenance of genetic diversity will be discussed in this chapter.

Maintenance of the critical genetic diversity that crop germplasm represents is the basic factor that motivates the establishment of germplasm collections. Maintenance, along with acquisition, documentation, distribution, and evaluation are the primary functions of all crop germplasm collections. Constant vigilance is required to preserve the genetic diversity represented by the barley seed stored *ex situ* in germplasm collections around the world.

WORLD BARLEY GERMPLASM RESOURCES

International organizations became actively involved in genetic resources when the United Nations (UN) Food and Agriculture Organization (FAO) called a technical meeting on plant exploration and introduction in 1961 (Harlan 1975). The Crop Ecology and Genetic Resources Unit of FAO started collecting and distributing genetic resources in 1968. In 1972, the UN Conference on the Human Environment at Stockholm recommended the establishment of a program to identify unique genetic resources such as the major progenitors of barley, sorghum, and millet (Harlan 1975). The Consultative Group on International Agricultural Research (CGIAR) established the International Board for Plant Genetic Resources (IBPGR) in 1974 as an autonomous international scientific organization to promote an international network of genetic resource centers to further the collection, conservation, documentation, evaluation, and use of plant germplasm. In July 1981, IBPGR convened an ad hoc Working Group on Barley Genetic Resources to review existing collections of barley, to identify collection priorities, and to discuss the distribution of global base centers for barley germplasm.

In Europe, the coordination of genetic resource activities began in 1983 with the establishment of a Barley Working Group of the UN Development Program/IBPGR European Cooperative Programme for Plant Genetic Resources (ECPGR). The European collaborative effort resulted in the development of a European Barley Core Collection (BCC) and the European Barley Database (EBDB) (Knüpffer 1988). The regional initiative was later extended to the global level when an International Barley Genetic Resources Network (IBGRN) was established

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and an international BCC committee was formed (Knüpffer and van Hintum 1995). Since then, the international BCC committee has organized business meetings and open workshops to monitor progress and to coordinate activities, in connection with the International Barley Genetics Symposia.

After 7 years of negotiations, the FAO Conference adopted the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) in November 2001. This legally binding Treaty is in harmony with the Convention on Biological Diversity and provides a legally binding framework for access and benefit sharing in relation to plant genetic resources for a defined range of crops, including barley.

In a joint initiative of FAO and Bioversity International (formerly IPGRI) on behalf of the International Agricultural Research Centers of CGIAR, the Global Crop Diversity Trust (GCDT or the Trust) was established in 2004 as an endowment fund to support the conservation and availability of crop diversity over the long term. The Trust is recognized as an essential element of the Funding Strategy of the International Treaty in relation to the *ex situ* conservation and availability of plant genetic resources for food and agriculture.

The Trust is supporting the development of a set of conservation strategies that will guide the allocation of resources to the most important and needy crop diversity collections. The crop strategies are identifying collections, on a crop-by-crop basis, which are critically important at a global level, and they will be used to guide funding decisions of the Trust. The strategies should be largely driven by experts and holders of genetic resources of the crop in question. The process begins with a preliminary period of research into the state of diversity in the collections of that crop.

In the initial phase of the development of "the Global Strategy for the *ex situ* Conservation and Use of Barley Germplasm," the International Center for Agricultural Research in the Dry Areas (ICARDA) in Aleppo, Syria, was commissioned by the Trust with major input from the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany, to coordinate the global inventory of *ex situ* collections of barley genetic resources in order to asses the collection content and status of conservation. The barley strategy was planned with CGIAR and FAO assistance, and significant input was provided by a group of national and international barley experts and collection managers.

The inventory was primarily based on direct responses from institutional holders of major barley collections. Additional information was obtained from different national, regional, and international information sources, such as Global Inventory of Barley Genetic Resources (GIBGR, hosted by ICARDA), EBDB (hosted by IPK), World Information and Early Warning System (WIEWS on Plant Genetic Resources for Food and Agriculture, hosted by FAO), EURISCO (hosted by Bioversity International), and GCDT regional strategy reports.

The GCDT inventory showed that the total of barley germplasm holdings at 47 major barley collections (those with more than 500 accessions) was 402,000 accessions. If 28 minor collections are included, then the global total would be 405,000 accessions. This total is 16% lower than the FAO's estimate (FAO 1996) and 8% higher than a more recent report by van Hintum and Menting (2003). In general, there is no major discrepancy between the gene bank collection totals identified in the GCDT report and Hintum and Menting's data.

Information on 20 globally major barley collections is presented in Table 7.1. The largest collection of barley germplasm is held by Plant Gene Resources of Canada (PGRC). Its holdings represent 10% of the world total. This collection grew from 13,000 to 36,000 accessions in 1989 when the United States Department of Agriculture (USDA) collection was duplicated there. Consequently, there is a large overlap between the PGRC and USDA collections. According to van Hintum and Meeting (2003), considerable duplications exist among the four largest collections, that is, PGRC, USDA, Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA, Brazil), and ICARDA.

The GCDT inventory also provided information on the structure of the global barley

Table 7.1 Major barley collections as of September 2007 based on the Global Crop Diversity Trust (2008) inventory

Country	Gene Bank/Institute	Total No. of Accessions
Canada	Plant Gene Resources of Canada, Saskatoon	39,852
United States	USDA-ARS National Small Grains Collection, Aberdeen, Idaho	29,870
Brazil	Recursos Geneticos e Biotecnologia, EMBRAPA/CENARGEN, Brazil	29,227
CGIAR	ICARDA, Aleppo, Syria	26,117
United Kingdom	John Innes Centre, Norwich	23,603
Germany	Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben	22,106
China	Institute of Crop Germplasm Resources, CAAS, Beijing	18,818
Republic of Korea	National Institute of Agricultural Biotechnology, Suwon	18,764
Russia	N.I. Vavilov Institute of Plant Industry (VIR), St. Petersburg	17,850
Ethiopia	Institute of Biodiversity Conservation (IBC), Addis Ababa	15,360
Japan	Research Institute for Bioresources, Okayama University	14,106
Sweden	Nordic Genetic Resources Center, Alnarp	13,435
Australia	Australian Winter Cereals Collection, Calala, New South Wales	12,600
CGIAR	CIMMYT, El Batan, Mexico	11,202
Japan	National Institute of Agrobiological Science, Tsukuba	8806
India	National Bureau of Plant Genetic Resources (NBPGR), New Delhi	8384
Iran	National Gene Bank of Iran, Genetic Resources Division, Karaj	7600
Israel	Institute for Cereal Crops Improvement, Tel Aviv University	6662
Poland	Plant Breeding and Acclimatization Institute (IHAR), Radzików	5942
France	Station d'Amélioration des Plantes, INRA, Clermont-Ferrand	5517

germplasm holdings. Of the total of 290,820 accessions with germplasm type known, 15% are wild relatives, 44% are landraces, 17% include breeding materials, 9% are genetic stocks, and 15% are cultivars. Thus, a larger proportion (59%) of global barley holdings includes germplasm that has evolved in a long-term interaction with the local environment and farmers' practices (land races and wild relatives), while the other three categories (41%) are products of modern plant breeding and research (breeding materials, cultivars, and genetic stocks).

Wild relatives

The majority of the wild relative collections are represented by the barley wild progenitor, *Hordeum vulgare* subsp. *spontaneum*. Holdings held in public domain in the major collections include 20,700 accessions. The largest collections are maintained in Israel, Canada, ICARDA, the United States, and Germany. The ecologically and geographically most diverse collection has been assembled at ICARDA, as its 1900 accessions originate from 20 countries and 730 different collection sites. Of the 5900 accessions of wild barley species belonging to the secondary and tertiary gene pool, the largest collections are maintained in Canada and in Sweden.

Landraces

Landraces of cultivated barley represent the largest part of barley germplasm conserved in gene banks worldwide. Of the germplasms with type known, 130,000 accessions (44%) are landraces. The largest landrace collections are held in seven gene banks: ICARDA (Syria), Chinese Academy of Agricultural Sciences (CAAS), Institute of Biodiversity Conservation (IBC, Ethiopia), PGRC (Canada), USDA (United States), IPK (Germany), and Research Institute for Bioresources (RIB, Japan), each having more than 10,000 accessions. ICARDA, with 15,500 landrace accessions leads the group.

Breeding materials

Breeding materials are the second most frequent category of barley germplasm held in gene banks globally with 49,000 accessions. The largest collection is conserved at Centro Internacional de

Mejoramiento de Maiz y Trigo (CIMMYT) in Mexico with 11,000 accessions, followed by PGRC (Canada), ICARDA (Syria), USDA (United States), National Institute of Agrobiological Research (NIAR, Japan), and Institut National de la Recherche Agronomique (INRA, France) with more than 3000 accessions.

Genetic stocks

The most extensive collection of genetic materials is held at the Nordic Genetic Resources Center or NordGen (formerly Nordic Gene Bank) in Alnarp, Sweden. It comprises about 10,000 accessions that resulted from a joint Scandinavian mutation research program and 685 translocation and 58 duplication lines. Large collections of genetic stocks are also maintained at PGRC (Canada), USDA (United States), and NIAR (Japan).

Cultivars

This category includes finished products (both advanced and obsolete) of plant breeding programs. There are many duplications of this type of barley germplasm among gene banks worldwide, as well as internal duplications within a gene bank. The largest cultivar collections are held at N.I. Vavilov Research Institute of Plant Industry (VIR, Russia) with 9600 accessions, IPK (Germany), PGRC (Canada), and USDA (United States).

Germplasm distribution

Major global providers of barley seed to scientists and other users are collections held at USDA, ICARDA, and IPK Gatersleben with an annual distribution of 8000, 5400, and 4000 seed samples, respectively. A significant portion of these total amounts are distributed internationally: 38%, 48%, and 57%, respectively, by the above collections.

Germplasm documentation

Passport information is computerized in most collections; however, the availability of characterization and evaluation data electronically is lower. A number of collections may be accessed via the Internet, but only some are searchable online. In addition to individual gene bank data management systems, a number of global, regional, and specialized systems have been developed to link different sources of locally curated data. These include the CGIAR System-wide Information Network for Genetic Resources (SINGER), the GIBGR, and the International Barley Information System (IBIS); EURISCO and the EBDB developed by ECPGR at IPK; and the Database for Barley Genes and Barley Genetic Stocks maintained at NordGen.

International BCC

To improve the accessibility of large crop collections and to rationalize the evaluation of plant genetic resources, the concept of core collections was developed (Frankel and Brown 1984). The international BCC has been assembled since 1989 by an international consortium as a voluntary activity of the participating institutions (Knüpffer and van Hintum 1995). It attempts to create a common set of barley genotypes for use mainly in research, allowing the compilation of a large set of data on the genetic diversity in barley.

The BCC is developed in an international network, which consists of a coordinating committee and subset coordinators, responsible for the selection of BCC subsets, the creation of BCC accessions via single seed descent, the initial multiplication of BCC accessions, and the distribution to the "active BCC centers." These active BCC centers are major research institutions, which are responsible for the distribution of BCC samples to bona fide users in their respective regions:

- IPK in Gatersleben, Germany, for Europe;
- ICARDA in Aleppo, Syria, for the Central and West Asia and North Africa (CWANA) region;
- RIB, Barley Germplasm Centre in Kurashiki, Japan, for South and East Asia;
- USDA National Small Grains Collection (NSGC) in Aberdeen, Idaho, for the Americas; and
- Australian Winter Cereals Collection (AWCC) in Tamworth, Australia, for Australia and New Zealand.

In 2007, the size of the BCC was about 1500 accessions. Given the maximum size of 2000 accessions, this leaves room for future additions.

A global strategic approach to conserving the barley gene pool

The crop gene pool includes genetic diversity conserved within all unique accessions held *ex situ* in gene banks. These genetic materials are, in general, readily accessible for utilization in breeding and research. The other part of the crop gene pool occurs under *in situ* conditions on farms or in the wild and remains to be collected.

Regarding the barley material that is already conserved within *ex situ* collections, there are some collections that represent a large part of genetic variation (i.e., most of the major collections listed in Table 7.1) that are well maintained with seed readily available under the terms of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). Such key collections may be a basis of a global network providing genetic variation to the international community for crop improvement.

A rational approach to conserve the barley gene pool would be to provide sustainable support to the international community through GCDT via a global network of the key collections to ensure that they meet and maintain international conservation standards and are able to distribute high-quality seeds in a timely manner to users worldwide. There may also be other significant and large collections holding a unique, and in many cases, local genetic diversity of barley. These collections might be upgraded with targeted international support to meet the criteria of the key collections. Therefore, smaller and unique collections may be integrated into the key collections.

BARLEY GERMPLASM IN THE UNITED STATES

History

The United States is a country with very few native crop species. Early colonists necessarily brought their favorite seeds with them. Native Americans had, at the time of colonization, small fruits, some nuts, sunflowers (including artichoke), as well as introduced crops of maize, beans, tobacco, cotton, and squash. As Native Americans migrated northward from Mexico and Central America, they brought seeds with them. (White et al. 1989)

The first barley cultivars in North America were probably introduced by Columbus on his first voyage in 1492. Subsequently, immigrants from several countries introduced various types of barley cultivars. Via the East Coast of North America, the English most likely introduced the late-maturing two-rowed cultivars, while the Dutch brought six-rowed cultivated barleys from continental Europe. Barley was introduced via Mexico and the West Coast of North America by the Spanish, who brought six-rowed types from Spain and North Africa (Moseman and Smith 1985).

The U.S. government has recognized the need for collecting and preserving plant material from the beginning. Thomas Jefferson, who was truly a Renaissance man, had a galaxy of interests and accomplishments. He traveled extensively to Europe in the late 1700s, where he collected many botanical specimens including seeds of agronomic and horticultural crop species for utilization in the United States (Jewett 2005). He once said "The greatest service which can be rendered any country is to add a useful plant to its culture …" In 1827, President John Quincy Adams instructed U.S. Foreign Service officers to "collect and transmit seed and plants to the United States." Cultivar tests with barley, oats, and wheat began on USDA land on the Mall in Washington, DC in 1866 and 1867. It was not until 1898 that a unit within the U.S. government was formally established with the responsibility for collection and management of germplasm resources. This unit became known as the USDA Seed and Plant Introduction Section. Since that time, some unit within the USDA has been responsible for the collection and distribution of germplasm.

The USDA Barley Collection began in the Cereal Investigations Section of the USDA in Washington, DC with the introduction of four accessions in 1894 and 11 additional accessions in

1895. USDA cerealist M.A. Carleton soon increased the size of this collection with accessions he gathered on a plant exploration trip to Russia in 1898 and on a trip to the Paris Exposition in 1900. A special appropriation of the Research and Marketing Act of 1946 provided the funds for grouping the small grains into one collection under the management of a USDA Agricultural Research Service (USDA-ARS) curator. Appropriations arising from this 1946 act were intended to be used to (i) grow introductions in special quarantine nurseries, (ii) ensure the maintenance of viable seed, (iii) accumulate data on adaptation and reaction to pests, (iv) catalog other information of use in improving the crop, and (v) respond to requests for seed from researchers. In 1948, the NSGC was organized as an official USDA project at the Beltsville Agricultural Research Center in Beltsville, Maryland (Moseman and Smith 1985). In 1988, a new facility was dedicated at Aberdeen, Idaho, and the collection was moved to its present location.

H.V. Harlan served as leader of the USDA barley investigations from 1912 to 1945 and, with assistance from Mary Martini, M.N. Pope, and G.A. Wiebe, collected and conducted many studies on the accessions in the USDA collection. In 1923 and 1924, Harlan made extensive plant exploration trips in which he collected barleys in Russia, Ethiopia, North Africa, and many other areas. G.A. Wiebe was responsible for the management and operation of the collection from 1945 to 1969 and, with D.A. Reid and J.G. Moseman, continued to develop and evaluate the collection. J.G. Moseman served as leader of the USDA barley investigations from 1969 until 1972 and was responsible for the management of the collection during that period. In 1972, the USDA Barley Collection was incorporated into the National Plant Germplasm System (NPGS).

The NPGS

As late as the 1940s, only a few institutions had the facilities to provide minimal conditions for long-term preservation of seeds. Few scientists at that time sought the yet unknown requirements for refrigerated and dehumidified conditions now recognized as crucial for the storage of viable crop seeds. A study conducted in 1943 by the National Research Council identified the need for more organized regional activities relating to plant conservation, and through the legislation of the 1946 Research and Marketing Act, the Regional Plant Introduction Stations, the National Potato Introduction Station, and NSGC were established (Shands 1995).

The 1972 National Academy of Sciences report on the genetic vulnerability of the nation's crops, and internal reviews suggested that a National Board for Plant Genetic Resources be established to advise the Secretary of Agriculture on plant germplasm issues and to recommend appropriate policies (Anonymous 1972). In 1976, the NPGS Committee consisting of federal, state, and industry representatives evolved from the previous New Crops Coordinating Committee to advise the NPGS.

The NPGS today is coordinated by the USDA-ARS and involves cooperation with a number of federal, state, and private organizations. The 27 active sites of the NPGS acquire, maintain, regenerate, distribute, document, characterize, evaluate, and conduct research on seed, clonal, and genetic stock collections. The National Center for Genetic Resources Preservation (NCGRP, renamed in 2002 from the National Seed Storage Laboratory) provides long-term backup storage for the NPGS, as well as for some non-NPGS collections and foreign countries, and conducts research on preservation methodologies (Shands 1995). All the NPGS sites are linked through the centralized Germplasm Resources Information Network (GRIN) database, which contains passport, taxonomic, descriptor, observation, evaluation, and inventory data important for managers and curators responsible for the accessions. The National Germplasm Resources Laboratory (NGRL) in Beltsville, Maryland, manages the GRIN database. Scientists in the plant germplasm user community can access GRIN online at http://www.ars-grin.gov/npgs/index.html to learn about the NPGS and its component active sites, as well as view passport and evaluation data, make queries, and order germplasm. The NGRL facilitates the activities of the

40 Crop Germplasm Committees (CGCs), composed of scientist users with expertise on a specific crop or group of crops, which provide advice to the NPGS on germplasm activities, including acquisition. The NGRL also administers the Plant Exploration Program (Williams 2005).

The NPGS is one of the largest distributors of germplasm in the world. USDA maintains a policy of free and unrestricted exchange of germplasm for research, breeding, and education. For the years 1995–2003, an average of more than 126,000 samples of germplasm was distributed yearly to a total of 153 nations (Smale and Day-Rubenstein 2002).

The NSGC barley collection today

Today, the NSGC maintains a barley germplasm collection totaling nearly 29,900 accessions representing 20 *Hordeum* species obtained from more than 100 countries. The collection includes old and new cultivars and breeding lines, landraces, and genetic stocks (see Tables 7.2–7.4).

Acquisition

New barley germplasm incorporated into the NSGC is obtained from several sources: (i) collection expeditions to important centers of diversity of barley; (ii) exchanges with other gene banks, institutes, and individual scientists; and (iii) new cultivars or selected improved breeding materials developed by breeding programs in the United States, Canada, and other countries. Acquisition priorities include cultivated barleys and *Hordeum* species to fill species and ecogeographic gaps in the collections. Geographic regions of special interest are Caucasia and central Asia.

Maintenance

A core activity of the NSGC is to carefully conserve the germplasm. Seeds are held in medium-term storage (see Fig. 7.1) under controlled temperature (6°C) and relative humidity (25%). Seed is provided to the NCGRP at Fort Collins, Colorado, for safety backup. (Figs. 7.2 and 7.3)

Table 7.2 Number of *Hordeum* accessions in the USDA-ARS National Small Grains Collection by taxonomy (June 2008)

Taxon	Total
Hordeum bogdanii	16
Hordeum brachyantherum subsp. *brachyantherum*	9
Hordeum brachyantherum subsp. *californicum*	2
Hordeum brevisubulatum	10
Hordeum brevisubulatum subsp. *iranicum*	6
Hordeum brevisubulatum subsp. *nevskianum*	1
Hordeum brevisubulatum subsp. *turkestanicum*	1
Hordeum brevisubulatum subsp. *violaceum*	21
Hordeum bulbosum	183
Hordeum capense	1
Hordeum chilense	10
Hordeum comosum	4
Hordeum hybrid	7
Hordeum jubatum	26
Hordeum lechleri	2
Hordeum marinum	8
Hordeum marinum subsp. *gussoneanum*	14
Hordeum marinum subsp. *marinum*	1
Hordeum murinum	21
Hordeum murinum subsp. *glaucum*	29
Hordeum murinum subsp. *leporinum*	16
Hordeum muticum	3
Hordeum parodii	2
Hordeum procerum	3
Hordeum pusillum	9
Hordeum roshevitzii	2
Hordeum secalinum	4
Hordeum sp.	22
Hordeum stenostachys	13
Hordeum vulgare subsp. *spontaneum*	1507
Hordeum vulgare subsp. *vulgare*	27,917
Total	29,870

Table 7.3 Number of *Hordeum* accessions in the USDA-ARS National Small Grains Collection by improvement status (June 2008)

Improvement Status	Number
Breeding materials	3733
Cultivars	4395
Genetic stocks	3208
Landraces	13,138
Wild relatives	1948
Others[a]	3448
Total	29,870

[a]Status unclear due to poor documentation in the passport data.

Table 7.4 Number of *Hordeum* accessions in the USDA-ARS National Small Grains Collection by country of origin (June 2008)

Country	Count	Country	Count
Afghanistan	442	North Korea	27
Albania	15	South Korea	324
Algeria	68	Kyrgyzstan	18
Ancient Palestine	7	Latvia	6
Argentina	106	Lebanon	20
Armenia	38	Libya	14
Asia	1	Lithuania	7
Australia	153	Macedonia	289
Austria	183	Mali	2
Azerbaijan	110	Mexico	49
Belarus	9	Moldova	3
Belgium	40	Mongolia	177
Bhutan	5	Morocco	168
Bolivia	34	Myanmar	2
Bosnia and Herzegovina	39	Nepal	620
Brazil	13	The Netherlands	102
Bulgaria	115	New Zealand	14
Cameroon	1	Norway	47
Canada	527	Oman	16
Chile	54	Pakistan	291
China	2186	Paraguay	2
Colombia	681	Peru	275
Croatia	46	Poland	162
Cyprus	20	Portugal	35
Czech Republic	88	Romania	122
Czechoslovakia	83	Russian Federation	504
Denmark	186	Saudi Arabia	17
Ecuador	4	Slovakia	28
Egypt	373	Slovenia	36
Eritrea	27	South Africa	157
Estonia	5	South America	6
Ethiopia	4217	Spain	318
Europe	4	Sudan	8
Finland	129	Sweden	895
Former Soviet Union	51	Switzerland	741
Former Yugoslavia	33	Syria	305
France	328	Taiwan	3
Georgia	111	Tajikistan	65
Germany	925	Tunisia	126
Greece	231	Turkey	1911
Guatemala	9	Turkistan	3
Honduras	1	Turkmenistan	62
Hungary	263	Ukraine	242
India	747	United Kingdom	768
Indonesia	3	United States	5322
Iran	480	Unknown	212
Iraq	74	Uruguay	12
Ireland	12	Uzbekistan	30
Israel	1161	Venezuela	11
Italy	58	Western Asia	8
Japan	509	Yemen	27
Jordan	45	Yugoslavia	179
Kazakhstan	24	Zimbabwe	6
Kenya	2		
		Total	29,870

Fig. 7.1. Medium-term storage in the National Small Grains Collection, USDA-ARS, Aberdeen, Idaho. Rooms are maintained at 5–6°C and at 25% relative humidity (RH). *Source:* H. Bockelman. For color details, please see color plate section.

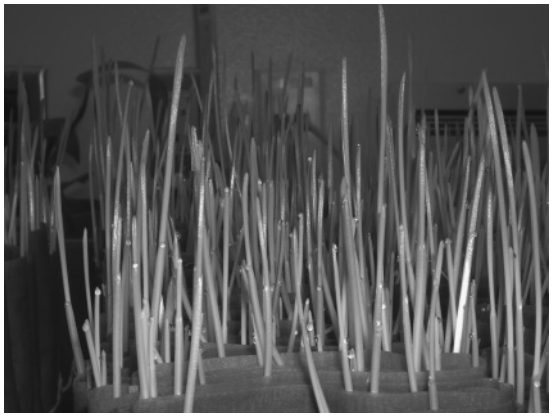

Fig. 7.2. Viability testing of barley accessions in the National Center for Genetic Resources Preservation, USDA-ARS, Fort Collins, Colorado. *Source:* D. Ellis, NCGRP. For color details, please see color plate section.

Fig. 7.3. Cryogenic storage tank in the National Center for Genetic Resources Preservation, USDA-ARS, Fort Collins, Colorado, holding safety backup samples of NSGC barley accessions and other germplasms throughout the NPGS. *Source:* D. Ellis, NCGRP. For color details, please see color plate section.

Table 7.5 Number of distributions of *Hordeum* accessions from the USDA-ARS National Small Grains Collection by year

Year	Samples Distributed
1996	5029
1997	13,136
1998	5729
1999	13,137
2000	5630
2001	2810
2002	1825
2003	5612
2004	2280
2005	3174
2006	7419
2007	8867

Regeneration

Accessions in need of regeneration are grown in fields of the University of Idaho Research and Extension Center and in USDA-ARS greenhouses at Aberdeen, Idaho, and in Parlier, California, at the USDA-ARS National Arid Land Plant Genetic Resource Unit. Accessions are scheduled for regeneration using a priority matrix based on seed viability and inventory quantities. Viability tests are scheduled for each accession every 5 years.

Distribution

Seed samples from NSGC are made freely available in response to requests from scientists involved in bona fide research nationally and internationally. In the past 10 years, *Hordeum* distributions have ranged from 1800 to 13,000 accession samples per year (see Table 7.5). More than 30% of distributions are to scientists in other countries.

Evaluation

The systematic evaluation of barley accessions in the USDA-ARS NSGC is coordinated at Aberdeen. Data obtained from evaluations of NSGC germplasm are entered in GRIN. Data available on GRIN for barley are summarized in Table 7.6. More than 452,000 individual records of barley evaluation data are currently in GRIN. An illustrative example of diversity for lemma and pericarp colors and hull type is shown among Ethiopian landraces in Fig. 7.4.

Geographic information system (GIS)

Many phenotypic traits, especially disease and insect resistance, can be found more frequently in certain ecogeographic regions. Studies have been conducted on the geographic origin of various disease and insect resistances in barley accessions (Bonman et al. 2005). Acquisition of new accessions will benefit from analyses of ecogeographic data and will aid in planning future collection endeavors. A more detailed analysis of the collection helps scientists choose limited numbers of accessions for specific studies. Country, state/province, locality, and latitude/longitude data for NSGC barley accessions are maintained in GRIN. Traits of interest are mapped and analyzed using GIS software and appropriate statistical techniques (see Figs. 7.5–7.7).

Genetic stocks

More than 10% of the NSGC barley collection consists of genetic stocks of several types, including morphological mutants, genetic male sterile stocks, and various cytogenetic stocks, for example, trisomic, inversion, and translocation stocks. Many of these are extensively described in the *Barley Genetics Newsletter* (Lundqvist et al. 1997) including all of the designated "barley genetic stock" (BGS) numbers with identified genes. The newest additions to the genetic stocks are mapping populations designed to link molecular markers to economic traits.

Core subset

A core subset of the cultivated barley (*H. vulgare* subsp. *vulgare*) accessions in the NSGC has been established and marked in GRIN. It consists of 2764 accessions (approxmately 10% of the total) from every country of origin. The core subset was chosen using the following steps: (i) the number

Table 7.6 List of descriptors for barley in the USDA-ARS National Small Grains Collection with data in the Germplasm Resources Information Network (GRIN)

Descriptors	
Aleurone color	Neck breakage
Awn deciduousness	Net blotch (*Pyrenophora teres*)[b]
Awn roughness	Plant height
Awn type	Ploidy
Beta-glucan[a]	Protein[a]
Barley stripe mosaic virus (BSMV) free	Rachilla hair length
Barley yellow dwarf virus (BYDV)[b]	Russian wheat aphid (*Diuraphis noxia*)[b]
Cereal leaf beetle (*Oulema melanopus*)[b]	Scald (*Rynchosporium secalis*)[b]
Days to anthesis	Shattering
Growth habit	Spike angle
Hull cover	Spike density
Kernel plumpness	Spike row number
Kernel weight	Spot blotch (*Cochliobolus sativus*)[b]
Kernels per spike	Straw breakage
Leaf rust (*Puccinia hordei*)[b]	Barley stripe rust (*P. striiformis* f. sp. hordei)[b]
Lemma color	Test weight
Lipid[a]	Yield

[a]Grain content.
[b]Resistance/susceptibility to pest (causal agent).

of accessions from each country of origin was counted; (ii) the log of the number of accessions for each country of origin was calculated; (iii) accessions were chosen randomly within each country of origin based on the relative log numbers, with a minimum of one per country; and (iv) obvious duplications were removed. Theoretically, the core subset should represent the majority of the diversity in the barley collection. It is already proving useful for preliminary screening for new traits and high-cost molecular analyses. The core subset is being refined as further evaluation data (including genotyping) are gathered to better characterize the genetic diversity of the core accessions.

Barley CGC

CGCs are an important connection between the U.S. germplasm user community (or stakeholders) and the working germplasm collections. A 1981 report on the NPGS (Murphy 1981) called for crop advisory committees to provide guidelines for various phases of germplasm management for a specific crop, including such activities as germplasm exploration and acquisition, germplasm storage, germplasm regeneration and distribution, and standards for germplasm evaluation. The committees evolved over time into the present CGC structure. The Barley CGC is composed of federal, state, and private sector research scientists, including the NSGC curator. Current membership can be found on the GRIN Web site. The Barley CGC developed a complete descriptor list, including agronomic, pathological, morphological, and quality traits, to aid in the barley germplasm evaluation process. These descriptors are utilized to characterize the barley accessions for an array of traits, greatly enhancing the value of the germplasm collection. The Barley CGC also provides input on acquisition needs through the development of exploration proposals.

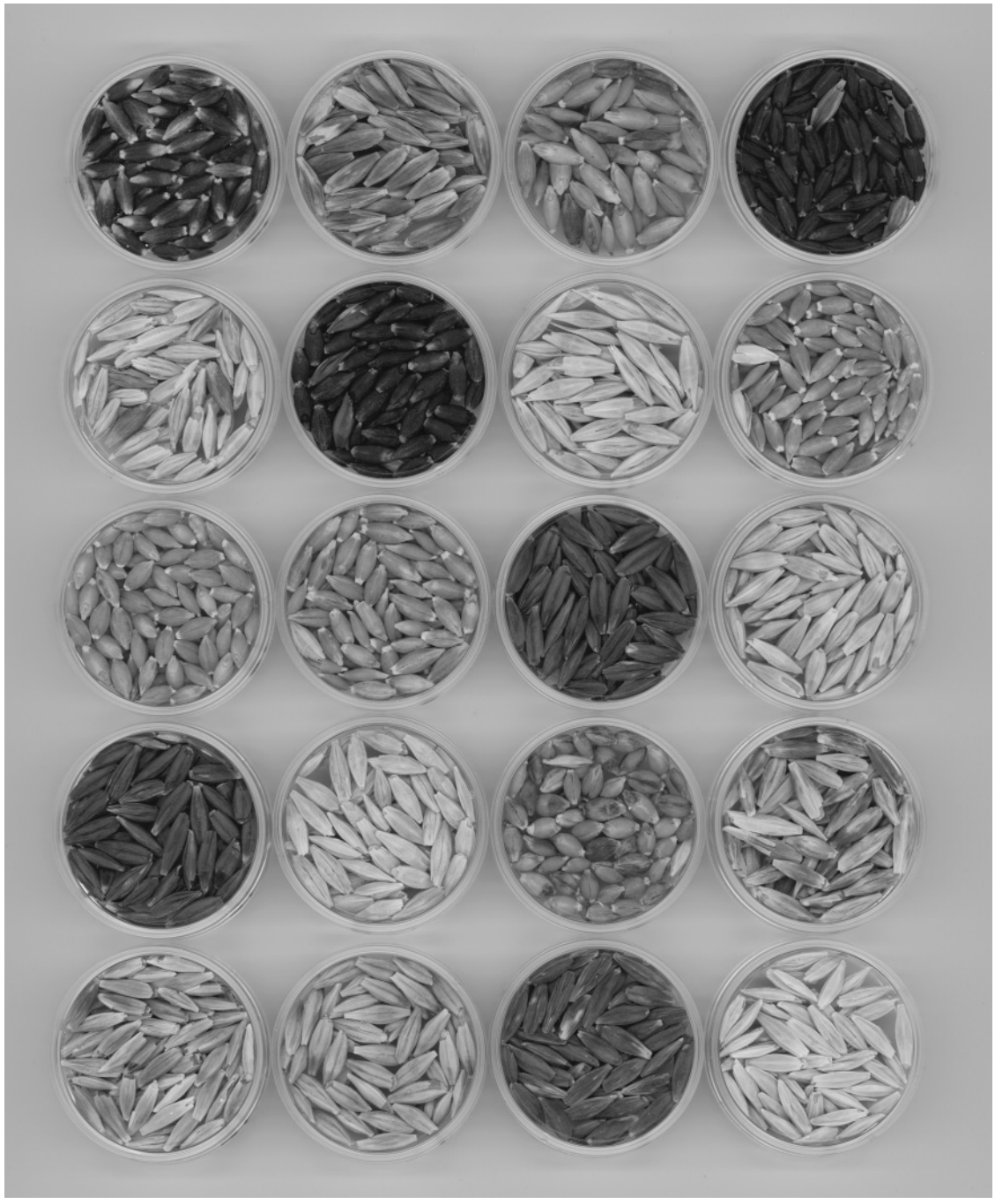

Fig. 7.4. Seeds of 20 different landrace barley accessions from Ethiopia—an illustration of diversity from the USDA-ARS National Small Grains Collection. *Source:* H. Bockelman. For color details, please see color plate section.

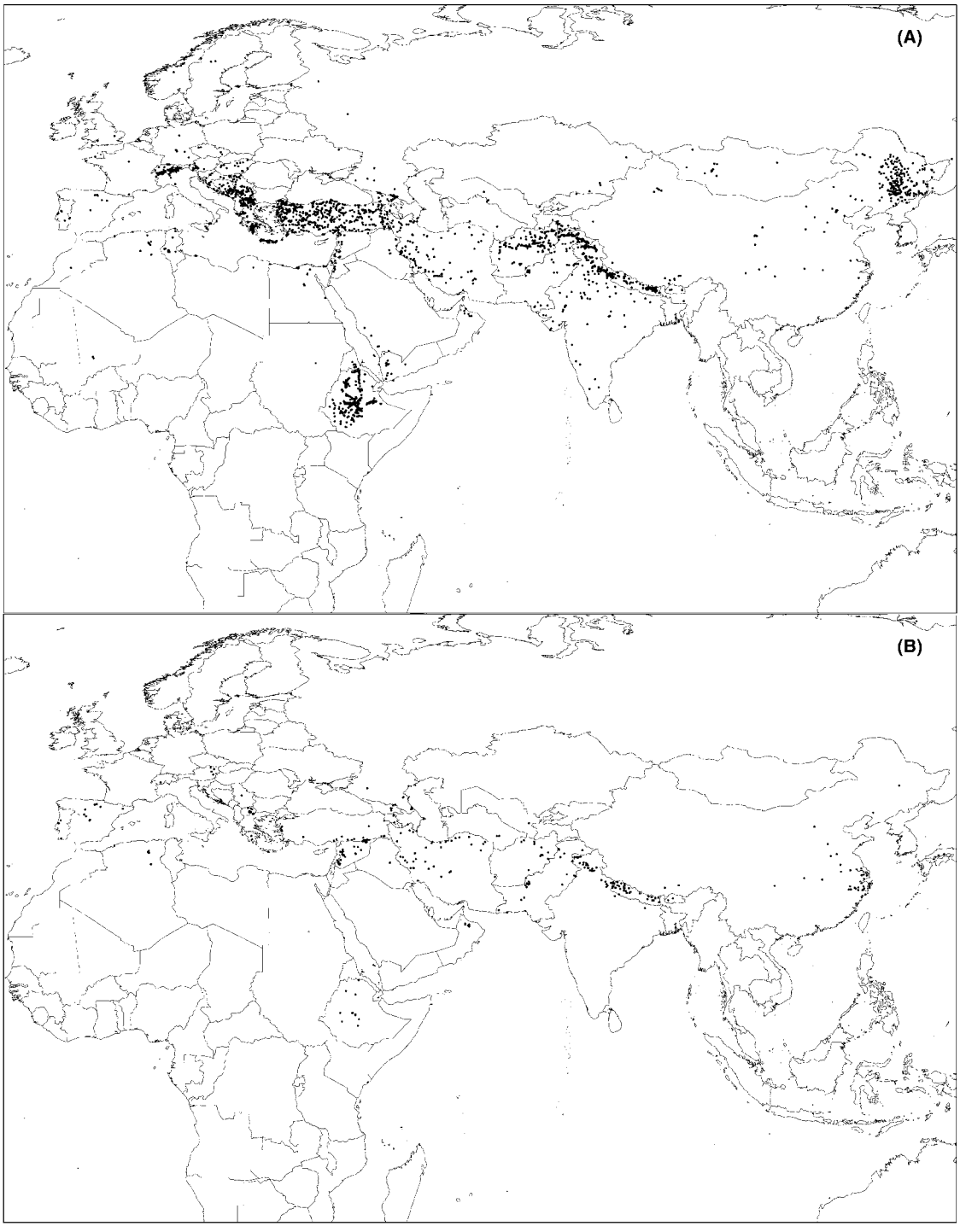

Fig. 7.5. Origin of landrace barleys in the USDA-ARS National Small Grains Collection by growth habit: (A) spring and (B) winter. *Source:* H. Bockelman.

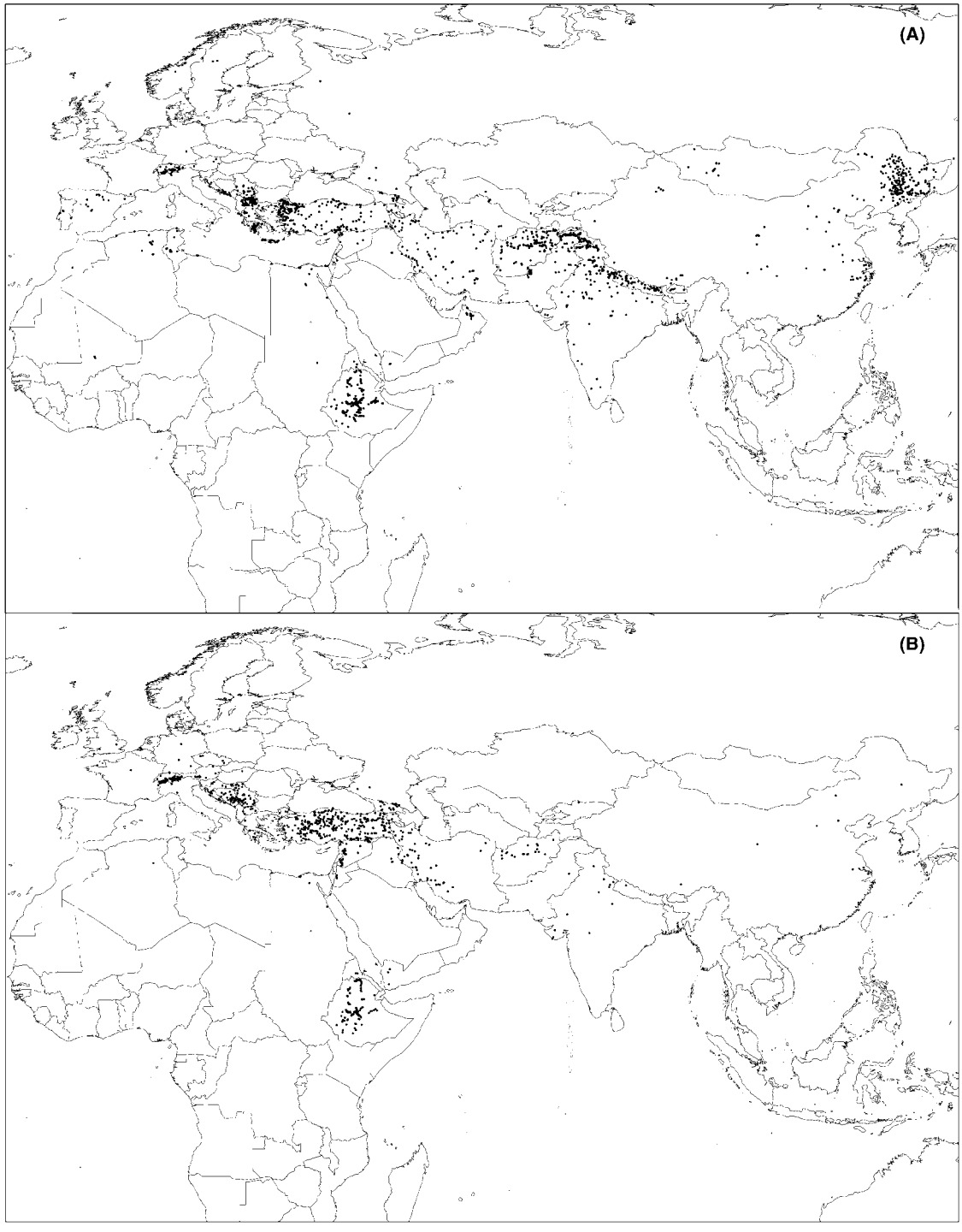

Fig. 7.6. Origin of landrace barleys in the National Small Grains Collection by spike row number: (A) six-rowed and (B) two-rowed. *Source:* H. Bockelman.

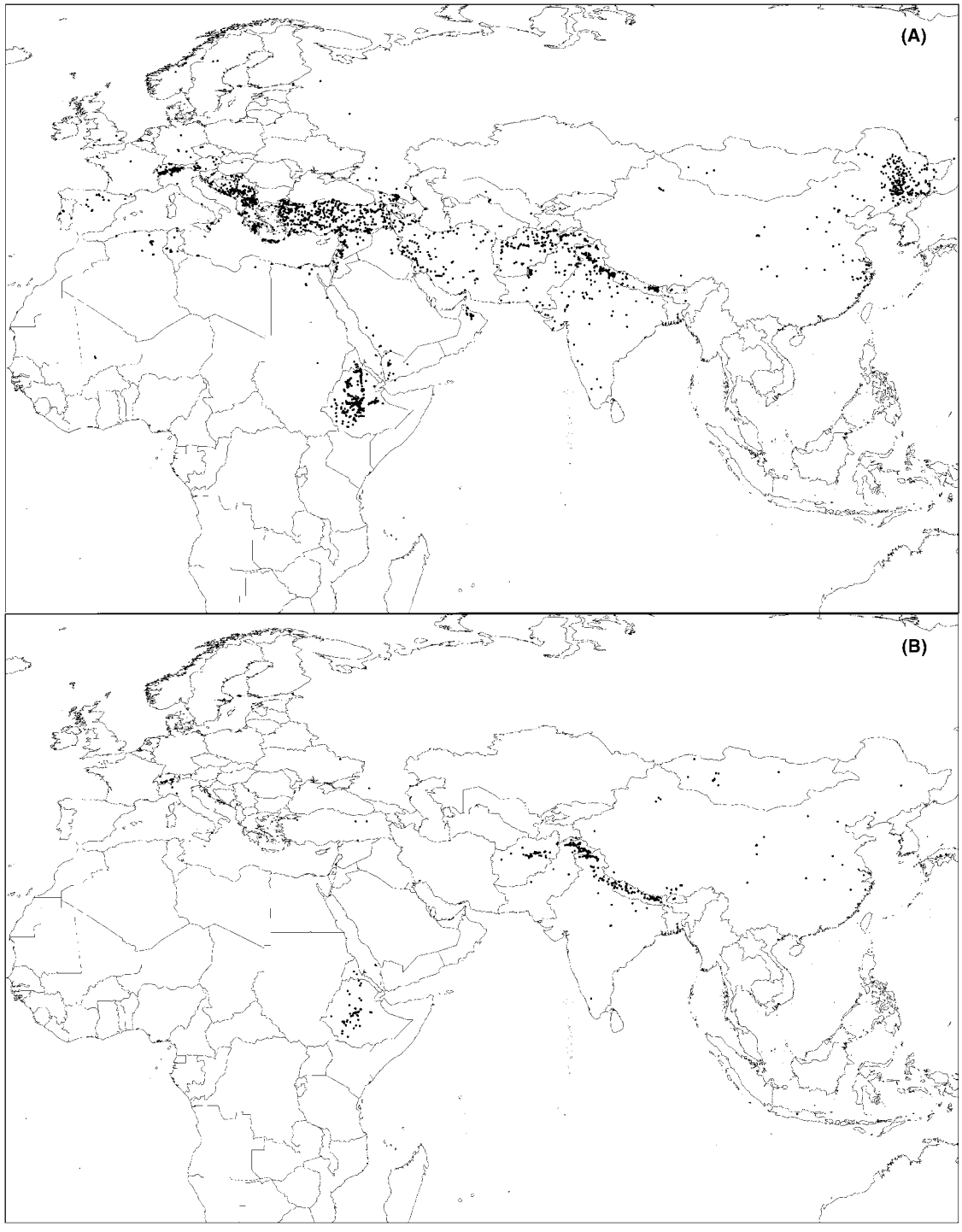

Fig. 7.7. Origin of landrace barleys in the National Small Grains Collection by hull cover: (A) hulled and (B) hulless. *Source:* H. Bockelman.

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Chapter 8
Barley Breeding History, Progress, Objectives, and Technology

EUROPE

Wolfgang Friedt

POSITION OF BARLEY PRODUCTION AND BREEDING

Barley (*Hordeum vulgare*) is one of the leading cereal crops of the world and it is clearly number 2 in Europe, next to bread wheat (*Triticum aestivum*). For example, in Germany, approximately 1.9 million hectares of spring and winter barley contrast to 3 million hectares of winter wheat. The remaining small grain cereals, that is, oats, rye, and triticale, together cover around 1 million hectares.

On a global scale, the barley area harvested worldwide has declined from a maximum of more than 80 million hectares in the 1970s to nowadays less than 60 million hectares (Table 8.1). During the same time, the cultivation area in Europe went down from approximately 53 to 29 million hectares in 2008 (Table 8.2). This represents 51% of the world barley hectarage (56.8 million hectares). The major European barley-growing countries, France, Germany, Russia, Spain, Ukraine, and the United Kingdom, account for about three-quarters of the total annual barley grain production of about 105 million tons, representing 67% of the world production (157,644,721 t in 2008). Almost two-thirds (62%) of the total European barley production comes from the European Union (EU) member states (EU 27) covering 50% of the cultivation area in Europe. The average grain yield per unit area in the EU (27) is 25% higher than the European grand mean (45.4 vs. 36.2 dt/ha; cf. FAO 2009). These figures demonstrate the outstanding importance of barley for Europe and of the European crop for global barley grain production.

The high yield potential of modern cultivars and improved cultivation practices basically allow continuously increasing barley production in all areas. For example, in Germany, the average grain yield of spring barley on a farm scale has grown from less than 2.0 t/ha in the early nineteenth century to a current level of 6.1 t/ha. Over the last four decades, barley yield in Europe has been increased by 60%, in Western Europe by 80% even. The corresponding relative yield increases worldwide amount to 53% (Table 8.1; FAO 2009).

In recent decades, the productivity of barley cultivars has risen by an annual rate of approximately 1%–2%, which is due to (i) genetic breeding progress in terms of more productive cultivars, (ii) more efficient disease and insect control, (iii) improved fertilization schemes, and (iv) improved agricultural production technology (harvest, storage, etc.) in general. Yield increases go along with a substantial improvement of malting quality and significant enhancement of agronomic traits leading to better yield stability, for example, due to resistances against lodging, diseases, and insects. Barley cultivars released and grown in Europe today are characterized by a considerable spectrum of resistance against

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Table 8.1 Barley production: comparison of global production figures with those of Europe (total and western), 1966–2008 (FAO2009)

	World		Europe (Total)		Western Europe	
Period[a]	Area Harvested (M ha)[b]	Grain Yield (t/ha)[c]	Area Harvested (M ha)[b]	Grain Yield (t/ha)[c]	Area Harvested (M ha)[b]	Grain Yield (t/ha)[c]
1966–1968	61.6	1.7	34.3	2.0	5.1	3.3
1976–1978	81.1	2.1	53.4	2.3	6.3	3.8
1986–1988	77.6	2.2	48.7	2.4	5.3	4.9
1996–1998	62.0	2.4	30.9	2.9	4.3	5.9
2006–2008	56.2	2.6	28.7	3.2	4.0	5.9
Change (%)[d]	91	153	84	160	78	179

[a]Averages of three consecutive years each.
[b]10^6 ha.
[c]Metric tons per hectare.
[d]Relative values 2006–2008 versus 1966–1968.

virus diseases—particularly soil-borne yellow mosaic viruses—and fungal pathogens such as powdery mildew (*Blumeria graminis*), leaf rust (*Puccinia hordei*), or scald (*Rhynchosporium secalis*). The number of resistant cultivars has been significantly increased throughout the past few decades (Table 8.3).

For example, a comparison of rankings for powdery mildew attack and grain yield of current winter barley cultivars with those on the market in Germany two or three decades ago clearly shows the drastic change in the varietal performance: some modern winter barley cultivars even combine a very high yield potential with a pronounced mildew resistance, which was not achievable before (cf. Friedt et al. 2000; Beschreibende Sortenliste 2009). The main reason for strongly improved mildew resistance particularly in the spring cultivars is seen in the wide use of effective resistance genes. Various genes have been exploited throughout the decades with some emphasis on the *Mla* locus complex. Recently, however, the interest of breeders has focused on the *mlo* gene. Today, probably more than one third of the spring barley cultivars in Europe carry this gene conferring practically complete field resistance against powdery mildew.

A similar situation also exists with regard to resistances against other diseases, such as scald (*R. secalis*), leaf or brown rust (*P. hordei*), and yellow mosaic-inducing viruses (barley mild mosaic virus [BaMMV] and BaYMV), some of the most important diseases of winter barley in Europe. Whereas in the 1980s only a few cultivars showed satisfactory resistance, many cultivars expressing a high level of resistance, that is, low susceptibility (1–3 on a 1–9 scale according to the German Plant Variety Office) have been released more recently (Table 8.3). For improving rust and scald resistance, various genes have been used. For example, *Rph7* provides fairly stable leaf rust resistance and has been transferred to various cultivars. New genetic sources for scald resistance have been identified showing low disease incidence in different regions of Europe (cf. Friedt and Rasmussen 2004). Also, *Pyrenophora* net blotch and *Ramularia* leaf blotch have to be mentioned as spreading fungal diseases, which occasionally and regionally cause high infections and damage to European barley crop.

The increased level of disease resistance in present-day cultivars also reflects a general tendency in agricultural practice, as farmers are under a constantly increasing pressure to reduce the use of agrochemicals and pesticides for environmental reasons, thus enforcing focus on the resistance properties of varieties. Therefore, breeding for resistance in barley is a very important task on a global scale, since average yield losses worldwide of up to 30% have to be faced due to fungal and viral diseases along with insect

Table 8.2 Major figures of barley production in European countries in 2008 (FAO2009)

Country	Area Harvested (ha)	Grain Yield (dt/ha)	Production (t)
Albania	1400	25.0	3500
Austria	185,857	52.1	967,921
Belarus	612,639	36.1	2,212,480
Belgium	55,016	77.0	423,800
Bosnia and Herzegovina	22,723	33.4	75,841
Bulgaria	222,659	39.4	878,000
Croatia	61,200	44.5	272,100
Czech Republic	482,395	46.5	2,243,865
Denmark	717,300	47.3	3,396,000
Estonia	141,200	24.7	349,100
Finland	585,500	36.4	2,128,600
France	1,799,300	67.6	12,171,300
Germany	1,961,700	61.0	11,967,100
Greece	150,000	25.3	380,000
Hungary	332,000	44.5	1,478,200
Ireland	181,200	69.0	1,249,700
Italy	330,067	37.5	1,236,697
Latvia	131,200	23.4	307,100
Lithuania	332,500	29.2	970,400
Luxembourg	9739	54.2	52,816
Malta	400	40.0	1600
Moldova	130,179	27.1	353,124
Montenegro	790	15.2	1200
The Netherlands	50,200	61.8	310,200
Norway	128,240	41.3	530,000
Poland	1,206,560	30.0	3,619,460
Portugal	43,100	23.2	99,800
Romania	386,706	31.3	1,209,410
Russian Federation	9,420,800	24.6	23,148,450
Serbia	92,417	37.3	344,141
Slovakia	213,050	41.8	891,317
Slovenia	19,229	39.9	76,788
Spain	3,462,400	32.5	11,261,100
Sweden	407,700	44.2	1,801,000
Switzerland	33,112	61.2	202,700
Macedonia[a]	47,351	34.4	162,779
Ukraine	4,167,200	30.3	12,611,500
United Kingdom	1,032,000	59.5	6,144,000
Total or average	**29,157,029**	**36.2**	**105,533,089**
European Union (EU) (EU 27)	**14,473,752**	**45.4**	**65,662,080**

Source: http://faostat.fao.org/.
[a]Former Yugoslav Republic of Macedonia.
Major seed producers (1000 t): Belarus (250), Czech Republic (104), Denmark (132), Finland (136), France (235), Germany (322), Poland (235), Russia (3000), Spain (850), Ukraine (700), and the United Kingdom (143). The global annual seed production varied from 9.5 to 9.8 Mt in 2006–2008. Of the total global annual seed production, 6.8 Mt (70%) is generated in Europe and 2.8 Mt (29%) in the EU (27).

pests. Therefore, in order to avoid necessary applications of chemicals for the prevention of respective yield losses and to ensure economic grain production, future barley breeding for disease and pest resistances should even be further intensified.

CURRENT SPRING BARLEY CULTIVARS COMBINING HIGH QUALITY AND DISEASE RESISTANCE

Modern European spring barley varieties trace back to European landraces from Bavaria (south-

Table 8.3 Number of winter barley cultivars listed in Germany expressing pronounced resistances to major pathogens and diseases[a]

Year	No. of Listed Cultivars	Mildew Resistant[a]	Scald Resistant[a]	Brown Rust Resistant[a]	Yellow Mosaic Resistant[a]
1980	35	3	3	0	n.d.
1986	46	3	1	0	n.d.
1994	69	14	5	3	19
2002	84	32	28	18	49[c]
2009	67[b]	33	18	19	52[c]

Source: Beschreibende Sortenliste, Bundessortenamt, Hannover, Germany (German Plant Variety Office).
[a]Low susceptibility: scores 1–3 (scale 1–9: 1=minimum susceptibility, 9=maximum susceptibility) for powdery mildew (*B. graminis*), scald (*R. secalis*), brown rust (*P. hordei*), and yellow mosaic viruses (BaYMV and BaMMV).
[b]Comprising 34 six-row and 33 two-row cultivars.
[c]Four cultivars each are also resistant against BaYMV-2.
n.d., not determined.

ern Germany), Moravia (today Czech Republic), Sweden, and the United Kingdom. Cycles of cross-breeding first focused on hybridizations among the landraces, leading, for example, to early varieties like "Isaria" (1924) and "Kenia" (1931). Later, more distant and exotic barley materials were included, for example, *Hordeum laevigatum* and "arabische," two major sources of disease resistance finally leading to outstanding spring varieties like "Aramir" (released in Germany 1974) and the *mlo*11-carrying variety "Apex" (1983). A third cycle of cross-breeding comprised the Czechoslovakian short-straw, heavy-tillering mutant "Diamant" (Fig. 8.1), which also contributed to better malt quality due to high proteolytic enzyme activity (Fischbeck 1992, cit. Friedt et al. 2000). This led to the important cv. "Trumpf" (1973), also named "Triumph," which acquired a wide distribution in Europe. Numerous progenies of crosses between Trumpf and representatives of the "Aramir group" became popular cultivars. Major members of this cycle were "Blenheim" (UK, 1985), "Carmen" (A, 1985), "Prisma" (NL, 1985), "Natasha" (F, 1986), and "Cheri" (D, 1987). An outstanding exponent of this variety group is "Alexis" (D, 1986), a widely grown cultivar with additional genes for mildew resistance, for example, the *mlo* gene of the Diamant mutant "Helena" (Fig. 8.1). On the other hand, the improved quality of Alexis as compared to Trumpf was probably derived from parents like "Proctor" or Isaria (Fischbeck 1992, cit. Friedt et al. 2000). More recent outstanding spring barley cultivars were "Scarlett" (1995) and "Barke" (1996), both classified at maximum malt quality (score 9) and thereby representing an improvement in comparison to Alexis (score 8). "Marnie," a later spring malt barley cultivar, combines very high grain yield (ca. 10% superior to Barke) with improved malting quality (superior lytic parameters) and resistances against powdery mildew, leaf rust, scald, and net blotch. Marnie's outstanding disease resistance derives from Israeli *H. spontaneum* used as a cross parent (J. Breun, pers. comm.).

Malting quality is an economically very important but also genetically complex trait, comprising a number of different quality parameters. For a reliable description of this complex and its stability, multilocation trials are necessary, which need to be confirmed over several years of cultivation. As many as 29 out of 47 (62%) spring barley cultivars in Germany are classified by very high malting quality (extract content) represented by a score of 8–9 (Beschreibende Sortenliste 2009). Malt quality is defined by the following specific criteria (desired expression): Kolbach index, that is, enzyme activity (high), extract difference (low), extract content (high), and protein content (low). Results of EBC trials with European varieties released during the past few decades clearly show that modern, high-yielding cultivars have a drastically increased Kohlbach index and extract

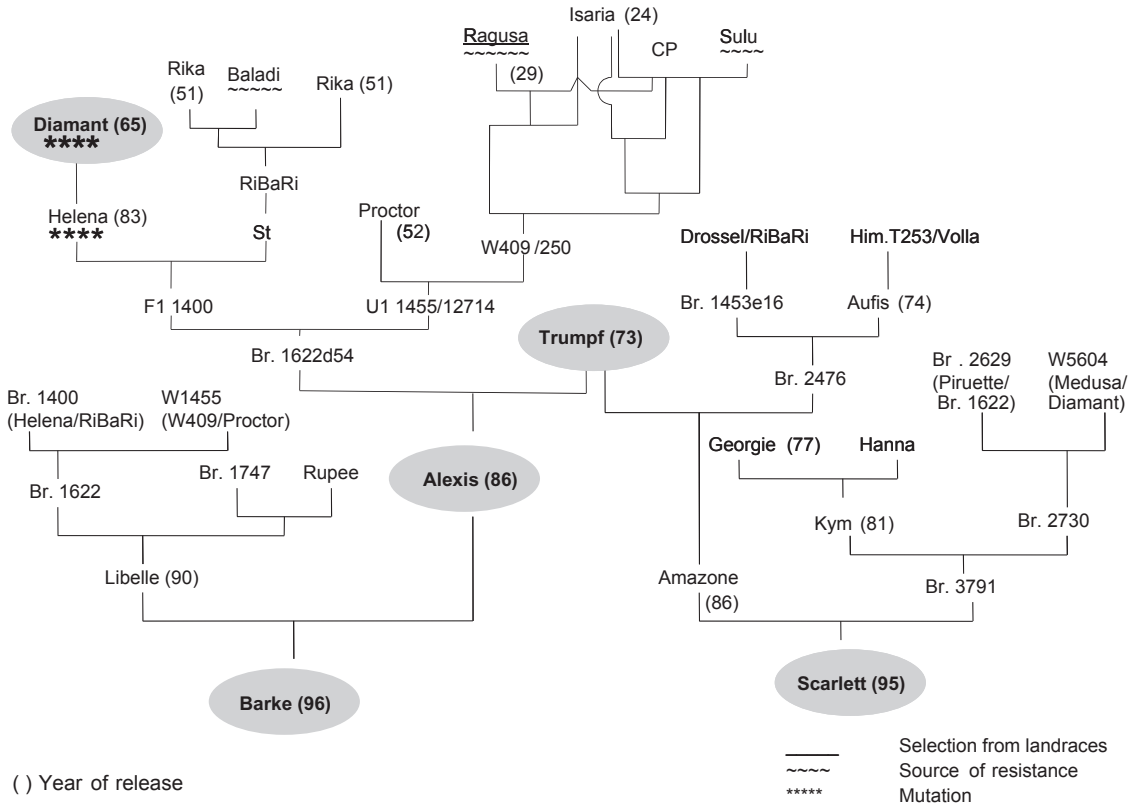

Fig. 8.1. Pedigree of major German spring barley cultivars as important parents of modern high malting quality varieties.

content along with a strongly reduced extract difference and a lower protein content (R.S. Schildbach, pers. comm.). These are the reasons for the observed progress regarding extract content as the major overall quality trait.

However, the barley grain is not only used for feeding and beer production but also for producing malt whisky. Breeding lines have been developed, which combine all the relevant trait expressions desired for malt whisky production, for example, sprit yield, enzymatic potential (diastatic power), and low content of glycosidic nitrile. Cultivar "Optic," derived from the cross Chad × (Corniche × Force), has been the leading spring barley in England and in Scotland. Its successors, "Publican" and "Forensic," are considered to be particularly suitable for British (Scottish) conditions, helping growers with extra yield and also providing quality advantages to end users, for example, distillers (http://fwi.firstlightera.com/EN/Microsites/1/Syngenta+Seeds+Cereals/Springbarley).

Modern variety breeding aims at the development of cultivars with high adaptability and elasticity so that they may be successfully grown in widely different environments. Therefore, cultivars developed under continental climatic conditions as prevalent in large parts of Germany should also be competitive in other European countries and elsewhere. Such successful modern cultivars represent optimal genetic complexes determining the necessary trait combinations providing high crop stability and performance. For example, the German spring barley cultivars depicted in Table 8.4 are characterized by such optimal trait combinations, such as broad resistances against abiotic and biotic stresses, high yield potential, and superior malting quality.

Table 8.4 Major positive traits of spring barley cultivars listed in Germany in 2009

Cultivar (Year of Release)	Disease Resistance	Crop Stability	Grain Yield	Malt Extract
Annabell (1999)	Moderate BR and NB resistance		Average	High
Braemar (2002)	Resistant: PM (high), BR (moderate)	Low culm and ear cracking	Average	High–very high
Grace (2008)	Broad partial resistances	Low lodging, ear and culm cracking	High-very high	High
Marthe (2005)	Broad resistance, particularly to PM and NB	Low lodging, ear and culm cracking	Average-high	Very high
Quench (2006)	Resistant: PM (high), scald (moderate)	Low lodging, ear and culm cracking	High	High–very high
Streif (2007)	Broad resistance, particularly against PM and NB	Low lodging, ear and culm cracking	Average-high	Very high
Tocada (2008)	Moderate NB and BR resistance	Low lodging, ear and culm cracking	Average-high	High–very high

BR, brown rust (*P. hordei*); NB, net blotch (*Pyrenophora teres*); PM, powdery mildew (*B. graminis*).

CURRENT WINTER BARLEY VARIETIES COMBINING HIGH YIELD AND MALTING QUALITY

Modern European winter barley varieties are mainly traced back to two sources of six-row barley landraces, one from The Netherlands and one from the Canadian winter barley sort "Mammut." The variety "Friedrichswerther Berg" (1904), a progeny of hybridization between these two pools, occupies a central position in Central European winter barley breeding. Subsequent breeding cycles based on combinations of six-row winter types and both two- and six-row spring barleys, including, for example, the mildew-resistant Dalmatian landrace "Ragusa," led to a series of important varieties such as "Dea" (1953) and "Dura" (1961). Much later, it became obvious that Ragusa also imparted resistance against BaMMV to the German winter barley gene pool. "Vogelsanger Gold," another major variety derived from a cross of a mildew-resistant spring barley and repeated backcrosses, together with cv. Dura, were major cross parents for later widely grown six-row winter barleys like "Corona" (1980) and more recent cultivars (cf. Fischbeck 1992, cit. Friedt et al. 2000). Varieties like Dea were also parents of crosses with two-row spring barley cultivars like "Ingrid," which finally in 1968 led to the development of cv. "Malta" (see Fig. 8.2), the major parent of subsequent widely distributed two-row winter-type malting barley cultivars such as "Sonja" (1974), "Igri" (1976), "Marinka" (1985), "Trixi" (1987), and progenies thereof.

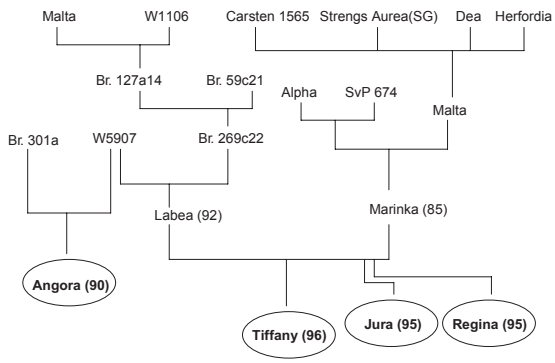

Fig. 8.2. Pedigree section of important two-rowed German winter barley cultivars as major parents of modern, quality-improved two-row winter barley varieties (J. Breun, pers. comm.).

A more recent cross between the two-row winter barleys "Labea" (1992) and Marinka led to cultivars like "Jura" (1995) and "Tiffany" (1996) (Fig. 8.2; cf. Friedt et al. 2000). Particularly the latter two closely related cultivars rapidly gained importance in Germany in the late 1990s, since they combined a fairly good malt quality (extract content 7, equivalent to spring barley Apex) with a sufficient yield performance (cf. Friedt and Rasmussen 2004). Winter malting

barley cultivation in Western Europe is still expanding at the expense of spring barley. Among the currently leading commercial winter two-row varieties are "Campanile," "Finesse," "Finita," "Malwinta," "Passion," and "Reni." Examples of currently leading six-row winter types are "Fridericus," "Highlight," "Lomerit," and "Naomie." It is worth mentioning that hybrid breeding is gaining importance in European winter barley breeding. For example, the high-yielding six-row hybrid cv. "Zzoom" was added to the German variety list in 2008 (Beschreibende Sortenliste 2009).

BROAD-BASED VIRUS-RESISTANT WINTER BARLEY CULTIVARS

Considerable breeding progress has been made with regard to virus resistance of European barley varieties. Besides barley yellow dwarf virus (BYDV), particularly yellow mosaic-inducing soil-borne viruses transmitted by the plasmodiophorid *Polymyxa graminis* have recently expanded due to the extension of cereal cultivation and infested acreage. They cause damage to barley crops in many European growing regions. Among the insect-transmitted viruses vectored by aphids, BYDV has an outstanding relevance for barley cultivation and is expected to gain importance with advancing global warming. There is no doubt that breeding of virus-resistant cultivars is the best, cost-effective, and environmentally compatible approach to prevent virus infection. Numerous sources of resistance or tolerance to the major barley viruses were identified in the past few decades and have been used in classical breeding as well as by molecular breeding approaches (cf. Ordon et al. 2009).

Since these viruses were identified in the late 1970s, the breeding goal of high-yielding virus-resistant cultivars has been achieved quite rapidly (cf. Werner et al. 2000), starting from the identification of resistant sources within exotic cultivars or unadapted germplasm and followed by usually biparental crosses to combine resistance with other agronomic and quality traits. At the beginning of the 1990s, some important cultivars like "Jana" were already resistant to BaMMV and BaYMV. Furthermore, extensive screening and genetic analysis enabled the release of cv. "Tokyo" ([(Fallon × 13060) × 87 – 5381 B] × Swift]; R. Hemker, pers. comm.), only a few years after the first detection of the resistance-breaking strain BaYMV-2 in Europe. The resistance of "Tokyo" was derived from line number 13060, which has "Mokusekko 3," the donor of *rym5* in its pedigree. Due to its agronomic shortcomings, susceptibility to scald, and the relatively limited distribution of BaYMV-2, this cultivar has not achieved great acceptance by growers. However, resistant lines derived from Tokyo with better agronomic performance are being released.

Winter barley is particularly attractive to growers due to its high yield potential as compared to spring types. On the other hand, due to the more recent history of this crop, the variation for disease resistance or malting quality is rather limited in comparison to spring barley. Nevertheless, great improvements have been achieved as may be recognized from Table 8.5. Recently listed German winter barley cultivars such as "Jade" (two-row) and "Christelle" (six-row) exhibit superior trait compositions, combining quantitative resistances against major diseases with outstanding yield and promising malting quality. From that, it can be expected that future varieties will combine their maximal yield potential with sufficient stability (e.g., lodging and stress resistances) as well as sufficient malting quality. Ultimately, such a trend can be expected to entirely replace spring barley with winter barley in many European barley-growing areas.

BIOTECHNOLOGY-BASED AND MARKER-ASSISTED APPROACHES: EVOLUTION OF BREEDING METHODS

Acceleration of barley breeding via haploidy

Combinations of different resistance genes or the introgression of novel resistances from non-adapted germplasm into adapted cultivars' background are classically achieved by sexual

Table 8.5 Major positive traits of new winter barley cultivars, released in Germany in 2009

Cultivar	Disease Resistance	Crop Stability	Grain Yield	Grain Quality
Christelle (six-row)	Mildew, net blotch, brown rust, BaYMV	Low culm cracking	Very high	Moderate test weight
Kathleen (six-row)	Mildew, brown rust, BaYMV	Low spike cracking	Very high	Low–moderate test weight
Semper (six-row)	Mildew, BaYMV		Very high	Moderate–high test weight
Souleyka (six-row)	Mildew, net blotch, scald, brown rust, BaYMV		Very high	Low–moderate test weight
Anisette (two-row)	Mildew	Low culm and spike cracking	High-very high	Moderate test weight
Canberra (two-row)	Mildew, BaYMV	Lodging resistant	High	High test weight
Jade (two-row)	Mildew, net blotch, scald, BaYMV	Lodging resistant, low culm cracking	High	Moderate–high test weight
Lucie (two-row)	Mildew, scald, brown rust, BaYMV	Low spike cracking	Moderate-high	Moderate–high test weight
Zephyr (two-row)	Scald, BaYMV	Low culm cracking	High	Moderate test weight

BaYMV, resistant against barley yellow mosaic viruses.

recombination, that is, crosses between selected parental lines followed by phenotypic selection in the segregating offspring. In this case, the success of breeding entirely depends on extensive field and/or greenhouse tests for resistance to the respective pathogen(s). However, since barley is damaged by many pathogens, which often show a rapid adaptation to their hosts' resistance, breeding for resistance is a very complex task, and the identification of desired recombinants by phenotypic selection, for example, in pedigree selection schemes, has almost reached the limits of manageability. Thus, methods of plant biotechnology like anther and microspore culture allowing the rapid production of homozygous doubled-haploid (DH) lines and cultivars were highly welcome and have been implemented into barley breeding schemes. For example, via anther culture, the spring barley cv. "Henni" (D, 1995), the two-row winter barley "Anthere" (D, 1995), and the six-row cultivars "Uschi" (D, 1997), "Sarah" (D, 1997), "Carola" (F, 1997) and "Nelly" (D, 1998) had been released earlier than expected and became widely grown in Europe (E. Laubach, pers. comm.). It is obvious that a substantial time gain can be achieved by the application of this biotechnology step in barley breeding (Fig. 8.3). Consequently, the "haploid breeding method" is widely applied now and has gained great importance for barley breeding as demonstrated by the growing number of DH varieties released (E. Laubach, pers. comm.).

Year	Breeding	Activities
1	Crossing (May–June)	*In vitro* culture: doubled-haploid (DH) plant production
2	Field propagation and observation of DH lines (100–200 per cross)	Disease assessment at different locations (3), seed increase (4–5 kg)
	Seed multiplication	Propagation in winter nursery (Southern Hemisphere)
3	First yield assessment	Field trials at different locations (in Germany, e.g., five)
4	Second yield assessment	Multilocation field trials (e.g., eight locations)
	Maintenance and seed multiplication	Application for registration: official trials for performance and DUS

Fig. 8.3. Generalized breeding scheme for spring and winter barley assisted by the application of the "haploid method" (E. Laubach, pers. comm.). DUS, distinct, uniform, and stable.

Molecular markers and marker-assisted selection

In addition, the development of molecular markers, which to some extent allow the transfer of selection steps from the phenotypic (field) to the genotypic (laboratory) level, offers new

opportunities for a more efficient barley breeding aiming at desired combinations of resistance, yield, and quality (Friedt et al. 2002).

For example, to enhance the resistance against BYDV, Habekuss et al. (2009) used DH lines and molecular markers to combine resistance genes *Ryd2* and *Ryd3* and a quantitative trait locus (QTL) from cv. "Post" on chromosome 2H. DH lines combining *Ryd2* and *Ryd2* are reported to show lower virus titer and reduced symptom expression as compared with parental materials.

Another example is yellow mosaic-inducing viruses: barley cv. Taihoku A has been described as resistant to yellow mosaic viruses reported in Germany (i.e., BaMMV, BaYMV, BaYMV-2, and BaMMV-Teik). This cultivar carries the BaMMV resistance gene *rym13* on chromosome 4H, which may be responsible for the resistance against the whole virus complex. By bulked segregant analysis using simple sequence repeats (SSRs) and amplified fragment length polymorphisms (AFLPs), Humbroich et al. (2010) constructed a map with the closest marker linked to the BaMMV/BaMMV-Teik resistance locus at a distance of 1 cM. These markers are useful tools to introduce resistance to BaMMV-Teik into adapted breeding lines carrying other resistance genes, which are not effective against all BaMMV/BaYMV strains known in Europe (Werner et al. 2005, 2007).

For a long time, powdery mildew has been known as an important pathogen of barley in almost every growing region so that resistance against mildew is of utmost importance. To identify molecular markers, Korell et al. (2008) used a cDNA-AFLP approach to study near-isogenic barley lines differentiated by alleles of the resistance gene *Mlg* located on chromosome 4H. Based on the identification of a short differential fragment (37 bp), which turned out to be part of a nucleoside diphosphate kinase, a cleaved amplified polymorphic sequences (CAPS) marker cosegregating with *Mlg* was developed. Due to its codominance, clear banding pattern, and close linkage, this marker is well suited for marker-assisted selection procedures.

While DNA marker analyses gain increasing importance in plant breeding and become more widely adopted in cultivar development, the capacity for high-throughput analyses at low cost is crucial for its practical application. Automation of the analysis processes is a way to meet these requirements. For this purpose, the company Svalöf Weibull AB, Sweden, has developed a fully automated polymerase chain reaction (PCR) system. It was evaluated on barley lines and was shown to be capable of analyzing up to 2200 samples per day at costs of EUR 0.24 per analysis for marker-assisted selection and quality control of genetically modified (GM) plants (Dayteg et al. 2007).

Genome analysis and genetically modified barley

Today, sequencing of the barley genome has become a realistic option. Necessary steps to establish and improve genomics tools have been initiated and assembled, coordinated by an international consortium (cf. Schulte et al. 2009). A suitable reference genome sequence will be an excellent foundation for "selection with markers and advanced recombination technology" (SMART) or marker-assisted breeding, leading to future genomics-based barley improvement.

In the foreseeable future, access to highly sophisticated breeding tools with a broad genetic diversity (cf. Fig. 8.4) as an absolute basis for gain of selection will probably become the major limiting factor for further breeding progress. Therefore, the conservation, maintenance, and evaluation of plant genetic resources are urgently needed social tasks to build the foundation for future reasonable plant breeding, crop improvement, and entirely successful agriculture. This will be even more the case if expected climate changes will lead to additional requirements of new varieties such as drought or heat resistances. Within the international network of gene banks, for example, the Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany, plays a major role not only for barley conservation but also for barley genomics (see Stein et al. 2007; Schulte et al. 2009; cf. Sato et al. 2009).

Genetic transformation is not only necessary for developing new cultivars with specifically modified traits such as improved disease and stress resistance or grain quality. Stable genetic

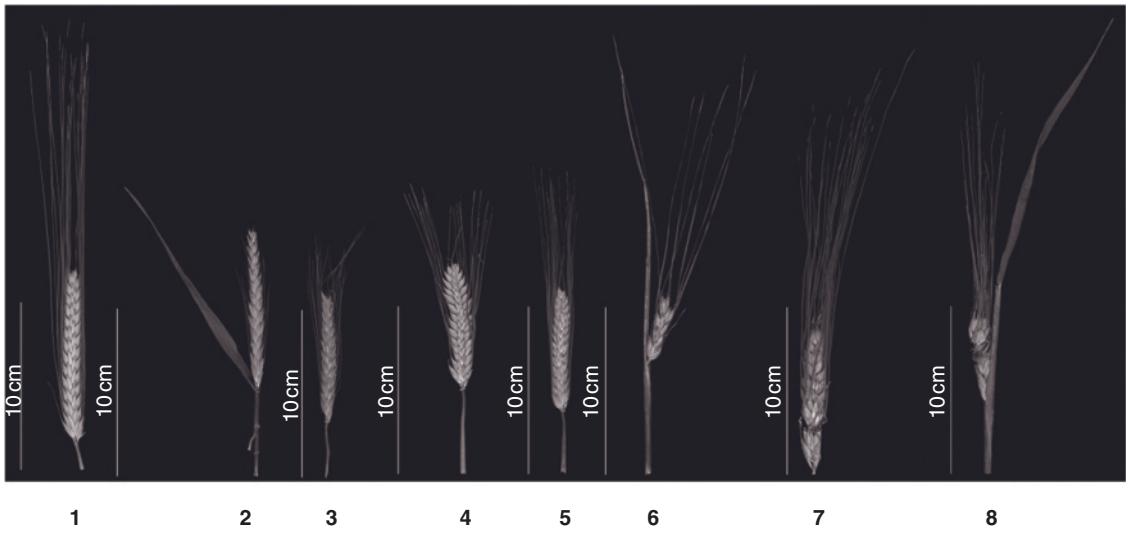

Fig. 8.4. Barley spike phenotypes: 1, two-rowed cv. "Barke" (wild type); 2–8, mutant phenotypes; 2, awnless; 3, intermedium erectoides; 5, brachytic; 6–8, "irregular" spikes. *Source:* Dr. S. Gottwald.

transformation represents also an optimal approach to the detailed elucidation of plant gene function(s). This is considered particularly relevant in barley, a widely used experimental model plant. So, Hensel et al. (2008) presented details of the establishment of a protocol for *Agrobacterium*-mediated gene transfer to immature embryos, enabling the highly efficient generation of transgenic barley. Advancements were made by comparing the influence of various treatments and cocultivation conditions, leading to transgenic lines expressing the respective transgene product at low T-DNA copy numbers. In particular, the newly established protocol turned out to be useful for transformation of various spring and winter barley genotypes besides the highly amenable cv. "Golden Promise." A very useful tool is now available for functional gene analyses as well as genetic engineering approaches in barley cultivar development.

Nevertheless, it cannot be expected that transgenic barley will play a major role in European agriculture in the near future. Strong reservations against GM crops and GM food among some consumers and farmers have caused political decisions by European authorities to the disadvantage of the application of GM technology up to the present.

CONCLUSIONS AND PERSPECTIVES

Barley breeding in Europe has been extremely successful throughout the last century. Cycles of cross-breeding, which first made use of hybridization between European landraces, and later exploiting more distant germplasms providing valuable disease resistances, and finally combining high-yield and high-quality varieties, have led to highly productive modern cultivars, both in spring and winter barley. In most cases, the practical breeding techniques comprised little more than manual hybridization, careful observation, precise testing, and conscious selection. More recently, breeding programs have been enhanced by the implementation of modern biotechnology tools, like the "haploid method." Now, it is becoming obvious that marker-assisted selection procedures—particularly PCR-based techniques combined with fast and high-throughput analysis, also called SMART breeding—will further enhance the process of selecting resistant varieties with superior agronomic performance in the future. As a consequence, existing breeding schemes including "fast track" procedures will even be accelerated and carried out more efficiently than up to now.

In addition to methodological progress, "new" traits including special qualities ("functional barley") for alternative, for example, chemical purposes, such as high amylose and waxy barley, would potentially allow the diversification and extension of barley cultivation and use. Furthermore, hulless barley may help to improve feed quality regarding digestability and nutrient contents, probably combined with phytase activity or low phytate content for better phosphate use. In this regard, a better understanding of gene and genome functions will enable more straightforward breeding approaches like the direct transfer of donor genes into suitable receptor cultivars.

In this context, it is worth mentioning that a new ethyl methane-sulfonate (EMS)-induced mutant population comprising 10,279 M_2 individuals of two-rowed spring barley cv. Barke was described recently (Gottwald et al. 2009). This new targeting induced local lesions in genomes (TILLING) resource has potential for use in fundamental research as well as in applied breeding.

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NORTH AMERICA

Richard D. Horsley and Bryan L. Harvey

Large breeding programs for barley in North America exist in Canada, Mexico, and the United States. This portion of the chapter will focus on the programs in Canada and the United States because of the working knowledge and associations the authors have with these programs and the individuals working on them. The two largest programs in Mexico are the national program, which is the Instituto Nacional de Investigaciones Forestales, Agricolas, y Pecurias (INIFAP), and the program overseen by the International Center for Agricultural Research in the Dry Areas (ICARDA). For many years, the ICARDA program was located at the headquarters of the International Maize and Wheat Improvement Center (CIMMYT) in El Batan, Mexico. The national program has been successful in developing malting barley cultivars for Mexico, such as Esmerelda, and the ICARDA program has been successful in developing germplasm with multiple disease resistance for use around the world. In 2007, the ICARDA barley breeder responsible for Mexico and South America was moved to the ICARDA headquarters in Aleppo, Syria; however, the responsibilities of this breeder for the region did not change.

BARLEY PRODUCTION IN CANADA

Barley in Canada was introduced to New France by the first governor, Samuel de Champlain, in 1606. Canada's first brewery was built in Quebec city in 1668 (Metcalfe 1995). Later, the British brought two-row brewing barleys to Upper Canada. Trade between Canada and the United States thrived and brought good returns to Ontario growers for their two-row malting barley. This trade was brought to an end by U.S. protectionist measures in the form of the McKinley Tariff Act in 1890. Therefore, Ontario growers were forced to turn to feed markets for their barley. Although the two-row barley cultivars were highly suited to malting, they were low yielding, and thus improvements were required to facilitate viable barley production in that area.

The Hudson Bay Company introduced cereal grains, including barley, to their trading posts throughout Northwest Canada in the 1600s in an attempt to attain food self-sufficiency at these posts. However, significant commercial production did not begin in Western Canada until the arrival of Selkirk settlers in southern Manitoba in 1812. Barley has been a major cereal grain in Western Canada since that time.

Over 90% of Canada's barley acreage is in Western Canada (Table 8.6). However, it is still an important crop in Eastern Canada where growing conditions are highly variable with a multiplicity of agro-ecoregimes.

Canada today can be divided into specific barley-growing regions (Fig. 8.5). Below are

Table 8.6 Canadian barley production in thousands of tons, 2004–2008 (calculated from data provided by Statistics Canada; http://www.statcan.gc.ca; verified July 22, 2009)

	Year						
Area	2004	2005	2006	2007	2008	Mean	%
Canada	12,567	11,678	9573	10,984	11,781	11,314	100
Maritimes	171	170	113	146	123	146	1
Quebec	368	340	302	308	258	315	3
Ontario	339	292	291	218	192	266	2
Manitoba	1278	603	1035	1195	1121	1122	10
Saskatchewan	4681	4969	3397	3945	4594	4317	38
Alberta	5628	5232	4405	5114	5448	5165	46
British Columbia	90	72	31	58	47	60	<1

Fig. 8.5. Barley production regions in Canada: 1, Maritimes; 2, Quebec; 3, Ontario; 4, Southeastern Prairies; 5, South Central Prairies; and 6, Northern Montane.

specific characteristics and breeding priorities for each region.

Maritime region

This region is characterized by abundant moisture, high humidity, and moderate temperatures. Acid soils are typical for this area. Foliar diseases are common and lodging is often a problem. There are no longer any barley breeding programs based in this area; thus, growers in the region rely on germplasm developed elsewhere. The Agriculture and Agri-Food Canada (AAFC) program in Ottawa has responsibility for this area.

Québec region

The eastern portion of this region is similar to the Maritimes, while the western portion has commonalities with Ontario. In both areas, resistance to foliar diseases and strong straw are important selection criteria. Breeding for this region is carried out by the private sector since all of the public breeding programs have been terminated. The Ottawa program also has responsibility for this area.

Ontario region

This region also is favored by adequate moisture; however, temperatures are considerably higher and midsummer drought is often encountered. Thus, in addition to disease resistance and straw strength, early maturity is desirable to avoid the hot dry periods of July and August. A small area of winter barley is produced in the southwestern portion of this province. This region is serviced by breeding programs at AAFC's Ottawa station, the private sector, and a small program at the University of Guelph.

Western Canada

Western Canada is a vast area comparable in size to continental Europe. Clearly, such a large area has many ecozones contained within its borders. It can, however, be divided into three broad areas: the warm, moist Southeastern Prairies region; the cool Northern Montane region; and the drier South Central Prairies region.

Southeastern Prairies

The Southeastern Prairies region is characterized by adequate moisture levels, in most years, high temperatures and humidity in midsummer. Diseases such as stem rust (incited by *Puccinia graminis* f. sp. *tritici* [Eriks. and E. Henn.] D.M. Henderson), fusarium head blight (FHB, incited by *Fusarium graminearum* Schwabe), and spot blotch (incited by *Cochliobolus sativus* [Ito and Kuribayashi] Drechs. ex Dastur) are favored by such conditions. The AAFC's Brandon programs play a key role in servicing this area. The University of Saskatchewan's Crop Development Centre also provides cultivars for this area. Cultivars developed for Minnesota and North Dakota also do well here.

South Central Prairies

The South Central Prairies region is characterized as having reduced rainfall, high evapotranspiration rates, and frequent droughts. Foliar diseases are less common and net blotch (incited by *Drechslera teres* [Sacc.] Shoemaker f. *teres* and *D. teres* f. *maculata* Smedeg.) is the most frequently found. This area is less suited to six-row cultivars and short-statured cultivars for those producers who windrow their crop prior to combining. This is prime two-row malting barley production territory. This area is the prime focus of the Crop Development Centre in Saskatoon but is also serviced by the Brandon and Lacombe programs.

Northern Montane

The Northern Montane region typically also has adequate moisture but has cooler temperatures and shorter growing seasons. Scald (incited by *Rhynchosporium secalis* [Oudem.] J.J. Davis) is a serious leaf disease in this region. Lower temperatures generally do not favor spot blotch and FHB. The Alberta Field Crop Development Centre, in Lacombe, is strategically located to service this area. The area is also serviced by the programs in Brandon and Saskatoon.

Of the 11–12 million tons of barley produced in Canada each year, over 90% is grown in Western Canada. Approximately 80% is used for feed, most of that domestically. Canada's exports of barley are typically in the range of 2–3 million tons. The largest portion of this is malting barley. Major customers for malting barley are the United States, China, Colombia, and South Africa. United States and Japan are major importers of Canadian malt, amounting to several hundred thousand tons annually. Markets for feed barley are more variable with Saudi Arabia, Japan, and several Middle Eastern countries as the most consistent buyers. Food barley consumes a very small but growing amount of barley and it constitutes an important niche for some growers. Detailed statistics can be obtained from the Canadian Grain Commission reports (http://www.grainscanada.gc.ca/; verified July 22, 2009).

BARLEY PRODUCTION IN THE UNITED STATES

Barley was introduced into the United States along the Eastern Coast shortly after the first settlers arrived in the seventeenth century and

its production moved west with the growth of the population (Poehlman 1985). One factor hastening the movement of barley to the west was the disease FHB. This pathogen attacks not only barley but also wheat (*Triticum* spp.) and maize (*Zea mays* L.). As maize production would move into an area, the level of FHB in the barley would increase; thus, its production would move west to areas where maize was not grown. This movement of barley west to avoid FHB continues today. The Red River Valley region of North Dakota and Minnesota was the center of six-row barley production in the United States from the 1940s to the 1990s. In the 1990s, above-average precipitation and an increase in maize production in the Red River Valley provided the conditions conducive to FHB in barley. Thus, many growers in the region quit growing barley because of the high susceptibility of the crop to FHB and other market factors. The center of six-row barley production in the United States is now north central North Dakota, and new cultivars are being developed that are adapted to the more arid regions of western North Dakota and eastern Montana.

The area of barley production in the United States can be subdivided into four regions: the East, Upper Midwest, West, and Southwest. The Midwest and West regions account for over 80% of the area sown to barley (United States Department of Agriculture National Agricultural Statistics Service [USDA-NASS]; available at http://www.nass.usda.gov/index.asp; verified May 23, 2009). Specifics mentioned below on barley production and cultivars grown in the United States and in many states can be obtained from the Web site of the USDA-NASS (available at http://www.nass.usda.gov/index.asp; verified May 23, 2009).

North Dakota is the largest barley-producing state in the United States followed by Idaho and Montana (USDA-NASS). Over 90% of the barley sown in the Upper Midwest region of Minnesota, North Dakota, and South Dakota are six-row malting barley cultivars. Production in this region is typically done under dryland conditions, and the reason for the preponderance of six-row barley goes back to the origin of the barley improvement efforts in the early 1900s. The barleys found to be best adapted to the region and to have acceptable malt quality at the time were six-row barley introductions that could be traced back to the Manchuria region of China (Rasmusson 1985).

In the West region, two-row barley cultivars are typically grown, and barley for malting is often grown under irrigation. Of the early barley introduced into much of this region, two-row barley accessions from central Europe were best adapted and formed the germplasm pool from which many malting barley cultivars were developed. The area sown to malting versus nonmalting types varies by state. Idaho and Montana, the two largest barley-producing states in the region, and Wyoming sow between 65% and 80% of their barley area to malting cultivars. Other states in the region may sow less than 10% of their barley area to malting cultivars.

In the East region, nonmalting winter barley cultivars are typically grown. Maryland, Pennsylvania, and Virginia sow the most barley in the region; yet, their combined production area accounts on average for only 3.6% (5-year average, 2004–2008) of the U.S. total. In the Southwest region, nonmalting spring barley is typically grown and most of the production is in the irrigated desert regions of Arizona and Southern California. The estimated total area sown to barley in this region is around 1% of the U.S. total.

Unlike Canada, most of the barley produced in the United States is used domestically. From 2004 to 2007, less than 15% of the annual U.S. production of 4.8 million tons is exported, and most of the barley exported is feed barley (http://usda.mannlib.cornell.edu/usda/fas/grain-market//2000s/2008/grain-market-12-11-2008.pdf and http://www.nass.usda.gov/QuickStats/; verified July 22, 2009).

BREEDING PROGRAMS IN CANADA

Early barley improvement was carried out through introductions, initially from Europe and later from Manchuria. Selection carried out on

these Manchurian six-row landraces, first at Ontario Agricultural College in Guelph and later at Macdonald College in Québec, gave rise to important cultivars in Canadian barley history. Most notable of these was OAC 21, which was widely grown throughout both Eastern and Western Canada. It was the standard for malting quality for six-row barley for many decades. Successful hybridization did not start until the 1920s; among the first products of this program from Macdonald College was Montcalm. This cultivar was the first Canadian malting barley developed by hybridization. It was widely adapted and high yielding and had excellent malting quality for its time. Not surprisingly, it soon dominated the malting barley acreage and held that position until the mid-1960s.

In Western Canada, agricultural colleges were established in all three Prairie Provinces by 1918. As in the East, initial focus was on the introduction of cultivars from other sources. OAC 21, for example, was widely distributed by these colleges. Hybridization programs were quickly established, however, aimed at producing cultivars suitable for prairie conditions. The AAFC research stations also became active at this time. Notable among these was the station at Brandon, Manitoba, a program that has remained extremely important to this day.

Barley breeding in Canada is primarily carried out by public sector agencies due to the lack of profitability of private sector breeding in self-pollinated crops like barley. Typically, less than 10% of the barley acreage is sown with pedigreed seed; thus, royalty collection is insufficient to generate profitability. These public programs have a long history of collaboration with each other and with programs in the United States. A number of annual and periodic meetings within Canada provide networking opportunities for Canadian barley scientists. The triennial North American Barley Researchers Workshop is a venue in which barley scientists from both countries exchange views and information, setting the stage for ongoing shared targets and collaboration. Specifics on the public breeding programs in Canada are presented below.

AAFC, Ottawa

This program originally served eastern Ontario and western Quebec but has expanded its original mandate area to include the Maritime Provinces, Quebec, and Ontario where programs have been eliminated or reduced in scope. This is an enormous geographic area even though it only constitutes about 6% of the Canadian barley production. Production here is used exclusively for feed except for very small amounts of malting barley in Quebec. Thus, the program has the daunting task of servicing a diversity of production conditions. In this feed market, no premiums are paid for quality, and thus the emphasis is on yield and yield stability through disease resistance, straw strength, and nutrient use efficiency. Important diseases here include powdery mildew (incited by *Erysiphe graminis* DC. f. sp. *hordei* Em. Marchal), scald, FHB, stem rust, net blotch, *Septoria* speckled leaf blotch (incited by *Septoria passerinii* Sacc. and *Stagonospora avenae* f. sp. *triticea* T. Johnson), and barley yellow dwarf virus. This program and its predecessors have provided excellent varieties, primarily of six-row cultivars, to service the needs of producers in this region.

University of Guelph

As indicated earlier, this program was one of the original barley improvement programs in Canada and provided cultivars like OAC 21, which had wide importance for many years. This program, in addition to cultivar improvement, developed the *Hordeum bulbosum* method of doubled-haploid barley production that had widespread use in barley genetics research (Kasha and Kao 1970). The program now is the responsibility of one breeder for a portion of his time. The focus has narrowed to primarily six-row, spring, feed types. Objectives are high yield, disease resistance, and early maturity. Despite the reduction in resources, this program continues to play an important role for southwestern Ontario producers.

AAFC Brandon

The AAFC program in Brandon, Manitoba, is another program that has been historically

important to barley production in Western Canada. This program has an excellent record of producing widely adapted six-row cultivars for both feed and malting uses. Conquest and Bonanza are the best known of these cultivars and dominated the six-row malting area for many years. This station also provided valuable service by incorporating sources of resistance for a number of diseases into adapted materials. More recently, this role has expanded to two-row barley lines, and the broadly grown AC Metcalfe is an example of the important cultivars emanating from Brandon.

University of Saskatchewan

Since its inception during the early part of the twentieth century, this has been the only Saskatchewan-based breeding program. Its primary focus has been the dryer portions of the prairies. The program was given a significant boost with the establishment of the Crop Development Centre in 1971. This recognized the importance of barley in the province where the largest acreage and the second largest production in Canada exists. The program has been preeminent in the production of high-quality two-row malting barley as typified by Harrington and a number of its successors. Harrington set the standard for quality in high enzyme activity, rapid modification barley cultivars. It was also highly suited to the harsh environment of the dry prairies. The feed and food portion of the program pioneered a number of barley utilization areas in Canada including hulless feed barley, both two- and six-row; hulless waxy food barley; two-row forage barley; semidwarf six-row feed barley; pure amylopectin hulless, waxy food barley; hulless barley with resistance to both surface-borne and loose smut (incited by *Ustilago tritici* [Pers.] Rostr.) for organic production; hulless malting barley; and low phytate barley. The program is one of the few programs to date to utilize molecular markers routinely for breeding, especially disease resistance, and for purification of breeder seed.

Alberta Agriculture Field Crop Development Centre, Lacombe, AB

This program is the result of the evolution of AAFC's programs at Lethbridge, Beaverlodge, and Lacombe and at the University of Alberta, all of which have been terminated (except Lacombe). Soon after its inception, a federal provincial agreement was signed and resources from both governments were contributed. The vast majority of its efforts have been devoted to developing high yield potential cultivars for Alberta's hog and cattle industries. A number of cultivars have been released with good disease resistance and adaptation to high manure rates and adequate rainfall.

Supporting facilities

In addition to the above breeding programs, there are several units that are important contributors to the breeding programs. Pathology support is provided by AAFC's scientists with the lead role taken by the station in Winnipeg and support from Lacombe, Charlottetown, and Ottawa. These scientists have developed testing protocols for all of the important barley diseases in Canada and supervise and collaborate in screening for resistance. This is of course a vital resource for breeders. Plant Genetic Resources Canada, with headquarters in Saskatoon, contains Canada's gene bank. Canada has global responsibility for barley and oat, and thus has a large collection of cultivated and wild species of barley. There is excellent collaboration between breeders and gene bank scientists in the collection characterization and utilization of barley germplasm.

Malting barley is an important cash crop for Canada, and thus malting cultivars are sown on far more area than end usage would justify. Given Canada's significant role in the export market for malting barley and malt, the development of high-quality potential cultivars is essential. Malting barley breeders have their own screening laboratories but rely on others for screening advanced materials. This role is carried by several organizations. The Grain Research Laboratory of the Canadian Grain Commission develops testing

protocols, screens advanced lines from all programs, and collates the data for cultivar registration testing evaluation. Members of the industry also contribute to this testing through their own laboratories. The Brewing and Malting Barley Research Institute (BMBRI) is an industry-based organization that coordinates collaborative testing of candidate malting lines for registration in Canada. They also assist in arranging plant-scale testing post registration. The Canadian Malting Barley Technical Centre is an independent membership-based facility that provides pilot-scale malting and brewing tests on a fee for service basis. The Canadian Wheat Board is the marketing agency for Canadian malting barley and facilitates plant-scale testing of newly registered cultivars before final acceptance in the domestic and export markets.

All of these agencies located at Winnipeg collaborate to provide quality targets for breeders that can be accessed at the BMBRI Web site (http://www.bmbri.ca/; verified May 23, 2009). These are similar to those published by the American Malting Barley Association (AMBA) in the United States and are listed elsewhere in this chapter.

There are unfortunately no similar networks for feed and food barley; thus, breeders are forced to set up any necessary testing on a case-by-case basis.

BREEDING PROGRAMS IN THE UNITED STATES

Barley breeding in the United States is largely done by public institutions located in the northern tier of states. Institutions with breeders working solely on barley include Montana State University, North Dakota State University (NDSU), Oregon State University, the University of California, Davis, the University of Minnesota, Washington State University, and the United States Department of Agriculture Agricultural Research Service (USDA-ARS) at Aberdeen, Idaho. Public programs with breeders working on barley as well as other small grains are located at the University of Georgia, University of Maryland, University of Nebraska, Utah State University, and Virginia Polytechnic Institute and State University (VPI). All of these programs have development of wheat (*Triticum aestivum* L.) cultivars as their top priority and have much smaller efforts in barley. The programs in California, Minnesota, Montana, North Dakota, and Washington work primarily on developing spring barley cultivars; the programs in Georgia, Maryland, Nebraska, Oregon, and Virginia work almost exclusively in developing winter barley cultivars; and the USDA-ARS program in Idaho works on developing both spring and winter barley cultivars. In addition, the Minnesota, North Dakota, and Idaho USDA-ARS programs work primarily on developing malting barley cultivars. Unlike many areas of the world where only malt made from two-row barley is used for brewing, the brewing industry in the United States uses substantial amounts of malt made from six-row barley. The breeding program at the University of Minnesota focuses on the development of six-row barley cultivars, whereas the NDSU and USDA-ARS programs work on the development of two- and six-row cultivars. The public programs in California, Georgia, Maryland, Nebraska, Utah, and Virginia develop primarily nonmalting barley cultivars, and the Montana, Oregon, and Washington programs work on barley for malting and nonmalting uses. The VPI, Idaho USDA-ARS, Oregon, and Washington breeding programs also work on hulless barley for food, feed, and industrial uses. The program at VPI is particularly working on hulless barley for ethanol production in collaboration with the USDA-ARS Eastern Regional Research Center at Wyndmoor, Pennsylvania.

Breeding in the private sector is dominated by three companies that develop cultivars for production across more than one barley-growing region in the United States. The companies and the headquarters of their breeding programs are Busch Agricultural Resources, LLC (BAR, a member of Anheuser-Busch InBev), located in Fort Collins, Colorado; MillerCoors, located in Burley, Idaho; and Westbred, LLC, headquartered in Bozeman, Montana. The BAR and MillerCoors programs breed malting barley,

whereas Westbred develops feed, hulless food, and forage-type barley cultivars. The MillerCoors program develops cultivars almost exclusively for use within their company, whereas BAR develops cultivars that are used by brewers in addition to Anheuser-Busch InBev.

Breeding priorities in the United States

Malting

The choice of parents to use for crossing is critical for developing acceptable barley cultivars because barley cultivars often are adapted to specific production regions and are designed for particular uses, such as malting and brewing. Designation of a cultivar as a "malting-type" is assigned by a particular company that would use the cultivar or by an organization such as AMBA, located in Milwaukee, Wisconsin. AMBA is a nonprofit trade association composed of dues paying malting and brewing companies. A primary goal of AMBA is to ensure an adequate supply of high-quality two- and six-row malting barley for the malting and brewing industries in the United States (http://www.ambainc.org/about/bh.htm; verified May 23, 2009). This is accomplished, in part, by providing grant funds to public breeding programs to support their cultivar development efforts. AMBA also coordinates pilot-scale and plant-scale evaluation programs of advanced breeding lines or cultivars to determine their suitability for their member companies. These two programs will be discussed in greater detail later in the chapter. Evaluation of malt quality of breeding lines prior to entering into AMBA's testing programs is done by the USDA-ARS Cereal Crops Research Unit (CCRU) in Madison, Wisconsin, and in limited cases in industry laboratories. Evaluation of breeding lines developed by the public breeding programs at a single site by the USDA-ARS allows AMBA's members and other stakeholders to make comparisons between lines that are all malted and evaluated under similar conditions.

Malting barley in the United States is unique from most other crops in the country in that it is marketed and stored on an identity preserved basis. Malting barley cultivars are kept segregated by cultivar, and often times a cultivar is additionally segregated based on production area or by a specific quality trait such as grain protein level. Segregation is done because cultivars used for malting and brewing must meet a long list of specific criteria before they are accepted. In addition, malting is done by cultivar and often by cultivar grown at a specific location, and many beer brands have specific compositions of different barley cultivars in their brewing blend. A unique feature of malting barley in North America is that the life of cultivars can easily be 10 years or more. Examples of this are Larker, Morex, and Robust six-row barleys, and Klages and Harrington two-row barleys. Larker, Morex, Robust, and Klages were developed in the United States, and Harrington was developed at the University of Saskatchewan in Saskatoon, Canada.

Guidelines for specific quality traits, which include measurements on barley and malt, are provided to the public breeding programs by AMBA (Table 8.7). Quality traits that receive the most attention in malting barley breeding programs are barley grain protein and kernel plumpness, and malt extract, enzymatic activity (α-amylase and diastatic power), wort protein, measures of carbohydrate modification (β-glucan content and wort viscosity), and protein modification (Kohlbach index) in the malt. A detailed description of these malt parameters and others can be found in Kunze (2004).

Because of the long list of parameters that must be met, development of malting barley cultivars is typically done using crosses between parents that already have acceptable quality. Shortfalls in malt quality of either parent usually appear in the progeny. This need to make crosses between parents with acceptable quality has led to very narrow germplasm bases because variability already has been severely restricted by adaptation to specific production regions. For example, Horsley et al. (1995) stated that in the early 1990s, all cultivars developed by six-row barley breeding programs in the Upper Midwest United States and the eastern Prairie Provinces of Canada could be traced back to 15 accessions obtained in the early 1890s. However, even within the narrow germplasm bases of malt barley programs, gains still are being made. Rasmusson and Phillips (1997) theorized that gains are made in the narrow

Table 8.7 Barley and malt quality specifications provided to barley breeders in the United States by the American Malting Barley Association, Inc., Milwaukee, Wisconsin, in May 2008 (http://www.ambainc.org/feeds/item/quality-guidelines-for-malting-barley-breeders/92; verified 22 July 2009)

	Two-Row Barley	Six-Row Barley
Barley factors		
Plump kernels[a]	>90%	>80%
Thin kernels[b]	<3%	<3%
Germination[c]	>98%	>98%
Protein	≤13%	≤13.5%
Skinned and broken kernels	<5%	<5%
Malt factors		
Total protein	≤12.8%	≤13.3%
On 7/64″ sieve	>70%	>60%
Measures of malt modification		
β-Glucan (ppm)	<100	<120
Fine–coarse extract difference	<1.2	<1.2
Kohlbach index	40%–47%	42%–47%
Turbity (NTU)	<10	<10
Viscosity (absolute cP)	<1.50	1.50
Congress wort		
Soluble protein	4.4%–5.6%	5.2%–5.7%
Extract (fine grind db)	>81.0%	>79.0%
Color (°ASBC)	1.6–2.5	1.8–2.5
Free amino nitrogen	>190	>200
Malt enzymes		
Diastatic power (°ASBC)	>120	>140
α-amylase (20° DU)	>50	>50

[a]Kernels retained on a sieve with 0.24 x 1.9-cm slotted openings; [b]kernels passed through a sieve with 0.20 x 1.9-cm slotted openings; [c]percent germination after 72 h on a petri dish with 4 mL of water. db, dry basis.

germplasm bases due to de novo variation and elevated epistasis. In addition, for an excellent review on gains and diversification in barley obtained through breeding, the reader is encouraged to read Fischbeck (2003).

Feed and food

In developing cultivars designed for livestock feed, the choice of parents is of less importance because growers are usually not paid premiums for specific quality parameters as is done for malting barley. However, feed quality attributes are being developed for several classes of livestock. Using marker-assisted selection (MAS), cv. Valier was developed to incorporate excellent agronomic performance and improved feed characters for beef cattle (Blake et al. 2002). Incorporating disease resistance genes becomes especially important because application of expensive fungicides to feed barley can be cost prohibitive.

Breeders developing hulless barley for human consumption look to develop cultivars that are high in soluble fiber to reduce the risk of cardiovascular disease (Behall et al. 2004). The trait that receives the most attention is breeding for increased grain β-glucan content. Breeders developing hulless barley for the poultry feed market work to develop cultivars with reduced grain β-glucan content (Newman et al. 1987). Unlike the central USDA-ARS-CCRU laboratory available for testing malt quality, there is no central laboratory available for determining the feed or food quality of breeding lines. Each of the breeding programs is responsible for determining the quality of their lines themselves. Likewise, there is no single organization such as AMBA that is providing desired quality characteristics to breeders for feed and food barley.

Resistance to diseases

A high priority in many breeding programs is the development of cultivars with resistance to one or more pathogens. Because barley is considered a low-input crop, the easiest and most cost-effective method of disease control is growing resistant cultivars. The list of barley diseases caused by bacteria, fungi, and viruses is plentiful (Mathre 1997). Good sources of genes for resistance to diseases and other pests are often unadapted accessions or the wild ancestors of barley, such as *Hordeum vulgare* subsp. *spontaneum*. The USDA-ARS National Small Grains Collection (NSGC) in Aberdeen, Idaho, is the part of the USDA-ARS's National Plant Germplasm System that has a large collection of cultivated and wild ancestors and relatives of barley and other small grain cereals from around the world. In June 2009, the

USDA-ARS-NSGC had 27,925 accessions of *H. vulgare* subsp. *vulgare*, 1507 accessions of *H. vulgare* subsp. *spontaneum*, and over 440 additional *Hordeum* accessions (http://www.ars-grin.gov/cgi-bin/npgs/html/site_holding.pl?NSGC; verified July 22, 2009).

Another important utility that the USDA-ARS provides to barley breeders, pathologists, and other stakeholders is tracking and identification of different rust pathogens in the United States, and screening and identification of new sources of resistance to new rust pathotypes. This role is overseen by the USDA-ARS Cereal Disease Laboratory (CDL) located in St. Paul, Minnesota. Each year, the USDA-ARS-CDL publishes a series of bulletins that report on the current situation of several cereal rust diseases in the United States (http://www.ars.usda.gov/Main/docs.htm?docid=9757; verified July 22, 2009).

Each barley-growing region of the United States has different disease profiles and priorities when breeding for disease resistance. In the Upper Midwest United States, the high-priority prevalent diseases are wheat stem rust, FHB, spot blotch, and the net form of net blotch (incited by *Pyrenophora teres* Drechs.). All cultivars developed for this region must have resistance to the prevalent pathotypes of wheat stem rust due to the devastation this disease can cause. Since 1993, major breeding efforts have been ongoing in the region to develop cultivars with improved resistance to FHB and accumulation of the mycotoxin deoxynivalenol (DON) that is produced by the causal organism. The best sources of resistance to FHB and DON accumulation being used by Midwestern U.S. breeding programs have originated from China, Japan, and Switzerland (Prom et al. 1996; Urrea et al. 2005).

The list of foliar diseases causing significant losses in the western and southwestern United States is much smaller than that in the other regions due to the much drier environment. The disease receiving the most attention since the late 1980s is stripe rust, incited by *Puccinia striiformis* f. sp. *tritici*. In 1975, a pathotype capable of attacking most cultivars in the United States spread from Europe to Colombia, and the first disease caused by this pathotype was first seen in the United States in winter 1991 in Texas (Roelfs et al. 1992). From Texas, the disease spread quickly to California, Idaho, Montana, Oregon, and Washington (Chen 2004). Because breeders and pathologists were aware that this disease would eventually reach the United States, they were proactive and were able to identify and incorporate resistance into their germplasm (Roelfs and Huerta-Espino 1994; Chen 2004). Complexes of diseases that are more problematic in the western United States are a series of different root and crown rots (e.g., rhizoctonia, pythium, and fusarium). The effects of this group of diseases are especially noticed in reduced tillage and direct seed systems as well as during dry years or in dry areas of the field such as hilltops because roots are unable to take up sufficient water (Cook and Veseth 1991).

In the eastern United States, the predominant diseases are barley leaf rust (incited by *Puccinia hordei* Otth.), powdery mildew, and barley yellow dwarf virus. A difficulty in breeding for barley leaf rust is that multiple leaf rust resistance genes have been overcome by changes in pathogen races of *P. hordei*.

Resistance to insects and nematodes

In the United States, the aphids bird cherry-oat aphid (*Rhopalosiphum padi* [L.]), green bug (*Schizaphis graminum* [Rondani]), and Russian wheat aphid (RWA) (*Diuraphis noxia* [Kurdjumov]) are the major insect pests of barley (Porter et al. 1999). Breeding for resistance to RWA receives the most attention, and damage from this pest occurs predominantly in the intermountain west states of Colorado, Idaho, Montana, and Wyoming. A large screening effort for identification of accessions in the USDA-ARS-NSGC with resistance to RWA was conducted in the glasshouse by USDA-ARS scientists at Stillwater, Oklahoma (Webster et al. 1991). Over 100 lines with RWA resistance were identified and germplasm lines were rapidly developed and made available to breeders (Mornhinweg et al. 1995, 1999). In a collaborative effort

between USDA-ARS researchers at Stillwater, Oklahoma, and Aberdeen, Idaho, additional RWA germplasm lines and cultivars have been developed. The cultivar Burton (Bregitzer et al. 2005) is an example of this successful collaboration of ARS scientists with university experiment station researchers in the region.

Three nematode genera (*Heterodera*, *Meloidogyne*, and *Pratylenchus*) are known to cause economic damage to barley (Mathre 1997). However, breeding for resistance to nematodes in the United States is not a priority.

Resistance to abiotic stresses

As stated earlier, barley is considered by growers to be a low-input crop; thus, breeding for resistance to abiotic stresses is a high priority for many barley breeders. A partial list of these stresses includes drought and flooding, high and low temperatures, mineral deficiencies and toxicities, poor soil tilth (structure), and preharvest sprouting (PHS). A difficulty when breeding for abiotic stresses is that plants often are exposed to multiple stresses at the same time, and it is not unusual that common responses to the stresses are provoked in plants (Langridge et al. 2006). Thus, breeding for abiotic stresses generally is considered more difficult than breeding for diseases or other pests. Internationally, functional genomic technologies are being used to gain a better understanding of how plants respond to abiotic stresses (Langridge et al. 2006). However, establishment of effective breeding strategies based on this information will be challenging.

SELECTING SOURCES OF RESISTANCE TO ABIOTIC AND BIOTIC STRESSES IN CANADA AND THE UNITED STATES

After sometimes exhaustive searches, genotypes, including cultivars, unadapted genotypes, landraces, and wild barley accessions that are resistant or tolerant to most economically important biotic and abiotic stresses usually can be found (Ullrich et al. 1995). However, sources of the resistance genes are often poorly adapted to their intended region, and desirable genes may show linkage drag to undesirable traits. When a new disease or pest arises or resistance breaks down in currently grown cultivars, breeders can develop improved cultivars in a short time if they have been taking a proactive approach to the problem. Examples of this approach include work done by USDA–ARS researchers on RWA and barley stripe rust beginning in the late 1980s. The length of time needed to develop improved cultivars also is reduced if the sources of resistance can be found in adapted germplasm. When a loss of resistance is unanticipated, it can easily take more than 10 years to incorporate new resistance into acceptable cultivars. In the case of malting barley, this time frame is grossly underestimated because stringent quality parameters must be maintained. This is the current case for developing improved cultivars with resistance to FHB and DON accumulation. Breeding for these problems in the Midwest United States began in 1993, and the first breeding lines that combined acceptable agronomic performance, disease resistance, and malt quality did not enter advanced testing by the malting and brewing industries until 2008.

Not only is barley considered a low-input crop by producers, but many barley breeders are expected to develop improved cultivars using limited financial and physical resources. The two-row barley breeding program at NDSU in the United States is an example where the existing barley genetic resources were poorly adapted to the production area when the program was started in the early 1970s (Horsley et al. 2009). Improved two-row lines were developed by making crosses between unadapted two-row germplasm and adapted Midwest U.S. six-row malting cultivars. The most important selection criteria in early generations were low grain protein (Foster et al. 1967) and high kernel plumpness. The first cultivar released from this program was Bowman (Franckowiak et al. 1985). Additional two-row cultivars released by this program are Stark, Logan, Conlon, Rawson, and Pinnacle. Conlon was added to the list of cultivars recommended for production as a malting barley by AMBA in 2000.

BREEDING METHODS AND TECHNIQUES IN CANADA AND THE UNITED STATES

No two breeding programs use the same breeding scheme. A detailed example of a breeding scheme used to develop malting barley cultivars at NDSU in the United States can be found in Horsley et al. (2009). The scheme uses a modified-pedigree breeding method and off-season nurseries to reduce the length of time needed to develop new cultivars by up to 3 years. Variations to the scheme above can be made at any stage to accommodate specific resources and breeding goals. For example, doubled-haploids or MAS based on DNA markers can be used to shorten the length of time needed to develop new cultivars by several years. These methods, as well as barley transformation, are described in greater detail in other chapters. Off-season or glasshouse nurseries also can reduce the length of time needed to develop new cultivars by providing for two or more field generations per year. Locations used for off-season nurseries by breeders in Canada and the United States include Arizona and California in the southwestern United States, Argentina, and New Zealand. Finally, nonmalting or feed barley cultivars typically are developed as much as 3–4 years faster than malting barley cultivars because extensive quality testing is not required.

Currently, MAS is gaining wider use and research continues to identify opportunities to incorporate this promising method. As stated earlier, MAS is being used at the University of Saskatchewan disease resistance breeding and purification of breeder seed. In the United States, MAS is being used for identifying lines for resistance to such diseases as barley stripe rust, FHB, and *Septoria* speckled leaf blotch. A 4-year (2006–2010) multistate collaborative project in the United States titled the Barley Cooperative Agricultural Project (CAP), funded by the USDA Cooperative State Research, Extension, and Education Service (CSREES), has as its primary goal to identify single nucleotide polymorphism (SNP) markers associated with economically important traits using association genetics methods. Information from this project will be used to generate PCR primers pairs for MAS at the USDA-ARS high-throughput molecular marker laboratories located in Fargo, North Dakota, or Pullman, Washington. These laboratories are currently screening breeding lines using molecular markers for traits such as FHB and barley stripe rust resistance.

COLLABORATION BETWEEN BREEDING PROGRAMS IN THE UNITED STATES AND AMBA FOR DEVELOPING MALTING BARLEY CULTIVARS

A long history of collaboration has existed between malting barley breeders in the Canadian and U.S. breeding programs and members of the malting and brewing industries. Development of improved malting barley cultivars is dependent on barley breeders knowing the desired levels of the numerous malt quality parameters required by maltsters and brewers. Communication of the maltsters' and brewers' needs is conveyed to the breeders through the Web and periodic meetings. As described earlier, AMBA provides breeders in the United States with baseline criteria that must be met by new cultivars before they will be recommended for use by their members. This collaboration is accomplished through industry testing of the malting and brewing qualities of advanced breeding lines and new cultivars that is coordinated by AMBA. This evaluation, referred to as pilot-scale and plant-scale evaluations, requires a minimum of 4 years of testing. Before a cultivar can be added to the "AMBA List of Recommended Malting Barley Varieties," it must be rated as satisfactory in 2 years of pilot-scale evaluation and in 2 years of plant-scale evaluation. Breeding programs participating in the AMBA evaluation programs include the public programs in Idaho, Minnesota, Montana, North Dakota, Oregon, and Washington, and BAR.

AMBA pilot-scale evaluation

Based on favorable agronomic and malt quality characteristics, breeders are allowed to submit up to eight lines (four six- and four two-row lines) to

AMBA for pilot-scale evaluation. Seed for this evaluation is obtained from two of the best six locations of large increase plots that are grown specifically for AMBA's tests by collaborating breeders. For this evaluation, 17 kg of each entry is divided among the malting and brewing members of AMBA, and malt quality is compared to the check cultivars. No brewing evaluation is done as part of the AMBA pilot-scale evaluation. Lines must be rated as satisfactory in 2 of 3 years of pilot-scale evaluation before they can be considered for advancement to the AMBA Plant Scale Evaluation Program.

AMBA plant-scale evaluation

Typically, no more than one six-row and one two-row lines from a breeding program are allowed in the plant-scale evaluation program at any one time. The plant-scale evaluation program includes evaluation of malting and brewing quality. Grain of the candidate for plant-scale evaluation is grown on approximately 250 ha in the region where it is intended to be grown. The production is done by local producers with the goal of producing a total 435–655 t of grain. Malt for plant-scale brewing evaluation is typically produced by one of AMBA's malting members. In subsequent years of the AMBA Plant Scale Evaluation Program, a different maltster will be responsible for producing the malt. This process ensures that multiple maltsters have input during the AMBA plant-scale evaluation process.

The methods of brewing evaluation of plant-scale candidates are different for each brewing company. Some companies may use small inclusions of the candidate line in their brewing blend, while others may use inclusion rates of 50% or more. Ultimately, each brewing member wants to identify any deficiencies the candidate line may have during the malting and brewing processes and in the finished malt and beer. Even though the addition of a cultivar to the AMBA List of Recommended Malting Barley Varieties requires support from only one of AMBA's member companies, very few cultivars get added to the list because of the stringent quality requirements. For example, only three cultivars were added to the list in the 1980s (Azure, B1601, and Robust) and 1990s (Excel, Foster, and Stander) for production in the Midwest United States. All six of these cultivars are six-row. In the 2000s, seven new cultivars (Conlon, Drummond, Lacey, Legacy, Rasmusson, Stellar-ND, and Tradition) have been added to the list for production in the Midwest United States. Conlon is the only two-row cultivar. In addition, German cv. Scarlett was added to the list in 2007 based on input from AMBA's malting members.

CULTIVAR RELEASE IN CANADA

Cultivar registration is compulsory in Canada for a number of field crops including barley. Registration is administered by the Variety Registration Office of the Canadian Food Inspection Agency (CFIA; http://www.inspection.gc.ca/; verified July 22, 2009). This office recognizes and relies on registration recommending committees to develop testing procedures, to evaluate data, and to make recommendations on the suitability of candidate cultivars for registration. In Western Canada, for example, the recognized committee is the Prairie Registration Recommending Committee for Oat and Barley. Details of testing protocols are posted on the Web site http://www.pgdc.ca/ (verified July 22, 2009). Membership on this committee includes scientists from government, universities, and the private sector; industry specialists; provincial government specialists; and representatives of farmer organizations. This committee sets the standards for testing and tests are conducted under its auspices. It also determines its operating procedures subject to ratification by the CFIA. The committee is divided into three evaluation teams for data evaluation purposes: the agronomy team evaluates field performance; the disease team evaluates disease resistance; and the quality team evaluates suitability for end-use processing and manufacturing. The composition of these yield trials, including checks, is approved by the committee annually and the tests are administered by a test coordinator. There are three yield trials: the

Western Six-Row Barley Cooperative, the Western Two-Row Barley Cooperative, and the Hulless Barley Cooperative. Entry into these tests requires six station years of data from tests in Canada with appropriate checks. Cooperative preregistration trials are conducted by breeders and other cooperators at a number of locations, and candidates are evaluated over a 2-year period before consideration for registration. Data are collected on agronomic performance including yield, lodging, maturity, seed weight, and so on. In the case of malting barley, an additional year of collaborative malting tests is required. Quality evaluation is conducted on seeds from selected sites of the yield trials. Micro malts are produced and wet chemistry analyses are conducted by the Grain Research Laboratory. In parallel to these tests, a collaborative malting trial is coordinated by the BMBRI. Promising entries from first-year cooperative testing plus successful entries from previous collaborative testing are grown in separate plots, with commercial checks, at several sites across Western Canada. Seed from selected sites is malted and analyzed by the Grain Research Laboratory and by industry laboratories. Disease resistance data are collected on the yield trials when infections justify and separate nurseries are grown at various sites. For example, a stem rust nursery is grown at the Winnipeg AAFC station; a scald nursery is grown at Lacombe; and an FHB nursery is grown at the Brandon AAFC station. Coordinators are assigned for each disease and are responsible for test coordination and data collation.

When considering candidate cultivars, each evaluation team passes expert judgment on their area of expertise. A final judgment is then made by the committee in plenary on the basis of all the data as a total package. Thus, these experts now consider all data as opposed to their respective discipline data so they are able to balance weaknesses in one area with strengths in another to reach a final recommendation to either support or not support the application for registration. This information is transmitted to the registration office and a final decision is made there, usually taking the advice of the committee.

Postrelease adaptation and commercial acceptance testing in Canada

Adaptation testing generates more information on cultivar adaptation than is typically necessary for a breeder to feel comfortable in releasing a new cultivar and for cultivar registration purposes. However, this information is found to be very useful by many producers who use it to assist in their decision to adopt new cultivars. The type of testing in these yield trials is similar to advanced generation yield trials but with a greater number of stations involved and less data are recorded. These trials are usually conducted by agencies other than breeding organizations.

Commercial acceptance testing is necessary for malting cultivars and for special use food or feed cultivars. The malting and brewing industry, for example, will not take on new cultivars without such testing, in which they support and actively participate. Typically, they have seen several years of micromalting data on new cultivars but no brewing information. Thus, commercial scale fields are contracted and selected barley is malted and brewed under commercial conditions. Most maltsters and brewers want to see at least 2 years of successful malting and brewing before they will begin to incorporate a new cultivar into their blends. Again, these tests, while critical to the success of a cultivar, are beyond the purview of the breeder other than to provide seed sources and to take a keen interest in the process.

Seed production in Canada

Pedigreed seed production in North America is among the best in the world. The role of the professional seed grower is vital to the success of any new cultivar. They increase the seed that the commercial farmer uses and maintain the genetic integrity of the cultivar in doing so. The Canadian Seed Growers Association (CSGA) and the CFIA are coregulators of the pedigreed seed certification system. The CSGA sets the standards and the CFIA oversees production to ensure that standards are met. For barley, there are the following classes of seed:

1. *Breeder seed*, produced under the supervision of a recognized breeder;
2. *Select seed*, produced from breeder seed by qualified select growers;
3. *Foundation seed*, produced by registered seed growers;
4. *Registered seed*, and
5. *Certified seed*.

The reader is referred to the CSGA Web site, http://www.seedgrowers.ca (verified July 22, 2009), for more detailed information.

CULTIVAR RELEASE AND INTELLECTUAL PROPERTY ISSUES IN THE UNITED STATES

Unlike many areas of the world, there is no formal process in the United States administered by the federal government for determining if a line can be released as a named cultivar for commercial production. Each university or private breeding program has its own criteria and process for releasing cultivars. While an experimental line is usually evaluated in one or more Cooperative Regional Yield Trials coordinated by the USDA-ARS, this is not required before it is released as a named cultivar. The determination of whether a cultivar will be added to the AMBA List of Recommended Malting Barley Varieties is described previously.

In general, most cultivars from the public programs are released to seed grower organizations (e.g., Crop Improvement Associations) in their states. The breeder and foundation seeds of these releases are handled by the State Crop Improvement Association or by the university or state agricultural experiment station that released the cultivar. Production of the registered and certified seed classes is handled by growers often contracted by seed companies, but fields and seed are inspected and certified by the State Crop Improvement Association or State Department of Agriculture. Production of the foundation seed class for cultivars released by private companies is not consistent. Some companies may contract the production of the foundation class themselves with local growers or they may contract a university to handle this production.

Protection of intellectual rights for public and private breeders can be done by obtaining a plant variety protection (PVP) certificate administered by the Plant Variety Protection Office of the USDA Agricultural Marketing Service (http://www.ams.usda.gov/AMSv1.0/; verified July 28, 2009). Less commonly, utility patent protection may be obtained through the United States Patent and Trademark Office of the United States Department of Commerce (http://www.uspto.gov/main/patents.htm; verified July 28, 2009). The decision to obtain a PVP certificate or a utility patent for a new barley cultivar, especially in the public sector, is not consistent across universities, agencies, programs, or even cultivars nor is the institution of royalties for growing protected cultivars.

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AUSTRALIA

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HISTORY—BARLEY PRODUCTION IN AUSTRALIA

Barley is the second most important cereal crop, after wheat, in Australia. It has expanded from the first crop of 3.24 ha, sown at Farm Cove after the arrival of the First Fleet in 1788 (Sparrow and Doolette 1975), to an industry that annually produces 4.5–5.5 million tons of grain (Lovett 1997; Powell 1997; Stuart 1997; Healy 2001). Lovett (1997) stated that the net value of Australian barley production was greater than A$1 billion per year.

The barley-growing regions are confined to the southern temperate cereal-growing regions with rainfall varying from 250 to 600 mm (see Chapter 9, Fig. 9.8; Paynter and Fettell 2008) The figure indicates regional production as tons per square kilometer based on the Australian Bureau of Statistics 2006 Agricultural Census data and is for the 2005/2006 harvest. The areas of greatest regional production are southern Western Australia; the southern Eyre Peninsula, Yorke Peninsula, midnorth and upper southeast of South Australia; the "Mallee" and Wimmera regions of Victoria; New South Wales; and southern Queensland. Other production regions include northern New South Wales and Tasmania.

Most Australian barley cultivars are two-row spring types with white aleurone. In most areas,

the crops are sown in late autumn or early mid-winter, flower in spring, and are harvested in early summer.

Australian barley production over the 5-year period 2002–2006 inclusive averaged 7 million tons of which 2.619 Mt was used domestically and 4.590 Mt was exported (Table 8.8). Only 0.169 Mt was used for malt and other human uses, and most (2.257 Mt) are used for feed. Of the exported grain, 2.461 MT was feed, 1.485 Mt was malting barley, and 0.627 Mt was malt (in grain equivalents). This malt is mostly exported to Asian countries, vis-à-vis, Vietnam, Japan, South Korea, the Philippines, and Thailand (E-malt.com 2008). So, in total, malt production was 0.796 Mt. Although Australia is not a major malting barley producer, its exports represent approximately 25% of the world trade.

The largest barley production is in South Australia (SA), closely followed by Western Australia (WA), then Victoria (Vic), New South Wales (NSW), Queensland (Qld), and Tasmania (Tas). Yields are highest in the southern regions and lowest in New South Wales and Queensland (Table 8.9).

For practicality of research management, the Grains Research and Development Corporation (GRDC) has divided the Australian cropping environment into three regions, namely, the northern region, the southern region, and the western region (Davies 1993). The northern region includes Queensland and northern New South Wales. Southern New South Wales, Victoria, South Australia, and Tasmania make up the southern region. The dividing line between the northern and southern regions is drawn at Dubbo (latitude 33° S). Only Western Australian cropping areas are included in the western region.

During the 1990s, approximately 63% of the Australian barley crop was produced in the southern region (Healy 2001). The western region contributed 23% of the crop, while the northern region produced approximately 14%. These figures often vary, as seasonal conditions in Australian barley-growing areas can fluctuate widely (Powell 1997). In most seasons, 40%–50% of the Australian barley crop is accepted as malting barley (Lovett 1997; Stuart 1997; MacLeod 2001). However, only 700,000 t or 35% of malt barley production is malted within Australia (MacLeod 2001). Approximately two-thirds of the Australian malt production is exported annually (Powell 1997). Thus, approximately 88% of the total Australian malting barley crop is exported, making the country the third largest exporter of malt, after Europe and North America (MacLeod 2001). Consequently, the viability of the Australian barley industry is heavily reliant on maintaining its share of the international market (Stuart 1997).

Table 8.8 Australian barley production and disposal (5-year average, 2002–2006)

Australian Barley Production and Use	5-Year Average (2002–2006) Mt
Production	7.115
Domestic use	2.619
As malt and other human use	0.169
Feed	2.257
Seed	0.193
Export	4.590
Feed barley	2.461
Malting barley	1.485
Malt (grain equivalent)	0.627

Source: Australian Bureau of Agriculture and Resource Economics (ABARE) and Australian Bureau of Statistics (ABS).

Table 8.9 Australian barley production by state (5-year average, 2001–2005)

State	Units	Qld	NSW	Vic	Tas	SA	WA	Australia
Area	Hectares ('000)	113	778	793	8	1176	1160	4030
Production	Tons ('000)	175	1356	1477	26	2242	2126	7402
Yield	Tons per hectare	1.55	1.74	1.86	3.06	1.91	1.83	1.84

Source: Australian Bureau of Agriculture and Resource Economics (ABARE) and Australian Bureau of Statistics (ABS).

The main export market for Australian malting barley is the Asia Pacific region. China buys approximately 50% of the Australian malting barley crop each year (Stuart 1997). Furthermore, Sewell (1997) predicted that Chinese import requirements of malting barley from Australia are likely to reach 1.36 million tons by the year 2010. This has arisen through increased demand for beer within China and increased foreign investment in Chinese breweries (MacLeod 2001). Because brewhouse technology has been refined, quality specifications for the Chinese market have become more stringent (Spiel 1999).

Japan is also a key market for Australian malting barley and commands premium prices. In the early 1980s, Australia's share of the Japanese malting barley market was approximately 33% (Powell 1997). However, by 1997, significant improvements in the quality of Canadian and European cultivars led to Australian malting barley exports to Japan being reduced to 124,226 t, or 15.5% of Japan's malt barley intake (Fukuda et al. 1999). Other important markets for Australian malting barley include Taiwan, the Philippines, South Korea, and several other Asian and South American countries (Stuart 1997).

Australian malt is principally exported to Japan, Thailand, the Philippines, and South Korea (MacLeod 2001). While the Japanese market for Australian malt has declined in a similar pattern to that seen for malting barley, there have been significant increases in sales to Thailand, Vietnam, and South Korea (E-malt.com 2008). These three countries account for 47% of Australian malt exports. Increases in brewing capacity in Thailand, Vietnam, and South Korea should increase demand for Australian malt and malting barley but will also necessitate stable grain supplies and improved variety performance.

In response to the above market signals, the Australian industry has taken several steps to improve the quality and reliability of its export barley and malt supplies. The accreditation of Australian malting cultivars is coordinated by the Malting and Brewing Industry Barley Technical Committee (MBIBTC) (Hawthorne 1999). This committee has established technical guidelines, which are available to all breeding programs. Those guidelines include detailed experimental schedules and specifications to be met by potential cultivars before domestic and/or export malting accreditation is awarded (MBIBTC 1998).

Malting barley usually attracts a price premium of A$30–A$50 per ton over feed barley in Australia. To qualify for malting grade, the grain must firstly be obtained from an accepted malting cultivar. Point-of-delivery assessment must also prove that the grain meets appropriate specifications, typically, a low proportion of small grain or "screenings," protein between 8.5% and 12.0%, bright grain color, and absence of contaminants such as smut, sclerotes, weed seeds, and soil (ABB Grain 2008; CBH Group 2008; Grainco 2008).

HISTORY—BARLEY IMPROVEMENT IN AUSTRALIA

The early history of Australian barley improvement was comprehensively described in review papers written by Sparrow and Doolette (1975, 1987 loc. cit.). Most early Australian cultivars were obtained from Europe. These tended to be late maturing and befell the consequences of terminal droughts or the hot dry finishes to the seasons so typical in southern Australia. A South Australian farmer, Samuel Prior, developed the first Australian barley cultivar in 1903 from a selection of imported seed line of Chevalier (or alternatively, Spratt Archer). Prior's Chevalier selection was recognized for its superior malting quality and subsequently proved to be well adapted to the dry South Australian environment. It became known as Prior and led to a massive expansion in barley production. Prior became the backbone of the Australian barley industry until the end of the 1960s.

From the 1920s to the 1950s, barley breeding was conducted on a part-time basis in Victoria, New South Wales, and South Australia. Early breeding programs led to Prior A bred by Albert Pugsley and Research from the program at Werribee in Victoria, bred by A.R. Raw in 1942. However, in 1956, a barley improvement scheme

was established with funds provided by maltsters, brewers, and growers and with matching funds from the commonwealth government. This scheme led to the development of full-time breeding programs in South Australia and Victoria, and consequently, the release of several barley cultivars, which finally replaced Prior. The most significant of these was the malting barley Clipper, released from the Waite Agricultural Research Institute in 1968. The genotype × environment interaction studies of Finlay and Wilkinson (1963) had a significant influence on the South Australian program. Improved yield over a range of environments was found in introductions from North Africa. This material was early maturing, tolerant of alkaline and sodic soils, disease resistant (especially cereal cyst nematode [CCN], mildew, and other leaf diseases), and tolerant of hot dry finishes. A similar well-adapted material was sourced from the United States, but this originally came from North Africa.

New breeding programs were established in Western Australia in 1962 and in Queensland, New South Wales, and Tasmania in the late 1960s and early 1970s. Barley improvement in Queensland effectively began in 1967 when Dr. R.P. Johnston of the Department of Primary Industries initiated a series of trials to evaluate breeding lines from the Waite Institute (Poulsen 2001). A breeding program was designed to breed high-yielding malting and feed barley cultivars, targeting Queensland barley-producing regions.

Funding management for barley research and development went through a series of iterations from the late 1970s through to the present day (Joppich 1985; Purse and McNee 1985; Smith 1987; Davies 1993). Originally, funding for different crops was governed through a series of research councils. However, in 1990, 4 federal research councils and 10 state wheat and barley committees were amalgamated to form the GRDC, with the overarching responsibility to drive research and development across the entire Australian grains industry (Davies 1993). This has led to the development of a coordinated barley research and development program for Australia, including strong barley improvement programs in each of the southern, western, and northern regions (Lovett 2001). Further agronomic research of French and Schultz (1984a,b) has drawn attention to the importance of water use efficiency in water-limiting environments and the importance of understanding abiotic and biotic constraints, which may limit productivity and quality improvements.

Nationally focused projects have been established. For example, the National Barley Molecular Marker Program (NBMMP), later to be amalgamated with the equivalent wheat program to become the Australian Winter Cereal Molecular Marker Program (AWCMMP), was set up to develop and implement molecular technologies on a national scale (Langridge 1997; Barr et al. 2001).

The effects of developing focused barley breeding programs became clear during the 1980s, as new malting cultivars with specific regional adaptation were released, replacing Clipper (Sparrow 1984). Schooner was released from the Waite Institute in 1983 and quickly dominated the southern States. In Western Australia, the locally bred cv. Stirling similarly took over from Clipper. A third cultivar, Grimmett, replaced Clipper in Queensland and in northern New South Wales.

The significant investments from the GRDC, together with inputs from the malting and brewing industries and from marketing and grain handling organizations and authorities, led to a substantial improvement in the number and quality of malting and feed varieties released in the 1990s and early 2000s. Other cultivars have since been released in each of the GRDC regions, including high-yielding feed types.

A representative malting barley pedigree chart is shown in Fig. 8.6, where possible varieties are listed in chronological order. Caution should be taken in attempting to determine the order of each cross; special expediency sometimes prevented the convention of putting the female parent first. Some observations can be made. Early programs were sparse and based on a limited number of European parents. There was a limited use of backcrossing to incorporate disease resistance traits. Cycle times were often 12 or more years. The 1970s and 1980s saw the use of North African landraces providing both an appropriate

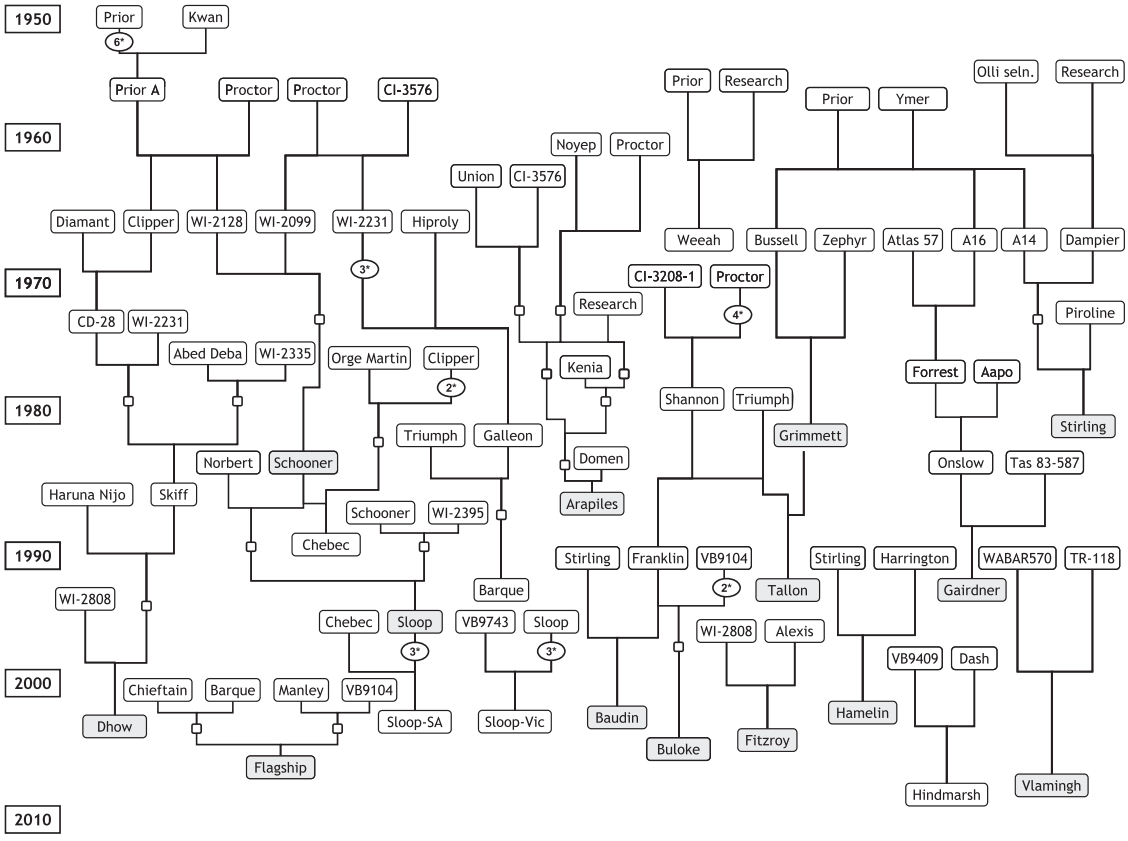

Fig. 8.6 Pedigree tree of selected Australian barley varieties.

phenological adaptation and disease resistances (such as CCN, mildew, and alkaline soil). New introgressions came from Europe, North America, and Japan. More varieties have been released with shorter cycle times. The use of molecular genetic markers has enabled more complex crosses to be managed more easily and with greater efficiency (e.g., Flagship, Sloop-SA, and Sloop-Vic). Barley Australia accredited varieties are shown as shaded rounded rectangles.

The newer malting varieties include Western Australia: Gairdner, Baudin, Hamelin, and Vlamingh; South Australia: Sloop and the back-cross derivative Sloop-SA; Tasmania: Shannon, Franklin, and Vertess; Queensland: Tallon and Lindwall; Victoria: Arapilies, Sloop-Vic, Buloke, and Hindmarsh; and New South Wales: Cowabbie.

Feed varieties and some malting lines are shown in Table 8.10. The table has been sorted by date of release (or introduction) and by name.

RECENT PROGRESS IN AUSTRALIAN BARLEY IMPROVEMENT PROGRAMS

Barley Breeding Australia (BBA)—a new paradigm

BBA began operating on July 1, 2006 as the Australian national barley breeding program implementing a national plan for breeding improved varieties to benefit the barley industry. BBA is an unincorporated joint venture between the GRDC, the Queensland Department of Primary Industries and Fisheries, the New South Wales Department of Primary Industries, the

Table 8.10 Australian feed barleys and other significant varieties

Name	Year Released	Pedigree	Breeder
Trabut	1916	Algerian introduction selection	DANSW
Abyssinian	1920	Abyssinian introduction	NSWDA
Commander	1930	Coast selection	DANSW
Maltworthy	1938	Prior × Beaven's Special	RAC
Deputy	1939	Hero selection	DANSW
Ablyn	1952	Abyssinian × Flyn	NSWDA
Noyep	1959	Prior's Chevalier selection	RAC
Anabee	1962	Research selection	VicDA
Resibee	1962	Research selection	VicDA
A14	1968	Prior × Ymer	DAWA
A16	1968	Prior × Ymer	DAWA
Bussell	1968	Prior × Ymer	DAWA
Ketch	1969	Lenta × Noyep	WARI
Lara	1971	Research × Lenta	VicDA
Cutter	1975	Prior A × Proctor	WARI
Corvette	1976	Bonus × CI-3576	WARI
Parwan	1979	((((Plumage Archer × Prior) × Lenta)) × (Research × Lenta))	VicDA
Bandulla	1981	((Prior × Lenta) × (Noyep × Lenta))	VicDA
Beecher	1981	Atlas × Vaughn	
Cantala	1981	Kenia × Erectoides 16	VicDA
Malebo	1981	Pallidum selection (CPI-11083)	NSWDA
Waranga	1981	((Plumage Archer × (Prior × Lenta)) × (Research × Lenta)) × Clipper	Vic-DPI
O'Connor	1984	WI-2231 × (Atlas 57 × A14)	DAWA
Moondyne	1987	((Dampier × A14) × Kristina)) × (Clipper × Tenn 65-117)	DAWA
AB6	1988	CPI-71283 × 4* Clipper	CSIRO-PI
Windich	1989	(Atlas 57 × A16) × Parwan	DAWA
Yerong	1990	M22 × Malebo	NSWDPI
Gilbert	1992	(Armelle × Lud) × Luke	Qld-DPI
Brindabella	1993	((((Weeah × CI-17115) × HCB27) × Jadar II) × Cantalla)	NSWDPI
Kaputar	1993	((5604 × 1025)) × ((Emir × Shabet) × CM67)	CIMMYT
Namoi	1993	(Sultan × Nackta) × (RM1508 × Godiva)	NSWDPI
Dash	1995	(Chad × Joline) × Cask	NFC
Mundah	1995	O'Connor × Yagan	DAWA
Molloy	1996	(Golden Promise × WI-2395) × 72S:267	DAWA
Picola	1996	VB75031 × Elgina	Vic-DPI
Fitzgerald	1997	Onslow × Tas 85-466	DAWA
Tilga	1997	Forrest × Cantala	NSWDPI
Unicorn	1997	54C25 × 51C38	Kirin Australia
Doolup	1998	75S:323 × 74S:314	DAWA
Lindwall	1998	Triumph × Grimmett	Qld-DPI
Tantangara	1998	AB6 × Skiff	NSWDPI
Wyalong	1998	Schooner × Stirling	NSWDPI
Yambla	1998	Skiff × FM 437	NSWDPI
Keel	1999	(CPI-18197 × Clipper) × WI-2645	WARI
Binalong	2001	Blenheim × (Skiff × O'Connor)	NSWDPI
MacKay	2001	Cameo × Koru	Qld-DPI
Cowabbie	2002	(((AB6 × Franklin) × Franklin)) × (Rubin × Skiff)	NSWDA
Milby	2002	(((AB6 × Franklin) × Franklin)) × (Rubin × Skiff)	NSWDA
Tulla	2002	Skiff × FM 437	NSWDPI
Capstan	2004	(Waveney × WI-2875) × (Chariot × Chebec)	Uni Adelaide
Maritime	2004	74S:314 × 74S:309	Uni Adelaide
Grout	2005	Cameo × Arupo "S"	Qld-DPI
Urambie	2005	(Yagan × Ulandra) × Ulandra	NSWDPI
Vertess	2005	Franklin × Cooper	UTas and TASDPIWE
Yarra	2005	(VB9108 × Alexis) × VB9104	MBQIP
Fleet	2006	Mundah × Keel × Barque	Uni Adelaide
Hannan	2007	B28719 × (Windich × Morex)	DAFWA
Lockyer	2007	Tantangara × VB9104	DAFWA
Roe	2007	Doolup × (Windich × Morex)	DAFWA

Department of Primary Industries, Victoria, the University of Adelaide, the Department of Agriculture and Food, Western Australia, and the Tasmanian Institute of Agricultural Research. BBA is governed through an advisory board formed by the parties and coordinated through a management committee. The formation of BBA rationalized the previous six state-based barley breeding programs into one national program with three regional nodes: BBA-North (QDPI-Warwick, Queensland), BBA-South (University of Adelaide, Urrbrae, South Australia), and BBA-West (DAFWA, Kensington, Western Australia). The restructure aims to use resources clearly and efficiently in barley breeding so as to provide the foundation of a future vibrant barley industry (Lance et al. 2007; Reading 2007). The previous programs in Victoria, New South Wales, and Tasmania will be focusing their efforts on germplasm development and barley prebreeding. The breeding nodes are to focus on the development, release, and adoption of varieties meeting specific market and regional needs. As such, they must achieve operational efficiencies through the adoption of a "best practice in breeding" framework.

BBA-North is actively changing its primary focus from breeding malt varieties to breeding feed varieties, thereby meeting the future demand for feed grain in northeastern Australia.

BBA-South has extended its trial network to cover the neutral to alkaline soils in New South Wales and Victoria and the alkaline soil regions of southern Western Australia. The most adapted barleys are mid season to earlier maturing.

BBA-West, in addition to conducting trials in Western Australia, conducts yield trials and nurseries in New South Wales, Victoria, and Tasmania, on neutral to acid soils. As such, the focus is on medium- to later-maturing varieties suited to the medium- to high-rainfall zones.

Germplasm from the NSWDPI, Victorian and Tasmanian breeding programs is being progressed and integrated into the operations of BBA-West and BBA-South.

Barley breeding in Australia will continue to evolve. From July 2011, the BBA agreements will come to an end and are likely to be replaced by a more commercially driven, self-funding arrangement. This follows an independent review commissioned by BBA which recommended that a more commercial approach was required for Australian barley breeding to be sustainable. It seems highly likely that although a more commercially focused system will occur at the business end of barley breeding, a mixture of public and private investment in barley genetic improvement will be required for the foreseeable future. Ultimately, the future success and sustainability of barley breeding in Australia will rely on improved industry productivity and the ability to capture end point royalties to fund the breeding efforts.

Barley Australia—malting barley accreditation

Barley Australia was established in 2005 as the peak body for the Australian barley industry. It was the recognition of a future deregulated marketing environment for Australian barley that was a catalyst for its creation as it was recognized by the maltsters and marketers (purchasing the barley crop from the Australian farmers) that there was a need for a body to deal with "industry good" functions that had previously been undertaken by the statutory marketing companies that preexisted the new market environment, such as cooperative industry decisions on quality standards, variety suitability to malting, and a coordinated approach to industry-wide research.

Barley Australia is entirely self-funded by the maltsters and marketers of Australian barley and is a testament to the members in their ability to cooperate for industry benefit even though in the Australian marketplace they are staunch competitors. Now in its fourth year, Barley Australia serves a significant purpose in the Australian industry as a "shop window" for the industry and disseminates information to the Australian barley industry stakeholders as well as to the international marketplace. Barley Australia is responsible for creating and managing the national varietal accreditation system as well as the Barley Australia Assured Quality trademark for Hazard Analysis Critical Control Point (HACCP)-certified barley and malt out of Australia. More information on Barley Australia can be sought from the Web site http://www.barleyaustralia.com.au/.

The MBIBTC

The MBIBTC has technical malting and brewing experts representative of Australia's major malting and brewing companies. Varieties submitted to Barley Australia are evaluated by an MBIBTC member for the first year of commercial evaluation. If deemed suitable, then the malt from the evaluation passes to Pilot Brewing Australia (PBA).

PBA

PBA conducts the last step in the process of evaluation of malting barley for either domestic or export market suitability. PBA is malting and brewing industry funded and also cofunded by the GRDC. Samples for pilot brewing are taken from commercial-scale malting evaluation trials. The facility is able to replicate the requirements of both domestic and export brewers utilizing sugar or starch adjuncts.

For a new malting variety to be accredited, it must pass two successful years of commercial malting evaluation and pilot brewing or commercial-scale brewing with all stages being reviewed by the MBIBTC.

The major proportion of the northern region barley crop is purchased for domestic stock feed. Most northern feed barley is used for cattle since the region contains the highest concentration of feedlots in Australia (Barr and Kneipp 1995). Feed for the monogastric industries is also a significant market, with approximately 30% of the grain purchased by stock feed manufacturers for pig and poultry rations (M. Covaceveitch, pers. comm.).

AUSTRALIAN BARLEY BREEDING OBJECTIVES

Barley breeding objectives have changed substantially in Australia over the past century. While the basic agronomic objectives of grain yield, lodging resistance, and appropriate maturity have changed relatively little, the evolution of on-farm cultural practices has resulted in a significant increase in the number of diseases affecting the crop. Objectives relating to grain quality have also increased in number and detail, as knowledge of grain quality for both malting and feed use has become more refined. See Table 8.11 for a comprehensive summary of breeding objectives/traits.

Agronomic traits

The principal agronomic traits of interest for Australian adaptation are grain yield, lodging resistance, straw strength, height (short straw, medium height straw), phenology (late maturity, short straw for longer seasons; early maturity, taller straw for drier shorter seasons), head loss, and stem breakage.

Improved grain yield has been the primary objective of Australian barley breeding activities since the nineteenth century (Sparrow and Doolette 1975). From the outset, modern Australian programs were clearly designed with yield as the primary selection criterion (Ellis 1983; Johnston 1983; Portman 1983; Read 1983; Sparrow 1983; Vertigan 1983). However, it is considered extremely important that yield advances are made together with improvements in grain quality to protect the Australian share of the world barley market (GRDC 2001). Johnston (1974) had concluded that a combined malting and feed breeding program could service both markets, improving both yield and quality.

As well as grain yield, Australian barley breeders have selected material for a range of agronomic traits. All of the programs made resistance to lodging or a high level of straw strength a key agronomic breeding objective (Portman 1983; Read 1983; Vertigan 1983). Johnston (1983) placed a very high priority on lodging resistance as the inability of barley cultivars to stand up in the field was seen as a major limitation to the further expansion of the crop in Queensland. Plant height was established as another objective of the Australian programs. For example, short cultivars were desirable in the high- and medium-rainfall zones of Western Australia, while taller cultivars suited low-rainfall areas (Portman 1983). Controlling head loss caused by stem breakage was another significant agronomic trait targeted, in particular, for Western Australia, New South Wales, and Tasmania.

Crop phenology targets of the different Australian programs were diverse and were driven

Table 8.11 Differences in the grain production environments and key selection targets and traits for GRDC breeding programs in northern, southern, and western regions

Southern Region	Western Region	Northern Region
Latitude 33° S–43° S Predominantly winter rainfall Lighter, shallower soils, sands in WA Winter cropping only	Latitude 28° S–35° S Predominantly winter rainfall Lighter, shallower soils, sands in WA Winter cropping only	Latitude 22° S–33° S Predominantly summer rainfall High proportion of heavy clay soil types Winter/summer crop rotations Double cropping
Agronomic traits Yield, grain weight, plumpness, hectoliter weight, seedling vigor, phenological adaptation (*bvp*, *ppd*, *vrn*)		
Malting quality Malt extract, diastatic power (DP), apparent attenuation limit (AAL), low viscosity, increased free alpha amino nitrogen (FAAN)		
Feed quality Digestible energy (DE), metabolizable energy (ME), animal intake, low bloat, low phytate		
Resistance/tolerance to biotic stress		
Net form net blotch Spot form net blotch Scald Powdery mildew Leaf rust (part of SA, S-NSW, NE, and SW-Vic, Tas) BYDV Barley grass stripe rust Rhizoctonia	Net form net blotch Spot form net blotch Scald Powdery mildew Leaf rust BYDV	Net form net blotch Spot form net blotch Scald Powdery mildew Leaf rust Stem rust Common root rot Crown rot
Major pests		
Cereal cyst nematode (SA, Vic)	Root lesion nematode—*Pratylenchus neglectus* and *Pratylenchus* sp.	Root lesion nematode—*Pratylenchus thornei* and *Pratylenchus* sp.
Resistance/tolerance to abiotic stress		
Drought/terminal drought Alkaline soil/boron toxicity tolerance (SA, Vic-Mallee) Acid soil/aluminum toxicity (sNSW, NE, and SW-Vic) Frost Salinity/sodicity tolerance Manganese deficiency tolerance Waterlogging tolerance	Drought/terminal drought Alkaline soil/boron toxicity tolerance (WA-Mallee) Acid soil/aluminum toxicity Frost Salinity/sodicity tolerance Waterlogging tolerance	Drought/terminal drought Frost
Preharvest spouting tolerance Blackpoint/kernel discoloration tolerance Screening tolerance Winter cropping only	Preharvest spouting tolerance Blackpoint/kernel discoloration tolerance Screenings tolerance Winter cropping only	Preharvest spouting tolerance Blackpoint/kernel discoloration tolerance Screening tolerance Winter/summer crop rotations, double cropping

by the amount of available moisture and the duration of optimal growing conditions. Read (1983) explained how longer-season, short-straw material was being developed for parts of southern New South Wales where early-sown cultivars were suitable. These types were also suitable for Tasmania and eastern Victoria. In Western Australia, longer-season cultivars were more suitable for the high- and medium-rainfall zones, while quick maturing barleys were required for the parts of the cropping area with low rainfall (Portman 1983). Most areas of South Australia

and parts of Victoria required medium-quick maturing genotypes. Data from Queensland and northern New South Wales revealed that medium to medium-slow maturing European-type germplasm gave better grain yield and quality in eastern environments, but that quicker maturity was required in western areas (D.M.E. Poulsen, unpublished data).

Malting and feed quality

Quality attributes include both physical and biochemical grain characteristics. Sparrow and Doolette (1975) suggested that early assessment of malting quality was aimed at identifying lines with plump and uniform grain, high malt extract, and quick and even germination when malted. That definition remains true today, and a host of individual parameters is recognized as contributing to each of the above attributes.

In 1983, the MBIBTC was formed to provide industry guidance to the breeding programs (Armitt and Way 1990). It has provided Australian barley breeders with guidelines to develop new malting cultivars (MBIBTC 1998) and has developed a rating system for malting barleys, which takes into account weighted factors for malt extract, protein modification, diastatic power (DP), wort viscosity, and dimethyl sulfide content (Armitt and Way 1990). The relative activities of specific enzymes such as α-amylase, β-amylase, and β-glucanase and cellular constituents such as β-glucan and starch are also recognized as target parameters for malting barley improvement programs. Grain dormancy is essential to protect against preharvest sprouting caused by summer storms (Poulsen et al. 1995b). Sprouting interferes with the malting and brewing processes by causing uneven starch and cell wall degradation in the grain. The southern and western regions are less concerned about dormancy as those areas have predominantly winter rainfall patterns; however, the southern coast of Western Australia is prone to preharvest sprouting problems and a degree of dormancy is required.

While some quality specifications are required for all malting barleys, there is now a dichotomy in domestic and export market requirements due to the different sources of carbohydrate adjuncts used in the brewing process (MBIBTC 1998). The domestic brewing industry requires moderate levels of DP; however, many Asian breweries require high DP values.

It has gradually become recognized that specific quality attributes can enhance livestock performance. Johnston (1974) commented that barley was seen as a source of energy and protein for both the ruminant and nonruminant feeding industries. He concluded that high protein requirements of the feed industry could be better dealt with through agronomic management than breeding. Recent research has begun to define attributes of barley affecting animal feeding performance. Bowman and Blake (1996) identified considerable genetic variation in barley ruminant feed quality. Dry matter digestibility, starch digestibility, starch content, starch particle size, ground grain particle size, and grain hardness were identified as key traits in cattle nutrition.

Resistance to biotic stresses

Early Australian priorities for disease resistance breeding were scald (*Rhynchosporium secalis*), covered smut (*Ustilago segetum* var. *hordei*), and powdery mildew (*Blumeria graminis* f. sp. *hordei*) (Sparrow and Doolette 1975). New South Wales Agriculture produced scald-resistant lines from the cross Commander × Prior during the 1940s. However, named varieties were not released. In the same era, covered smut and powdery mildew resistant (*Mlk*; now susceptible) (from the Indian variety Kwan) backcross-derived lines based on Prior and Research were produced at the Waite Institute (Pugsley and Vines 1946; Pugsley 1951), leading to Prior A (=Kwan × 6* Prior; Kwan was thought to have at least three genes conferring resistance to covered smut), which has proven to be a cornerstone of barley adaptation and improvement in southern Australia. Additionally, Prior was one of the earliest of the European introductions and contains the *Eam1* gene, which is prevalent in Australian germplasm (J.D. Franckowiak, pers. comm.) and is the source of earliness in Erbet (=Prior × 7* Betzes). This inherent

"earliness" enabled grain fill to be completed in a hot dry spring.

In the 1970s and early 1980s, disease resistance was considered less important than grain yield or grain and malting quality. Most programs used disease-resistant parents, but effective resistances were seen as desirable, rather than essential, characteristics in new varieties (Johnston 1985; Gilmour 1993). Disease resistance breeding took higher priority in the New South Wales breeding program. Resistance to leaf diseases was seen as necessary for early-sown cultivars grown in the southern parts of the State (Read 1983). Development of scald-resistant material was also occurring in Western Australia (Portman 1983, 1985), South Australia (Sparrow 1983), and Victoria (Ellis 1983).

By 1985, diseases were recognized as restricting barley production throughout Australia. Hirsch (1985) estimated yield losses from diseases in southern Australia between 1973 and 1983 to be more than 16% of the overall crop value, with a net cost of A$20–A$25 million. Rees (1985) noted that considerable changes had occurred in Queensland, and several diseases had become significant, including stem rust (*Puccinia graminis*), leaf rust (*Puccinia hordei*), scald, net-type net blotch (*Pyrenophora teres* f. sp. *teres*), spot-type net blotch (*P. teres* f. sp. *maculata*), crown rot (*Fusarium pseudograminearum*), and common root rot (*Cochliobolus sativus*). This was in direct contrast to an earlier statement that the only regular problem in northern barley crops was powdery mildew (Rees et al. 1981). The increasing disease levels were believed to have been due to changes in seasonal conditions, pathogen genetic diversity, cultivars, and agricultural practices.

Severe stem rust occurred in Queensland barley crops during 1982–1984 when the highly susceptible cv. Galleon entered production on the Darling Downs (Dill-Macky 1992). Localized epidemics occurred in 1982 and 1984; however, a more general epidemic occurred in the Darling Downs and Burnett regions during the 1983 season. Shortly afterwards, Rees (1985) suggested that the predominance of a single cultivar in Queensland, Grimmett, was making the State's crop generally vulnerable to disease, in the event that new pathogens or pathotypes became established. This threat was confirmed in 1988, when new leaf rust pathotypes caused a significant epidemic in Grimmett crops. A combination of cultivar susceptibility and suitable weather conditions led to highly damaging epidemics of leaf blotches in 1998 (Poulsen et al. 1999; Rees et al. 1999). The changing disease pressures in the northern region led to changes in the regional breeding objectives. A stem rust research program was initiated after the 1982–1984 epidemics (Johnston 1985), and other expansions to the program occurred after the 1988 leaf rust and 1998 net blotch epidemics (Poulsen 2001). In each case, projects were developed to assess the impact of the diseases and to identify useful sources of genetic resistance (Cotterill et al. 1992; Dill-Macky 1992; Platz 2001).

The most significant nematode pest of barley in Australia has been CCN (*Heterodera avenae*), which occurs naturally in South Australia and Victoria. The southern programs have bred for CCN resistance for many years (Ellis 1983; Sparrow 1983). The first Australian CCN-resistant barley cultivar was Galleon, a South Australian feed variety with the CCN resistance gene *Ha4* (Karakousis et al. 2003c) introgressed from a North African landrace, CIho-3576 (Gall eon = [Clipper × Hiproly] × 3* WI-2231, where WI-2231 = Proctor × CIho-3576). The second resistant cultivar was Chebec, which contained the CCN resistance gene *Ha2* (Barr et al. 1998) from Orge Martin (Chebec = [Orge Martin × 2* Clipper] × Schooner). Barleys are generally tolerant toward CCN, but resistance varieties reduce the number of CCN in the soil, which has a significant and positive effect on subsequent intolerant and susceptible wheat crops.

There are no major insect pests at present in Australia that can be addressed by genetic tolerance or resistances. Some pests such as Russian leaf aphid are regarded as "quarantine threats," and there are prebreeding programs to overcome potential incursions.

Resistance to abiotic stresses

Breeding for abiotic stress has generally been associated with soil characteristics. In the 1980s, the Waite Institute breeding program was select-

ing for manganese-efficient cultivars to address deficiency problems in some South Australian soils (Sparrow 1983). Conversely, the New South Wales program, now followed by the BBA-West program, has been developing cultivars adapted to aluminum and manganese toxicity for production on acid soils (Read 1985). Parts of Victoria, South Australia, and Western Australia have alkaline and sodic soils of marine origin, and these are associated with boron toxicity. The breeding programs in those States have developed conventional and molecular screening methodologies and have incorporated boron tolerance into elite germplasm (Jefferies et al. 1997; Barr et al. 2000).

INTEGRATION OF TECHNOLOGIES IN AUSTRALIA

Barley breeding methods in Australia

Many barley breeders use pedigree breeding systems. Specific parents are hybridized by controlled pollination, and individual lines are selected from segregating material in the F_2 and subsequent generations (Anderson and Reinbergs 1985). Selection usually commences with simple traits and progresses to multienvironment yield trials. Pedigree breeding strategies form the core component of most, if not all, of the Australian barley improvement programs. Various modifications may be applied to pedigree breeding strategies. Riggs et al. (1982) designed a pedigree program in which F_4 bulks were tested in field trials to bring yield evaluation forward by 1 year and to improve resource use efficiency in their program. The NBIP uses a variation of this technique in its cross-evaluation strategy (Johnston 1983; Poulsen et al. 1995b).

The Western Australian program uses several breeding strategies that are utilized where appropriate (Fig. 8.7).

The basic "conventional" method is an F_2 progeny method with a reselection phase at the F_5. Almost 50% of the stage 2 program is derived from doubled haploids (DHs). Marker-assisted selection (MAS) coupled with DH, single-seed descent (modified, SSDm) and male sterile facilitated recurrent selection (MSFRS) are seen as the methods of choice for increasing the rate of genetic gain and for reducing the time to release of new varieties.

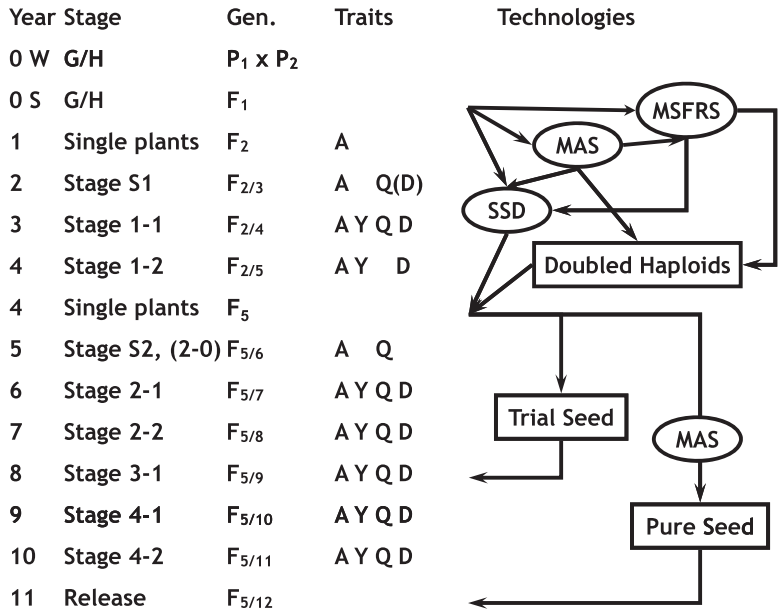

Fig. 8.7. Flow diagram showing application of breeding and selection strategies in the West Australian barley breeding program. A, agronomic; Y, yield; Q, quality; D, disease.

Backcross breeding aims to recover as much of a desirable recurrent genotype as possible while introgressing a new gene from a less-adapted parent (Harlan et al. 1922, cited by Anderson and Reinbergs 1985). In South Australia and Victoria, a backcross strategy utilizing molecular markers has been used to introduce disease resistance genes and boron tolerance into the malting cultivars Sloop and Gairdner without significantly changing their malting attributes (Barr et al. 2000, 2001).

Mass selection strategies are relatively inexpensive methods of early-generation screening (Anderson and Reinbergs 1985). Australian programs using this technique have included New South Wales (B. Read, pers. comm.) and Queensland (Poulsen et al. 1997).

Recurrent selection is an effective breeding strategy used in self-pollinating crops to improve complex characteristics such as yield, grain quality, and partial disease resistance (Stuthman et al. 1996). It involves repeated cycles of intercrossing and selection in a restricted gene pool. Carver and Bruns (1993) concluded a review paper by stating that "A review of genetic gains (ΔG) reported for grain yield and related traits since 1985 indicates that recurrent selection has been equally, if not more, effective than traditional breeding methods." While ΔG in traditional pedigree programs was estimated at 1% per year, recurrent selection programs with at least two completed cycles achieved ΔG of 3.4% per annum. In barley, a modification of the technique has been used in conjunction with a system of genetic male sterility to make significant yield improvements in North American feed barleys (Falk 1996, 2001).

DH production through either anther culture (Clapham 1973, cited by Kasha and Reinbergs 1982) or isolated microspore culture (Kasha et al. 1992) is used in Australia. However, DH production from the microspore-based techniques is more attractive because of improved recovery of DH lines, difficulties in managing *Hordeum bulbosum* plants, and spontaneous chromosome doubling, eliminating the need to use colchicine (Broughton and Poulsen 1997).

The principal reason breeding programs use DH systems is to save time (Logue et al. 1993). As DH lines are, by nature, homogeneous, the need for several generations of self-pollination to achieve the same ends is eliminated. It is anticipated that 2–4 years can be saved from cultivar development cycles in the Australian spring barley breeding programs through the use of DH techniques. For example, the DH cv. Tantangara was released in 8 years as opposed to the conventional breeding cycle of 10–12 years in the New South Wales Agriculture barley program (B.J. Read, pers. comm.).

Interspecific and intersubspecific hybrids have been used on several occasions to bring new genes into cultivated barley. *Hordeum vulgare* subsp. *spontaneum* has been used as a source of disease resistance genes in many breeding programs (Feuerstein et al. 1990; Abbott et al. 1991, 1992; Chicaiza et al. 1996). Translocations between *H. bulbosum* and *H. vulgare* subsp. *vulgare* have also been used as a source of new genetic variations for barley breeding (Xu and Kasha 1992; Pickering et al. 1995, 1998, 2000). The scald-resistant variety Tantangara, bred by Barbara Read at NSW-DPI Wagga Wagga, has a resistance gene from an *H. vulgare* ssp. *spontaneum* source (CPI-71283), which was developed by Dr. Tony Brown (Brown et al. 1993, 2000) as a Clipper backcross (BC_3) line, AB6 (Brown et al. 1988).

Disease resistance research and breeding in Australia

Australia has diverse barley cropping environments, and consequently, the occurrence of barley diseases is also diverse (Boyd and Dube 1989; Murray and Brennan 2001). The soil-borne diseases, crown rot and common root rot, are widespread throughout Australia and have become recognized in recent years as a national problem (Murray and Brennan 2001). Scald and CCN (*H. avenae* Woll.) have been major pathological constraints to barley production in southern Australia for many years, and resistance has been virtually mandatory in any new cultivars for the region. However, net blotch and other stubble-borne diseases have become increasingly significant throughout the entire continent in recent years (Murray and Brennan 2001).

There are five rust diseases capable of infecting cultivated barley; leaf rust (*P. hordei*), stem rust (*P. graminis* f. spp.), and barley grass yellow rust (BGYR) (*Puccinia striiformis*) are the major cereal rust pathogens of barley in Australia. Barley stripe rust (*P. striiformis* f. sp. *hordei*) and crown rust (*Puccinia coronata* Corda) have not been recorded in Australian barley crops. Stripe rust is considered to be a significant quarantine threat to the Australian industry and preemptive breeding strategies have been developed.

The sexual stages of *P. hordei* have been reported on "Star of Bethlehem" (*Ornithogalum umbellatum*) growing on the Yorke Peninsula in South Australia (Wallwork et al. 1992). The apparent sexual recombination of virulence genes was described in this work, following isolation of single leaf rust pustules collected from the area and differential testing on appropriate barley genotypes. This potential for genetic recombination of virulence factors and the consequent development of complex new pathotypes is likely to have a significant impact on resistance breeding and on the deployment of resistance genes.

The Australian National Cereal Rust Control Program (NCRCP) conducts annual surveys of rusts in wheat, barley, oats, and other cereals (McIntosh et al. 1995). The information is used to monitor the development of new pathotypes, recommend withdrawal of susceptible cultivars, and strategically deploy resistance genes for the most effective control of the diseases.

Severe localized epidemics of barley leaf rust have been reported in Australia (Dill-Macky et al. 1989) associated with a predominance of leaf rust-susceptible cultivars. In Australia, leaf rust is a significant disease of barley in southern Queensland, in northern New South Wales, and in parts of South Australia, West Australia, and Tasmania (Murray and Brennan 2001).

The sporadic nature of Australian leaf rust epidemics is due to climate variability and fluctuation in the relative production areas of susceptible cultivars. The most recent epidemics of barley leaf rust in Queensland and in northern New South Wales occurred in 1978, 1983, 1984, and 1988 (R.G. Rees, pers. comm.). The 1988 epidemic resulted from the development of two new pathotypes ($210P^+$ and $253P^-$) with virulence to the resistance gene in the predominant cultivar, Grimmett (Cotterill et al. 1994), causing substantial yield losses of up to 30% (Dill-Macky et al. 1989). Two new and potentially damaging pathotypes of leaf rust ($4610P^+$ and $5610P^+$) with virulence on most major cultivars were first identified in Tasmania during 1991 (Cotterill et al. 1992) and in West Australia in 1997 associated with the varieties Triumph and Franklin (Loughman 1998). Park and Williams (2000) reported detection of the pathotypes in the northern region during 1999 and that they made up over 60% of the barley leaf rust isolates collected from across Australia in the annual rust survey of that season. The pathotypes are virulent on most Australian barley cultivars and have caused economic damage to crops in Western Australia and South Australia.

Until 1999, *Rph12* was effective against all pathotypes of leaf rust found in Queensland and Northern New South Wales. The first Australian report of virulence to *Rph12* was confirmed with pustules isolated from a Tasmanian crop of Franklin barley during 1991 (Cotterill et al. 1992). The new pathotype spread to South Australia by 1993 (H. Wallwork, pers. comm.). In 1998, a different pathotype, also with *Rph12* virulence, was reported from Western Australia (Loughman 1998). The two pathotypes designated $4610P^+$ and $5610P^+$ by octal notation were identified in South Queensland during 1999 (G. Platz, pers. comm.). *Rph12*, derived from European cv. Triumph, is present in barley cv. Tallon (Cotterill et al. 1994) and Lindwall (R.G. Rees, pers. comm.). Both of these are major commercial cultivars grown in the Queensland and northern New South Wales winter cropping areas, and thus, the northern region barley crop is at greater risk from the disease. The release of Gairdner and later Baudin, derived from either "Franklin sib" or Franklin and both of which carry the *Rph12* gene, has further exacerbated the problem of having a longer-season, higher-rainfall barley crop with a high proportion of susceptibility.

Backcrossing has been one of the more widely used resistance breeding strategies in barley. This is because the technique is simple and has a high probability of developing well-adapted cultivars.

Backcross lines of Prior and Research were developed by the Waite Institute breeding program during the 1940s and were used as parents (Pugsley and Vines 1946; Pugsley 1951). Later, Western Australian breeders used backcrossing to introduce net blotch resistance genes from unadapted material from Ethiopia, Manchuria, and Turkey into locally adapted genotypes (Boyd et al. 1981). CCN resistance is expressed as a dominant trait, and a limited backcrossing approach has been utilized in both South Australian and Victorian programs producing such varieties as Galleon, Chebec, Sloop-SA, and Sloop-Vic.

Recurrent selection is another strategy that can be employed in barley to develop disease-resistant germplasm (Sharp 1985). However, as opposed to backcrossing, recurrent selection techniques increase general resistance levels through several cycles of recombination and selection in which genes of minor effect are accumulated. Major genes must not be present for recurrent selection to work effectively, as they mask the minor gene effects and interfere with the selection process. In barley, recurrent selection has been successfully used to improve resistance to leaf rust and powdery mildew (Parlevliet and van Ommeren 1988). The MSFRS system developed by Falk (1996) is now used routinely in the BBA-West program utilizing both genotypic and phenotypic screening methods. Genetic male steriles were first used in composite crosses (CC XIV) to facilitate hybridization (Ramage 1987 loc. cit.). Ramage gave Eslick the credit for coining the term "male sterile facilitated recurrent selection" (Ramage 1975).

It would seem that the best breeding strategy for long-term control of barley leaf rust would combine most of the systems described in the two previous sections because of the diversity of leaf rust pathotypes and the potential for sexual recombination of virulence genes in the pathogen. Australian barley pathologists and breeders have had to deal with the problem that the alternate host for barley leaf rust, Star of Bethlehem (*O. umbellatum*), is present on the southern end of Yorke Peninsula in South Australia (Wallwork et al. 1992) and is probably responsible for outbreaks of leaf rust in the region.

The NBMMP and AWCMMP

In Australia, researchers participating in the Australian NBMMP (Langridge 1997; Langridge and Barr 2003, "Better Barley Faster: the Role of Marker Assisted Selection" loc. cit.) produced maps for 10 major populations and conducted marker discovery in 44 minor populations (Barr et al. 2001).

The major populations were selected to represent Australian genotypes and key parent lines. They were Alexis × Sloop (DH/recombinant inbred lines [RIL]) (Barr et al. 2003a), Amagi Nijo × WI-2585 (Pallotta et al. 2003), Barque*2 × *H. vulgare* subsp. *spontaneum* 71284-45 (advanced backcross quantitative trait locus [ABQTL]) (J. Eglinton, pers. comm.), Chebec x Harrington (DH) (Langridge et al. 1995; Barr et al. 2003b), Clipper × Sahara (Karakousis et al. 2003b), Galleon × Haruna Nijo (DH) (Langridge et al. 1995; Karakousis et al. 2003c), Mundah × Keel, (Long et al. 2003), Sloop × Halcyon (DH) (Read et al. 2003), Tallon × Kaputar (DH) (Cakir et al. 2003b), Tallon × Scarlett (DH) (D. Poulsen, unpublished data), VB9104 × Dash (DH) (D. Moody, unpublished data), VB9524 × ND11231 (DH) (Emebiri et al. 2003), and Baudin × AC-Metcalfe (DH) (R. Lance, pers. comm.). Maps of Chebec × Harrington and Galleon × Haruna Nijo have been published on the Graingenes Web site along with a Clipper × Sahara map (http://wheat.pw.usda.gov/ggpages/maps.shtml).

One of the NBMMP objectives was to utilize and improve new technologies for Australian barley molecular geneticists. Simple sequence repeat (SSR) markers were introduced into the Australian barley research groups (Ablett et al. 2003; Karakousis et al. 2003a), and their application improved the efficiency and utility of markers in breeding programs.

The availability of a substantial number of markers common across individual barley maps facilitated the alignment of markers across mapping populations and the creation of consensus maps of the barley genome. Mapping data from six populations were combined to produce the first consensus map of 587 markers (Langridge et al. 1995). The majority of the markers were obtained from the Steptoe × Morex,

Igri × Franka, and Proctor × Nudinka maps but were linked together by common markers used to map three Australian populations: Clipper × Sahara 3771, Haruna Nijo × Galleon, and Chebec × Harrington. Overall, the consistency in gene order across the six original maps was high. Discrepancies only occurred where markers were tightly linked and were subject to the random error normal to mapping experiments. A second consensus map was constructed by Qi et al. (1996) from the Proctor × Nudinka, Igri × Franka, Steptoe × Morex, and Harrington × TR306 maps. The most recent barley consensus maps were constructed by Karakousis et al. (2001, 2003d) using restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), and SSR markers.

Among the early successes of the NBMMP were the mapping of genes and markers for resistance to CCN, a major soil-borne parasite of barley in southern production areas. Kretschmer et al. (1997) reported the association of a single dominant CCN resistance gene, *Ha2*, with flanking RFLP markers. The gene mapped to chromosome 2H. Molecular analysis of CCN-resistant Australian cultivars demonstrated that all possessed the *Ha2* gene. A second gene for CCN resistance, designated *Ha4*, was mapped to chromosome 5H in Australian cv. Galleon (Barr et al. 1998). RFLP markers for both genes have been used for selection in the South Australian program (Barr et al. 2001).

QTLs for genes associated with high malt extract were identified and validated in populations between Australian and overseas barleys (Collins et al. 2003). QTLs controlling kernel discoloration were described by Li et al. (2003a). This information has been invaluable to select for a difficult to measure trait associated with weather damage at grain maturation and prior to harvest. Similarly, QTLs controlling preharvest sprouting have been identified and implemented in breeding programs (Li et al. 2003b).

Box et al. (1997) reported evaluation of markers with putative linkage to the gene for hulless grain (*nud*) on chromosome 7H, and marker assays were ultimately developed (A. Box, pers. comm.). Markers for the hulless trait enable the more efficient selection of progeny as heterozygotes from various crossing situations. Further studies identified QTLs affecting coleoptile length on 7H and 5H in Proctor × Nudinka and Galleon × Haruna Nijo, respectively (Box and Barr 2000).

Four QTLs influencing response to boron toxicity were mapped in Clipper × Sahara 3771 (Jefferies et al. 1999). Boron toxicity is a major problem for barley crops in low-rainfall areas in southern Australia. Two QTLs, located on chromosomes 4H and 6H, were associated with reduced boron uptake. The 4H QTLs also appeared to affect root length, dry matter production, and symptom expression. Leaf symptom expression was affected by another QTL on 2H, and suppression of root growth by high boron levels was influenced by a QTL on 3H.

In another series of molecular studies of nutrient–plant relationships, a region on chromosome 4HL was identified as contributing to aluminum/acid soil tolerance in several genotypes (Raman et al. 2001, 2002, 2003). The Australian data matched a locus mapped elsewhere (Tang et al. 2000). Raman et al. (2001) identified closely linked SSR markers for the trait, with the intent of developing an alternative to biological screening assays.

Mel1, a gene affecting the efficiency of manganese uptake in barley, was mapped to the distal portion of chromosome 4HS (Pallotta et al. 2000, 2003). Bulked segregant analysis (BSA) of F_2 plants from the cross Amagi Nijo × WI-2585 was used to identify several closely linked RFLP markers for the trait, which was inherited from Amagi Nijo. Assessment of 95 genotypes used as parents in the South Australian breeding program indicated that two RFLP markers could be used to distinguish the presence of the Amagi Nijo locus in all cases.

Consensus mapping was used to identify 62 SSR markers linked by less than 10 cM to 14 traits of interest to most Australian barley breeding programs (Karakousis et al. 2000). The traits included resistance to powdery mildew (*mlo*), net blotch (*Rpt4*), barley yellow dwarf virus (BYDV) (*Yd2*) and CCN (*Ha4*). The markers were validated with populations from the NBMMP and successfully predicted plant phenotypes. Further analysis with breeding lines

from the South Australian Barley Improvement Program demonstrated that allelic variation was sufficient for the majority of the markers to be used for routine screening.

Coventry et al. (2001, 2003a) examined QTLs associated with yield and yield components in the Australian mapping populations Alexis × Sloop, Chebec × Harrington, and Mundah × Keel. Several yield and yield component QTLs were coincident with chromosomal regions known to be associated with phenology and development, including the 2H *Ppd-H1* photoperiod response gene and another locus on the same chromosome. The *denso* gene is present in the Alexis × Sloop population and was associated with major effects on grain size, yield, and plant height. The phenological development patterns of Australian barleys are an important aspect of adaptation to Mediterranean and East Coast environments. Boyd et al. (2003) enhanced the classical understanding by investigating the molecular genetic control of the basic vegetative period or earliness per se and photoperiod response as they apply to adaptation in the Australian context.

Apart from the loci discussed above, the NBMMP mapped and developed markers for a significant number of agronomic, quality, and disease resistance characteristics. A mixed model approach to better improve the QTL analyses from multienvironment trials was investigated by biometricians (Verbyla et al. 2003). Quality research with respect to molecular approaches was reviewed by Fox et al. (2003). Furthermore, biometrics analyses of quality within breeding programs and through quality laboratories from a strategic point of view were studied by Cullis et al. (2003). Other traits targeted by minor populations included grain staining, acid soil tolerance, preharvest sprouting, boron tolerance, grain size, and water sensitivity. Additional work conducted as part of the NBMMP has been the validation of markers for routine use in Australian breeding programs. For example, Collins et al. (2001) reported the validation of QTLs associated with the expression of malt extract, DP, α-amylase, and β-amylase in six representative breeding populations from the South Australian breeding program. The work clearly demonstrated that QTLs on chromosomes 1H, 2H, and 5H could be used to improve malting quality through the implementation of MAS.

Mapping barley disease resistance genes in Australia

As a demonstration of the scope of the NBMMP activities, disease resistance loci targeted in the major mapping populations are described by Williams (2003) and compared with other known genes for resistance. In a more recent phase of barley improvement in Australia, significant attention has been placed on the development of elite malting and feed barleys with multiple disease resistances pyramided from complex crosses.

Minor mapping populations were also constructed by the NBMMP and were analyzed by BSA to identify markers linked to resistance to scald (Genger et al. 2003), powdery mildew (Paris et al. 2003), spot-type net blotch (Williams et al. 2003), net-type net blotch (Cakir et al., 2003a, b; Gupta et al. 2003; Raman et al. 2003), common root rot, stem rust, leaf rust (Park et al. 2003), crown rot, covered smut, spot blotch, and the exotic quarantine diseases, such as stripe or yellow rust (Cakir et al. 2003c).

Scald (*R. secalis*) has also been a significant target for marker development as both the pathotype structure and the genetics of resistance are complex, making conventional selection techniques difficult. *Rh*, a gene conferring resistance to scald, was mapped to chromosome 3HL by BSA with random amplified polymorphic DNA (RAPD) markers in a set of near-isogenic lines (Barua et al. 1993). It has been suggested that the 3HL locus, now designated *Rrs1*, is multiallelic as is scald resistance in several other unrelated genotypes and has been mapped to the same location (Williams et al. 2001). One of the alleles confers resistance to 22 of the 23 known Australian *R. secalis* pathotypes. The multiallelic nature of *Rrs1* has significant strategic implications in designing crosses to combine scald resistance genes.

Pathotype-specific seedling resistance to net-type net blotch was mapped to chromosome 6H in a DH population derived from the Australian cross Tallon × Kaputar (Cakir et al. 2001, 2003a).

The resistance was inherited from Kaputar, a reselection of the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) cv. Arupo. Further work is being carried out on the population to identify the genetic location of adult plant resistance to net-type net blotch inherited from Tallon.

A dominant resistance gene to spot-type net blotch from Australian barley cv. Galleon was mapped to chromosome 7H and was designated *Rpt4* (Williams et al. 1999). Flanking RFLP markers were identified for the locus. Validation of the marker demonstrated greater than 90% accuracy in predicting the occurrence of resistance to spot-type net blotch. Karakousis et al. (2000) subsequently identified SSR markers in the vicinity of *Rpt4*, which have since been used for selection in at least three Australian barley breeding programs.

Other reports of marker development for barley disease resistance genes include identification of specific markers and/or QTL for resistance to BYDV (Collins et al. 1996; Paltridge et al. 1998). Stripe, leaf, and stem rust resistance genes have all been targets for marker development and genetic study in barley. In general, the development of markers has been seen as leading toward the implementation of better breeding strategies to develop long-term control of the diseases. The information obtained in these studies has contributed to the development of a greater depth of knowledge of the genetics and mechanisms of rust resistance.

Resistance to South American stripe rust pathotypes was mapped to chromosomes 2H and 5H in Australian cv. Tallon (Cakir et al. 2001, 2003c). The data were obtained from a field assessment of the NBMMP Tallon × Kaputar DH population at CIMMYT. Stripe rust resistance was also mapped in the NBMMP Arapiles × Franklin mapping population, with the resistance derived from Franklin (Cakir et al. 2003c). The Franklin resistance was also located on 2H and 5H. The data suggested that the two regions were common to both cultivars. It was suspected that both Tallon and Franklin had inherited the same resistance gene from Triumph, situated on chromosome 5H. The location of the QTL was not a perfect match for the 5H resistance mapped by Chen et al. (1994), and further study was required to determine the relationship between the loci. The resistance QTL on 2H from Tallon and Franklin appeared to be located in the vicinity of a gene related to plant maturity (Cakir et al. 2003c). It therefore appeared that differences in maturity may have influenced the expression of resistance in the mapping populations at the time of observation.

Several leaf rust resistance genes from the *Rph* series have been mapped and linked to molecular markers including *Rph2*, *Rph9/12*, and *Rph16* (Borovkova et al. 1997a,b, 1998; Graner et al. 2000). Poulsen et al. (1995a) identified an RAPD marker linked to the *RphQ* resistance gene from barley accession Q21861. RFLP and sequence tagged site (STS) markers were subsequently used by Borovkova et al. (1997a,b) to map *RphQ* to the centromeric region of chromosome 5H, in the same region as *Rph2*, and it was postulated on the basis of marker and phenotypic data that *Rph2* and *RphQ* were allelic. Nine RAPD markers, two RFLP markers, CDO749 and Rrn2, and one STS marker, ITS1, were identified as closely linked to *RphQ* (Borovkova et al. (1997b).

Molecular genetic marker implementation in Australian barley breeding programs

One of the major difficulties encountered in the implementation of marker-assisted breeding in Australian barley breeding programs has been the initial availability of only a limited number of suitable markers. Although RFLP markers were used fairly extensively for CCN resistance and boron tolerance by the Waite Institute program in the late 1990s (A. Barr, pers. comm), other Australian programs have taken longer to routinely adopt the technology. This has been partly due to time requirements of developing and validating suitable polymerase chain reaction (PCR) markers. Most Australian programs are not fully equipped for the large-scale operation of RFLP systems. However, with the maturation of the NBMMP, the situation is changing and all of the

programs are reaching the stage where routine marker implementation can occur.

The use of markers in Australian barley breeding programs was succinctly described in two review papers (Barr et al. 2000, 2001). The majority of those markers were developed and/or validated within the NBMMP strategic initiative.

It was also reported that MAS had been used in Australian breeding programs to select for malt extract QTL on chromosomes 1H, 2H, and 5H (Barr et al. 2001). In a particularly elegant example, the South Australian program had used selection for these loci in conjunction with a "defect elimination" backcross breeding strategy to develop improved versions of the cultivars Sloop (Barr et al. 2000) and Gairdner (Barr et al. 2001). Markers were used to assist recovery of the recurrent parent genomes while tracking genes for BYDV resistance (*yd2*), spot-type net blotch resistance (*Rpt4*), and CCN resistance (*Ha2*). After the single genes had been introgressed, selected lines were intercrossed with the aim of developing versions of the cultivars possessing all of the target genes.

However, the highest rate of usage of MAS in Australian barley breeding has been the enrichment of complex crosses by the South Australian program (Barr et al. 2000). By selecting F_1 plants with markers linked to a range of specific traits, the program is making more efficient use of material derived from three- and four-way crosses. Only progeny lines with desired combinations of loci are selected for field assessment. F_1 plants from complex crosses requiring additional crossing can also be identified. The new malting variety Flagship from the University of Adelaide program, accredited in 2006, is a testimony to the efficiency and expediency of utilizing this approach.

Marker use for the selection of QTL is also progressing in the Australian breeding programs. Collins et al. (2000, 2001) validated QTL related to malt extract, DP, α-amylase, and β-amylase and recommended markers for routine use in the southern region breeding programs. Validation of the markers in the northern and western region programs has also commenced.

It is anticipated that the adoption of marker technology by the Australian barley breeding programs will rapidly increase over the next few years, especially with increased identification of PCR-based markers linked to traits of interest to the individual programs.

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NEAR EAST, NORTH AND EAST AFRICA, AND LATIN AMERICA

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NEAR EAST

Barley is grown on about 8 million hectares in 16 different countries in the Near East. However, 98% of the area is in six countries, namely, Turkey, Iran, Syria, Iraq, Afghanistan, and Pakistan (http://faostat.fao.org/, searched in 2008). There is a large variability in the types of barley grown in this area. Two-row types predominate in Syria, Turkey, the northern part of Iraq, and the rainfed areas of Iran, while six-row types predominate in Pakistan and Afghanistan and in the irrigated areas of Iraq and Iran. In most of the dry areas of Syria and Iraq, and in some of the rainfed areas of Northern Iran, farmers have a strong preference for black-seeded types. Most of the barley grown in the areas is either winter or facultative and with a strong photoperiod sensitivity. With the exception of the irrigated areas, barley is predominantly grown in the semiarid and arid areas where rainfall is too low and erratic to grow wheat. In the Near East, the predominant use of barley is as animal feed. However, it is also used as food in Iraq and Iran.

Syria

Formal agricultural research in Syria began in the early 1940s with the establishment of experiment farms at Deir Elhajar and Kharabo, close to Damascus. After independence, in 1946, various Ministry of Agriculture and Agrarian Reform (MAAR) directorates (such as horticulture, forestry, animal resources, and plant protection) began to conduct limited agricultural research.

In 1964, the Directorate for Agricultural and Scientific Research (DASR) was established, taking on responsibility for research activities under MAAR. In 1982, DASR formed a technical committee to develop new varieties of cereals, legumes, and forage crops and to coordinate research activities with the Department of Agricultural and Scientific Research (DASR), the International Center for Agricultural Research in the Dry Areas (ICARDA), and the Arab Center for Studies of the Arid Zones and Dry Lands (ACSAD). This could be considered as the beginning of the barley breeding program, with training programs conducted on crossing techniques and breeding methods.

In 2002, the nine existing agricultural research entities under MAAR were merged to form the General Commission for Scientific and Agricultural Research (GCSAR), which is now

responsible for all the breeding programs (wheat, barley, chickpea, lentil, faba bean, cotton, etc.).

The breeding methods used vary from the introduction of international nurseries from international centers such as ICARDA and from other countries and their direct release as cultivars (examples in barley are "Furat 1" and "Furat 2"released directly from ICARDA's international nurseries); the introduction of segregating populations from ICARDA; the execution of crosses followed by classical pedigree and the induction of mutations (wheat, barley, chickpea, and soya beans). Plant breeding programs give special importance to traits such as drought resistance, earliness, disease resistance, and high yield. The barley breeding work is conducted at the stations and centers of GCSAR, and neither foreign companies nor the private sector conducts plant breeding activities in Syria.

The final stage of the breeding program is the on-farm trials, which are used to eventually recommend varieties for release. As a result of breeding work since the early 1970s, seven barley varieties have been released, but their rate of adoption is very low partly because of their lack of adaptation to the farmers' real conditions and partly because of the limited capacity of the General Organization of Seed Multiplication (GOSM), which is only able to produce 10% of the barley seed required for planting, while the corresponding figure in the case of wheat is 60% (Al-Ahmad et al. 1999).

Jordan

Formal agricultural research in Jordan began in the early 1950s with the creation of the first agricultural research station in the Jordan Valley. During that decade, various other research stations were established throughout the country. Research was initially carried out by the technical divisions of the Ministry of Agriculture, which were transferred to the Department of Scientific Agricultural Research when it was created in 1958, and then in 1970, the department was merged with the Ministry's extension unit to form the Department of Scientific Research and Agricultural Extension. The Ministry of Agriculture was restructured in the mid-1980s, resulting in the creation of the National Center for Agricultural Research and Technology Transfer (NCARTT). In 2007, NCARTT was renamed as the National Center for Agricultural Research and Extension (NCARE). The Center developed over the years, in part through the National Agricultural Development Project (NADP), which was cofunded by the United States Agency for International Development (USAID) and the Government of Jordan.

Agricultural research within the higher education sector began in the early 1970s with the establishment of the Faculty of Agriculture (1972) and the Marine Science Station (1974) at the University of Jordan (UoJ). Even if research in barley breeding is occasionally conducted at the university, NCARE has the main responsibility for breeding and releasing varieties (Taimeh and Sunna 1999).

The barley breeding program in Jordan is entirely based on introduced germplasm mostly from ICARDA and ACSAD. The material is tested in two research stations for 4 years before being tested in on-farm trials and eventually recommended for release. In addition to the local landrace (two-row), the most popular improved variety has been Rum (six-row). Recently, the program has released three additional varieties, namely, Athroh (six-row), Yarmouk (Esp/1808-4L//Harmal, two-row), and Muta'a (Roho/A. Abiad/6250, two-row).

During the last 4 years, the barley breeding program in Jordan has gradually been converted into a participatory program following the methodology described in Ceccarelli and Grando (2007).

Iran

Agricultural research in Iran dates back to 1925 when the Razi Institute, the "father" of the current Razi Serum and Vaccine Research and Production Institute (RSVRI), began its research activity in the district of Karaj, about 35 km west of Tehran. In 1926, the first agricultural college, affiliated with the Ministry of Agriculture, was founded in Karaj. Important changes occurred in the 1950s

and 1960s. New colleges of agriculture were established and institutes were created with technical support from international agencies, such as the United Nation's Food and Agriculture Organization (FAO). Among these, the Seed and Plant Improvement Institute (SPII) was established in Karaj in 1959 to conduct research and seed multiplication on the main crops (cereals, oil crops, cotton, rice, horticultural crops, forages, etc.). In 1991, the Dryland Agricultural Research Institute (DARI) was established in Maragheh (Azerbaijan province) to relieve SPII from its heavy responsibilities and to implement extensive research on some of the major commodities and noncommodity items of economic importance. In particular, DARI conducts basic research on mechanisms of abiotic (cold and heat) tolerance from the physiological, genetic, and agronomic points of view, and on developing strategies to overcome these stresses and to increase productivity.

Barley breeding started in Iran around 1930 with the exploitation of local germplasm. This was followed in the 1950s by the beginning of hybridization of local germplasm with exotic germplasm and the extensive use of the pedigree method, bulking in F_6 and multilocation testing in about 22 stations throughout the country. The final testing is done in the Uniform Regional Yield Trials, which are specifically targeted to the warm, cold, and rainfed regions of the country.

After the establishment of DARI, SPII kept the responsibility for barley breeding in the irrigated areas of the country, while DARI focused on the rainfed areas (Roozitalab 1996).

Both breeding programs (SPII and DARI) collaborate actively with ICARDA: they receive, test, and use both international nurseries as well as special nurseries specifically targeted to the growing environments in Iran. About 80% of the barley germplasm is either a direct introduction from ICARDA or is derived from crosses with the ICARDA germplasm.

Iraq

In the 1920s, a Directorate General of Agriculture, affiliated with the Ministry of Economics and Transport, started agricultural research activities and established the first experimental stations at Abu Ghraib, near Baghdad and at Neinevah, near Mosul.

In the 1940s, agricultural research activities were conducted by the Directorate General for Agricultural Research and Extension (DGARE) until in 1958 the Directorate General for Agricultural Research and Projects (DGAREJ) was established. During the 1970s, research activities were expanded as several specialized research stations and centers were established including the Iraqi Atomic Energy Commission (IAEC). In 1980, the State Board for Applied Agricultural Research (SBAAR) was established, which in 1987 was renamed as the State Board for Agricultural Research and Water Resources (SBARWS). In 1990, SBARWS was terminated and replaced by the State Board for Agricultural Research (SBAR) and the Center for Water and Soil Resources (CWSR).

Barley breeding in Iraq used to be divided into two subprograms: one addressing the irrigated areas around Baghdad and based in Abu Ghraib, and the second addressing the rainfed areas of the North, which, from an agroecological viewpoint, are very similar to the areas in Northeast Syria.

The first subprogram, based on six-row types mostly bred for dual purpose as cattle feed, fully exploited the segregating populations received from ICARDA and released successful cultivars such as IPA 7, IPA 9, and IPA 265. The second subprogram, although addressing an area where farmers' preferences are for two-row black-seeded barley, attempted the introduction of six-row types. The major success of the program was the release of Rihane-03, a straight introduction from ICARDA, which in the late 1990s was cultivated on about 250,000 ha.

Currently, due to the war since 2003, Iraq is conducting a limited amount of variety trials using material introduced from ICARDA.

Yemen

Agricultural research in Yemen dates back to the 1940s during the rule of the British Colonial Government. El-Kod Research Station was established in 1955 about 50 km from Aden, and

Seiyun Research Center was established in Wadi Hadramout in 1972 to cover the mid-altitude region of South Yemen. Research activities were later developed in most parts of the country, especially through numerous projects supported by the United Nations Development Program (UNDP)/FAO and the International Development Association (IDA). They were introduced in North Yemen starting in 1970, later developing into a central research station in Taiz in 1978. In 1980, the Ministry of Agriculture and Agrarian Reform of South Yemen created the Department of Research and Extension (DRE), based at Aden, which was transformed in 1986 to the Directorate of Research and Extension (Ministry of Agriculture and Fisheries 1989). At the same time, the Agricultural Research Authority (ARA) was established in Dhamar in North Yemen for conducting research and applied studies to improve agricultural production. In 1990, after the unification of North and South Yemen, the Agricultural Research and Extension Authority (AREA) was formed by merging DRE and ARA and their respective research centers and stations.

As in the case of other countries in the region, Yemen's barley breeding program is based on introductions from international centers, particularly ICARDA and ACSAD, and more recently from the International Atomic Energy Agency (IAEA). Most of the breeding work takes place at the research station of Al Erra, just outside the capital city Sana'a. Two varieties, introduced through ICARDA nurseries, Beecher and Arivat, were released in 1986. In 1999, ICARDA and AREA conducted a 2-year project introducing participatory plant breeding in Yemen: two barley varieties were adopted by farmers at the end of the project, and currently, AREA is using participatory methodology to evaluate barley germplasm obtained by mutation breeding from IAEA.

NORTH AFRICA

Cereal cultivation in North Africa dates back to ancient times. It seems that it preceded the Phoenician colonization, which took place about the twelfth century BC. The founding of Utica at the delta of the Médjerda valley, particularly suited to wheat cultivation, had the only objective of supplying food for the long sea route from Tyrus to Guadalquivir. Carthage eventually replaced Utica as the export harbor near the present site of Tunis.

When the Roman Empire, after destroying Carthage, spread over North Africa, cereal cultivation was already established in the plateau of Setif (Algeria) and in Numidia, which was later occupied by the Romans because of the soil fertility and the abundance of cereals they could provide. "The soil of Africa," wrote Pliny, the Elder in the first century of our era, "has been given as gift from Ceres: oil and wine were almost refused; all the glory of the country is in the harvest."

Today, barley is second only to wheat in the five North African countries where it is grown on about 3.5 million hectares (average of 2002–2006) of which about 60% is in Morocco (http://faostat.fao.org/). It is predominantly grown in the semiarid and arid areas where rainfall is too low and erratic to grow wheat. These include the Northwest coast of Egypt, where high humidity allows growing a barley crop with about 100 mm annual rainfall, and in some provinces of Western Algeria. In the latter, barley is an established crop because of high demand for livestock feed.

Contrary to the Near East, the majority of the barley landraces and improved varieties grown in North Africa are six-row with very few exceptions. The release of superior two-row cultivars has not been followed by any significant adoption because of religious reasons (two-row types are associated with beer production).

As in the Near East, the main use of barley is as animal feed. However, the use of barley as human food is much more widespread than in the Near East, and in fact, Morocco has the world's largest per capita human consumption of barley. In recent times, the consumption of barley as human food has increased also in Algeria and Egypt (Grando and Gomez Macpherson 2005).

The history of barley breeding in North Africa varies among the countries, but, as already described in the case of the Near East, in all countries is much more recent than for wheat. As a

consequence, and for many years, barley and wheat breeding were done by the same scientist, in the same research stations, and with the same philosophies and methodologies. This situation did not consider that the two crops are grown in distinct agroecologies by farms differing in size, by farmers differing in wealth with access to differing amounts of information.

Tunisia

The history of barley breeding in Tunisia is emblematic of most of North Africa and the Near East. In Tunisia, cereal breeding started with wheat at the beginning of the last century in the then Service Botanique et Agronomique de Tunisie (SBAT). Wheat breeding evolved from the utilization of the variability existing within local varieties of durum wheat, followed by mass selection until 1930, when the first crosses were made. It was about at this time that bread wheat was introduced from France and Algeria. Later in the 1970s, the introduction of germplasm from Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) occurred, when several varieties were released, both using direct introduction and selection from segregating populations. The introduction of these varieties was accompanied by the wider use of inputs such as fertilizers and chemical weed control.

The barley breeding programs are much more recent as the local varieties were used until 1950 when "Martin" and "Cérès" were introduced in the North of the country. Problems with seed production of these two varieties did not allow replacing the local germplasm in the center and the south of the country.

The first serious attempt to start a barley breeding program was made in 1973 (M. Harrabi, pers. comm.) with crosses and selection of early material for the semiarid environments. The materials obtained were tested in central Tunisia and in the high plateau of the Northwest. With the establishment of ICARDA in 1976, Tunisia, where ICARDA had a regional office, was used as a testing site for ICARDA's barley breeding program, thus receiving more genetic material than other countries. A large number of lines were screened, and already in 1984, the first promising lines, such as ER/Apm, Roho, and WI 2198 (from the Waite Institute in Adelaide, South Australia) were identified and eventually released in 1985 with the names of Faiz, Roho, and Taj, respectively. The collaboration with ICARDA has continued over the years with the exchange of germplasm (both finished lines and segregating populations) and the release of varieties based on those nurseries.

Algeria

Wheat (bread and durum) and barley are the main winter cereals in Algeria. Among the wheats, it is possible to find small amounts of *Triticum turgidum*, *Triticum dicoccoides*, and *Triticum polonicum* in the local landraces of wheat and *Triticum spelta* particularly in the oases.

As in Tunisia, farmers have initially grown local landraces. Cereal breeding started in 1905 by Prof. L. Ducellier, who produced the first pure lines of durum wheat. After his premature death, his work was continued by his students at the Station Centrale d'Essais de Semences et d'Amélioration des Plantes de Grande Culture de Maison-Carrée and by other botanists as Trabut, Bœuf, and Laumont.

The history of breeding can be divided into two periods:

1. The first period (1900–1962) was characterized by the utilization of the existing material within local landraces with collections and characterization of durum wheat. Laumont and Ducellier started from 1930 the production of varieties selected by mass selection procedure and then the first crosses were made; these crosses aimed at higher productivity and better quality.
2. The second period (1962–1990) was characterized by the introduction of germplasm from Spain, Italy, Greece, Syria, Russia, and the United States through European projects, FAO, CIMMYT, and then ICARDA. Several varieties were released either by direct introductions or selection from segregating populations. These varieties were characterized by the need for more inputs

(fertilizers and weed control) than landraces. During this second stage, a limited crossing program also started.

This first stage was followed by the introduction of durum wheats and by hybridization between the local landraces and the introduced varieties. Already at that time, the Station Centrale de Maison-Carrée could count on a network of a dozen regional experiment stations corresponding to the main cereal regions of Algeria.

The improvement of bread wheat, which started being grown in Algeria only in 1930, followed the same route as durum wheat.

Similarly, in the case of barley, the initial cultivation of mixtures introduced from the Near East was followed by pedigree selection, which led to the development of locally adapted cultivars such as Saida 183 and Tichedrette, the latter particularly adapted to the mountainous areas. The majority of cultivated barley has white kernels; however, farmers remember cultivating black types considered resistant to drought. Malting barley did not become important in Algeria because of its susceptibility to shattering and because of the indifference of the local industry.

As a result of the collaboration with ICARDA, several varieties were released, but the two locally adapted types are still the most widely grown.

At the moment, Algeria's barley breeding program is almost entirely based on ICARDA's germplasm and in the last 3 years has started being organized in a participatory way (Ceccarelli and Grando 2007).

Morocco

The beginning of barley breeding in Morocco dates back to 1920 and was based on the improvement of local landraces and on the introduction of two-row foreign varieties. Among hundreds of cultivars from Europe, the United States, and Australia, about a dozen were selected including Chevalier, Hannchen, Combesse, Guldkorn, Princesse, and Prior (Grillot 1939). Selection within the local populations initially identified two lines, 077 and 071, later named Rabat 77 and Merzaga 71, respectively (Saidi et al. 2005).

The introduction of two- and six-row varieties characterized barley breeding until 1970 and led to the release of cultivars such as Arig 8, Tamelalt, Asni, and Azilal. Starting from 1980, the introduction of collections of barley from the United States was the beginning of another phase of barley breeding with the objective of improving earliness and harvest index. The germplasm generated in this period was used for multilocation testing to identify widely adapted lines (Amri 1993). Selection was conducted in the research stations for disease resistance and yield. A dozen varieties were released, but despite their performance, they were grown on no more than 5% of the barley growing area. The poor adaptation to farmers' conditions was considered to be the cause of the lack of adoption (Saade 1994). This imposed a change of strategy, which consists of breeding for wide adaptation in the case of favorable environments and for specific adaptation in the case of marginal environments. In the latter, the barley breeding program in Morocco is now also using participatory plant breeding.

Libya

Agricultural research in Libya dates back to the early part of the twentieth century during the Italian colonial era, when the "Centro Sperimentale Agrario e Zootecnico della Libia" at Sidi El Masri near Tripoli was established to serve the Italian agricultural settlers.

In the early 1950s, the Ministry of Agriculture was established and agricultural research became affiliated with the Directorate of Plant and Animal Production, with major changes in goals and organization. In 1981, the General People's Committee (Minister's Cabinet) initiated the National Authority for Scientific Research (NASR) to formulate and supervise the national research policy, to fill in gaps in research not tackled by any existing research institutes and centers, and technically to coordinate research carried out at research centers. New research centers were established under the umbrella of NASR, including the Agricultural Research

Center (ARC), where most of the plant breeding is conducted. The Center has the following four "Regional Agricultural Research Centers" (RARC), supported by 13 experimental stations:

1. The RARC for the western area (which encompasses the largest part of agriculture and of the population) located at Tajura, 20 km east of Tripoli, has six research stations and three experimental sites and focuses mainly on fully irrigated and supplementary-irrigated crops such as vegetables, cereals, fruits, and forages, with some emphasis on rainfed agriculture as well.
2. The RARC for the eastern area, located at Al-Marj, 1100 km east of Tripoli, covers the areas from Sirte to the Egyptian borders (four stations)and focuses on rainfed agriculture (cereals, legumes, and fruits), forestry, and range.
3. The RARC for the central area, located in Musrata, 200 km east of Tripoli, covers the areas from Khomes to Sirte (two stations). Its 10 researchers are working mainly on salinity research.
4. The RARC located at Sebha (700 km south of Tripoli) covers the southern area of the country (one station) and works in the areas of hot/dry climate and fully irrigated crops (cereals, legumes, forages, and palm trees).

The breeding programs are mostly based on testing germplasm introduced from international organizations: in the case of barley, the main supplier of germplasm has been traditionally ICARDA, which, as described below, in the last 10 years has begun distributing very early germplasm particularly suitable to the short growing season of most areas where barley is grown in Libya. Eight varieties have been released between 1992 and 2005.

Egypt

The first school of agriculture in Egypt was established in 1869 and the first directorate of agriculture in 1875.During the nineteenth century, agricultural research was carried out by the Egyptian Royal Society, and as early as 1897, a number of experimental farms were established at various locations. In 1910, the Agricultural Authority was established with the responsibility for conducting research and producing seed, extending methods in crop production to farmers, especially soil analysis and use of fertilizers, pest control, and production of scientific and technical publications. The Ministry of Agriculture was established in 1913. In 1957, research departments were formed, and one of the first was the Department of Plant Breeding (Rivera and Elkalla 1997).

The Ministry of Agriculture has undergone several reforms in the past decades. It has grown from only 7 departments in 1913 to 28 in 1950 to 194 in 1963 and to 92 at present, dealing with various aspects of agricultural production. Early on among the major departments were Agriculture, Horticulture, Plant Protection, Soil, Animal Production, Veterinary Laboratories, and Seed Production. These research departments were reorganized in 1971 into one research body within the Ministry of Agriculture and Land Reclamation named the General Authority for Agricultural Research, which was later (1983), renamed the ARC, and evolved as the major institution for agricultural research and extension in Egypt until today and has the major responsibility for crop breeding.

Barley breeding in Egypt has a very long history, which started more than a century ago. This has attracted the attention of international organizations for the Egyptian genetic resources, especially in relation to adaptation to abiotic stresses such as drought, salinity, and poor soil fertility. Many landraces and local varieties have been collected from the desert areas of the Northwest Coast, Siwa, and Sinai. These collections are maintained in gene banks and utilized by different barley breeding programs for their valuable attributes.

Until the beginning of the last century, farmers relied on their own local varieties developed by selecting superior plants from existing landraces. Each tribe had its own local variety (known as Bedouin variety) selected and maintained by the members of the tribe.

The first organized efforts in barley breeding started with the establishment of the Department of Plant Breeding at Bahteem Agricultural Research Station in 1898 as a part of the Sultanic Agricultural Organization (Royal Agricultural Society). Until the 1940s, barley breeding relied mostly on the collection and selection from local varieties and landraces known as "Baladi varieties." These efforts resulted in the selection of several high-yielding and disease-resistant varieties, such as Baladi 16, Bahteem 52, and Giza 24. Starting in the 1940s, barley breeders started to introduce germplasm from other countries, such as Abyssinia 12, Hungaria 1, and Palestine. Selection from this introduced germplasm resulted in the release of two new varieties, Giza 68 and Giza 73, in 1948. In 1956, a new extremely early barley variety (Hybrid 100) was developed, which is also known as Saharawi 100. This variety has been extensively used in the breeding program for earliness until now. It was the first barley variety developed through crossing between Baladi-16 and Atsel. Another important variety was California Mariout, used side by side with Sahrawi 100 in the rainfed areas of Egypt.

Barley breeders continued to cross local varieties and introductions to produce new varieties with better adaptation to low rainfall areas. For example, the variety Giza 117 was produced in 1959 as a selection from the cross Baladi 16 × Palestine 10, and the variety Giza 119 and Giza 121, produced in 1973 and 1980, respectively, as selections from the cross Baladi 16 × Gem.

Another wave of barley varieties was developed in 1980s, such as Giza 121, CC-89, Giza 123, and Giza 124. The last two varieties replaced all the old varieties because of their high yield potential, especially in saline soils and under heat stress conditions, respectively.

Barley breeding activities focus on screening of barley genotypes for drought and salinity tolerance and for disease and aphid resistance as well as heat tolerance. The germplasm is based on introductions from ICARDA and on local breeding material developed from local crosses.

The program addresses three major environments, that is, the rainfed areas of the Northwest Coast and Sinai, the new reclaimed land (irrigated) and saline soils, and the old irrigated areas. The program follows a traditional pedigree method with yield testing conducted on station in micro and macro yield trials in several locations. Seed multiplication is conducted at Sakha research Station under irrigation.

In collaboration with the Desert Research Center (DRC), ICARDA has conducted for the last 8 years a participatory barley breeding program in the Northwest Coast: this involves eight villages and has so far generated five varieties, which are being multiplied by farmers (by law, they cannot be officially multiplied), and this is so far the main factor limiting their wider adoption.

EAST AFRICA

The two most important countries for barley cultivation in East Africa are Ethiopia and Eritrea, two of poorest countries in the world. In both countries, agriculture is almost the only source of living for the majority (85%) of the population. Since Eritrea gained independence from Ethiopia in 1991, the history of barley breeding was common to the two countries before that date.

Barley is one of the most important staple food crops and has a significant role in the diet of many millions of people. Cultivation of slightly more than 1 million hectares of barley occurs in the highlands of the two countries. The farmers of both countries grow barley because it has several advantages over other cereals: (i) barley can be grown in marginal areas where the choice of other cereals is limited; (ii) it offers the farmer an earlier crop harvest than most cereals, providing relief of food shortages, which frequently occur during the long rainy season; (iii) it has better stability of production over other cereals; (iv) it is a dependable food crop as it is grown in different seasons and production systems; (v) it is the preferred crop for the preparation of traditional drinks and beer; and (vi) its straw is a good source of feed and bedding for animals and thatching of roofs.

Barley is believed to have been cultivated in Ethiopia and Eritrea as early as 3000 BC. (Gamst 1969). Both countries, but Ethiopia in particular,

have diverse climates, soils, topography, social environments, vegetation cover, and livestock. The long history of barley cultivation and the diverse agroecological and cultural practices have resulted in a large number of landraces and traditional agricultural practices. All the different types of barley are grown: hulled, hulless, six-row, two-row, irregular forms, dense, lax, hooded, long awn, short awn, and rough and smooth awn (Asfaw 1988). In addition, barley landraces vary in their characteristics such as maturity, seed color, seed size, seedling vigor, straw strength, and in disease and insect pest resistance. Landraces are also grown in mixtures, often with other crops; the best known of these mixtures, called *hanfets*, is a barley–wheat mixture very popular in Tigray, the Northern part of Ethiopia, and in the highlands of Eritrea (Woldeamlak et al. 2001, 2008).

Ethiopia and Eritrea are two of the centers of diversity of barley, but also of other important crops such as durum wheat, and therefore they have a very long history of barley breeding if we also include the millennia of selection conducted by farmers.

Ethiopia

In Ethiopia, barley research started in the early 1950s by the former Alemaya College of Agriculture and Mechanical Arts at its experimental station in Debre Zeit, with the evaluation of landraces and introduced nurseries. After the establishment of the Institute of Agricultural Research (IAR) in 1966, barley research was transferred from Debre Zeit to Holetta Research Center.

After the restructuring of agricultural research with the establishment of a federal center (Ethiopian Agricultural Research Organization [EARO]) and of agricultural regional centers, barley research has been coordinated from the Holetta Research Center in collaboration with the regional research centers (at Kulumsa, Ambo, Sheno, Adet, Sirinka, Mekele, and Sinana), and other Ethiopian institutions. International collaborations include FAO, the United States Department of Agriculture (USDA), and the Swedish Agency for Research and Education Cooperation (SAREC). Since the mid-1970s, the collaboration with ICARDA has been valuable for the exchange of information and germplasm and for capacity building.

Between 1970 and 1990, nearly 14,000 local landraces were evaluated. Most of the entries were found susceptible to scald (caused by *Rhynchosporium secalis* Oud.), net blotch (caused by *Helminthosporium teres* Sacc.), spot blotch (caused by *Helminthosporium sativum* Pum.), leaf rust (caused by *Puccinia hordei* Otth.), and lodging. However, from this work, some outstanding varieties such as Shege, Misratch, and Abay were released in late 1990s (Lakew et al. 1997; Yitbarek et al. 1998).

Between 1966 and 2001, nearly 30,000 exotic entries were evaluated. Most of them were found to be highly susceptible to scald, net and spot blotch, and barley shoot fly (*Delia flavibasis*) and had poor plant vigor and small grains. About 6% were selected for further study. In the early 1970s, the major contributors of germplasm were the FAO Near East Regional Program, the USDA, and the Arid Land Agriculture Development (ALAD), and from the mid-1970s, ICARDA. Germplasm has been also received from Brazil, Colombia, the former Czechoslovakia, Egypt, India, Kenya, Peru, Sweden, and the former Republic of Yugoslavia (Gebre et al. 1996). From these efforts, one hulled variety, AHOR 880/61, was released, and some other elite lines are still being used in the national crossing program as sources of genes for desirable agronomic traits such as grain quality and stiff straw and for disease and insect pest resistance.

Between 1974 and 2001, over 1600 crosses (single, double, and three-way) were made between exotic and local germplasm and among local germplasm to improve their resistance to lodging and to major diseases such as scald, net blotch, spot blotch, and leaf rust. F_2 progenies have been evaluated at a number of locations in the country. Only one outstanding hulled barley variety, HB 42, has been identified from this program.

Currently, some regional centers have started programs of participatory barley breeding.

Eritrea

In Eritrea, barley research started with a germplasm collection conducted by the staff of the Department of Agricultural Research and Human Resource Development (DARHRD) in 1997 (shortly after independence) and with the repatriation of local germplasm held in the Ethiopian Gene Bank. Most of this work was funded by the government of Denmark.

The barley breeding program started formally in 1998 with the evaluation of the landraces collected in 1997 and with targeted crosses made at ICARDA. In 1999, the first field trials were conducted in three villages, and three potential varieties were identified. After an interruption caused by the war with Ethiopia, the barley breeding program started again and, since 2004, was funded by a project of the Challenge Program on Water and Food of the Consultative Group on International Agricultural Research (CGIAR).

In this second phase, after the systematic evaluation of all the landraces available in the gene bank, and of a number of experimental hanfets, the National Agricultural Research Institute (NARI), the new denomination of DARHRD, has started the field testing of new breeding material derived from crosses made at ICARDA between local landraces and exotic germplasm.

LATIN AMERICA

Barley was introduced in the Americas by Columbus as early as his second trip to the New World. Barley was first planted in 1493 by the Spaniards who stayed at Isabela, Puerto Rico and from there it was introduced to Mexico and the United States. It is hard to document the first barley and wheat crops in South America, but it most probably was in the Fort of Sancti Spiritus in 1527, in the present day province of Santa Fe in Argentina (Arias 1995). From there, it spread to the Andes and the countries that share that mountain range. The first scientific works in barley and wheat in South America were carried out starting in 1912 in Uruguay by Drs. Alberto Boerger and Enrique Klein, selecting pure lines resistant to leaf rust (gene Pa7) from populations being used by farmers.

Barley production areas in Latin America can be divided in two main groups, based on ecological regions and end use: (i) the Andean region, with Bolivia, Ecuador, Colombia, and Peru, where the main uses of barley are for food, feed, and forage; and (ii) the countries of the Southern Cone of South America, with Argentina, Brazil, Chile, and Uruguay, plus México, where almost exclusively barley is made into malt for beer production. The production in the first group is stable, whereas in the second, the production is increasing due to the higher demand of malt because of the rise in consumption of beer and malt derivatives in the continent and worldwide.

After the establishment of the first barley research program in the subcontinent in La Estanzuela, Uruguay, research has been carried out at different times in different countries in the region. Barley breeding at public institutions has always been an appendix to wheat breeding and has always counted on less economic and human resources. Despite this, an important volume of research and selection has been done in the Latin American countries where barley is cultivated at commercial scale (Arias 1995). At present, several public and private long-term, well-established, and successful breeding programs are working in Argentina, Brazil, Chile, Ecuador, México, Peru, and Uruguay. As the main varieties released were for malting, very few local genetic resources have been used in crossings because they were forage and feed barleys. Almost all cultivated barleys have spring habit, except in Chile, where some winter or facultative barleys have been released. Almost all cultivars are covered, except in Ecuador and Peru, where a few hulless varieties have been released.

In the Andean countries, epidemics of stripe rust (caused by *Puccinia striiformis* f. sp. *hordei*) introduced from Europe in the late 1970s affected all varieties planted at that time, severely decreasing the production in all the highlands (Dubin and Stubbs 1985). All the native cultivars were highly susceptible, and the local breeding programs had to produce resistant cultivars in a relatively short period of time. At present, all released

cultivars must show resistance to this disease in the Andean countries and in Mexico.

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Chapter 9
Cultural Practices: Focus on Major Barley-Producing Regions

EUROPE

John R. Garstang and John H. Spink

EUROPEAN PRODUCTION AND YIELD TRENDS

In the 4-year period from 2002 to 2005, the 27 member States of the European Union (EU) produced 41% of the world barley crop from 25% of the 56.7 Mha under cultivation globally (FAO 2008). The 58.4 million tons of grain from the EU was produced from 13.9 Mha, giving an average yield of 4.2 t/ha. About half of the world's malt production of 18 Mt is produced by European maltsters (Euromalt 2008) with the majority of grain used being spring barley of EU origin.

The countries of the EU 27 range from the maritime west, characterized by Ireland and the United Kingdom, to the central continental conditions in Hungary, Romania, and Bulgaria, and from the dry and hot states bordering the Mediterranean to the cool northern Scandinavian states around the Baltic. In the main, barley is grown in the cooler temperate zones where moisture levels are adequate for both autumn and spring planting and are adequate during the early summer months to enable grain to grow and fully mature. Over the last 45 years, yields have increased at an average of about 37 kg/ha per annum (Fig. 9.1)

The drop in yields in 1992 was caused by poor weather across much of mainland Europe, the United Kingdom and The Netherlands being the only countries to show an increased yield compared to the previous year. Were it not for the high yield in 2004, there would be little evidence of a year or year increase for a decade. This slower rate of yield increase has also been noted in other cereals from the mid-1990s (Legg 2005).

CROP MANAGEMENT

This section outlines the main cultural practices across Europe that have supported the progressive improvement of yields and relates them to local growing conditions. Unlike other regions of the globe, EU farmers operate within the common agricultural policy in which support payments are linked to compliance with Council Regulation 1782/2003 (European Commission 2003), a so-called cross-compliance. This compliance entails the farmer meeting the management requirements of 18 directives covering, among others, environmental and plant health issues. Additionally, farmers must comply with standards that provide "good agricultural and environmental conditions." These include, among other items, the avoidance of erosion and the preservation of soil organic matter. This legislation impacts on European barley growers, and we will refer to it where it affects the possible options growers may take.

Types of Barley Grown

The 295 barley cultivars granted community plant variety rights with the EU Plant Variety

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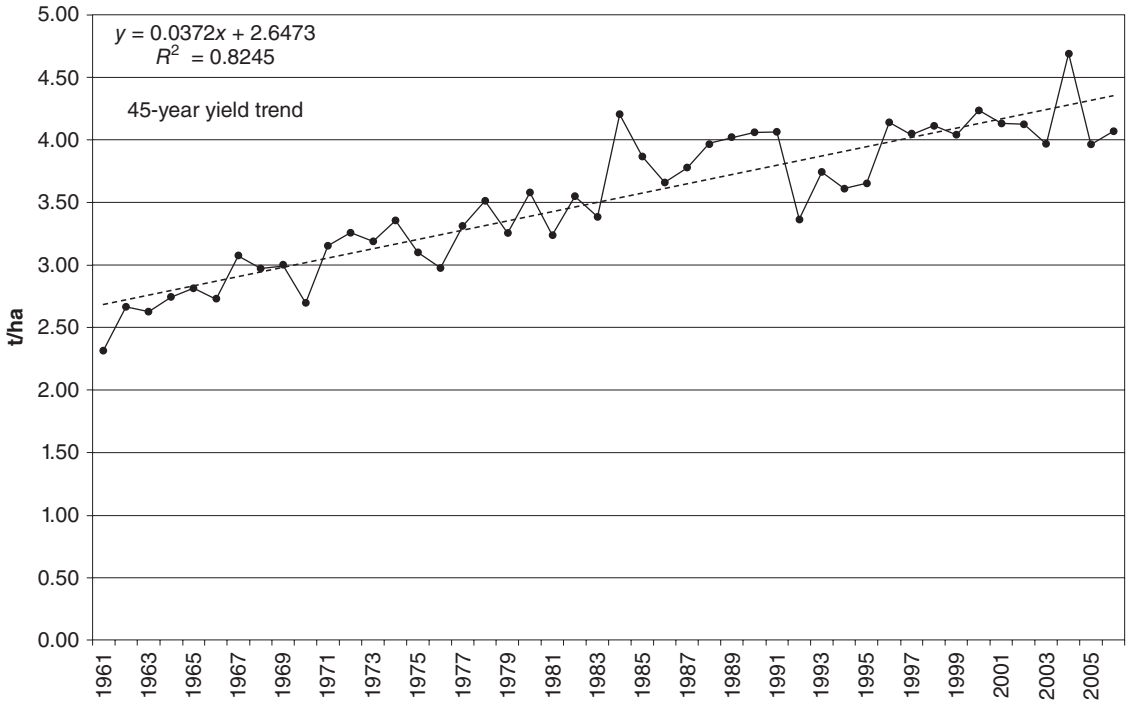

Fig. 9.1. EU 27 barley yield, 1961–2006 (ton per hectare, mean of all types). *Source:* FAO (2008).

Office are all classified as *Hordeum vulgare* L. sensu lato (CPVO 2008). This grouping covers all the six- and two-row types of winter and spring cultivars that show value for cultivation and use, while at the same time being distinctly uniform and stable.

Across Europe, the balance of winter and spring barley is determined by the requirements for winter hardiness and early maturity and ripening, where drought would otherwise impact on yield and quality. The effect of location can be seen by comparing production in Ireland and in Spain. Spain, with the largest area of barley in the EU (3:1, spring : winter), grows about 80% of its crop in main arable areas in Aragon and Castilla y Leon in the north and La Mancha to the south of Madrid, harvested in June and July. From 2002 to 2005, the average spring crop yield was 2.58 t/ha. By way of contrast, Ireland (8:1, spring : winter), with its constant supply of moist air from the Atlantic, produced 6.23 t/ha, the highest spring barley yield in the EU (Table 9.1). The effect of the different types of climate on yield variability is shown in the coefficients of variation (CV%) for annual yield per hectare. For the years 1996–2006, the CV% for the annual winter and spring barley yields in Ireland and in Spain are 8.64% and 10.48%, and 20.77% and 22.53%, respectively. Crop management practices aim to reduce this variation between years as well as to produce high and profitable yields.

In the warm southern and the mild western areas of Europe, the winter and spring categorization becomes less important. In the absence of harsh winters or with the threat of spring drought curtailing yield, spring barley is often autumn-sown. This increases potential yield but also the period of exposure to pests and disease. While the switch to earlier sowing moves the crop toward some of the management practices normally associated with winter barley, the change does not produce a complete change of phenotype.

The general market perception across Europe favors spring barley for the malting market, although there are some exceptions. In an analysis of supply for the 2006 season, Malteurs de France

Table 9.1 Barley area, production, and yield in European countries 2002–2005

	Winter Barley Area ('000 ha)	Spring Barley Area ('000 ha)	Total Area ('000 ha)	% as Spring Barley	Winter Barley Production ('000 t)	Spring Barley Production ('000 t)	Total Barley Production ('000 t)	Winter Barley Yield (t/ha)	Spring Barley Yield (t/ha)	Mean Yield (t/ha)
Germany	1349	644	1993	32	8425	3108	11,533	6.24	4.82	5.79
France	1082	576	1658	35	7109	3436	10,545	6.56	5.98	6.36
Spain	777	2356	3134	75	1965	6073	8038	2.51	2.58	2.57
United Kingdom	452	581	1033	56	2880	3107	5988	6.38	5.33	5.79
Denmark	127	607	734	83	749	3072	3821	5.87	5.07	5.20
Poland	140	908	1048	87	497	2842	3338	3.52	3.13	3.18
Czech Republic	121	387	507	76	490	1607	2097	4.05	4.17	4.13
Finland	0	553	553	100	0	1816	1816	0.00	3.28	3.28
Sweden	6	378	384	100	29	1623	1652	5.35	4.29	4.30
Ireland	20	157	177	89	150	978	1128	7.48	6.23	6.38
Hungary	183	157	340	46	634	481	1115	3.47	3.11	3.28
Romania	266	189	454	41	691	356	1047	2.60	1.90	2.30
Austria	76	123	199	62	391	516	908	5.15	4.22	4.56
Lithuania	8	321	329	98	25	870	895	3.17	2.73	2.72
Bulgaria	281	32	313	10	822	72	894	2.82	2.44	2.85
Slovakia	22	201	224	90	68	721	789	3.12	3.62	3.52
Norway	0	164	164	100	599	0	599	3.69	0.00	3.65
Italy*	0	0	320	—	0	0	1145	0.00	0.00	3.58
The Netherlands	3	49	52	94	19	295	315	6.50	6.00	6.02
Belgium	33	8	41	19	260	45	305	7.82	5.53	7.40
Estonia	0	133	133	100	0	290	291	1.30	2.18	2.18
Latvia	4	133	136	97	8	281	290	2.32	2.11	2.12
Greece	95	0	95	0	207	0	207	2.20	0.00	2.18
Cyprus*	0	0	57	—	0	0	109	0.00	0.00	1.92
Luxembourg	5	5	10	47	31	22	53	6.00	4.92	5.48
Slovenia	6	1	7	14	22	3	25	1.75	1.47	3.45
Portugal	18	0	18	0	21	0	21	1.34	0.00	1.17
Total/means	5075	8663	14,115	62	26,093	31,615	58,962	3.75	3.15	3.90

Source: Eurostat (2008).
*FAO data.

showed Esterel, a six-row winter variety, to be the most popular variety grown in five out of eight regions across central and northern France. Moisture is required for spring barley to mature and ripen with well-filled endosperms giving high yields while avoiding excessive nitrogen content. In the United Kingdom, around 50% of all spring barley production is in Scotland where long, cool days and adequate moisture produce high yields and relatively low nitrogen content.

Spring barley is also grown to avoid loss of the crop over winter. Barley is less tolerant of severe cold than wheat. It also tends to be grown on lighter soils, the top layers of which can lift on freezing, separating the top of the plant from its roots. The most northerly member states around the inner Baltic grow spring barley almost exclusively.

The marked effect of the Gulf Stream is shown by the areas of winter barley grown in Denmark, at the same latitude as the inner Baltic States.

Severe late frosts also present a hazard to winter barley. Once stem extension starts and the young ear is raised 20 cm or more above the ground, late air frosts colder than around −6°C can damage or kill the developing ear. Mild winters associated with climate change can encourage early growth, making late March and April the main period of susceptibility.

In each country, there are also a wide range of agronomic and economic factors that affect the ratio of spring and winter barley. In Denmark, the lower nitrogen requirement of spring barley and its performance on lighter soils make it a better "fit" with the country's environmental legislation and for collocating on dairy farms. The demands of the livestock sector for feed have favored higher-yielding winter barley in Germany and France. The Netherlands, despite an intensive livestock sector, favors spring barley, with 43% of its domestic consumption being used in beer production (Eurostat 2008)—the highest use for brewing in the EU.

In addition to the split of barley into winter and spring types, the winter group can be split into two- and six-row types, and the six-row varieties can now be grouped as conventional and hybrids. Under normal production conditions, six-row barley varieties produce high yields of relatively small grains with low specific weights (Beschreibende Sortenliste 2007). Hybrids appear to be addressing the latter problem, although the grain size does not yet match two-row cultivars (HGCA 2007).

The breakdown of the plantings into malting and feed varieties varies from country to country as markets change. As earlier comments show, six-row winter malting types are popular in parts of France. National variety listings of barley indicate the value of varieties for malting by presenting data showing the malting performance of suitable varieties. The predominance of spring types for malting is clearly shown in the German and U.K. listings. In Germany, out of 37 winter six-row varieties, only 1 has malting data presented, while 10 out of 39 two-row varieties are listed with malting data. In contrast, 41 out of 53 spring varieties are presented with their malting ratings. In the U.K. listings, only 3 winter varieties (all two-row) out of 22 are rated with malting information, while 17 out of 21 spring varieties have hot water extract data presented and 12 are listed as malting varieties. Recent years have seen that wet harvests cause significant malting barley supply problems. Inevitably, market preferences and purchasing standards may change when supply-side problems intervene.

Tillage Practices

Previous descriptions of barley tillage techniques (Briggs 1978) highlighted the role of the plough in seedbed preparation. Recent years have seen a move toward minimum cultivation techniques (min-till), which rapidly prepare a surface tilth of only a few centimeters depth and sow the crop often in a single or reduced number of operations (Fig. 9.2).

Min-till has been widely adopted across Europe as the agricultural industry has responded to low prices by reducing fixed costs through amalgamation of farms with fewer workers resulting in the need to drill more hectares in a fixed drilling window. Surface tillage of the light friable soils

Fig. 9.2. Modern drills can go from stubble to drilled crop in a single pass (photo courtesy of Vaderstad Verken AB). For color details, please see color plate section.

on which barley tends to be grown produces a good seed–soil interface with less risk of smearing associated with high clay fraction soils. Also, in dry autumns, avoiding soil inversion conserves soil moisture. Minimum tillage, however, is poor at providing the weed control associated with the burial of seed achieved by ploughing. The implications of this are discussed under weed control later in this section.

Lighter soils have also traditionally been favored for malt production in some areas as the soil nitrogen supply tends to be lower. Changes in brewing technology and market preferences have allowed higher nitrogen grain to be purchased, and the production of malting barley from heavier soils where wheat may be the preferred crop has been shown to be feasible (Garstang and Giltrap 1999). High yield and good malting premia are needed to make malting barley as financially viable as wheat on heavier soils. Selecting the best soils for malting barley production is a compromise between lighter soils with low nitrogen content but with adequate available moisture. The latter is more important in areas where summer drought affects grain quality.

The majority of crops are drilled using a range of machines from simple tined drills to complex combination drills that can put seeds into a range of seedbeds from min-tilled soil to a fully prepared seedbed. Broadcasting of barley is occasionally used for winter barley planting where delays mean a lot of ground must be planted quickly. Broadcast winter barley can produce similar yields and quality to crops drilled in equivalent conditions. Spring barley in northern sites produces satisfactory yield and quality only after ploughing and drilling (Ball 1985).

Studies to optimize the production of barley in the western European conditions of the United Kingdom suggest relatively high seed rates are used with a benchmark spring population of 305 plants/m^2 (HGCA 2006). With only one floret per spikelet, barley is less able to compensate for lower seed rates than wheat, an effect further compounded if spring barley is late drilled. Monitored crops showed 50% emergence after an elapsed thermal time of 150°C days. This period is extended when dry soils slow initial imbibition and the start of germination. The time needed to accumulate 150°C days ranges between 10 days to about 7 weeks depending on the time of year and the location. The net result of varying assumptions about survival rates from planting to the final established population and different 1000 grain weights gives seed rates ranging from about 100 to 250 kg/ha for light seeds (<35 g/1000) with a high survival rate (>90%) and heavy seeds (>49 g/1000) with a low survival rate (60%).

The crop thus reaches the start of the spring period of rapid growth with a sufficient number of healthy plants ready to respond to any applied fertilizer. We will look at this aspect after we have considered weed control measures.

Weeds and Weed Control

Of the broad-leafed weeds found in Europe, Hanf (1983) reported that 33% of the species could be found throughout Europe. A further 15% could only be found on the mainland roughly to the south of The Netherlands and northern Germany, while a further 23% were restricted to the south of a line drawn approximately from La Rochelle on the west coast of France to Budapest. Another 12% were exclusive to lands to the west of a line from Venice to Friesland, and 6% were confined to the Iberian Peninsula, while the remaining 11% were found in various regions in Eastern Europe and in Italy. The weed flora of Europe is thus diverse, with only one-third of the species being common to the whole continent.

Herbicide use is the main method of weed control in barley across Europe. Removing broad-leafed weeds from cereal presents few problems if weeds are tackled at the seedling stage, while grass weeds are the most difficult target weeds to control. The first selective chemical herbicides were developed to remove broad-leafed weeds from cereals over 60 years ago. Now, active substances like diflufenican and pendimethalin have very broad spectra of annual dicotyledon control in barley, while pendimethalin and other materials also control seedling grasses. In common with many active substances, best control is achieved when used during the preemergence of the weeds or during early postemergence. The use of some materials varies between winter and spring barley. Pendimethalin can be used up to the end of tillering on winter barley but must be used during preemergence of the crop on spring barley. Sulfonylureas are now one of the largest groups of herbicides and work by inhibition of acetolactate synthase (ALS). They control many broad-leafed weeds, and some have been specifically targeted at grass weeds. However, their use in barley is not straightforward as some are suitable only for use on wheat, while some can be used on winter wheat and on spring barley. As with all herbicides, a degree of skill is required by the end user when seeking to optimize their use.

The difficulty with grass weed control is heightened as the portfolio of grass weed herbicides is less well developed for barley than for wheat. For example, in the U.K. 2008 grass weed herbicide market, there were 36 active substances and combinations of substances approved for use on wheat, but only 22 were approved for use on barley. Increases in the distribution of herbicide resistance across most EU countries, and reviews of pesticide safety, point to the need in the future to rely more on the integration of control measures: crop rotation, cultivation techniques, and herbicides, all of which need to contribute to reducing weed presence.

Controlling grass weeds in cereals, which are also grasses, is the main challenge. In European cereals, *Avena fatua* (wild oat) is the most competitive weed on an individual plant basis. It is a widespread weed across Europe with *Avena sterilis* subsp. *ludoviciana* (winter wild oat) becoming more important to the south and on higher land there. *Lolium rigidum* and *multiflorum* (ryegrasses), along with *Phalaris minor* and *paradoxa* (canary grass), are major noncereal grass weeds in southern countries. In the north, particularly on heavier soils with wheat/barley in the rotation, *Alopecurus myosuroides* (blackgrass) is a problem, while in less-intensive arable areas *Poa* spp. (bluegrass) and *Lolium* spp. can become prominent weeds. However, the presence of a mixed rotation with short-rotation forage grass and livestock gives more scope for integrated control measures using grazed pastures and ploughing. Small-seeded grass weeds like *A. myosuroides* and *Poa* spp. can easily exceed 1000 plants/m^2 in severe infestations. At these numbers, yield is reduced; crop lodging increases; and moisture content at harvest can be increased due to extra green material.

The more restricted portfolio of grass weed herbicides available for barley becomes more of a problem where herbicide-resistant weeds are found. To date, herbicide-resistant *A. myosuroides* has been found across northern Europe from the

United Kingdom to Germany and in Spain (Weed Science 2008), while resistant *A. fatua* has been confirmed in France, Belgium, and the United Kingdom. Herbicide-resistant *L. rigidum* has been found in Greece, France, and Spain including resistance to glyphosate in the latter two. Some broad-leafed weeds resistant to ALS inhibitors have also been found in a range of European countries.

Fallow, either as set-aside or for moisture conservation, can allow cultivation or nonselective herbicide to be used for weed control. Ploughing provides the best burial of weed seeds and is the recommended seedbed preparation where herbicide-resistant weeds have become established. In some situations where dormant seeds remain viable in the soil, ploughing can bring to the surface viable seeds shed in previous years. This is more likely to occur where the very high plant populations mentioned above have been allowed to develop. Minimum tillage techniques can provide ideal conditions for grass seed germination, providing a relatively shallow soil cover and good moisture conductivity in undisturbed soil. Repeated use of the technique can build up high populations of grass weeds like *A. mysuroides*.

Stale seedbeds are another commonly used method of weed control. This requires moist soil to promote the germination of weeds for removal by nonselective herbicides or cultivation before drilling the next crop. The relatively early sowing of winter barley compared to wheat can restrict the success of stale seedbeds, particularly following a dry late summer period, which promotes little weed growth. Although seedbed consolidation improves water transfer for the germinating seeds, stale seedbeds need to be produced long enough before drilling to allow weed growth. Weeds resistant to glyphosate as the *L. rigidum* mentioned above may require a switch to other nonselective herbicides like glufosinate-ammonium or inversion ploughing to bury the germinated plants. Where barley follows wheat in the rotation, early germination of tailing corn wheat provides one of the best routes for avoiding wheat volunteers in the following barley crop. Dry soils in late summer and autumn can give poor germination of tailings from the previous crop with a consequent increase in wheat volunteers over winter. In contrast, the drilling of spring barley follows the wetter winter period, so predrilling weed growth is stronger in warm regions. Further north, cooler conditions and early drilling may result in weed growth being delayed by cold weather and developing simultaneously with the spring crop. Late-drilled spring barley produces fewer tillers than an early-sown spring or winter barley, so it competes less well with weeds.

Barley needs to be relatively weed free during establishment and early growth so individual plants can develop in adequate numbers and size. Once crop plant survival is assured, weed competition should then be avoided during the grand period of growth and subsequent grain filling. There is some evidence that in northwestern European growing conditions, winter barley can produce greater responses to herbicides than wheat, which in turn produces greater responses than spring barley (Davies 1997). Timeliness of herbicide application are of major importance as are the health and vigor of the crop and thus its ability to compete with the weeds.

Fertility

Efficient nutrient management requires the total nutrient input to be matched to the anticipated total nutrient offtake by the barley crop, taking into account the supply of nutrients already in the soil at the start of the growing season. The legislation and nutrient requirements underpinning this system of nutrient management is well documented (MAFF 2000; De Clercq et al. 2001). In common with the other main cereal crops, nitrogen, phosphate, and potash are the main elements required, with sulfur being required more routinely in parts of Europe where atmospheric deposition rates have fallen and soil supplies are inadequate. The lighter soils stretching from Eastern England and across Holland, Germany, Poland, and the Czech and Slovak Republics are expected to show the greatest changes in sulfur availability as the amount of coal-fired heavy industry diminishes.

The soil nitrogen supply must take into account available nitrogen and variation in the mineralization of soil organic nitrogen supplies during the growing period. High soil nitrogen supply at planting boosts early vegetative growth and the transfer of nitrogen from the stems and leaves to the ear during grain formation, increasing the protein value of feed barley. The same protein increase can also reduce the quality of malting barley if applied fertilizer rates have not been adjusted to take account of soil nitrogen supplies. In southern Europe, the relatively warm soils support higher levels of nitrogen mineralization, which, combined with the effects of drought, can produce grain with relatively high nitrogen content.

Organic fertilizer, whether as slurry or higher dry matter manures, adds to the soil nutrient pool and needs to be considered along with the soil nitrogen supply, with mineralization adding more available nitrogen as the season progresses. Slurry should not be applied in conditions where runoff or leaching occurs (European Commission 2003). Soluble nutrients in the aqueous phase are ready for immediate uptake by the plants, like inorganic fertilizers.

Once estimates of the nutrient supply from the soils and manures have been made, inorganic fertilizer can be applied to support production of the anticipated crop canopy and grain yield. A barley crop requires about 28 kg N/ha of green area index (GAI), that is, to produce 1 ha of leaf (one side only) per hectare requires 28 kg of nitrogen (HGCA 2006). As a typical crop may approach a GAI of 6, the canopy will require about 168 kg/ha of nitrogen from applied fertilizer and soil sources, and crop offtake will be about 132 kg N/ha in winter barley. Spring barley with a shorter crop growth period and generally lower total biomass will have a maximum nitrogen offtake of 25%–30% less than the winter crop. Over half the nitrogen is taken up by the time the flag leaf is fully emerged (Zadoks GS 39; Zadoks et al. 1974). Both winter and spring malting barley require most of their nitrogen to be applied not much later than the start of the main period of stem extension, Zadoks GS 31. This ensures nitrogen is available for the main period of growth but avoids late surges in supply that can increase grain nitrogen content. In regions where dry weather delays nitrogen uptake, mid-season rains can produce increased late uptake and elevate grain nitrogen content. The pattern of seasonal rainfall means this can happen almost anywhere but is least likely in cool moist production regions (e.g., Ireland, Scotland, The Netherlands, and Scandinavia). Phosphorus application to barley in very low rainfall areas has been shown to improve water use efficiency (WUE) on phosphorus-deficient soils. Nitrogen also gives very significant increases in WUE but also increases absolute water use.

Crop development

Yields of both winter and spring barley in the United Kingdom are the fifth highest in Europe, a performance that could be classified as typical of more intensive national agricultural practices. How the crop grows, develops, and is influenced by inputs is outlined in the Barley Growth Guide (HGCA 2006), which shows that in the United Kingdom, crop yield is sink limited; that is, yield is limited by grain number and potential size rather than the crop's ability to fill the grain. In this section, we look at how this varies across Europe and the impact of management practices. Most of the uncontrolled variation arises from differences in temperature and water supply in the rainfed majority of crops.

Once the crop is fully emerged and established, leaf numbers increase. The time taken for each leaf to emerge (phyllochron) is measured in thermal time; a phyllochron averages 108°C days in winter barley in the United Kingdom. Cooler conditions in the north tend to produce fewer leaves (~13), while the warmer conditions in the south can produce around 15 leaves. At a given latitude, late-sown crops accumulate less thermal time and produce fewer leaves, but late-sown crops can compensate by reducing the phyllochron and increasing the rate of leaf emergence. Overall, the net effect is to partially offset the effects of late drilling rather than to remove the effect completely. There is also a significant genetic variation in phyllochron with reports of

variation in experiments from 50 to 97°C days (Frank and Bauer 1995).

In spring barley, the phyllchron is about 10% less than the winter crop, and the crop generally produces 8–10 leaves rather than 13–15. Tillering is similarly affected by temperature, but as water and nutrient supply also affect the final outcome, the farmer has more scope for controlling tiller number. Tillering is quite rapid in the autumn. The areas in Europe where winter barley is grown are those where autumn allows adequate growth before winter temperatures curtail growth and where the absolute winter conditions do not produce excessive losses over winter (see also Types of Barley Grown).

Tiller numbers reach a maximum just before the period of rapid stem extension. Early-sown crops have a higher number of potential tillers and can thus be sown at lower seed rates. If this is done, those extra potential tillers must survive, so drought and nutrient stress must be avoided during early stem extension when tiller numbers decline naturally. Maximum shoot numbers before the start of stem extension are around 1000–1300 per cubic meter. Once the demands of rapid stem extension and canopy production stress the plant, tillers are lost and numbers fall to between 750 and 800 per cubic meter or to about three per plant if nutrient and water supply is adequate. The final ear numbers are similar in winter and spring barley, but the latter with few tillers per plant needs a slightly higher plant population to compensate.

Tillers increase in height as the season progresses, reaching about half their height by the time of flag leaf emergence (Zadoks GS 39), and the majority of their height by full ear emergence (Zadoks GS 59). Drought-affected crops tend to be shorter, and when such crops reach harvest, the crops may stand little more than 30 cm in height with poorly finished grains and a low stem count. Most severely drought-affected crops occur in countries bordering the Mediterranean.

Crop height is one of the components that affect lodging. The taller the straw, the greater the leverage of wind and the weight of the ear itself. Lodging occurs through either root upheaval or buckling of the stem. Lighter soils with lower clay content give less anchorage. Such soils are widely found across Northern Europe and southern Scandinavia. The lightest sandy land with a tendency to have lower pH is often used to grow rye rather than barley.

Plant growth regulators are applied to barley and other cereals in order to shorten and thicken the stem, thereby reducing the risk of lodging. Two main modes of action are used. The inhibition of gibberellin production reduces apical dominance; if applied when the crop is tillering, it can produce more uniform crops and can increase tiller survival where subsequent growing conditions allow. If applied at the end of tillering and at the start of stem extension (Zadoks GS 30–31), it shortens the lower internodes of the growing stems. This effect is more pronounced in wheat than in barley; nevertheless, gibberellin inhibitor growth regulators are widely used on barley throughout Europe. Ethylene production and its restriction of cell expansion is the basis of the second mode of action used. Compounds producing these effects are typically applied in the latter stages of stem extension, but before the ear has emerged, shortening the upper internodes of the crop. The most effective stem shortening in barley has been achieved by using a sequence of gibberellin inhibitors and ethylene-producing compounds, the most common being chlormequat and 2-chlorothlyphosphonic acid (ethephon), respectively. Mepiquat chloride, another gibberellin inhibitor, is used as a formulated product with both chlormequat and ethephon. More recently introduced gibberellin inhibitors trinexapac-ethyl and prohexadione-calcium offer more flexibility in timing than chlormequat. There is some evidence to suggest that there is a degree of synergism when the two modes of action are used in succession. Late secondary tillering may be accentuated by ethylene-producing compounds if used on drought-stressed crops. This can adversely affect evenness of ripening and malting quality, and late growth regulators need to be withheld if drought stress occurs late in an otherwise normal growing season. Severely drought-stressed crops are unlikely to need additional stem shortening

Water management

With increasing demands for water from both agricultural and nonagricultural users, water use through irrigation is increasingly directed to higher-value horticulture crops and field crops like maize and sunflowers where the main growth period coincides with increasing summer moisture stress. The majority of barley grown in Europe is largely rainfed and indeed, compared with other cereals, it is well adapted to drier conditions through its WUE. Barley's earlier ripening and harvest also minimize its exposure to much of the hottest weather after the summer solstice. In dry seedbeds in either the autumn or spring, showers can provide sufficient water to trigger germination, but a return of drought conditions can cause considerable seedling loss through desiccation. Additional irrigation is useful in these situations. Over the whole growing season, however, the greatest WUE for grain yield in semiarid dryland winter barley in Spain was achieved from effective rainfall from stem extension to harvest (Moret et al. 2007). Throughout Europe, and not just in the main irrigating countries like France, Spain, and Italy, irrigation during this period often clashes with water use for higher-value crops, so where irrigation is used, it tends to be tactical for the correction of periodic severe water shortage and the prevention of total crop loss or major yield losses. As the values in Table 9.1 show, the highest yields are produced in countries with generally adequate rainfall during the principal phases of growth—establishment, stem extension, and grain filling.

Harvest

As harvest approaches, excessive moisture becomes a nuisance rather than desirable. In common with all cereals, barley grain needs to be at or below 14% moisture content for safe storage. This is a good general figure as the actual value varies with ambient temperature and the specific risk factor. Storage below 4–5°C will avoid in-store heating of grain at 19%–20% moisture, while around 12% moisture is needed if temperatures above 30°C are anticipated. Fortunately, the hotter southern countries are more able to produce drier grain ex-field. Retention of germination is crucial for malting barley production and because of this, drying may be done by the buyer and the cost factored into the sale contract. Grain at about 15% moisture content needs to be stored at less than 15°C if it is to be kept in store. Insect damage to stored barley is temperature rather than moisture dependent, and storage below 15°C is essential. In many areas of Europe, grain harvested in the heat of the day can require grain ventilation with cool night air to get large bulk stores below this threshold. In contrast to other insects, mites need drier grain and lower temperatures to subdue their activity and require grain to be less than 12% moisture or stored at 2°C or less.

In the regions of highest rainfall and high yields, weeds, particularly well-rooted perennial plants, can remain green during harvest. To reduce the risk of excessive wet green material being admixed with dry grain, nonselective herbicides (glyphosate) can be use to desiccate the weeds once the grain has dropped below 30% moisture. Such desiccation in the absence of weeds has been shown to produce lower grain moisture in spring barley harvested in Scottish conditions. Users are not recommended to use this technique on barley intended for seed and to consult their grain merchant before treating any crop grown for malting, distilling, or grown on contract. Green material admixed with grain not only gives an uneven distribution of moisture but also interferes with the even air flow in bulk dried grain. During drying, malting barley above 24% moisture content should not exceed 43 or 49°C when the moisture has dropped below 24%. Green material spread in patches though the bulk of grain makes such moisture assessment and temperature regulation difficult. Such uneven moisture distribution can lead to bands of spoiled grain if drying is inadequate.

TECHNICAL EXPERTISE AND INVESTMENT

Like most continents, Europe is climatically and agriculturally diverse. Barley is produced at varying levels of output in most of the arable areas. Until recently, there has been considerable disparity between some countries and regions in on-farm and infrastructure investment. The resources and techniques discussed in this section, long available to the original 15 EU member states, are now, where economics allow, available to all 27 member states. We should expect some of the lower yields shown in Table 9.1 to increase in many member states where climatic and soil factors are not limiting.

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RUSSIAN FEDERATION, UKRAINE, AND KAZAKHSTAN

Mekhlis Suleimenov

BARLEY TYPES PLANTED AND ECOREGIONAL DISTRIBUTION

Barley has been grown in all parts of the Russian Federation, Ukraine, and Kazakhstan (Fig. 9.3). Northwestern Asia (NWA) of the former Soviet Union (FSU) includes steppe and forest steppe zones of North Kazakhstan and West Siberia, as well as the eastern part of the South Ural areas of Russia. The NWA area lies between 48° and 56° N latitude and 52° and 110° E longitude. Northeastern Europe (NEE) of FSU includes dry steppes of the Lower Volga, semiarid steppes of the Volga area, North Caucasus, South and Central Ukraine, the Central Chernozem area, the forest steppe zone of Russia and Ukraine, and the non-Chernozem zone of Russia and Ukraine. The NEE area lies between 48° and 55° N latitude and 32° and 52° E longitude.

Kazakhstan and West Siberia

Spring barley in North Kazakhstan and West Siberia occupies on average 10%–15% of area

Fig. 9.3. Map of the Commonwealth of Independent States (former Soviet Union).

under small grains. The share of barley is increasing from dry steppe to forest steppe and from lowlands to hilly areas. In areas with shorter growing seasons, the share of barley may go up to 40%–50% of area under small grains.

Until recently, malting barley was not grown in Western Siberia because in the FSU, there were other agroecological regions more suitable for growing malting barley (Sharkov et al. 2003).

Northeast Europe of Russian Federation and Ukraine

In the steppe zone of North Caucasus, Rostov (Sokol and Beltyukov 1988) and Krasnodar (Vasyukov and Kuznetsova 1988), and of Southeast Ukraine, Donetsk (Logvinenko et al. 1998), spring barley is mainly grown as feed grain. The barley area sown is quite often larger than that of winter wheat and maize put together. On average, every third hectare of winter wheat is winterkilled because of low winter temperatures, icy soil cover, and dry soil during sowing. Also, spring barley is the best cover crop in mixture with dry pea for perennial forages.

In the steppes of the Central Chernozem area, Voronezh, barley mostly of malting type occupies 15%–18%, but in some years, with the high rate of winterkilled wheat, its area may go up to 50% of grain area (Gorshkova et al. 1988; Cherkasov et al. 2006).

In Tatarstan, in the forest steppes of Middle Volga, "Kazan" spring barley is the major feed grain, but since 1999, malting barley has also been grown (Blokhin 2006). Barley occupies 26% of the total area under grains.

In the foothill zone of the Krasnodar area, the first winter barley variety Novator was registered in 1992 (Shevtsov and Naidenov 1988). Winter barley has been grown in the steppes of Central Ukraine, at Dnipropetrovsk where annual precipitation is about 500 mm (Saiko 1993). In the forest steppe zone of the Middle Volga, climate change made growing winter barley possible because of very mild winters (Tupitsyn and Valyaikin 2005).

In the Upper Volga, non-Chernozem zone, "Ivanovo" barley is grown on brown grassland and gray forest soils for malting and as feed grain occupying a larger area than wheat (Meltsayev and Shramko 2006). In the Orel area on dark gray soils, barley is an important grain crop, but in the transition period after the fall of the Soviet Union, its sown area reduced and yields decreased from 2.68 to 1.01 t/ha (Lopachev et al. 2001).

BARLEY IN CROP ROTATIONS

Kazakhstan and West Siberia

In North Kazakhstan, feed barley is normally grown in a 5-year crop rotation of "summer fallow–wheat–wheat–barley–wheat." Spring wheat sown after barley often gives higher yields than continuous wheat because the seeding dates of barley are 1 week later, which gives possibility for better weed control (Suleimenov 1991). In areas with short growing seasons where barley occupies larger areas, growing continuous barley is possible with no significant difference of yields as compared to sowing after wheat.

In the forest steppe zone of West Siberia, Novosibirsk, the best crops to precede malting barley are winter rye, row crops grown for silage, and buckwheat (Sharkov et al. 2003).

Northeast Europe

In the dry steppe zone of the Lower Volga area, Volgograd, barley is grown in a 3-year crop rotation: "summer fallow–winter wheat–barley" (Belenkov 2006).

Maize grown for silage was found to be the best preceding crop for spring barley in Rostov area (Sokol and Beltyukov 1988). The average barley grain yield grown in 1984–1986 after maize for silage, winter wheat, maize for grain, and sunflower was 4.67, 4.52, 4.43, and 4.30 t/ha, respectively, as compared to 3.94 t/ha on continuous barley. Maize was also found to be the best predecessor for spring barley in the steppes of Southeast Ukraine (Logvinenko et al. 1998).

The best crop to precede spring barley in the northern steppe of Krasnodar area is green manure. On average during 1985–1986, the grain yields of spring barley after green manure, dry

pea, mixture of pea, and oats were 5.36, 4.91, 4.99 t/ha, respectively, as compared with 4.66 t/ha after winter wheat(Vasyukov and Kuznetsova 1988).

In the Central Chernozem zone, Voronezh, barley is placed after maize for silage and grain and after sugar beet (Gorshkova et al. 1988; Gulidova 2001). In Kursk, spring barley sown 5 years continuously improved productivity when it was double cropped with green manure as compared to growing in crop rotation (Kartamyshev et al. 2006)

In the foothills of the Krasnodar area, the most widespread crop to precede winter barley is winter wheat. The grain yield of winter barley sown after winter wheat was 0.3–0.9 t/ha higher as compared with barley sown after row crops (Shevtsov and Naidenov 1988). In the steppe of Central Ukraine, winter barley is grown after food legumes, melons, potatoes, and maize (Tsikov 1981). In the Ulyanovsk forest steppes, it is best to sow winter barley after summer fallow (Tupitsyn and Valyaikin 2005).

In Tatarstan, it is recommended to sow spring malting barley after winter wheat and rye, as well as after row crops, while it is recommended to sow feed grain barley after legumes (Blokhin 2006). In Orel, barley is normally placed after maize. The barley is also used as a cover crop for clover (Lopachev et al. 2001).

SEEDING DATES

Kazakhstan and West Siberia

North Kazakhstan is characterized by a continental climate with hot summers and very cold winters. July is the hottest time of the year combined with highest rainfall.

Studies were conducted during 1986–1989 at the Esil Experiment Station located 400 km west of Astana, on heavy clay loam calcareous dark chestnut soil with 3.2% organic matter (Kaskarbayev 1994). The annual average precipitation is 280 mm. Five seeding dates were tested from May 12 to June 9. Delayed seeding dates allowed better wild oat control, field seedling emergence rate, crop survival rates, plant density, and 1000 kernel weight. Spring barley grain yield increased significantly by sowing at the beginning of June (Table 9.2). The same conclusions were made on black soil as well. May 30 was the most adequate seeding date for spring barley Suleimenov (1991).

Table 9.2 Spring barley grain yield (ton per hectare) as affected by seeding dates in the dry steppes of North Kazakhstan, Esil (data of Z. Kaskarbayev and M. Suleimenov, published in Kaskarbayev 1994)

	Years				
Seeding Date	1986	1987	1988	1989	4-Year Mean
May 12	1.74	1.68	1.20	1.15	1.44
May 19	1.74	2.13	1.51	1.07	1.67
May 26	1.96	2.40	2.02	1.11	1.87
June 2	2.07	2.52	2.08	1.13	1.95
June 9	1.90	2.03	1.35	1.17	1.61
LSD_{05}	0.07	0.19	0.14	0.10	

LSD_{05}, least significant difference at p = .05.

In the southern forest steppe of West Siberia, spring barley seeding is best about May 10 (Alimov 1998). In northern forest steppe, malting barley should be sown from the end of April (Sharkov et al. 2003).

Northeast Europe

In NEE, the share of winter and spring precipitation is higher, while summer (July) has less rainfall. The annual average temperature is higher than in Astana: 4.9°C in Saratov and 8.6°C in Rostov. The earliest possible seeding dates of spring barley were found to be most adequate: end of February–March in Southeast Ukraine (Logvinenko et al. 1998) and in the Krasnodar steppe zone (Vasyukov and Kuznetsova 1988), and March until the beginning of April in the Rostov area, in the Central Chernozem area (Gorshkova et al. 1988), and in Tatarstan (Blokhin 2006). In the Rostov area, on average for 1977–1982, delayed sowing by 1 week reduced barley grain yield by 0.29–0.38 t/ha (Sokol and Beltyukov 1988).

In the Krasnodar foothill zone, annual precipitation is about 500–600 mm and winters are relatively mild. Winter barley should develop three to four tillers before winter. It is recommended to

sow between September 5 and October 5, and in the drier northern part of the region, from September 10 to 20 (Shevtsov and Naidenov 1988).

The seeding time of winter barley in the Ulyanovsk forest steppe was moved to the end of August until the beginning of September because of climate change. The optimum seeding time is from September 3 to 8 (Tupitsyn and Valyaikin 2005).

SEEDING RATE

North Kazakhstan and West Siberia

In North Kazakhstan, a study on seeding rates of spring barley was conducted during 4 years at the Esil Station in the dry steppe zone (Kaskarbayev 1994) and in the semiarid steppe zone during 1985–1989 at Shortandy, 60 km north of Astana (Kupanova 1988). The grain yield did not differ significantly under seeding rates from 2.5 to 3.5 million seeds per hectare in dry steppe and from 2 to 4 million seeds per hectare in the semiarid steppe. The barley grain yield under lower seeding rates was reduced because of higher weed infestation.

In the southern forest steppe zone, the seeding rates of barley tend to be higher: 5.0–5.5 million seeds per hectare (Alimov 1998). In the northern forest steppe, malting barley is typically sown with heavier rates of 6–6.5 million seeds per hectare (Sharkov et al. 2003).

Northeast Europe

In the dry steppe of the Lower Volga, seeding rates of spring barley are from 3.0 to 3.5 million seeds per hectare (Belenkov 2006).

In the semiarid steppes of the Rostov area, the recommended seeding rates of spring barley for early and delayed sowing are 4 and 5 million seeds per hectare, respectively (Sokol and Beltyukov 1988). In Southeast Ukraine, the recommended seeding rates of spring barley are 4.0–4.5 million seeds per hectare (Logvinenko et al. 1998). In the Krasnodar steppe zone, spring barley grain yields are usually not affected by seeding rates from 2 to 6 million seeds per hectare under favorable weather conditions. But in dry 1983, because of sparse stand, the low seed rate of 2 million seeds reduced grain yield to 2.70 t/ha as compared with 3.57 t/ha with a rate of 6 million seeds per hectare. The same situation was observed when sowing was delayed in 1985(Vasyukov and Kuznetsova 1988).

In the Central Chernozem zone, crop yields are not affected by seeding rates significantly (Gorshkova et al. 1988). Recommended rates are 5–6 million seeds per hectare. The seeding rates increase under unfavorable soil moisture conditions for seed emergence and shortage of fertilizers.

In Tatarstan, the recommended crop density for common varieties is 350–380 plants and for intensive varieties with stronger tillering capacity—300–320 plants/m^2, which is equivalent to 3 million seeds per hectare.

In the Krasnodar foothill area, winter barley grain yields are normally not affected by seeding rates from 3 to 6 million seeds per hectare. However, in the northern part of the area with more severe winters, it is recommended to sow 5–6 million seeds per hectare (Shevtsov and Naidenov 1988).

In Ulyanovsk, the seeding rates of winter barley are in the range of 3–5 million seeds per hectare. Lower rates are used in less risky areas where seed emergence in the field is more reliable (Tupitsyn and Valyaikin 2005).

In the Upper Volga on soddy-podzolic soil, it is recommended to apply seed rates to ensure the establishment of 350–400 plants/m^2 (~4 million seeds per hectare) (Meltsayev and Shramko 2006). In Orel, the recommended seeding rate on dark gray soil is 5 million seeds per hectare. When barley is used as cover crop for forages, its seeding rate is reduced by 1 million seeds per hectare (Lopachev et al. 2001).

SEEDING DEPTH

North Kazakhstan and West Siberia

A North Kazakhstan study of seeding depth was conducted in the semiarid steppe zone at

Table 9.3 Spring barley grain yield (ton per hectare) as affected by seeding depth and seed rate in the semiarid steppes of North Kazakhstan, Shortandy (average for 1985–1989) (data of L. Kupanova and M. Suleimenov, published in Kupanova 1988)

	Seed Rate (Million Seeds per Hectare)		
Depth of Seeding (cm)	2	3	4
4	2.46	2.60	2.46
6	2.40	2.49	2.51
8	2.35	2.49	2.53
10	1.67	1.87	2.19
LSD_{05} (t/ha)	Seeding depth—0.18, seed rate—0.17		

Shortandy (Kupanova 1988). On average, over 4 years, the grain yield of barley was not affected by seeding depths of 4–8 cm, but reduced significantly at sowing 10 cm deep especially under low seed rates (Table 9.3). The field seed emergence rate was higher when seeds were sown 4–6 cm deep (73%–71%).

In the southern forest steppe zone of West Siberia, it is recommended to sow spring barley 5–6 cm deep (Alimov 1998), while in the northern forest steppe zone, malting barley should not be sown deeper than 5 cm (Sharkov et al. 2003).

Northeast Europe

Seeding depth in the steppe of Southeast Ukraine normally is around 6 cm (Logvinenko et al. 1998). In Krasnodar, the seeding depth of winter barley can vary from 2 to 6 cm depending on soil moisture. On average, the best winter survival is obtained when barley is sown 4 cm deep (Shevtsov and Naidenov 1988). Sowing of winter barley in the Middle Volga area on summer fallow is recommended at 5–8 cm (Tupitsyn and Valyaikin 2005).

TILLAGE PRACTICES

North Kazakhstan and West Siberia

According to Akhmetov and Zinchenko (1976), in the semiarid steppe of North Kazakhstan, efficient depth of fall tillage depended on soil moisture. When soil was wet, deep tillage with sweeps at 25–27 cm provided significantly better snowmelt water infiltration in spring. When tillage was done in dry soil conditions, depth of tillage did not play an important role for water accumulation.

In Kostanai Province, recent research shows an advantage of no-till combined with leaving a 30-cm-tall stubble during harvest to trap snow (Dvurechenskiy 2008). This practice improved moisture accumulation in soil by spring barley seeding time by 30 mm, as compared to the traditional 15-cm-tall stubble and fall tillage with blades.

Tillage treatments in the fall for spring barley were studied in the southern forest steppe zone on leached black soils in Kurgan, West Siberia (Isayenko 2006). The factor of weed infestation was decisive in barley grain yield. Yield was highest under moldboard plowing compared to conservation tillage with blades.

Another study on leached black soil in the forest steppe zone in the Novosibirsk area was conducted during 1986–1994 (Alimov 1998). The combination of moldboard plowing with conservation tillage in a rotation was found most efficient for crop yield and energy conservation.

Northeast Europe

In Southeast Ukraine (Logvinenko et al. 1998) and in the Central Chernozem area, (Gorshkova et al. 1988) disking is done two to three times starting soon after harvest of the preceding crop. Main tillage by moldboard plows is done 20–22 cm deep at the end of September until the beginning of October. In the Lipetsk area, barley grain yield on conservation tillage was the same as after plowing but ensured considerable saving of energy (Gulidova 2001). In the Kursk area, green manure grown as double crop with continuous barley increased barley yield significantly when green manure was incorporated by plow or disk harrow (Kartamyshev et al. 2006). In Tatarstan, deep plowing has been replaced by minimum tillage with disks or duck foot cultivators, saving fuel by 20%–50% (Blokhin 2006).

In the Middle Volga area, winter barley is sown on summer fallow. Most important is to have a firm seedbed with tillage no deeper than 5–8 cm prior to sowing (Tupitsyn and Valyaikin 2005). In the steppes of Central Ukraine, early double disking is recommended for seedbed preparation for winter barley sown after maize (Tsikov 1981). However, no-till is being rapidly adopted due to considerable saving of energy and labor (Medvedev 2006).

In the Upper Volga, it is recommended to rotate tillage methods on soddy-podzolic soil. In dry years, minimum tillage is adequate, while in favorable years, moldboard plowing combined with application of adequate rates of fertilizers and pesticides ensured highest grain yields (Meltsayev and Shramko 2006). In Orel, on dark gray soils, deep tillage with moldboard plows can be replaced by minimum tillage when barley follows a row crop with low weed infestation (Lopachev et al. 2001).

WEEDS AND WEED CONTROL

There are three major classes of weeds: rootstock, perennial suckering, and annuals. These weeds are found everywhere and weed control practices are very important components of cropping systems. In the traditional Soviet cropping systems, tillage was the predominant control tactic for rootstock and suckering perennial weeds across all regions. The only widely applied herbicide in the Soviet Union was 2,4-D.

North Kazakhstan and West Siberia

In the southern forest steppe zone of North Kazakhstan, the most widespread weeds are Canada thistle (*Cirsium arvense*), field bindweed (*Convolvulus arvensis*), wild oat (*Avena fatua*), tartaric buckwheat (*Fagopyrum tataricum*), green foxtail (*Setaria viridis*), and redroot pigweed (*Amaranthus retroflexus*). The prevailing weeds in the semiarid steppe zone are Canada thistle, field bindweed, wild oat, goosefoot (*Chenopodium album*), redroot pigweed, green foxtail, field pennycress (*Thlaspi arvense*), and shepherd's purse (*Capsella bursa-pastoris*). In the dry steppe zone, the most troublesome weeds are couch grass (*Agropyrum repens*), sedge (*Carex caespitosa*), Canada thistle, field bindweed, gorchack (*Acroptilon picris*), wild lettuce (*Lactuca seriola*), green foxtail, and redroot pigweed (Suleimenov 2006).

Delayed seeding is always considered as one of most powerful means to control some weeds, especially wild oat (Suleimenov 1991). Seeding rates are of importance because weeds compete with crops. It is no doubt that widening of row spacing from 15 cm in a disk drill to 23 cm in a stubble (no-till) drill provides more space for weeds. Another factor is depth of seeding. Under delayed seeding, it is quite common to place seeds deeper than it is necessary for germination and emergence. Sometimes, this works, but delayed and sparse emergence favors weed infestation.

Recently, with wide adoption of no-till farming practices, weed control methods have changed dramatically (Dvurechenskiy 2008). In summer fallow, two sprayings of a glyphosate-type herbicide are recommended: the first in mid-May to control shepherd's purse, field pennycress, wild oat, and thistle, and the second in mid-July to control field bindweed, thistle, lettuce, wild oat, and redroot pigweed.

The most efficient herbicides to control perennial weeds in spring barley are Sekator-turbo (50–75 mL/ha), Musket (40–50 g/ha), and Granstar (12 g/ha) (Dvurechenskiy 2008). To control wild oat and green foxtail in spring barley, Puma Super (0.6–0.9 L/ha) and Bars Super (0.6–0.7 L/ha) are the best herbicides.

Northeast Europe

The most troublesome weeds in the Northern Caucasus and Southern Ukraine are the following: perennial weeds—Canada thistle, field bindweed, sow thistle (*Sonchus arvensis*), and Russian sweet sultan (*Centaurea picris*); rootstock weeds—couch grass; and annuals—redroot pigweed and knotweed (*Poligonum aviculare*) (Suleimenov 2006).

To control suckering weeds, an intensive tillage system after crop harvest is recommended:

double disking followed by deep plowing. After early harvested crops, it is recommended to combine disking with herbicide application. Although intensive tillage is quite common in NEE to control weeds, minimum tillage and no-till technologies are becoming adopted everywhere. Dnipropetrovsk in the Central Ukraine has been an important center for promoting and spreading minimum tillage technologies (Medvedev 2006).

SOIL FERTILITY MANAGEMENT

North Kazakhstan and West Siberia

The dry steppes of North Kazakhstan and West Siberia were developed for grain production in the 1950s. During the first 30–35 years, soils were rich in available nitrogen, and only phosphorus fertilizers were applied. The most widespread method of application was during the summer fallow period, once in 4 years at the rate of 60 kg P/ha (Lichtenberg 1995). The amount of N-NO_3 in the 0- to 100-cm soil layer during 1960–1980s decreased from 433 to 200 kg/ha in summer fallow and from 301 to 125 kg/ha in stubble. Presently, the application of nitrogen fertilizer on stubble fields is recommended based on soil analysis. In 1988, the application of 50 kg N/ha on stubble doubled barley grain yield on black soil (Filonov 2005).

Northeast Europe

The semiarid steppe belt of NEE is located on common black soils. In the Rostov area, spring application of fertilizers before seedbed preparation with duck foot cultivators is most efficient. In a study conducted during 1980–1984, application of 40–60–40 kg/ha of nitrogen/phosphorus/potassium (NPK) by cultivator in the fall, plow in the fall, and cultivator in spring gave grain yields of 4.08, 4.11, and 4.27 t/ha, respectively, versus 3.41 t/ha in the control with no fertilizer (Sokol and Beltyukov 1988). Higher rates of fertilizers increased grain yield, but the unit of yield per unit of fertilizer return was much lower.

In Southeast Ukraine, barley is able to use very well carry-over manure applied to the previous crop. When the previous crop was maize with 30 t/ha of manure applied, barley grain yield increased by 0.22 t/ha. Fertilizers may also be applied in the fall before deep tillage. The rate of 40–80–40 kg/ha of NPK fertilizer ensured a grain yield gain of 0.87 t/ha and a return of 5.4 kg of grain per 1 kg of fertilizer applied (Logvinenko et al. 1998).

In the steppe of the Krasnodar area in a 2-year study, spring barley sown after a mixture of pea and oat with the most common rate of 60–60–45 kg/ha of NPK yielded 5.72 t/ha versus 4.99 t/ha grain with no fertilizer (Vasyukov and Kuznetsova 1988).

In the Central Chernozem zone, an adequate fertilization rate for malting barley is 60–60–60 kg/ha of NPK (Gorshkova et al. 1988). Further increase of fertilizer rates may increase crop yields but with lower return. In the Kursk area on typical black soil with OM of 5.2%–5.4%, application of the same 60–60–60 NPK rate increased the grain yield of continuous barley by 56.1%, which was equal to barley grown in a crop rotation. When 3- to 5-year continuous barley was double cropped with green manure (rapeseed and clover), barley yield gain was 5.8%–7.4% higher than barley in a crop rotation (Kartamyshev et al. 2006). Double cropping of continuous barley with green manure during 3 years increased soil OM by 0.1%.

Also in the Kursk area on sloped land when barley was sown after sugar beet, the highest yield was obtained from a carry-over effect of 48 t/ha of manure applied for sugar beet; 3.46 t/ha versus 2.42 t/ha for control with no fertilizer (Deriglazova and Boyeva 2006). Combined applications of mineral fertilizers on the background of manure even reduced barley yield.

In the Tatarstan forest steppe, the recommended rates of fertilizers are 60–40–60 kg/ha of NPK. These rates of fertilizers increased protein content from 9.3% to 10.7% in control with no fertilizer up to 13.4%–14.5% (Blokhin 2006). In Penza Province, fertilizer rates recommended are 45–50 kg/ha of NP. The yield increase was 20% as compared to control with no fertilizer averaged

over 3 years and four varieties. Split application of nitrogen did not show any advantages (Koshelyayev 2006).

The south part of Krasnodar province is located in a foothill area with rich black soils and relatively high rainfall. The highest grain yields of winter barley (6 t/ha) were obtained with application of fertilizer at rates of 140–250–140 kg/ha of NPK. Nitrogen application was split with 70 kg/ha in the fall and 35 kg/ha both in spring and during the tillering stage (Shevtsov and Naidenov 1988). In the Middle Volga, nitrogen fertilization of winter barley is critical. It is recommended to do side dressing with ammonium nitrate early in the fall up to 40 kg N/ha and at the jointing stage at 60–80 kg N/ha (Tupitsyn and Valyaikin 2005). On dark gray forest soils, mineral fertilizers at the rate of 30 kg N/ha are applied prior to seedbed preparation with duck foot cultivators and 10–15 kg P/ha with seeds (Lopachev et al. 2001). On gray soil with 4.9% OM averaged over 2002–2004, the barley control (no fertilizer applied) grain yield was 1.82 t/ha; with 5 t/ha of straw applied, the yield was 2.19 t/ha; with 33–30–22 kg/ha of NPK, the yield was 2.74 t/ha; and with a combined application of straw and mineral fertilizer, the yield was 3.22 t/ha (Melnik et al. 2006).

COMPLEX OF CULTURAL PRACTICES

North Kazakhstan and West Siberia

In North Kazakhstan semiarid steppe, three levels of cultural practices were continuously applied during 19 years (1988–2006) in a "fallow–wheat–wheat–barley–wheat" rotation: low, common, and high inputs. The three levels included different relative levels of water, plant nutrition, and weed management. Most important was enhancement of water management by improved snow harvest technologies. Over the 19 years, one was wet, three were very dry, nine were dry, and six were favorable. Increasing levels of the combined cultural practices improved barley grain yield dramatically in all types of weather (Table 9.4).

Table 9.4 Spring barley grain yield (ton per hectare) as affected by three input levels of cultural practices and four different weather conditions in the semiarid steppes of North Kazakhstan, Shortandy (data of K. Akshalov and M. Suleimenov)

	Weather Condition			
Cultural Practice	Very Dry	Dry	Favorable	Wet
Low	0.66	1.43	1.89	1.05
Common	1.19	2.22	2.64	1.18
high	1.85	2.98	3.50	1.94
LSD_{05}	0.20	0.25	0.32	0.16

In the wet year, the grain yield was almost doubled from low-input (1.05 t/ha) to high-input practices (1.94 t/ha), although the potential was not realized because of high incidence of diseases. In the three very dry years, the grain yield tripled from the low- to the high-input cultural practices (0.66 t/ha vs. 1.85 t/ha). In the nine dry years, the grain yield more than doubled across management regimes and reached an average 2.98 t/ha for the high-input practice. And in the 6 years with favorable weather conditions, the grain yield was rather high even with low-input cultural practices. Application of the best cultural practices under favorable weather conditions almost doubled barley grain yield, reaching an average level of 3.5 t/ha.

In the southern forest steppe of West Siberia, 5 year (2001–2005) average barley yield gains obtained from fertilizers, herbicides, and fungicides amounted to 60%, 59%, and 7%, respectively. The combined application of the three factors increased grain yield by 109% and reached 3.23 t/ha (Kholmov and Shulyakov 2006).

Northeast Europe

In the Central Chernozem zone, three factors were studied: fertilizer, variety, and seed rate. The average effects of the factors on barley grain yield were fertilizer—37.7%, variety—13.6%, and seed rate—1.5%. For high-yielding cv. Olimpiyets, the share of the same factors was 35.0%, 24.3%, and 4.6%, respectively. It was further concluded that the effect of fertilizers increased in dry years up to 40.9%–43.2%, and

that of variety increased up to 33%–34% (Gorshkova et al. 1988).

In the Penza forest steppe, the influence of three factors on barley grain yield was studied: variety, fertilizer, and weather (Koshelyayev 2006). The highest average effect in grain yield was due to fertilizer—25.9%. Variety played an important role in some years, and on average, its effect was 12.6%. Weather's effect on grain yield was, on average, 23.7%. Interaction of weather with fertilizer was also important, affecting 18.9% of barley grain yield.

On dark gray soil, four levels of cultural practices were studied: (i) extensive, with no fertilizer and pesticides; (ii) intensive, with commercial synthetic fertilizers and pesticides plus carry-over effect of 50 t of manure applied to the previous crop; (iii) transitional to organic production, with reduced synthetic chemicals plus carry-over effect of applied manure and 5–6 t of straw to the previous crop and 6–8 t of green manure; and (iv) organic, with no synthetic chemicals but with applications of manure, straw, and green manure. The highest barley grain yields were obtained on intensive and organic transitional cultural practices (3.7 and 4.0 t/ha, respectively) (Lopachev et al. 2001).

SUMMARY

Major components of cultural practices ensuring remarkable yield increases are soil moisture, weed, and soil fertility management. The weakest point of farming practices in the three countries is very low rates of applied fertilizers for wheat and almost no fertilizers for barley. Soil conservation practices including no-till farming have been adopted on a large scale only in Kazakhstan. Weed management needs to be improved a great deal in all regions.

Research data on cultural practices for barley show that in all ecoregions of the Russian Federation, Ukraine, and Kazakhstan, there is great potential for increased grain yields. The expected yields under adequate cultural practices can reach the following, on average: in the steppes of North Kazakhstan and West Siberia—3.0–3.5 t/ha, in the Volga area—3.5–4.0 t/ha, in the North Caucasus and South Ukraine—4.5–5.0 t/ha, in the Central Chernozem area and Central Ukraine—5.0–5.5 t/ha, in the forest steppes of Russia and Ukraine—5.5–6.0 t/ha, and in the foothills of Krasnodar area—6.0–6.5 t/ha for spring barley and 6.5–7.0 t/ha for winter barley.

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NORTH AMERICA

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INTRODUCTION

Most barley (*Hordeum vulgare* L.) in North America is produced in the Canadian Prairie provinces of Alberta, Saskatchewan, and Manitoba and in the northern-tier U.S. states of North Dakota, Montana, Idaho, Washington, and Minnesota. There is limited barley production in Mexico, and essentially, none produced in Belize, Costa Rica, El Salvador, Guatemala, Honduras, and Panama. While barley is produced in most Canadian provinces and in 27 of the U.S. states (Table 9.5), our focus here is the cropping systems and cultural practices in the major barley regions of the Canadian and U.S. Northern Great Plains (NGP) and the U.S. Pacific Northwest (PNW). Although winter barley is produced in certain regions of North America with mild climates, winter barley cultivars that can withstand the cold winter temperatures of the NGP and most areas of the PNW have not yet been developed. Spring barley makes up the vast majority of NGP and PNW production and in this chapter, the term "barley" implies spring barley unless otherwise mentioned.

In the 10-year period from 1998 to 2007, barley was produced on an average of 3.92, 1.73, and 0.36 million hectares per year in Canada, the United States, and Mexico, respectively (USDA-NASS 2008) (Fig. 9.4A). Production area has remained relatively stable in Canada (Statistics Canada 2008) and in Mexico, but there has been a fivefold decrease in barley hectarage in the United States since its peak in the mid-1980s

Table 9.5 Barley production area and average barley grain yield in each province and state in Canada and the United States, respectively, in 2007. Data are from Statistics Canada (2008) and USDA-NASS (2008)

	Production Area (1000 ha)	Average Yield (kg/ha)
Canada		
Alberta	1729	3700
Saskatchewan	1660	3000
Manitoba	380	3900
Quebec	95	4100
Ontario	67	4100
Prince Edward Island	33	3600
British Columbia	20	3600
New Brunswick	13	4300
Nova Scotia	3	3300
	Total: 4000	Average: 3400
United States		
North Dakota	563	3800
Montana	292	3000
Idaho	223	5400
Washington	91	4000
Minnesota	44	3800
Colorado	23	8400
Oregon	21	3200
Wyoming	21	6000
Pennsylvania	17	4900
California	16	4000
Maryland	14	5600
Arizona	13	7700
Virginia	12	4800
South Dakota	12	2700
Wisconsin	9	3800
Utah	9	5200
Delaware	7	5200
Maine	7	4700
North Carolina	6	3600
Kansas	5	3200
Michigan	5	3800
New York	4	3100
All other states	51	3600
	Total: 1465	Average: 4000

(Fig. 9.4A). Reasons for the decline in barley hectarage in the United States include (i) conversion of cropland to perennial grasses and shrubs under the federal Conservation Reserve Program; (ii) the difficulty of producing malt-grade barley under rainfed conditions in many years; (iii) the grain price of wheat (*Triticum aestivum* L.), and more recently maize (*Zea mays* L.), generally being higher than that for feed barley; and (iv) soil carryover of some herbicides used for wheat production have plant-back restrictions for barley and other crops for certain time periods.

Average barley grain yields are generally slightly greater in Canada than in the United States, with yields increasing in a near-linear manner in both countries during the past 50 years (Fig. 9.4B). Historically, barley grain yields in Mexico have been considerably lower than those in Canada and the United States, but yields in Mexico have increased appreciably in the past 10 years (Fig. 9.4B).

Barley is the third most important crop grown in the NGP of Canada after wheat and canola (*Brassica napus* and *Brassica campestris*). In the NGP of the United States, barley is the fourth most important crop after wheat, soybean (*Glycine max* L.), and maize. Malt-grain cultivars make up about 75% of barley hectarage in the NGP, but selection of barley for malt quality is generally only 25%–30% of malt barley production. The remaining land area is devoted to feed-type barley cultivars. In the PNW, most barley produced under rainfed conditions is used for feed and that under irrigation is used for malt. Lesser amounts of barley are also grown for silage for the feedlot industry. In all regions, the majority of barley is produced under rainfed conditions. One exception is in Idaho, where 60% of the state's barley is grown under irrigation (NASS 2008). Malt-grade barley is often difficult to achieve under rainfed conditions due to the vagrancies of climate.

Historically, about 80%–90% of the malt cultivars grown in Canada are two-row types. Malt-grade barley has returned a premium of almost Can$125 per hectare over feed barley prices. The biggest challenge for farmers is production of malt barley cultivars with a careful focus on agronomic practices including crop rotations for disease management, planting dates, planting rates, and fertilizer management to ensure barley grain will meet malt quality standards.

CLIMATE IN RELATION TO BARLEY PRODUCTION

The precipitation patterns in the NGP and PNW are vastly different. The NGP has a continental

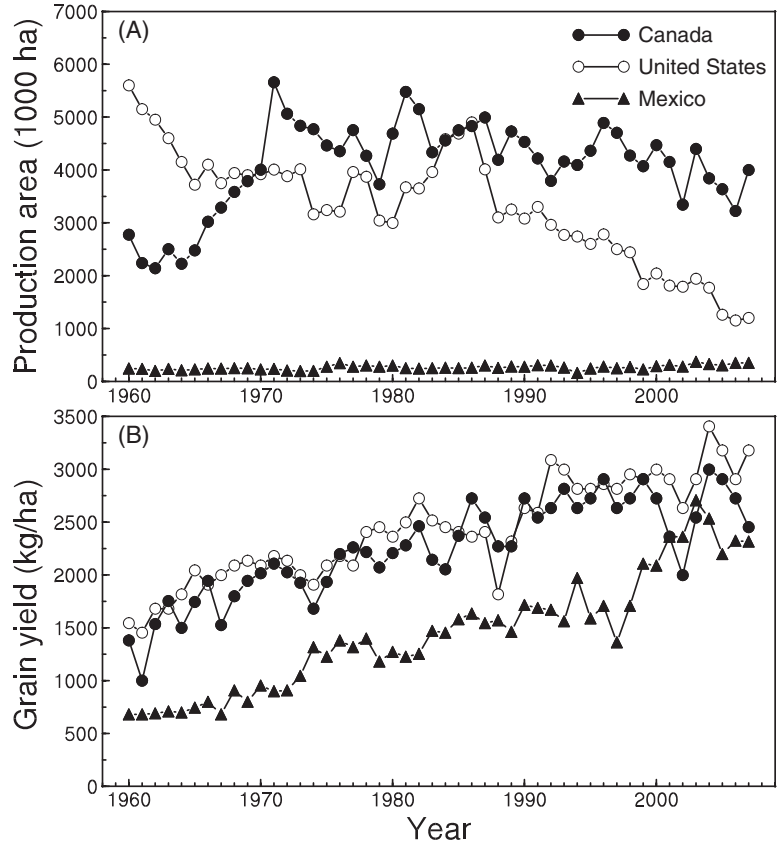

Fig. 9.4 Annual production area (A) and average grain yield (B) of barley in Canada, the United States, and Mexico from 1960 to 2007. Data are from the United States Department of Agriculture National Agricultural Statistics Service (USDA-NASS 2008) and Statistics Canada (2008).

climate with long, cold, relatively dry winters and short, warm, and generally wet summers. The annual average precipitation for NGP croplands ranges from 300 to 800 mm (Cochran et al. 2006). The greatest amounts of precipitation occur during the "growing season" months of May, June, and July (Fig. 9.5).

The PNW has a Mediterranean-like climate where the majority of annual precipitation occurs during the winter (Fig. 9.5). Winter temperatures are cool to cold but milder than those of the NGP. The annual average precipitation for PNW dry croplands ranges from 150 mm in South Central Washington to 1000 mm in the Willamette Valley of western Oregon. Crop production is heavily dependent on winter precipitation stored in the soil. High-pressure systems dominate during the summer leading to warm, dry conditions and low relative humidity. This climate is ideal for production of cool-season crops like wheat, barley, and grain legumes.

CROPPING SYSTEMS

Barley is a cool-season crop with early maturity that has a certain tolerance to drought and saline problems. Barley is a good rotational crop throughout the NGP and the PNW and fits well into conservation-till and no-till cropping systems.

No-till dynamic cropping systems are currently used in the major barley producing areas of the NGP (Tanaka et al. 2002). Many of these cropping systems have roots in the crop–fallow systems and evolved over the years to improve precipitation-use efficiency and to conserve natural resources. Barley fits well into dynamic

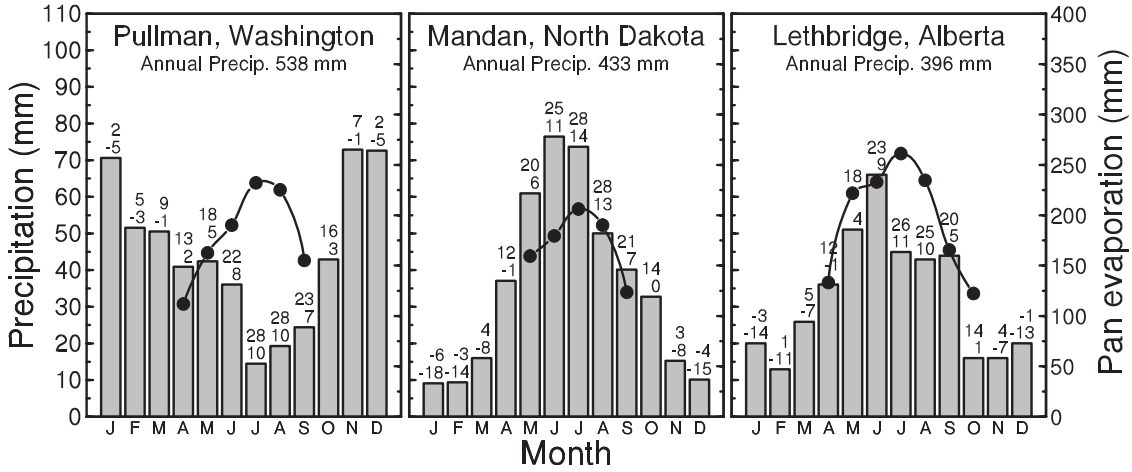

Fig. 9.5. Average monthly precipitation (bars) and pan evaporation (dotted line) from three representative barley production areas in North America. Lethbridge, Alberta, and Mandan, North Dakota, are located in the Northern Great Plains of Canada and the United States, respectively. Pullman, Washington, is in the Palouse region of the U.S. Pacific Northwest. The numbers above individual bars are the average monthly maximum and minimum (°C) air temperatures. The data provided are courtesy of Washington State University, Agriculture and Agri-Food Canada, and USDA Agricultural Research Service.

cropping systems (Tanaka et al. 2005). The crop sequence that usually results in the lowest barley grain yield is when barley is planted on its own crop residue. Factors associated with these lower yields were not obvious but may be associated with plant disease (Krupinsky et al. 2006). Some of the best barley crop sequences are when barley is planted on legume residues. Barley planted on spring wheat residue and vice versa improves crop yields and reduces disease pressure (Schillinger and Paulitz 2006) compared to planting barley or wheat on its own residue even though both are cool-season grasses and share many pest problems. In the NGP, crops planted on barley residue tend to equal or better the yields of crops planted on spring wheat residue (Krupinsky et al. 2006). For example, deep-rooted and high water-using crops such as sunflower (*Helianthus annuus* L.) and safflower (*Carthamus tinctorius* L.) do better after barley than after spring wheat.

Prior to 1990, barley was grown almost exclusively in tillage-based systems in the NGP with hard red spring wheat as the most common cereal grown followed by barley. Most barley was grown after a 21-month summer fallow period in the drier regions (<400-mm annual precipitation) and was recropped (i.e., no fallow) where annual precipitation was >400 mm. In drier regions, the recropping decision is largely based on the quantity of water stored in the soil over the winter; if the soil wetting front does not extend at least 45 cm deep (in the range of 50–75 mm of available soil water), then NGP farmers will generally leave the land fallow. During the 1990s, due to the development of efficient no-till drills and affordable nonselective herbicides such as glyphosate, there was a gradual shift from tillage-based farming to no-till, where seed and fertilizer are delivered in one pass into the undisturbed standing stubble of the previous crop. In Alberta, the total cropland area under no-till increased from 3% in 1991 to 47% in 2006 (Statistics Canada 2008). In Saskatchewan, 60% of all cropland was in no-till by 2006.

The shift toward no-till has resulted in increased soil and water conservation, which in turn has reduced the amount of land in summer fallow across the NGP with concurrent shift in cropping systems used by farmers. More diverse

crop rotations have been adopted that include other cereals, oilseeds, and legumes that have reduced disease pressure and allowed better weed control.

In the PNW, barley is commonly grown in a 3-year winter wheat–spring barley–spring legume rotation in the Palouse region that receives a 450- to 600-mm annual precipitation. Barley is at somewhat of a disadvantage (compared to legume) because it follows winter wheat, a high water user, in the rotation. Palouse farmers prefer to grow winter wheat after a spring legume crop because legumes use less water than barley, resulting in world-record rainfed winter wheat grain yields that average 6500 kg/ha. Average spring barley yields are 4500 kg/ha. Barley is also popular in the intermediate precipitation (300- to 450-mm annual) region of the Inland PNW where it produces an average 2800 kg/ha grown in a 3-year rotation of winter wheat–spring barley–summer fallow. Barley emerges quickly from the soil and has wide leaves and is therefore more competitive against weeds compared to spring wheat. Relatively little barley (or any spring crop) is produced in the low precipitation (<300-mm annual) region of the PNW where 95% of cropland is in a 2-year winter wheat–summer fallow rotation. However, when 125 mm or more over-winter precipitation is stored in the soil, many farmers will plant recrop spring barley or spring wheat with good results (Schillinger et al. 2007). Spring barley grain and residue yields are equivalent to those of spring wheat (Schillinger 2005), but barley residue decomposes at a faster rate than wheat residue. The rate of straw decomposition involves the interaction of several factors and compounds including the content of hemicellulose, cellulose, lignin, tannins, and nitrogen as well as the carbon : nitrogen ratio (Stubbs et al. 2009).

Barley yields in North America and throughout the world are greatly influenced by water availability. In a study with feed barley at 20 sites in Alberta, McKenzie et al. (2004) reported that the average water-use efficiency was 15 kg grain/ha/mm, similar to the 16 kg grain/ha/mm obtained in Alberta by Hoyt and Rice (1977) and higher than the average 10 kg grain/ha/mm reported by Bole and Pittman (1980). A higher water-use efficiency than observed previously in the NGP can be attributed to improved cultivars and agronomic practices including no-till and residue on the soil surface that conserves soil water, making the water available for transpiration through the plant. The relationship of barley grain yield to water use is affected by potential evapotranspiration and the timing, duration, and intensity of periods of water deficit (Heapy et al. 1976; Baldridge et al. 1985).

Fibrous rooted crops such as barley produce relatively high quantities of belowground biomass. Soil organic matter levels and soil quality can be maintained or even improved by including barley in the crop rotation. Spreading barley residue along the entire width of the combine header at harvest makes planting the next crop easier, reduces immobilization of nutrients during decomposition by microbes, increases the efficiency of herbicides, and reduces diseases.

CULTURAL PRACTICES

Much of the barley in the NGP is harvested by swathing or using preharvest desiccants to hasten drying after the grain reaches physiological maturity (<35% seed moisture); however, on the southern prairies, barley is often direct combined without swathing or use of desiccants. Direct combining is usually best if grain moisture level is <20%. Due to its dry summer climate, all barley is direct combined in the PNW when grain moisture content is <12%.

Barley water use from soil water depletion plus growing season precipitation is slightly greater than that of dry pea (*Pisum sativum* L.). Of 10 crops evaluated for water use in North Dakota, dry pea had the lowest soil water depletion (4.1 cm) followed by barley (4.3 cm) (Merrill et al. 2004). Soil water recharge during the noncrop period greatly influenced the next year's crop. Soil water recharge for barley residue was equal to spring wheat residue (Merrill et al. 2004). Some of the greatest soil water contents at planting in the spring were following dry pea, barley,

dry bean (*Phaseolus* spp.), crambe (*Crambe cordifolia*), spring wheat, and soybean.

Planting date

The highest spring barley grain yields in all regions are generally achieved when planted as early as possible in late winter/early spring. In Alberta, McKenzie et al. (2005) found that delaying planting from late April to mid-May reduced grain yield by 20%. This was consistent with other barley planting date studies in North America (Beard 1961; McFadden 1970; Ciha 1983; Lauer and Partridge 1990; Juskiw and Helm 2003). The average yield loss of 20% from first to latest planting in the McKenzie et al. (2005) study was less than observed in Minnesota (35%; Beard 1961) and central Alberta (47%, Juskiw and Helm 2003), likely due to the shorter time period between the first and last planting dates (≈3 weeks) versus the latter-mentioned studies (5–6 weeks). Early-planted barley has a grain yield advantage in all regions because the crop can capitalize on early spring moisture, longer spring days, and cool temperatures before the onset of hot summer temperatures.

Row spacing

Highest grain yields of barley are generally produced from rows spaced 250 mm or less. Low spike density generally occurs with rows spaced >250 mm and, although the plant will compensate with greater kernel number per spike and kernel weight, grain yield is reduced (Schillinger et al. 1999). Spike number per unit area is considered the most important yield component for barley under dryland conditions when severe water stress is not a factor (Arnon 1972). Weed growth tends to be more abundant in rows spaced more than 250 mm apart than in narrower rows.

When barley is swathed, support can be a problem as row width is increased. Drill openers that do not place the seed in distinct rows but rather scatter the seed in 5-, 7-, or 10-cm bands usually have good yield potential, and greater quantities of fertilizers with high salt index can be safely placed with the seed.

Planting rate

Optimum planting rate is influenced by many environmental and economic factors. Assuming that grain yield must increase four times the increase in planting rate to pay for the seed, McKenzie et al. (2005) recommended an optimum stand density of 200 plants/m^2 under rainfed conditions and 250 plants/m^2 under irrigated conditions in Alberta. A planting rate of 400 seeds/m^2 generally results in higher yields versus 200 seeds/m^2 at sites in Alberta and Manitoba. Further, barley is more competitive with weeds at the higher planting rate.

In a 4-year study in eastern Washington, Schillinger (2005) reported no differences in barley grain yield with planting rates of 120, 200, and 280 seeds/m^2. Although spike number per unit area was slightly reduced with low planting rate, the increased number of kernels per spike consistently compensated for reduced plant stand density. Planting rate had no effect on kernel weight or straw production. These results suggest that with precise placement of seed, farmers in the PNW could reduce planting rates by 50% or more from rates commonly used. These results (Schillinger 2005) somewhat contradict those of Lafond (1994), who observed that increased planting rates reduced kernel weight. Overall, the literature suggests that under dry conditions, lower than normal planting rates may actually increase barley yield by reducing plant competition for water, whereas when water is not so limiting, higher planting rates increase crop competitiveness and yield.

FERTILIZER MANAGEMENT

Achieving optimum yields of barley requires careful attention to nutrient requirements in all agroecological, soil, and climatic conditions. A balanced fertilizer program must be developed to achieve high barley yields.

Nitrogen (N)

N is by the far the most important nutrient required to ensure optimum barley grain yield and quality and almost universally increases grain yield on soils with low available N. Adequate N promotes vigorous plant growth and a larger leaf area with a deep green color. N in older leaves is redistributed to younger leaves to maintain growth. As a result, the older leaves first show the characteristic lighter green to yellow color followed by withering, indicating N deficiency.

The amount of N fertilizer required depends on the level of soil nitrate-N (NO_3^-), the mineralization potential of the soil, stored soil water, and expected precipitation. These conditions vary greatly across regions. As stored soil water and growing season rainfall increase, so too does the need for N fertilizer. As a general rule, feed barley requires about 38 kg of available N for every 1000 kg of expected grain yield. Franzen and Goos (2007) recommended about 30% less N for feed barley compared to malt barley grown in the NGP.

Phosphorus (P)

Native soils of the NGP often had total soil P levels in the range of 1100–1350 kg/ha (McKenzie et al. 2003). However, the portion of usable or plant-available P in native soils is very low; therefore, the majority of soils in the NGP are considered P deficient. Essentially all agricultural soils in the PNW are considered low in available P.

Barley response to applied P fertilizer depends to a large extent on placement and on the quantity of plant-available P already in the soil. The application of P can increase the retention of tillers and can hasten maturity. P levels in some soils have increased over the years as a result of repeated annual P fertilization.

Barley is frequently most responsive to seed-placed P followed by banded P fertilizer (McKenzie and Middleton 1997). Seed-and band-placed fertilizer P allows the roots of barley seedlings easy access to a concentrated quantity of P, whereas mixing P into the soil causes a dilution and in many cases causes P to be fixed in the presence of calcium or iron that reduces P availability. To obtain maximum P fertilizer efficiency, adequate rates of N and other nutrients must also be available to the barley crop. After P fertilizer has been applied for 10–20 years resulting in an increase of residual soil P, a relatively low annual maintenance application of P is generally all that is required to meet crop requirements, that is, to replenish soil P that was removed by the previous crop.

Potassium (K)

Barley takes up nearly as much K as nitrogen and therefore has a high K requirement. However, only 20% of the K is removed with the grain with the remainder in the leaves and stems normally returned to the soil. The majority of cropland soils in the NGP and PNW contain adequate K for barley production with extractable soil K levels in the range of 450 to over 1200 kg/ha. There is generally little to no response in barley to K fertilizer when soil test levels are greater than 250 kg/ha. In the few soils that test <250 kg K/ha, or on sandy soils or intensively cropped fields, some K fertilizer may be required.

Sulfur (S)

S is the second most deficient nutrient (after nitrogen) in the PNW (Rasmussen and Douglas 1992), whereas S deficiency in barley is less common in the NGP (Cochran et al. 2006). S deficiency is most commonly observed on black and gray wooded soils in the northern regions of the NGP. Barley requires about 1 kg of S for 16 kg of N, and application of 12–20 kg S/ha is common. A 4300 kg/ha barley crop (grain and straw) contains approximately 12–14 kg S/ha, of which 50% is contained in the grain.

In the PNW, sulfur is most commonly applied in combination with N as liquid ammonium thiosulfate or ammonium polysulfide. Granular ammonium sulfate (21-0-0-24) is most commonly used to correct S deficiencies in the NGP. Sulfate-S is water soluble and mobile within the soil; therefore, banding sulfur near the seed at the time of planting is normally the preferred method of application.

Micronutrients

Research on micronutrients since the 1960s has identified zinc (Zn), copper (Cu), manganese (Mn), and boron (B) as potential deficient micronutrients. Organic (peat) soils were also identified as a primary target for micronutrient deficiencies. Extensive work on the Canadian Prairies identified Cu as the micronutrient most likely to produce significant yield response in barley (Karamanos et al. 1983). Some 1.6 million hectares of cropland in Alberta and Saskatchewan is potentially Cu deficient (Kruger et al. 1985), primarily on the black and gray-black transition soils. Coarse-textured soils are most commonly Cu deficient, and wheat and barley are the two most sensitive crops to Cu deficiency. Copper fertilizer is commonly used in these deficient areas. To date, however, most farmers have applied very little of the other micronutrients for rainfed barley production.

WEEDS, DISEASES, AND INSECTS

Weed control

Many of the pest problems associated with wheat are also common to barley (Ransom 2005), and many of the herbicides used to control weeds in wheat are also registered in barley. Grass weeds that are a problem in barley include wild oat (*Avena fatua*), jointed goatgrass (*Aegilops cylindrica*), yellow foxtail (*Setaria glauca*), green foxtail (*Setaria viridis*), and Italian ryegrass (*Lolium multiflorum*). As a spring-planted crop, barley is effective in controlling downy brome (*Bromus tectorum*), by far the most problematic grass weed in the PNW and in other areas where winter wheat is produced. Downy brome is a winter annual with a growth cycle similar to winter wheat. Rotation to spring barley, spring wheat, legumes, oilseeds, or summer fallow is a prerequisite to control downy brome in the winter wheat-based cropping systems in the PNW (Thorne et al. 2007). Problematic broadleaf weeds include Russian thistle (*Salsosa kali*), common lambsquarter (*Chenopodium album*), horseweed (*Conyza canadensis*), kochia (*Kochia scoparia*), prickly lettuce (*Lactuca serriola*), redroot pigweed (*Amaranthus retroflexus*), mayweed chamomile (*Anthemis cotula*), field bindweed (*Convolvulus arvensis*), wild buckwheat (*Polygonum convolvulus*), and wild mustard (*Sinapis arvensis*).

Postemergence weed control in barley is usually less of a problem than in wheat because emerging barley seedlings tend to have a more vigorous and prostrate early growth habit with wider leaves to compete against weeds. Weeds can cause serious grain yield losses in barley, and their control is essential to attain optimum yield and quality. Reduction in grain yield and quality is generally proportional to the weed biomass in the crop. Weeds present in barley grain can result in major dockage and grade losses. Excessive weed biomass present in silage barley can reduce the quality of the fodder and affect palatability. Malting barley with excessive amounts of certain weeds will not achieve malt grade.

The cost of weed control must be balanced by the expected increase in return to the farmer from an increase in crop production, both in the current year and in future years. Crop competition and herbicides are the primary means of controlling weeds in barley. Most recommended herbicides are relatively cost-efficient and, other than crop competition, are the only widely applicable means of in-crop weed control. Various preventative measures include preplant application of a burndown herbicide such as glyphosate, using certified seed, and thoroughly cleaning combines after harvesting a weed-infested field. Physical control measures are used such as no-till planting to leave weed seeds on the surface where they are less likely to germinate compared to being incorporated into the soil with tillage. Cultural weed control methods include use of crop rotations with forage crops or fall-planted cereals to disrupt the life cycle of existing weeds. Increasing planting rates and planting early are other effective means used to increase crop competition with weeds. Cutting barley for green feed or silage can also be an effective form of weed control.

Some soil residual herbicides used in wheat and in other crops have barley plant-back restrictions ranging from a few months to 2 years.

These include some grass herbicides developed to selectively control downy brome and jointed goatgrass in wheat. In the past 5 years, the soil residual herbicide imazamox has been available for weed control in Clearfield™ wheat (Ball et al. 2003). Clearfield wheat cultivars were developed through mutation breeding for tolerance to imazamox herbicide. Imazamox selectively controls several grass and broadleaf weeds, but, due to its soil persistence, barley should not be planted for 12–18 months (or longer) after its application. Other soil residual herbicides used in wheat that have plant-back restrictions for barley include sulfosulfuron, imazethapyr, and propoxycarbazone. Soil texture, precipitation, soil organic matter, soil microbial activity, and soil pH are factors that influence persistence of herbicides in the soil. Farmers need to be aware of any carry-over problems and cropping restrictions of herbicides that they use.

Diseases

Numerous fungal and viral diseases affect barley. The major barley diseases in the NGP are generally not the same as those in the PNW because of the difference in precipitation pattern (Fig. 9.5). Barley diseases of greatest significance in the NGP are common root rot (also known as seedling blight) caused by *Cochliobolus sativus*, net blotch (caused by *Pyrenophora teres*), scald (caused by *Rhynchosporium secalis*), and fusarium head blight or scab (caused by *Fusarium graminearum*). An overview of these diseases is provided by Bailey et al. (2003).

The spores of several root rot fungi are ubiquitous in agricultural soils in both the NGP and PNW where they survive for many years. Common root rot, identified by the extensive brown discoloration of the subcrown internode and crown roots, damages the barley plant by plugging vascular tissue. Common root rot is the only consistently destructive soilborne disease of barley in the NGP. Rotations of several years with nonhost crops such as canola, alfalfa (*Medicago sativa* L.), flax (*Linum usitatissimum* L.), or legumes are necessary to reduce disease incidence.

Net blotch is a common foliar disease in the NGP that can reduce barley yields by 40% depending on the amount of affected tissue in the leaves and spike. The fungus overwinters on crop residue and infections occur in the spring from spores that are spread by rain and wind. Control is by crop rotation, foliar fungicide application, and planting barley cultivars that have some resistance to net blotch.

Scald is a major foliar disease of barley in the wetter regions of the NGP, particularly the black and gray soil regions in Canada. Scald is primarily a foliar disease but is also found on leaf sheaths and glumes. Large water-soaked, grey-green spots become bleached with brown margins as the leaves dry out. Spots often join and kill the entire leaf. The scald fungus overwinters on barley residue and produces spores in the spring. The disease is favored by cool (12–20°C) air temperatures, high humidity, and dense crop canopies where leaves remain wet for prolonged periods.

Scab infections have resulted in rejection of barley for malt and in some instances for feed grain. Crop sequence influences scab infections. The highest scab infections occur with cropping systems where previous crops are wheat, barley, and corn. Fusarium head blight is the most destructive fungal foliar disease of barley in the NGP. The highest severity of this disease occurs predominantly in the United States in North and South Dakota and Minnesota, as well as in Canada in western Manitoba and eastern Saskatchewan, with minimal levels in Alberta. Fusarium head blight has caused major economic losses due to reduced access to malt and feed markets.

In the PNW, fungi that infect roots, crowns, and stems are the primary pathogens affecting barley production. Rhizoctonia bare patch disease caused by *Rhizoctonia solani* AG8 is an important fungal disease of barley, wheat, and other crops in no-till fields. The disease results in patches of killed or stunted plants several meters in diameter. Rhizoctonia bare patch is unique to no-till fields; it is not a problem when tillage disrupts the fungal hyphal networks in the soil surface. At present, there are few management strategies for rhizoctonia bare patch (other than tillage), but breeding efforts are underway to introduce

genetic resistance into barley and wheat cultivars. A comprehensive overview of soilborne cereal pathogens in the PNW is found in Paulitz et al. (2002).

Insects

Insects are occasionally a concern in barley production but are usually not a major problem. The insect pests of greatest concern are wireworm (*Agriotes lineatus*) and cutworm (order Lepidoptera). In drier years, grasshoppers (order Orthoptera) are occasionally of significant concern. Insects that are normally of minor problem include aphids (order Hemiptera) and barley thrips (order Thysanoptera). In recent years, a new insect pest, the cereal leaf beetle (*Oulema melanopus*), has been identified but is considered only a minor problem in both the NGP and PNW where parasitic insects have been introduced for its control (Glogoza 2002).

FUTURE OUTLOOK

The future for barley may be very promising given the current global status of cereal grains. One shift that could occur is the greater use of winter barley, both feed and malting types, as more cold-hardy cultivars are developed. When not killed by cold, winter barley cultivars have demonstrated considerably higher grain yield potential than spring barley. Although winter barley cultivars so far developed do not have good low-temperature tolerance (Fowler 2008), there is potential for genetic improvement for this trait (Thomashow 2001). Development of cold-hardy winter barley cultivars would likely lead to a rapid expansion of barley hectarage in regions where winter wheat is typically grown.

Since 2007, barley grain price has been on par with that of wheat where before it decidedly lagged behind that of wheat in U.S. markets. Barley production in Canada is expected to remain stable and, overall, it is felt that farmers will continue to show interest in barley, a crop with many desirable agronomic characteristics that also provides valuable crop rotation benefits. To more consistently achieve malt-grade quality, farmers need to continue to fine-tune fertilizer applications in line with available soil water and climatic considerations and to practice effective crop rotations for disease control.

On a broader scale, barley may play a significant role for biofuel production and human nutrition and health. For example, 20% of U.S. corn grain is currently used for ethanol production. Barley is an effective addition to a healthy diet to lower cholesterol and glycemic index due to high grain soluble fiber content. Scientists are only beginning to understand the importance of barley for human nutrition and health maintenance.

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WEST ASIA AND NORTH AND EAST AFRICA

Salvatore Ceccarelli and Stefania Grando

INTRODUCTION

North Africa and West Asia countries (Fig. 9.6) are not only among the oldest areas where barley was cultivated but are also the areas where agronomic science developed during the Roman Empire, particularly through the *De Re Rustica* ("On Agriculture"), the 12-volume treaty written by Lucius Iunius Moderatus Columella. Columella introduced agronomic practices unknown at the time, the most famous of which being fallow.

Contrary to breeding, barley agronomy is relatively less variable among countries of West Asia and North Africa. This is probably the consequence of the fact that (i) barley is predominantly

Fig. 9.6. North Africa (Morocco, Algeria, Tunisia, Libya, and Egypt) and West Asia (Jordan, Lebanon, Israel, Turkey, Syria, Iraq, Iran, Saudi Arabia, and Yemen). *Source:* ICARDA GIS Unit.

grown in marginal areas, where, for example, there are fewer options for rotations, and is predominantly used as animal feed both as grain and as straw; and that (ii) barley is grown in winter (autumn planting and late spring-early summer harvesting) following a dry summer.

One of the major differences in the barley-based farming systems between West Asia and North Africa, particularly in the rainfed areas, is the almost complete disappearance of the fallow in West Asia, while a barley–fallow rotation is still common in North Africa, particularly in Algeria.

In East Africa, mainly Ethiopia and Eritrea (Fig. 9.7), barley is grown twice a year. The main cropping season, known locally as *meher*, relies on June-September rainfall, while the minor cropping season, known as *belg*, is during the short rainy season, March–April. Because of the increasing frequency of droughts, the belg has virtually disappeared in most of Eritrea and in the northern part of Ethiopia (Tigray). Barley is the most suitable crop for belg season production, and it accounts for about 30% and 28%, respectively, of the total major cereal areas and total cereal production during this season. Ethiopia grows over 50,000 ha of malt barley in the Arsi and Bale highlands.

FERTILIZER USE

Being a typical crop of marginal environments with low yield potential and high risk of crop failures, barley has been always and traditionally allocated low amounts of fertilizers, despite the demonstration by International Center for Agricultural Research in the Dry Area (ICARDA) of the effect of fertilizers, both nitrogen and particularly phosphate, in improving water-use efficiency (Cooper et al. 1987). In the semiarid areas, farmers are particularly reluctant to use phosphate because its maximum benefit is when applied before or at planting when farmers do not have any indication on how the cropping season will be. The high probability of either a low yield or of a crop failure explains the risk-averse strategy of keeping investments at a minimum. This applies only to a limited extent to nitrogen. As this fertilizer can be top dressed at the end of tillering, it is common in wet years to see farmers hand spreading nitrogen, usually urea or ammonium nitrate, to barley fields.

In high rainfall areas or under irrigation (such as Iran, Iraq, or Turkey), barley is regularly fertilized as expected yields are much higher and the risk of crop failures is remote. Therefore, in these areas, it is common for farmers to apply fertilizer regularly even though in doses lower than for wheat.

In Ethiopia and Eritrea, nutrient depletion and land degradation are commonly recognized as two of the main yield factors in barley production. However, because of the type of land tenure, the land is owned by the government and farmers cannot sell their land and have no incentives to use improved agronomic practices. However, in some potential barley growing regions of Ethiopia, farmers apply (mainly on malt barley) 60–60 kg/ha urea and diammonium phosphate (DAP). The rate of urea applications can vary based on the preceding rotation crop where less urea is applied if the rotation crop is a legume.

ROTATIONS

In West Asia and North Africa, barley is grown under a number of rotations, which mostly depends on rainfall pattern. In the past, the dominant rotation was barley–fallow, which several studies have shown as the best rotation in dry areas. However, with the exception of several areas in North Africa, the barley–fallow rotation has been almost completely replaced by continuous barley. This is mainly due to two reasons. The first is the increase in the human population with a consequent increase in the demand for animal products, such as meat, milk, and its derivates, and the increase in a number of small ruminants. This has created a continuously increasing demand for animal feed, and as mentioned earlier, barley is considered to be the ideal and most dependable animal feed in the majority of North Africa and West Asia countries. The second is the failure of forage and pasture research to find

(a)

Fig. 9.7. (a) Ethiopia. *(Continued on next page.)*

suitable and adapted legumes to be used as animal feed, which, like barley, can grow during the cold months of the Mediterranean winter, and to withstand the drought in the later stages of crop growth.

Besides continuous barley (no rotation), the most common rotations found in North Africa and West Asia are with lentil, cumin (particularly common in Syria, but depending on the market price of cumin), and, much less common, vetch.

(b)

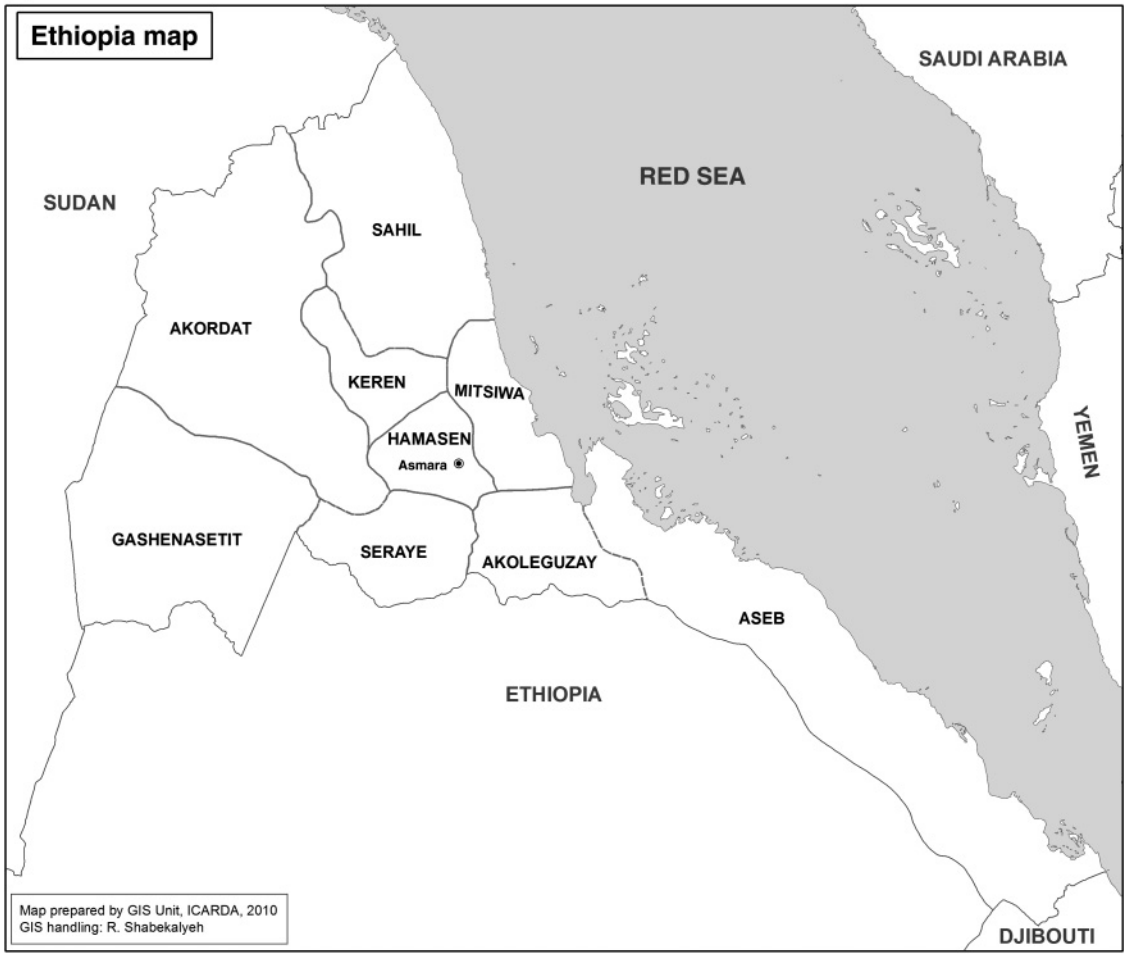

Fig. 9.7. (b) Eritrea and their administrative regions. *Source:* ICARDA GIS Unit.

Occasionally, in the wetter areas, it is possible to find barley following wheat or chickpea, but this is considered to be one of the worst rotations.

In the highlands of East Africa, farmers have practiced the barely–fallow cropping system, but this is now disappearing due to population and livestock pressure, as seen elsewhere. In the mid-altitude areas, farmers rotate barley with faba bean, field pea, and oil crops, mainly brassica and linseed, as well as wheat.

TILLAGE

Because of its importance as animal feed, in the majority of years, and in most of the barley areas, barley stubble is grazed during summer months until the soil is left barren. Therefore, in the areas with continuous barley, soil tillage consists usually of a disk plough, often delayed until later in the autumn.

Even when a rotation is used, in most of the rainfed areas, tillage is more often done after the

first rains and after the emergence of weeds and volunteers in order to insure a reasonably clean seed bed.

Preliminary experiments conducted recently at ICARDA's main research station with conservation tillage have shown its advantage in a number of crops.

In some countries, notably Algeria, some progressive farmers have an interest in experimenting with conservation tillage.

In the highlands of north and northwest Shewa where waterlogging is a major problem during the meher season, farmers use a soil preparation system locally known as *guie* (soil burning). This involves three to five plowings of fields that have been left fallow for at least 5 years. The practice is very tedious. Early maturing farmers' varieties, Demoye and Magie, are used in this system. Grain yield is higher in the first year (about 2.0 t/ha) but dramatically declines in subsequent years. Other cultural practices are similar to the late barley production system (Yirga et al. 1998a). In some parts of Bale, farmers, on average, plow their land five times.

SOWING

In most of North Africa and West Asia countries, and in most of the areas where barley is grown, sowing is done in autumn using spring or facultative types usually on a dry soil profile, following a summer with no rainfall and high temperatures.

The rainfed agriculture of these areas is therefore different from other rainfed and dry areas of the world because at the moment of sowing, farmers cannot count on any amount of stored moisture. As will be seen, this explains a number of farmers' choices in terms of agronomic practices.

In North Africa and West Asia, there has been a dramatic shift in the last 30–50 years from hand broadcasting to mechanical sowing with either imported or locally made drills (Turkey is one of the major producers of drills as well as other agricultural machinery).

Hand broadcasting, as it is still practiced, in mountainous areas and by poor farmers, is done after opening furrows with a disk plough and is followed by splitting the furrows with the same disk plough to cover the seed. The resulting arrangement of the plants in the field would be irregular as would be the seed depth. The lack of control of seed depth had its own advantages, particularly in conditions with an irregular and unpredictable opening of the rainy season. In the case of an early rain in the autumn (a false start of the rainy season) followed by a long period of drought, if seeds are at different depths, only a fraction of them will be reached by the wet front. These will eventually germinate and then dry out for lack of moisture. However, most of the seed will remain in the soil ready to germinate at the real start of the rainy season. In this situation, only a fraction of the sown seed will actually constitute the crop, and this might be a reason why in a number of dry areas, particularly of West Asia, farmers are using high seed rates (as high as 250 kg/ha); however, in other dry areas of the region, such as Jordan, farmers never use more than 100 kg/ha of seed.

In most of the highlands of Ethiopia and Eritrea, the crop is cultivated in two seasons per year. The main cropping season, locally known as meher, uses the June–September rains with harvest in December–January. The March–April rains provide moisture for a second season, locally called belg, with the crop harvested in June–August. In some regions, such as Gojam in Ethiopia, barley locally known as Belga is also produced from September to January under residual moisture. During the belg season, barley is the most widely grown cereal. It covers about 40% of the area and gives 46% of the total cereal production (Central Statistical Authority 1992).

SEEDING RATES

In Ethiopia and Eritrea, barley is planted by broadcasting and the optimum seeding rate is 100–125 kg/ha for broadcast sowing and 85–100 kg/ha for drill sowing. The seeding rate for malt barley is 100 kg/ha in Ethiopia.

In the Near East, the issue of seeding rate is one of the main arguments among farmers, and within a given country, it varies from as little as 60 to 100 kg/ha to as much as 250 kg/ha. Research at ICARDA has shown those seeding rates above 70 kg/ha to have no effect on yield; nevertheless, those farmers who are accustomed to use high seeding rates are very reluctant to change. This is probably a relic of the past when, as mentioned before, the lack of control of seed depth and poor seed quality justified higher seeding rates.

PLANTING DATES

While in most of the countries in North Africa and the Near East planting time for barley is between late October and mid-December and largely depends on the date of the first rain, planting date is much more variable in Ethiopia and to some an extent in Eritrea, where the date of planting determines different production systems as follows.

Late-barley production system

This is the most dominant system important in the high-altitude areas of both Ethiopia and Eritrea and is practiced during the main rainy season (June–October). Two variants of this system (Genbote and Sene gebs) are known based on the planting dates. In South Gonder, North Welo, and northwest Shewa, Genbote is planted in May and Sene gebs is planted between mid-June to early July. Sene gebs is the most common system in Eritrea. Different farmers' varieties are grown in the two subsystems (Yirga et al. 1998a,b). These varieties require 5–6 months to mature. Grain yields for this system vary from 0.6 to 2.0 t/ha (Yirga et al. 1998a).

Early-barley production system

This is also a main rainy season system and is important both in the mid- and high altitudes of Gojam and Gonder (Northwest Ethiopia) and in some parts of Shewa. Early farmers' varieties such as Semereta in Shewa and Gojam, Belga in North Gonder, and Tebele in South Gonder require 3.5–4.0 months to mature. The varieties are planted from mid-May to June and are harvested between early September and early October. Important early barley varieties are Aruso in Arsi and Bale and Saesa in Tigray (Negusse 1998). In Welo, farmers' varieties such as Ehilzer and Tebele, two-row types, are important for early growing areas (Yallew et al. 1998). The yield of early barley in a normal year varies from 0.7 to 1.5 t/ha (Yirga et al. 1998a).

Belg barley production system

This system is practiced in North and Northwest Shewa, North Welo, Bale, and a few areas in Arsi. Belg barley is planted in February and/or early March and is harvested in early July. Early maturing farmers' varieties that require 3–4 months to mature are usually cultivated. In this system, farmers do not apply fertilizer. Moisture stress and Russian wheat aphid (*Diuraphis noxius*) are the major threats to barley in this system. The yield of belg barley in a normal year varies from 0.8 to 1.2 t/ha (Yirga et al. 1998a).

Residual moisture barley production system

This system is important in some parts of Gojam, North and South Gonder, and West Shewa. Early maturing farmers' varieties, Belga in North Gonder and Semereta in Gojam, are important in this system. Planting is done between September and October, immediately after harvest of the main season barley crop. The seed of the main season barley is sown again in the same field or in any other field where the main season crop has failed. Fertilizer is not generally applied in this system. Harvesting is done from December to February. Grain yield from this system is generally low, less than 1.0 t/ha (Yirga et al. 1998a), and it is mainly used as seed for the next season.

WEED CONTROL

In the case of weed control, as in many of other agronomic practices, there is a considerable

difference between barley grown in wetter areas and the same crop grown in dry areas.

In wet areas, wild oat is one of the most common weeds. Because expected yields are higher, chemical weed control is often practiced particularly when heavy weed infestations occur using any available and cheap herbicide. In North Africa and for broadleaf weeds, the herbicide 2,4D has been frequently used.

In dry areas, one of the most common weeds is the wild brassica. In these areas where expected yields are lower and the demand for animal feed is high, a weed, particularly if highly palatable by small ruminants like the wild brassica, ceases to be a weed and it is rather considered an additional source of feed. Therefore, in those countries where labor is still cheap, or is available within the family, most of the weed control is done by hand.

In Ethiopia, research conducted on station showed that a single hand weeding (25 days after planting or 20 days after emergence) is optimum for barley. Results of recent studies indicated that diclofopmethyl, at a rate of 1.5 L/ha, is the most preferred herbicide if the grass weed population consists of *Avena fatua* and *Phalaris paradoxa* (EARO 2000). Broadleaf weeds are still well controlled with 2,4D herbicide at a rate of 1 L/ha.

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AUSTRALIA

Blakely H. Paynter and Neil A. Fettell

The paper of Sparrow and Doolette (1975) provides a good historical view of barley production in Australia between 1860 and 1975. There are sections on the history and development of the Australian barley crop, agronomy, and breeding. This chapter focuses essentially on the environment in which barley is currently grown but reflects on some of the changes that have occurred in the last 35 years since the Sparrow and Doolette (1975) paper.

CROPPING ENVIRONMENT FOR BARLEY IN AUSTRALIA

Barley (*Hordeum vulgare* L.) is grown in all regions of Australia except the Northern Territory. This includes summer-dominant rainfall zones in northern New South Wales and Queensland and winter-dominant rainfall zones in South Australia and Western Australia. The semiarid cropping zone also includes a transitional zone where rainfall is distributed about equally between summer and winter in parts of Victoria and southern New South Wales (Foster 2000; McKenzie et al. 2004). More than 80% of Australia has an annual rainfall

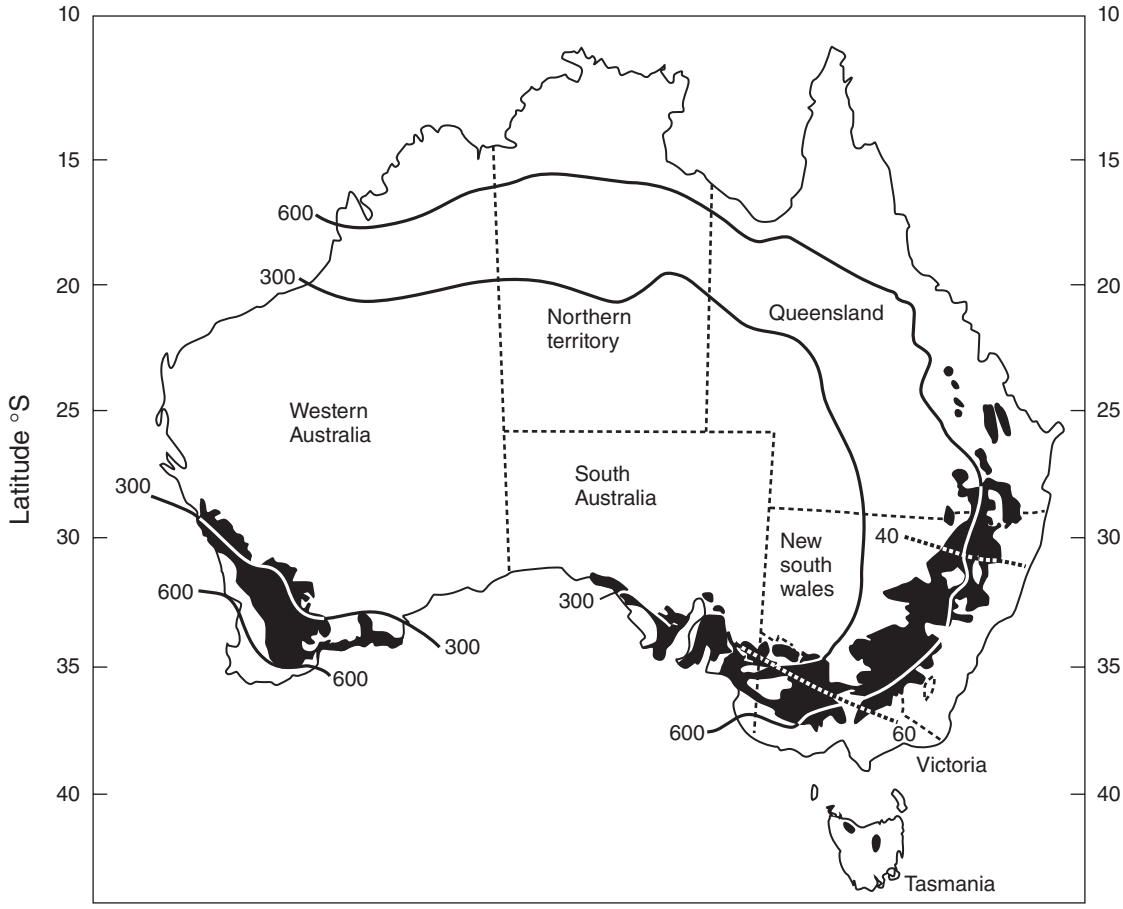

Fig. 9.8. Map showing the main cereal cropping areas of Australia (shaded) and annual rainfall isohyets in millimeters (solid lines). Agricultural land to the west and south of the 60% dotted line receives more than 60% of annual rainfall in the winter months. Agricultural land to the north of the 40% dotted line is the summer-dominant rainfall zone. Agricultural land between the 40% and 60% dotted line is the transitional rainfall zone. Adapted from Foster (2000) by W.K. Anderson and J.F. Angus (unpublished data).

of less than 600 mm, with most barley planting occurring between the 300- and 600-mm annual rainfall isohyets (Fig. 9.8).

In most areas of Australia, barley is produced in a dryland farming system and relies on stored soil moisture and/or in-crop rainfall. Small areas of irrigated barley are also grown in New South Wales and Queensland. The growing season for barley is winter and spring (May–October), and rainfall for this period ranges from 170 to 550 mm. Year to year variability is extremely high in most regions, with coefficients of variability of nearly 20%. This results in highly variable winter crop yields (Singh and Byerlee 1990).

Westerly air streams dominate the climate of southern Australia (Hobbs 1988; Foster 2000). The position of the subtropical band of high pressure in the Indian and Southern Oceans is responsible for much of the rain-generating disturbances. High rainfall-generating systems often occur when the westerlies combine with moist air from the tropics. The climate of eastern Australia is more complex where rainfall from the westerlies is supplemented by summer rainfall from moist air masses from the Pacific Ocean (Foster 2000). Cyclic warming and cooling of ocean temperatures in the central and eastern Pacific have a large bearing on the likelihood of summer rainfall

patterns in eastern Australia. This effect is known as the El Niño Southern Oscillation (ENSO). The ENSO state is determined by measuring differences in sea level pressure at Darwin and Tahiti and is reported as the Southern Oscillation Index (SOI). El Niño years (ocean warming, negative SOI) usually mean a lower likelihood of rainfall. La Niña years (ocean cooling, positive SOI) are usually associated with average to above-average rainfall. The more negative the SOI, the further south down the east coast of Australia the drought extends.

The ENSO effect is important but less significant in Western Australia. Rainfall patterns in Western Australia are more related to a dipole pattern of sea surface temperatures in the Indian Ocean, an effect known as the Indian Ocean Dipole (IOD). A positive IOD corresponds with above-average sea surface temperatures in the western Indian Ocean region, a cooling of waters in the eastern Indian Ocean, and a greater likelihood of lower rainfall in southern Australia. A negative IOD brings the opposite conditions and increases the chances of average to above-average rainfall.

Rapid advances are being made in the understanding of the ENSO and IOD systems, with a number of climate models predicting the ENSO state and giving a rainfall outlook for the season (Fairbanks 2006). Some of the models used in Australia include the operational models of the Bureau of Meteorology (http://www.bom.gov.au) and the Queensland Department of Environment and Resource Management (http://www.longpaddock.qld.gov.au) and the experimental models from the International Research Institute for Climate and Society (www.portal.iri.columbia.edu/portal/server.pt), European Centre for Medium-Range Weather Forecasts (http://www.ecmwf.int), and the Department of Agriculture and Food Western Australia (http://www.agric.wa.gov.au) experimental ENSO Sequence System. While the reliability and timing of seasonal predictions is not yet sufficient for farmers to make major management decisions based on these growing season outlooks, the predictive skill of the various models is improving. For example, the Department of Agriculture and Food Western Australia experimental ENSO Sequence System successfully picked 13 of 16 ENSO states for the period 1988–2003 (Tennant and Fairbanks 2004; Fairbanks 2006).

The agroclimatic regions in which barley is grown are described as being Mediterranean in Western Australia, South Australia, and northwestern Victoria and temperate in Tasmania, southern Victoria, New South Wales, and southeastern Queensland (Stokes and Howden 2008).

BARLEY PRODUCTION—AREA AND TONS

Wheat (*Triticum aestivum* L.) is the most important annual crop in Australia followed by barley (Fig. 9.9). The decision to grow more or less barley relative to wheat is often related to differences in their relative product prices. There are often small increases in barley production following a year of high barley price and, conversely, small decreases in production following a year of low relative price.

Despite these fluctuations in relative product price, the area sown to barley has mirrored the continued rise in area sown to wheat since the 1940s (Fig. 9.9). Before the 1960s, the total area sown to barley in Australia was less than 1 million hectares. It has since quadrupled to 4 million hectares over the last 40 years (Table 9.6). Subject to variations in seasonal conditions, annual barley production now averages over 7 million tons. The largest barley crop grown in Australia was in 2003/2004. In that year, over 10 million tons of barley was grown on 4.5 million hectares.

By state, the largest barley-producing areas in Australia are South Australia and Western Australia followed by Victoria and New South Wales (Table 9.6, Fig. 9.10). These four states account for 97% of Australia's barley production, with South Australia and Western Australia accounting for nearly 30% each. Smaller areas of barley production occur in Queensland and Tasmania. This is a significant change from 1906

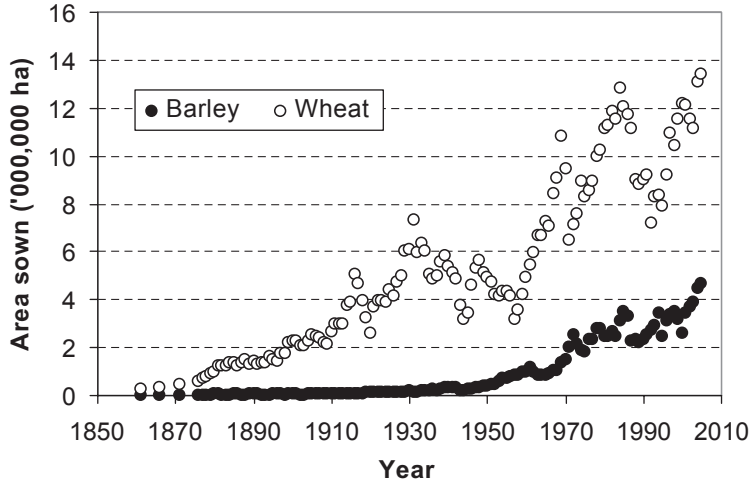

Fig. 9.9. Production ('000,000 ha) of wheat and barley in Australia since 1861. *Source:* Australian Bureau of Statistics (ABS 2006).

Table 9.6 Production by state and for Australia averaged over 5-year periods from 1906 to 2005 for (a) barley area sown ('000 ha), (b) average barley grain yield (ton per hectare), and (c) wheat area (hectare) sown for every 1 ha of barley sown

Period	Qld	NSW	Vic	Tas	SA	WA	Aust
(a) Area sown ('000 ha)							
1906–1910	3	4	23	2	14	2	50
1911–1915	3	5	26	3	24	3	63
1916–1920	2	3	34	2	46	4	91
1921–1925	3	2	34	2	76	4	121
1926–1930	2	3	36	2	103	6	152
1931–1935	3	4	36	3	116	8	169
1936–1940	4	6	60	3	168	22	262
1941–1945	4	8	55	2	151	26	247
1946–1950	8	9	70	3	235	27	351
1951–1955	22	9	105	3	378	56	572
1956–1960	78	32	133	4	494	140	880
1961–1965	77	86	90	7	494	164	916
1966–1970	154	163	131	10	498	215	1172
1971–1975	125	345	261	12	699	637	2079
1976–1980	216	460	364	11	967	525	2544
1981–1985	225	508	357	12	1050	691	2843
1986–1990	212	449	352	9	947	512	2481
1991–1995	164	515	536	13	993	608	2828
1996–2000	155	615	597	12	962	810	3152
2001–2005	113	778	793	8	1176	1160	4030
(b) Grain yield (t/ha)							
1906–1910	0.84	0.81	1.18	1.33	0.97	0.68	1.05
1911–1915	0.79	0.80	1.13	1.41	0.81	0.63	0.96
1916–1920	0.96	0.74	1.20	1.10	0.97	0.64	1.04
1921–1925	1.05	0.98	1.37	1.36	1.03	0.67	1.12
1926–1930	0.96	0.85	1.14	1.32	0.93	0.63	0.97
1931–1935	1.04	0.97	1.11	1.29	1.00	0.61	1.01
1936–1940	0.87	0.97	0.96	1.55	1.00	0.69	0.97
1941–1945	1.08	0.66	0.71	1.43	0.92	0.67	0.85
1946–1950	1.24	0.86	1.04	1.43	1.09	0.66	1.05
1951–1955	1.41	0.96	1.12	1.66	1.28	0.72	1.19
1956–1960	1.46	1.16	1.16	1.80	1.16	0.79	1.13
1961–1965	1.44	1.39	1.17	1.70	1.22	0.82	1.18
1966–1970	1.52	1.22	1.09	2.01	1.04	0.88	1.11
1971–1975	1.46	1.10	1.17	2.07	1.21	1.06	1.16
1976–1980	1.82	1.34	1.22	1.87	1.14	1.23	1.27
1981–1985	1.85	1.27	1.32	2.05	1.28	1.17	1.30
1986–1990	1.91	1.58	1.53	2.50	1.55	1.34	1.54
1991–1995	1.28	1.66	1.68	2.57	1.74	1.64	1.67
1996–2000	1.81	2.02	1.85	2.53	1.93	1.84	1.90
2001–2005	1.55	1.74	1.86	3.06	1.91	1.83	1.84

Table 9.6 *Continued*

Period	Qld	NSW	Vic	Tas	SA	WA	Aust
(c) Wheat area (ha) sown for every 1 ha of barley							
1906–1910	12	159	35	6	49	51	48
1911–1915	16	211	37	5	37	142	52
1916–1920	20	485	32	4	21	156	43
1921–1925	19	607	30	4	13	160	33
1926–1930	31	562	36	4	12	189	34
1931–1935	36	471	38	3	13	170	36
1936–1940	35	290	18	2	7	53	20
1941–1945	31	165	17	1	5	32	16
1946–1950	23	192	18	1	4	39	15
1951–1955	11	142	9	1	2	22	8
1956–1960	3	36	6	1	1	9	5
1961–1965	5	23	14	1	2	11	7
1966–1970	4	19	10	1	3	13	8
1971–1975	4	7	4	<1	2	4	4
1976–1980	3	7	3	<1	1	7	4
1981–1985	4	7	4	<1	1	7	4
1986–1990	4	6	3	<1	2	7	4
1991–1995	4	3	1	<1	1	6	3
1996–2000	6	5	2	<1	2	5	3
2001–2005	6	5	2	1	2	4	3

Source: ABS (2006).
Qld, Queensland; NSW, New South Wales; Vic, Victoria; Tas, Tasmania; SA, South Australia; WA, Western Australia; Aust, Australia.

to 1910 when Victoria represented 50% of the area sown to barley in Australia, South Australia 30%, and Western Australia only 4% (Table 9.6). South Australia has been the dominant barley-producing state since 1914, but this changed in 2001/2002. Since the 2001/2002 season, Western Australia has matched South Australia in area sown to barley. It is expected that these two states will continue to dominate production over the coming seasons.

The dominant areas of production in Western Australia include the Central Wheatbelt and the South Coast (Fig. 9.11). The major areas of production in South Australia are the Yorke Peninsula, southern Eyre Peninsula, and Murray Mallee, while in Victoria, these are the Mallee and the northern Wimmera. In New South Wales, barley production is concentrated along the western slopes and, to a lesser extent, the plains of the Dividing Range. In Queensland, the crop is largely grown on the Darling Downs and Goondiwindi border districts. There are growth areas into the western downs and central Queensland to supply the growing demand from intensive livestock industries.

Tasmania is the highest-yielding barley region in Australia with average grain yields of 3.0 t/ha over the period 2001–2005 (Table 9.6). Across the four major barley-growing regions of South Australia, Western Australia, Victoria, and New South Wales, the average grain yield of barley over the same period ranged between 1.7 and 2.0 t/ha.

The average rate of yield increase over the last 40 years has been just over 20 kg/ha/yr in the four main barley cropping areas in mainland Australia (Table 9.6). This has been associated with the release of over 40 new cultivars (see Chapter 8) and improved cultivar-specific management techniques (i.e., Paynter 1996; Paynter et al. 1999a,b; Smith and Paynter 2005; Russell et al. 2008a,b, 2009). This increase in yield is despite reductions in total annual rainfall across Australia during that period (Foster 2000; CSIRO 2007; Stokes and Howden 2008). Anderson et al. (2005) showed that over the period from 1985 to 2005, research to improve crop management contributed about two-thirds of the increased yield of Australian wheat crops, while genetic improvement contributed about

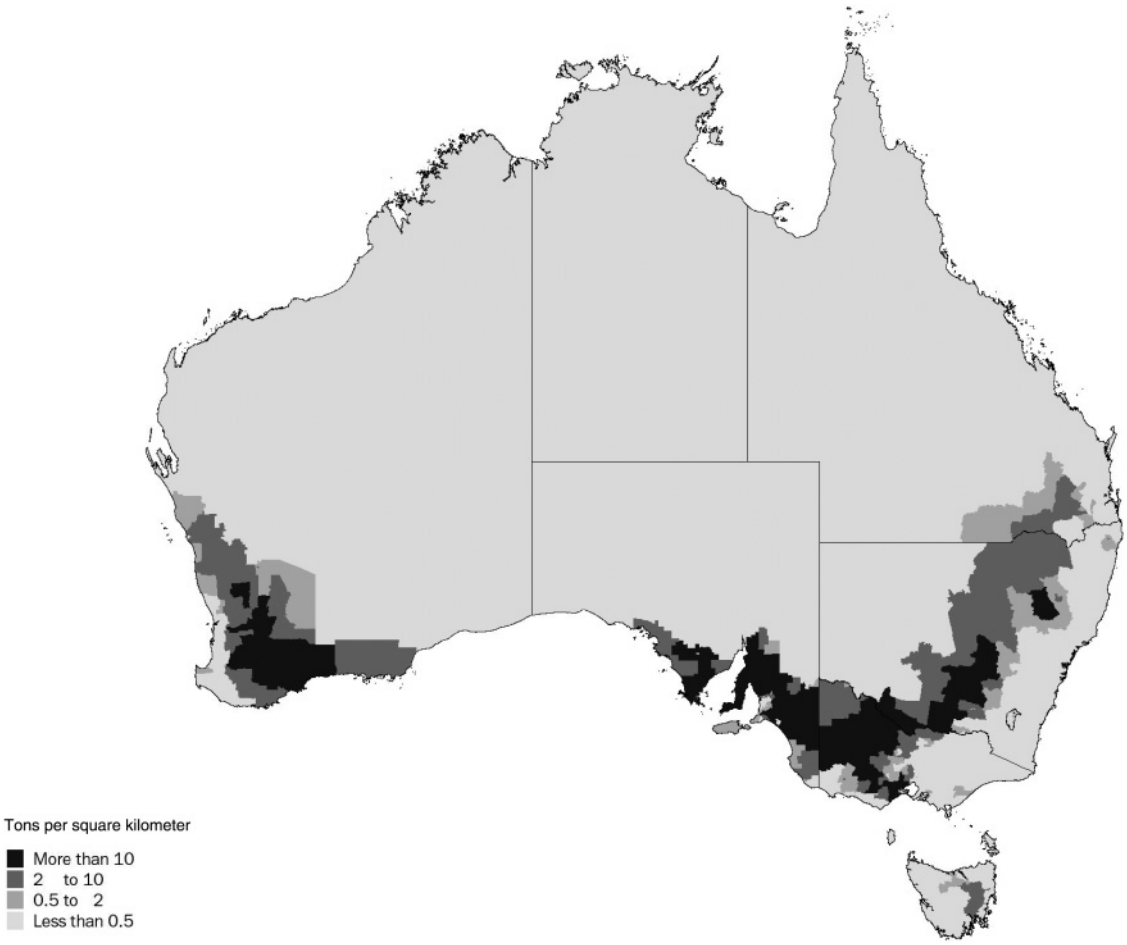

Fig. 9.10. Production density (ton per square kilometer) of barley in Australia for the growing season of 2005/2006. *Source:* ABS (2008).

one-third. A similar relationship would be expected in barley.

The Australian barley market is largely export focused, although the proportion of grain exported varies by state. Over the period from 2001 to 2005, exports of barley averaged 5.1 million tons. Of this average, 2.7 million tons were sold into feed barley markets; 1.8 million tons were exported to malting markets as grain; and another 0.6 million tons were exported as malt. In that same period, an average of 1.3 million tons was used by the domestic stock feed industry and 0.2 million tons by the domestic brewing industry. The use of barley by the domestic feed industry is likely to be an underestimate given on-farm use and local sales.

The Western Australian and South Australian barley industries are very much export focused with over 95% of production shipped as grain or malt to international markets. The main brewing markets for this grain and malt are China, Southeast Asia, Japan, South Africa, and South America. Grain exported as feed is sent to Japan, Taiwan, and a number of Middle Eastern countries such as Saudi Arabia.

In Victoria, New South Wales, and Queensland, there is a much smaller export focus and a larger domestic focus. Between 20% and 30% of total feed barley production in those three states is used by the domestic feed industry. The main end users are dairy in Victoria, beef in New South Wales, and both beef and pigs in Queensland. There is also a growing demand from the Australian brewing industry for malting barley to be grown in New South Wales and Queensland to cope with rising population demand.

TYPES OF BARLEY GROWN

The area sown to barley in Australia is dominated by cultivars with a two-row head, a white aleurone, and a hull. Dual-purpose grazing, forage, and hulless barley cultivars, including some with a six-row head, are grown, but usually only on small areas each year. There is a growing demand in the higher rainfall areas of New South Wales and Queensland for barley cultivars for use as a grazing, hay, and/or silage crop. The adoption of dual-purpose grazing and forage barleys outside that area is limited. The future growth of the hulless industry is dependent on the growth of the food barley market and demand from the nonruminant feed barley market.

Six-row barley cultivars for grain production were recommended for sowing in Western Australia right up until the 1980s (Shier and Reeves 1957; Fisher 1982). During the early 1980s, Beecher occupied 12% of the barley area in Western Australia, but it is now sown on only 0.1% or less than 2000 ha. Six-row grain cultivars have historically been more commonly grown in Western Australia than in eastern Australia (Sparrow and Doolette 1975). The two most common six-row cultivars grown in Western Australia were Atlas and Beecher. Beecher was recommended for sowing on the saline soils of the Eastern Wheatbelt (Fisher 1982) and has since been replaced by two-row cultivars such as Mundah (released in 1995). Six-row cultivars like Beecher are no longer accepted at receival due to their blue aleurone.

To meet the demand for premium malting quality barley, large areas of the Australian barley crop are sown to cultivars suitable for use in the brewing industry. In Western Australia, around 80% of the barley area is sown to cultivars with a malt rather than a feed classification (Table 9.7). Since 1985, this ratio of malt to feed cultivars has been fairly stable in Western Australia and has ranged between 75% and 90%. In South Australia, malting barley cultivars occupy about 60% of the barley acreage, in Queensland around

Table 9.7 Percentage (%) of the Western Australian barley area sown to different malting barley cultivars[a] and those with a feed classification averaged over a 3-year period since 1982

Cultivar	1982–1984[b]	1985–1987	1988–1990	1991–1993	1994–1996	1997–1999	2000–2002	2003–2005	2006–2008
Baudin							0.0	11.7	23.2
Clipper	25.4	5.3	0.8	0.9	0.0				
Dampier	11.3	0.9	0.2	0.0					
Franklin			0.0	0.5	6.2	3.7	0.1	0.0	0.0
Gairdner					0.0	4.6	18.2	26.4	21.2
Hamelin							0.0	3.9	10.5
Schooner	0.0	0.8	0.3	0.0	0.0	4.0	6.3	3.7	0.8
Stirling	22.2	69.2	82.8	78.5	72.8	66.1	61.3	35.0	15.8
Vlamingh								0.0	3.6
Other malt	0.0	0.0	0.0	0.0	0.4	2.5	3.0	1.5	1.0
Feed	41.1	23.6	16.1	20.1	20.7	19.1	11.1	17.8	23.9

Source: Cooperative Bulk Handling Pty Ltd, Perth, Western Australia.
[a]Other malt cultivars includes the area sown to Unicorn, Harrington, Buloke, and Flagship. Clipper, Dampier, Unicorn, and Harrington are, however, no longer segregated as malt cultivars in Western Australia.
[b]Data period 1982–1984 has no data for 1982.

50%, and in Victoria and New South Wales around 70%.

The decision whether to grow barley for malting or for feed depends on five main factors:

1. premium paid for cultivars that are segregated as malting,
2. relative yields of malting and feed-grade barley cultivars,
3. differences in input costs due to their agronomy and disease constraints,
4. likelihood that grain of a malting cultivar will meet the malt barley receival specifications, and
5. location of receival segregations for malt barley cultivars.

The selection for higher grain yield and improved malting quality using European semidwarf germplasm by Australian barley breeders has seen the proportion of the barley area sown to cultivars with a semidwarf habit increase (see Chapter 8).

This effect has been most obvious in Western Australia where semidwarf cultivars occupied only 10% (~68,000 ha) of the barley area in the early 1980s. Between 2006 and 2008, they occupied just under 50% (~500,000 ha). This change has occurred with the adoption of malting barley cv. Franklin (released in 1989) followed by Gairdner (released in 1997) and then Baudin (released in 2002) (Table 9.7). These cultivars have a higher grain yield potential than Stirling (released in 1980), the most common cultivar grown in Western Australia over the last 20 years. Their likelihood of achieving malt barley specifications, however, is lower (Paynter et al. 2004, 2008a; Paynter 2005a,b).

Gairdner is also widely grown in eastern Australia. It is estimated that Gairdner occupies some 10% of the malting barley area in South Australia, 30% in Victoria and southern New South Wales, and nearly 40% in northern New South Wales and Queensland. The dominant malting cultivars in South Australia, Victoria, and southern New South Wales during the last decade have been the tall cultivars, Schooner (released in 1983) and Sloop (released in 1997). They were also grown in northern New South Wales and Queensland in conjunction with Grimmett (released in 1982). The longevity of Schooner as a malting cultivar is a result of its higher likelihood of meeting malt specifications, particularly in drier environments (Fettell 2007). However, semidwarf feed barleys such as Skiff, Kaputar, Tantangara, Mackay, Grout, and Hindmarsh (released in 1988, 1993, 1998, 2001, 2005, and 2006, respectively) have performed well in eastern Australia. The adoption of Baudin in this region has been limited by its susceptibility to leaf disease, in particular barley leaf rust (caused by *Puccinia hordei*).

The recent approval of four new malting barley cultivars with a nondwarf habit—Buloke (released in 2006), Commander (released in 2008), Flagship (released in 2005), and Vlamingh (released in 2006)—is expected to see this increase in planting of cultivars with a semidwarf habit abated. A significant phasing out of Grimmett, Schooner, and Stirling (the backbone cultivars of the Australian barley industry since the early 1980s) will also occur.

Buloke and Flagship are expected to complement Baudin in the high-extract, high-diastase markets of China, Japan, South Africa, and South America. Commander and Vlamingh are better suited to markets where lower levels of starch adjuncts are used in the brewing process.

The adoption of new malting barley cultivars has been assisted through agronomic research trials and the subsequent release of cultivar-specific management packages. These guidelines describe details on how, when, and where to grow the cultivar. They include information on disease management, nutrition, seeding rates, dates of seeding, suitable soil types, herbicide tolerance, relative grain yield, and quality as well as details of the cultivars' main agronomic characteristics.

In Western Australia, for example, management guidelines have been released for the following malt barley cultivars: Harrington (Paynter 1996), Gairdner (Paynter et al. 1999a), Hamelin (Smith and Paynter 2005), Baudin (Russell et al. 2008a), Vlamingh (Russell et al. 2008b), and Buloke (Russell et al. 2009).

PLACE OF BARLEY IN THE FARMING SYSTEM

While product price drives the decision to grow barley or wheat, there are a number of reasons for growing barley that often override the product price differences. These include differences in tolerance to leaf disease, root disease, and waterlogging. In addition, barley offers growers advantages in integrated weed management (barley is more competitive against weeds and there are different herbicide options) and harvest management (barley is generally ready to harvest earlier than wheat). For example, Cousens (1996) and Walker et al. (2001) showed that barley gave greater weed suppression than wheat, and as a consequence, a lower herbicide rate was required for effective grass weed control.

In rotations with a double-cereal phase, barley is often a better choice as the second cereal for managing leaf diseases and for reducing input costs for fungicides, as well as for providing different weed management options. One of the reasons for this is that the leaf pathogens that attack wheat usually do not affect the productivity of barley (Mathre 1997). For example, wheat powdery mildew (caused by *Blumeria graminis* f. sp. *tritici*) and barley powdery mildew (*B. graminis* f. sp. *hordei*) are similar, but wheat powdery mildew does not infect barley. While *Septoria nodorum* (*Phaeosphaeria nodorum*) and *Septoria tritici* (*Mycosphaerella graminicola*) may infect barley, they do not usually cause any yield loss in Australia. Similarly, yellow spot (*Pyrenophera tritici-repensis*) rarely develops in barley.

Waterlogging is common on the duplex soils present in southern Australia (Moore 1998; McKenzie et al. 2004). Paddocks prone to waterlog are best not sown to barley unless there is a dry seasonal outlook. Barley has a lower tolerance of waterlogging than wheat under both saline and nonsaline conditions. Barley is very sensitive to waterlogging between germination and emergence, and when it is elongating before heading (Belford and Thomson 1981; Stepniewski and Labuda 1989a,b). The depth to the perched water table, the duration when it is within 30 cm of the surface and when it occurs in the life cycle of the plant influence the degree of yield loss likely in barley exposed to waterlogged conditions. High grain yields can still be achieved provided that the waterlogging does not persist for more than a few weeks.

The importance of barley in the farming system relative to wheat varies across states. Some of this is related to differences in soil type and fertility, domestic market demand, and the presence of other root pathogens such as nematodes. In South Australia and Victoria, there was 1 ha of barley sown to every 2 ha of wheat in the period 2001–2005 (Table 9.8). In Western Australia, New South Wales, and Queensland, wheat was more dominant, with only 1 ha of barley sown to every 4, 5, and 6 ha of wheat sown in that same period, respectively. In Tasmania, there was only a small area of both barley and wheat with barley being more widely sown.

Over the last 40 years, there has been a shift in the importance of barley in the farming system relative to wheat in New South Wales, Victoria, and Western Australia. In those three states, there has been an increase in area sown of barley relative to wheat compared to South Australia where there has been little or no change. Strong barley product price and higher-yielding cultivars along with research to support improved agronomic management are some of the reasons for the renewed interest in barley in Australia over the last 5 years.

While barley is grown in a wheat-dominated farming system, it is rotated with other crops. In southern Australia, other crops in the rotation include oats (for grain and hay) (*Avena sativa*), canola (*Brassica napus*), narrow-leafed lupins (*Lupinus angustifolius*), field peas (*Pisum sativum*), annual pasture legumes (predominantly *Medicago* spp., *Ornithopus* spp., and *Trifolium* spp.), and lucerne (*Medicago sativa*) depending on rainfall zone and soil type. In the northeast, sorghum (*Sorghum bicolor*) and chickpeas (*Cicer arietinum*) are also important rotation crops.

Common rotations in Western Australia are sequences of barley as the second cereal after pulse crops like lupins or field peas or as a first cereal after canola. In eastern Australia, barley commonly follows wheat and, to a lesser extent,

canola. Wheat and canola are usually grown following pulse crops or legume pastures to better utilize the higher soil nitrogen supply.

In all regions, growers are moving away from fixed rotations and are increasingly making decisions on when to sow barley based on the current and expected price of barley versus other commodities, the risk of not meeting target quality specification due to the seasonal outlook, input costs, paddock nutrient status, the likely cost of inputs, root disease risk, weed seed banks, and herbicide resistance issues. Malting barley is best sown in a rotation following a nonlegume such as wheat or canola or oats to assist with the management of nitrogen inputs in targeting a 10.5% protein crop (Paynter 1995; Paynter and Young 1996; Smith and Paynter 2005).

Genetically modified (GM) crops are still in their infancy in the Australian cereal farming system, although GM cotton is grown in the cotton-growing areas of New South Wales and Queensland. From 2008, the planting of GM canola has been allowed in Victoria and New South Wales and from 2009 in Western Australia. The area sown to GM canola is expected to increase significantly in subsequent years as growers and governments embrace the technology. In the other states, there are government moratoriums that currently prevent the commercial production of GM crops like canola, lupins, barley, and wheat. It is expected that these bans on GM crops in cereal-based farming systems will be gradually lifted by 2013. This is dependent on governments and the consumer developing a better understanding of the benefits and risks from growing these crops as well the development of strategies to manage the risks. In some states, the governments are also being cautious so as to not cause damage to Australia's international market share.

SOIL TYPES ON WHICH BARLEY IS GROWN

Soil types vary significantly across paddocks and between states in Australia (Leeper 1964; Moore 1998; Bolland 2000; Schoknecht 2002; McKenzie et al. 2004). Many of the soils in Western Australia, South Australia, Victoria, and parts of southern New South Wales contain a high proportion of kaolinite clays that have a low exchangeable cation capacity and low pH buffering capacity. Being highly weathered, they also have a low capacity to hold water and generally have low fertility. In northern New South Wales and Queensland, the soils contain more illite and montmorillonite clays that have greater exchange capacity, hold more water, and have inherently higher soil fertility. The soil type present in each region impacts on the relative performance of wheat versus barley and the capacity of growers to grow malting quality barley.

In southwestern Western Australia, there are 21 main soil groups that are grouped into nine broad categories (Moore 1998; Schoknecht 2002). The three dominant soils by area are duplex soils (texture contrast within top 80 cm), deep sandy soils (sand to at least 80 cm), and gravelly soils (dominated by ironstone gravel). Duplex soils with sandy surfaces and clay subsoils are also common in drier areas of southern Australia. Many have large amounts of calcareous material throughout the profile and are highly alkaline, particularly at depth, often with high levels of boron and salinity. More favorable soils, comprising neutral to acid surface loam over moderately alkaline clay subsoils, occur widely in the more temperate parts of this region. Finally, there are large areas of cracking clay soils used for cropping in the northern New South Wales, Queensland, and lesser areas in central Victoria.

The low clay content of sandy surfaced soils means that they have a low capacity to retain macronutrients such as nitrogen, sulfur, and potassium (Bolland 2000; Foster 2000; McKenzie et al. 2004). Those nutrients are readily leached and need to be regularly applied. The presence of iron oxides in many Australian soils leads to the fixation of applied phosphorus, which means that fertilizers containing phosphorus need to be regularly applied.

Barley can be grown on a wide range of soils found in Australia, although its yield is often restricted when sown on soils with a soil pH ($CaCl_2$) below 4.5 due to aluminum toxicity

(Dolling et al. 1991a,b, 2001) or above 7.5 due to boron toxicity (Cartwright et al. 1984; Riley and Robson 1994; Riley et al. 1994).

Soil acidity is a problem on many soils, either occurring naturally or induced by agricultural practices. Agricultural production increases hydrogen ions in solution through the application of nitrogenous fertilizer, the sowing of annual legume pastures, and the removal of plant material (hay, grain) (Helyar and Porter 1989; Dolling et al. 2001; Bolland et al. 2004). Australian soils are sensitive to increases in hydrogen ions due to their low buffering capacity. Most farming systems rely on applying lime to the soil at a rate of 100–200 kg/ha/year to maintain acceptable pH levels (Dolling et al. 2001). Australia-wide, some 12–24 million hectares of agricultural land is acidic at the surface (pH ≤ 4.8, 0–15 cm), of which 3.8 million hectares is also acidic in the subsoil (pH ≤ 4.8, 30–40 cm) (Dolling et al. 2001). Acidic soils with a pH ≤ 4.8 are found predominantly in New South Wales, Western Australia, and Victoria, with smaller areas in the other states.

The solution to improving barley productivity on acidic soils is to apply lime, although this is difficult and expensive if the soil is acidic at depth (Bolland et al. 2004). The application of 1–2 t/ha of lime can lift soil pH by 0.3–0.7 pH units (Dolling et al. 1991a,b, 2001). Subsequent increases in grain yield and productivity are due to improvements in rooting depth associated with the reduction in aluminum toxicity (Reid et al. 1971; Foy 1988). While breeding is not the solution, improved germplasm can minimize the problem and can assist in paying for the costs of a liming program. Improved germplasm can also improve yield and quality stability as soil pH (and aluminum toxicity) is not constant across paddocks and many paddocks have areas with marginal acidity. The first Australian cultivar with improved acid tolerance (Brindabella) was released in 1993 (Read and Oram 1995). More recently, the barley breeding program at the Department of Agriculture and Food Western Australia has identified a molecular marker for a gene that confers tolerance to aluminum called *Alt* (C. Li, pers. comm.). Elite germplasm containing the *Alt* gene are up to 20% higher yielding in small plot trials on highly acidic soils (Table 9.8). Not applying lime, even with the use of more tolerant germplasm, will eventually result in subsurface acidification, which is more difficult to fix than surface acidification (Dolling et al. 2001).

A number of the soils with clay at the surface or at depth have alkaline subsoils. On these soils, soil pH ($CaCl_2$) can be above 7.5 and boron toxicity may be a problem (Cartwright et al. 1986; Riley 1988; Rengasamy 2002). Soils with an alkaline subsoil (pH ≥ 7.0, 30–40 cm) occupy 74 million hectares of agricultural land (Dolling et al. 2001). These soils are located predominantly in South Australia and Victoria and in the eastern and southeastern areas of Western Australia. Unlike acidic soils, it is not possible to ameliorate

Table 9.8 Grain yield (% of their parent) of backcross lines (BC_4) of either Baudin or Hamelin relative to their parental line (Baudin or Hamelin expressed as ton per hectare) when sown at three sites in Western Australia with acidic subsoils in 2006 and 2007

	2006			2007			
Cultivar	Boscabel	Kalannie	HoltRock	Chittinup	Kalannie	Newdegate	Overall
Baudin (t/ha)	1.43	0.39	1.08	2.55	0.15	1.23	1.14
WABAR2473 (%)	139	116	124	102	147	133	121
WABAR2476 (%)	122	115	137	99	163	144	120
WABAR2478 (%)	139	133	136	86	157	134	114
Hamelin (t/ha)	1.92	0.32	1.16	2.82	0.17	1.37	1.30
WABAR2480 (%)	97	110	122	117	179	134	122
WABAR2481 (%)	99	139	127	122	142	130	111
WABAR2482 (%)	106	134	124	122	167	135	122

Source: C. Li, unpublished data.

soils with alkaline subsoils. Breeding is therefore the only solution to improving the tolerance of barley to soils prone to boron toxicity. Genetic resistance to boron toxicity exists, and the recent identification of the *Bot1* gene (Sutton et al. 2007), which works by preventing the entry and accumulation of boron in the plant, will allow commercial cultivars to be developed with tolerance to boron. Through conventional breeding, a number of cultivars have been developed with improved adaptation to boron toxic soils. Some of these cultivars also show little or no leaf symptoms due to the accumulation of boron in their leaf material. To date, however, the absence or presence of leaf symptoms has not always been associated with enhanced yield performance (Riley and Robson 1994; Riley et al. 1994; Bolland 2000; J. Eglinton, pers. comm.). Riley et al. (1994) observed that susceptible cultivars are able to compensate to some degree for the loss of photosynthetically active leaf areas due to boron lesions by increasing their leaf area on primary tillers. Yield loss caused by boron toxicity is greatest in lower rainfall years where susceptible cultivars are unable to extract moisture from the toxic subsoil. In wetter years, the impact of reduced rooting depth is less noticeable and a susceptible cultivar can yield similarly to more tolerant cultivars.

Aside from the issues of aluminum or boron toxicity, the selection of soil type can have a critical impact on the success of barley grown for the malting barley industry. As soil type varies (along with water-holding capacity) across a paddock, so too does the probability of a cultivar meeting malting barley receival specifications. Table 9.9 shows examples of how cultivars may differ in their grain plumpness or screening level when grown in a paddock with contrasting soil types over two dates of seeding. The primary reason for the difference in screening response is related to differences in grain shape and not differences in their average grain weight response. Baudin, Gairdner, and Vlamingh have a similar time to

Table 9.9 Screenings (% < 2.5 mm) of five malting barley cultivars when sown at three sites (growing season rainfall from May to October in brackets) over two dates of seeding in the 2002 season for two different soil types in the same paddock

Soil Group[a]	Loamy Earth		Sandy Duplex	
Location	Calingiri	(235 mm)		
Date sown	May 22, 2002	June 11, 2002	May 22, 2002	June 11, 2002
Baudin	27	23	7	10
Gairdner	30	37	20	16
Hamelin	10	23	9	7
Stirling	6	11	2	4
Vlamingh	12	13	3	6
Location	Brookton	(277 mm)		
Date sown	June 5, 2002	June 27, 2002	June 5, 2002	June 27, 2002
Baudin	13	13	16	25
Gairdner	12	30	18	36
Hamelin	8	17	20	18
Stirling	5	15	9	20
Vlamingh	4	7	12	13
Location	Katanning	(263 mm)		
Date sown	May 16, 2002	June 11, 2002	May 16, 2002	June 11, 2002
Baudin	39	49	18	18
Gairdner	43	48	27	23
Hamelin	17	30	12	11
Stirling	11	30	5	12
Vlamingh	20	27	15	9

Source: Paynter et al. (2008a).

[a]Soil type was assessed according to Schoknecht (2002).

flower, but Vlamingh has plumper grain than Baudin, which in turn is plumper than Gairdner. Therefore, it is important for Australian growers to match their paddocks (and soil types) with the cultivars they are growing.

DATE OF SEEDING

In Australia, winter temperatures are relatively mild (mean July temperatures between 7 and 15°C) and cold damage to vegetative barley is rare. Therefore, spring-type barleys are sown in autumn (May–June) with the aim of flowering in early spring (mid-August to late September). Harvest occurs between October and December. The optimum flowering date is complicated by the conflicting need to avoid frost damage around ear emergence and flowering and to complete grain filling before the high temperatures and frequent dry periods of late spring (Shackley 2000). Cultivars with a high photoperiod response are favored in most areas of Western Australia and in the medium and lower rainfall areas of South Australia, Victoria, and New South Wales (Young and Elliott 1994; Flood et al. 2000; Paynter et al. 2001; Paynter 2005b) as they have a shorter vegetative period when sown late.

Based on simulations using historical rainfall records, the median sowing date for cereals in South Australia ranges between May 15 and 30, a week earlier than currently practiced (Yunusa et al. 2004). In another simulation study by Sadras et al. (2002), the median sowing date for wheat was May 24 for the Mallee regions of South Australia, Victoria, and New South Wales. Simulations done in Western Australia have the median sowing date around May 23 (Abrecht and Balston 1996; Abrecht 2007).

Studies with wheat suggest that current sowing dates have moved forward some 3 weeks compared to the early 1980s. This shift has been more pronounced in Western Australia and Queensland (Stephens and Lyons 1998). Barley has also followed this same trend with the introduction of desiccant herbicides and minimum tillage seeding (direct drilling or no-tillage). In southern Australia, seeding was often delayed in the 1960s and 1970s until July in higher rainfall areas to prevent the development of rank crops (Sparrow and Doolette 1975). The release of later-maturity cultivars in conjunction with improved tillage practices has also allowed earlier sowing in these high-rainfall, generally longer seasoned environments.

Barley and wheat often need to be sown at a similar time on Australian farms, particularly in years when sowing rains are late. Delayed sowing has long been recognized as deleterious for both yield and likelihood of producing high-quality grain, with yield reductions as high as 30% (800 kg/ha) for a 3- to 4-week delay (Ridge and Mock 1975; Paynter and Hills 2007; Paynter et al. 2008a,b). In Western Australia, the yield loss due to delayed sowing was estimated to be just less than 20 kg/ha/day (calculated from Paynter and Hills 2007 and Paynter et al. 2008b).

In the 1980s, the usual practice was to sow barley later than wheat. This has largely changed in recent years with research showing the benefits of early sowing to the yield and quality of barley. Barley is now more commonly sown before wheat in Western Australia, although in eastern Australia, it largely depends on what cultivar is sown. Longer seasoned barley cultivars like Gairdner would be sown before main season wheats in eastern Australia, but the earlier-maturing cultivars like Schooner would usually be sown after wheat.

The most significant benefit of earlier sowing is an increase in the chance of grain meeting malt barley receival specifications (Paynter 1995, 2005a,b; Paynter et al. 2008a). This is mainly due to an increase in grain plumpness and a reduction in screenings. It may also be associated with the delivery of grain with a protein level between 9.5% and 12.5%. Delayed sowing often increases grain protein and may result in grain not meeting the current protein specification for malting barley. However, improved grain plumpness with earlier sowing is not a universal response (Table 9.9). In three comparisons (averaged across 10 cultivars) in eastern Australia, Fettell et al. (1999) found that kernel weight was greater with delayed sowing, a result of a higher rate of grain filling. Sowing time effects on grain set and hence

source–sink ratios are a likely cause of these differing responses, and there are strong across-season interactions due to rainfall distribution.

PLANT POPULATION AND SEED RATE

Target plant populations of up to 100 plants/m^2 were commonly recommended for barley before the 1990s (Sparrow and Doolette 1975; Young 1995; Paynter 1996). Recent research has shown that the optimum seeding rate can be increased to produce higher yield without major detrimental impacts on grain quality. The latest cultivar-specific management guidelines reflect this change in target plant population.

Current target plant populations are the same for malting and feed barley cultivars and vary around Australia:

- Western Australia—120–150 plants/m^2 (Paynter et al. 1999a,b, Smith and Paynter 2005; Russell et al. 2008a,b, 2009)
- South Australia and Victoria—120–140 plants/m^2 in rainfall zones below 350 mm, 140–160 plants/m^2 for annual rainfall between 350 and 450 mm, and 160–180 plants/m^2 above 450 mm.
- Southern New South Wales—80–130 plants/m^2, with the lower density used in drier areas (Fettell 2008; McRae et al. 2008).
- Queensland and northern New South Wales—100–120 plants/m^2 (McIntyre 2008).

At an average seed weight of 40–45 mg and assuming 80% establishment, 120 plants/m^2 is equivalent to a seeding rate of 60–70 kg/ha. In South Australia, this is nearly double the recommended seeding rates of the late 1960s and early 1970s (Sparrow and Doolette 1975).

In Western Australia, the yield benefit of 300 kg/ha has been achieved by lifting the target plant population from 100 to 150 plants/m^2 (Paynter and Hills 2009; B. Paynter, unpublished data). In northern New South Wales, Doyle and Kingston (1992) concluded that a sowing rate of 60 kg/ha was optimal for yield or equivalent to about 130 plants/m^2 for the cultivars they used. In southern Australia, Fettell et al. (1999) reported higher yields at populations of 160 compared to 80 plants/m^2, and in New South Wales, an optimum of about 120 plants/m^2 is suggested (Fettell 2007). Higher plant densities invariably result in lower kernel weights (Doyle and Kingston 1992; Young 1995) and often reduced grain plumpness, although the effect may be reduced in cultivars with inherently large grain size and under favorable conditions (Fettell et al. 1999; Paynter et al. 1999b, 2008a; Fettell 2007).

Other benefits of sowing barley at a higher target plant population include improved competitiveness with weeds, decreased impact of mid-season waterlogging and improved plant establishment where insects or soil moisture may be an issue (Young 1995; Paynter and Hills 2009; B. Paynter, unpublished data).

NUTRITION WITH A FOCUS ON NITROGEN

Due to the low inherent fertility of many Australian soils (particularly in Western Australia), application of nutrients is required to optimize production either on an annual basis for nutrients like nitrogen and phosphorus or every 3–5 years for micronutrients like copper and zinc (Bolland 2000).

For malting barley production, nitrogen is one of the more important nutrients. A focus on premium quality malting barley has seen the malting and brewing industry target grain protein levels in delivered barley. As a consequence, both upper and lower limits for protein exist on delivery. In Western Australia, the problem for many barley growers is delivery of grain with low protein, whereas in Queensland and in New South Wales, excessive protein is a major reason for downgrading. In seasons or environments with low in-crop rainfall during spring, high grain protein is an issue for most growers. In many environments, the nitrogen rate for maximum yields may cause grain protein concentrations to exceed the malting specification, particularly in cultivars with inherently higher protein concentrations (Fathi et al. 1997).

The four key factors influencing the management of grain protein include soil type, rotation (legume vs. nonlegume), fertilizer application, and yield potential (cultivar, seasonal outlook, stored soil moisture, and date of seeding).

Application strategies for nitrogen have changed over the last 20 years in Australia. This is to meet industry demand as well as to cope with increasing nitrogen input costs and the difference in grain plumpness of newer malt barley cultivars.

One of the key drivers impacting on the most cost-effective use of nitrogen is cultivar. While nitrogen is needed to meet grain yield and grain protein targets, it may also push Australian barley growers out of malting because of high screenings. Nitrogen application generally decreases average grain weight, and this may result in an increase in screenings depending on the grain shape of the cultivar. Malting barley cultivars with a narrow grain shape (i.e., Baudin, Buloke, Flagship, and Gairdner) are more sensitive to nitrogen application than cultivars with a broader grain shape (i.e., Hamelin, Stirling, Schooner, and Vlamingh) (Paynter 2005c,d; Fettell 2007; Hills and Paynter 2008; Paynter et al. 2008a; B. Paynter, unpublished data). It is in seasons where moisture conditions during grain filling are marginal that the impact of grain shape is most noticeable. Grain shape can be the difference between grain meeting the receival standard or being downgraded.

Traditionally, most nitrogen has been applied up front at seeding or as a two-stage application with some starter nitrogen at seeding and a follow-up application at 4–6 weeks after seeding. The availability of liquid nitrogen has meant that nitrogen application can now be spread over a greater part of the growing season. With the introduction of higher-yielding malting cultivars, the timing of nitrogen has been examined more closely. The aim has been to find ways of minimizing the impact of nitrogen application on screenings without compromising grain yield or grain protein.

The practice of canopy management—limiting crop nitrogen early to limit tillering—and applying nitrogen as late as stem elongation, flag emergence, or even flowering is designed to yield fewer but fatter grains (Poole 2005). The theory is that it reduces the risk of high screenings from too much nitrogen applied before stem elongation. The advantage of this method is a better assessment of the yield potential of the barley crop. This is because it allows the grower to take seasonal forecasts for spring rainfall into account as well as to review current disease pressure and growing conditions. Until the reliability of seasonal forecasting tools improve, it is still not possible to determine the best time to apply nitrogen to optimize grain protein and minimize screenings. Research by Paynter (2005d), Poole (2005), and Hills and Paynter (2008) has demonstrated that the concept of canopy management in barley is sound. Delayed application, however, is less effective in the summer-dominant rainfall areas where there is less in-crop rainfall to wash fertilizer into the root zone (Doyle and Shapland 1991; Kingston et al. 2001).

Australian barley growers are using a number of decision support tools to help determine nitrogen requirements for a given rotation, soil type, and expected grain yield. These include Select Your Nitrogen and Yield Prophet®.

Select Your Nitrogen (SYN) was developed by the Department of Agriculture and Food Western Australia and is a spreadsheet-based decision support tool for quantifying nitrogen availability and crop response. SYN is a weekly time-step, simulation model designed to give the user a quantitative feel for how different components of the farming system impact on available nitrogen, grain yield, and grain quality, as well as the dollar returns. The main purpose of SYN is not to recommend a fertilizer rate; rather, it is to show the consequences of any possible nitrogen management strategy in any cropping situation.

Yield Prophet (http://www.yieldprophet.com.au) was developed by the Birchip Cropping Group in collaboration with the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Yield Prophet is a web interface for the crop production model APSIM (http://www.apsim.info) developed by the CSIRO. It simulates crop growth based on paddock-specific inputs of soil type, presowing soil water and nitrogen,

rainfall, irrigation, and nitrogen fertilizer applications and climate data. Like SYN, Yield Prophet is a risk management tool for dryland farming systems in Australia, with an emphasis on decision support for nitrogen fertilizer inputs.

A rule of thumb that is applicable to the production of malting barley in southern Australia is that paddocks, rotations, and rates of applied nitrogen, which typically produce wheat between 7% and 10% protein, suit the production of malting barley (Paynter and Young 1996). Areas where high protein wheat is grown—either due to soil type, low rainfall, or rotation—are not likely to be suitable for malting barley production.

In Victoria, there is a rule of thumb based on testing for deep soil nitrogen (0–60 cm) (McLellan et al. 2001). Paddocks where soil mineral nitrogen is greater than 100 kg N/ha at seeding are less likely to be suitable for malting barley production. Similar rules of thumb based on deep soil nitrogen (0–90 cm) exist for New South Wales where Kingston et al. (2001) suggest that deep soil nitrogen should not exceed 120 kg N/ha for successful malting barley production.

In northern New South Wales and Queensland, a rule of thumb used for nitrogen application is not to apply more than 40% of the nitrogen needed to grow prime hard wheat (McIntyre 2008). For malting barley, this equates to 0.5 kg of nitrogen at sowing for every millimeter of available soil moisture between 0- and 120-cm depth (Dalal et al. 1997). Thus, if there is 150 mm of available soil moisture, the crop will require 60 kg N/ha to produce a 10.5% protein barley crop.

PEST MANAGEMENT

Root diseases such as take-all (*Gaeumannomyces graminis* var. *tritici*) and rhizoctonia (*Rhizoctonia solani* Kühn AG-8) are present in most Australian farming systems, while crown rot (*Fusarium pseudograminearum*) is a major disease in northern New South Wales and Queensland. Take-all and crown rot can be managed by rotation (MacNish and Nicholas 1987; MacNish 1995; Wallwork 1996; Macleod et al. 2008) and rhizoctonia by tillage (MacNish 1985, 1995; Jarvis and Brennan 1986; Wallwork 1996; Macleod et al. 2008). Both barley and wheat are similarly sensitive to rhizoctonia, but barley tolerates take-all better than wheat. MacNish (1995) demonstrated that for every 1% increase in take-all severity on wheat roots, barley yields relative to wheat increase by 1%. Barley can therefore be sown instead of wheat in rotations where low levels of take-all exist. In rotations where take-all is severe, oats, pulse crops, or annual pasture legumes are often sown. Fungicides applied either to the seed or in the planting furrow (carried on fertilizer) can also assist in suppressing take-all and foliar diseases.

Nematodes are also present in most Australian farming systems. The most common nematodes found are cereal cyst nematode (*Heterodera avenae*) and root lesion nematode (*Pratylenchus* spp.). Cereal cyst nematode is found in Victoria, South Australia, and Western Australia and, to a limited extent, in New South Wales (Vanstone et al. 2008). Since barley cultivars are tolerant to cereal cyst nematode, yield loss in barley is limited even when infection does occur. Barley cultivars, however, vary in their resistance to cereal cyst nematode. A resistant cultivar retards nematode development, leading to fewer cysts on the roots and lower nematode levels in the soil for subsequent cropping seasons. Cultivars such as Barque, Capstan, Commander, Doolup, Flagship, Hindmarsh, Keel, Maritime, and Yarra are resistant to cereal cyst nematode. So, where cereal cyst nematode is a problem, growers are advised to include nonlegume crops in their rotation and to sow a resistant cultivar. Where high levels of cereal cyst nematode are present, a break of at least 2 years will be required to reduce nematode levels below those that are yield limiting.

Several species of root lesion nematode are known to occur in cropping soils of southern Australia (Riley and Kelly 2002; Thompson et al. 2008; Vanstone et al. 2008). *Pratylenchus neglectus* is the most widely distributed species occurring in most cropping environments of Queensland, New South Wales, Victoria, South Australia, and Western Australia. *Pratylenchus thornei* is also relatively common. There have also been isolated

reports of other species, including *Pratylenchus penetrans*, *Pratylenchus brachyurus*, *Pratylenchus teres*, *Pratylenchus zeae*, and *Pratylenchus crenatus* (Riley and Kelly 2002; Vanstone et al. 2003, 2005). *P. teres* is not known to occur outside of Western Australia (Vanstone 2008). It is not possible to eradicate nematodes in dryland cropping systems, but their populations can be managed. Resistant barley cultivars can be used to reduce the nematode population over one or more seasons. The nematode population can quickly increase again when a susceptible crop or cultivar is sown. Barley cultivars and other crops may differ in their capacity to host *P. thornei* and *P. neglectus*. This means that where a mixed population occurs, a crop or cultivar may increase the population of one species and may reduce the population of the other. The rotation chosen and the cultivar sown, therefore, need to be tailored to match the nematode species present.

Barley cultivars differ in their resistance to the different species of root lesion nematodes. Some barley cultivars such as Barque and Flagship are resistant to both *P. neglectus* and *P. thornei*. Capstan, Doolup, Maritime, and Gairdner are resistant to only *P. neglectus*, while Hindmarsh, Keel, Schooner, SloopSA, and SloopVic are resistant to only *P. thornei*.

The favored rotational crops for malting barley are canola and wheat. These two species are generally susceptible to root lesion nematode and, as such, this rotational sequence is not ideal for root lesion nematode management. Where mixed populations of the nematode are present, field pea, narrow-leafed lupin, and rye (*Secale cereale*) could make good break crops as they are resistant to both species (Vanstone et al. 2008). In rotations where *P. neglectus* is present, faba bean (*Vicia faba*), lentil (*Lens culinaris*), and triticale (*Triticosecale*) are also good options. Oats are a useful rotational crop where *P. thornei* is present. It should be noted, however, that individual cultivars within a rotational species can still vary in their reactions to root lesion nematode. The crops that reduce nematode numbers may not suit rotational sequences for growing malting barley. For example, the increased nitrogen status would have to be taken into account following field pea, lupin, lentil, and faba bean. Triticale is usually grown on acidic soils that are not suited to the production of barley, due to barley's sensitivity to low soil pH and aluminum toxicity.

There is a significant variation in the major leaf diseases that affect barley production in the different barley-growing areas of Australia (Table 9.10). These differences are largely related to differences in environmental (rainfall patterns and temperature profiles) and cultural practices. Cultivars also differ in their leaf disease resistance profile from the different pathotypes of each disease in each environment. A cultivar may be rated as moderately resistant to a disease in one environment but susceptible in another environment.

Depending on the disease, there are a number of cultural (i.e., rotation, tillage, fungicides, date of seeding, and clean seed) and genetic (resistance) strategies that can be used to reduce both incidence and severity (Table 9.11).

Stubbleborne leaf diseases such as scald and net blotch are best managed by not sowing barley into previous season (6-month-old) barley stubble and sowing resistant cultivars. Even 18-month-old barley stubble can still cause net blotch infection (Jayasena and Loughman 2001). In the case of net blotch, growers are also advised not to sow susceptible cultivars on the downwind side of infected stubble. In addition, as both scald and the net form of net blotch can be transmitted via seed, harvesting seed from an uninfected paddock will reduce the likelihood of early infection. Should seed from an infected crop be used, then dressing the seed with a registered fungicide will be required.

The control of leaf disease in barley has been made easier with an increase in the range of registered active ingredients and a reduction in the cost of some fungicides. Less than 10 years ago, growers were reluctant to spray foliar fungicides on their barley crops as there was no perceived economic benefit. As productivity has increased during this time and the relative cost of the fungicide has reduced, there is now a much greater use of fungicides. Best practice disease management now includes the application of a fungicide

Table 9.10 Rating[a] of barley leaf disease importance in each state (region) of Australia should disease-susceptible cultivars be grown

Disease (Causal Organism)	State (Region) of Australia						
	WA	SA	Vic	NSW–S	NSW-N	Qld	Tas
Scald (*Rhynchosporium secalis*)	Medium	Medium	High	High	Low	Very low	High
Net-form net blotch (*Pyrenophora teres* f. *teres*)	High	High	High	High	High	High	Low
Spot-form net blotch (*Pyrenophora teres* f. *maculata*)	Medium	Medium	Medium	Medium	High	High	Low
Powdery mildew (*Erysiphe graminis* f. sp. *hordei*)	High	Medium	Medium	Medium	Medium	Medium	Medium
Leaf rust (*Puccinia hordei)*	High	High	High	Medium	Medium	Medium	Medium
Stem rust (*Puccinia graminis*)	Nil	Very low	Very low	Low	Low	Low	Very low
Barley grass stripe rust (*Puccinia striiformis* f. sp. unknown)	Nil	Low	Lon	Low	Very low	Very low	Very low
Spot blotch (*Bipolaris sorokiniana*)	Nil	Nil	Nil	Nil	Low	Low	Low
Wirrega blotch (*Drechslera wirreganensis*)	Low	Very low	Very low	Nil	Very low	Nil	Nil

Source: G. Platz, H. Wallwork, M. McLean, M. Zhou, and S. Gupta, unpublished data.
[a]Rating of disease importance: high: potential to cause moderate to heavy losses in susceptible varieties; medium: potential to cause low to moderate losses in susceptible varieties; low: unlikely to cause significant losses; very low: occasionally detected, of no commercial significance; nil: not detected.

Table 9.11 Effectiveness of management and cultural practices on barley leaf disease control, where 1 = very effective, 2 = moderately effective, 3 = not effective, and – = not known or no product registered for control

	Cultivar Resistance	Crop Rotation	Green Bridge Destruction	Stubble Destruction	Disease-Free Seed	Chemical—Seed	Chemical—Foliar
Scald	2	1	3	1	2	2	1
Net-form net blotch	1	2	3	1	2	2	2
Spot-form net blotch	2	2	3	1	2	3	2
Powdery mildew	1	3	3	3	3	2	1
Leaf rust	1	3	1	3	3	2	1
Stem rust	2	3	2	3	3	2	1
Barley grass stripe rust	1	3	2	3	3	2	1
Spot blotch	2	2	3	2	2	2	—
Wirrega blotch	2	2	3	2	—	—	—

Source: G. Platz and K. Jayasena, unpublished data.

to the seed or the fertilizer and a follow-up foliar spray at either stem elongation and/or flag leaf emergence and, in some cases, even during heading.

In terms of product range, over eight different fungicide products are now registered for foliar application on barley (subject to label registration) in Australia. These include triadimefon,

propiconazole, propiconazole + cyproconazole, azoxystrobin + cyproconazole, tebuconazole, flutriafol, and epoxiconazole. In addition, there are eight fungicide products that can be applied to the seed to control loose smut (*Ustilago tritici*) and covered smut (*Ustilago segetum* var. *hordei*). The registered seed dressing fungicide products include carboxin, carboxin + thiram, difenoconazole + metalaxyl-M, fluquinconazole, flutriafol, tebuconazole, triadimenol, and triticonazole. Some of these products also have activity against the seedborne net form of net blotch (carboxin + thiram and difenoconazole + metalaxyl-M) or the foliar diseases scald and powdery mildew (fluquinconazole, flutriafol, triadimenol, and triticonazole). Two fungicide products are registered for application to fertilizer, with flutriafol active against scald and powdery mildew and triadimefon active against powdery mildew. Fungicide products registered for barley for each state and for each disease can be found on the Australian Pesticides and Veterinary Medicines Web site (http://www.apvma.gov.au).

Damage from field insects is not generally a major factor for barley crops in Australia, although significant damage can occur if conditions favoring the buildup of insect populations occur. Seasonal factors, rotations, paddock management, and date of planting will influence the risk of loss from particular insects. The main insect pests of barley during the seedling stage include webworm (*Hednota* spp.), cutworm (*Agrotis* spp.), desiantha weevil (*Desiantha diversipes*), red-legged earth mite (*Halotydeus destructor*), and lucerne flea (*Sminthurus viridis*). Growers can reduce the risk of loss from seedling insect pests by appropriate paddock management. Factors that influence the level of damage include rotation, grass weed control in previous crop, soil type, date of seeding, and length of fallow before seeding (Grimm 1995). During tillering, the main insect pests are aphids. Corn aphid (*Rhopalosiphum maidis*) and wheat/oat aphid (*Rhopalosiphum padi*) can cause yield losses of up to 2 t/ha in crops averaging over 5 t/ha. Rice root aphid (*Rhopalosiphum rufiabdominalis*) and grain aphid (*Sitobion miscanthi*) are also found and may be important as virus vectors. Aphids, while seasonal, are becoming more important as our yield potential increases. Aphids affect barley by direct feeding and/or by transmitting the barley yellow dwarf virus. The use of insecticides on the seed (i.e., imidacloprid) or a foliar spray when 50% of tillers have at least 10–15 aphids per tiller can reduce the impact of aphids on barley production. Some cultivars have good resistance to barley yellow dwarf virus, such as Baudin and Gairdner, but are still susceptible to the feeding damage caused by the aphid. During flowering and grain ripening, the main insect pests of barley include aphids, armyworm (*Mythimna convecta*, *Mythimna loreyimima*, *Persectania ewingii*, and *Persectania dyscrita*), and the Australian plague locust (*Chortoicetes terminifera*). Native parasites can exercise good control of armyworm and spraying is not normally required every year. Spraying is usually undertaken when locusts are in plague proportions, and the locust plagues in Western Australia are rarely as severe as those in the eastern States. Outbreaks of locusts are very seasonal and usually occur when in seasons following strong periods of rainfall in inland Australia (nonagricultural).

Australia has a significant advantage over many barley-growing regions in the world in that it is free of a number of exotic insects and diseases. Biosecurity protocols therefore exist for the movement of barley (and other plant material) into Australia, between states, and from farm to farm. Shea et al. (2003), for example, describes the process of practical on-farm biosecurity. A number of exotic threats have been identified as being potential threats to the Australia barley industry. One of the main purposes of Australia's strict quarantine and biosecurity is therefore to protect Australia's favorable pest and disease status and enhances Australia's access to international markets.

Most of the highest category threats identified for barley are associated with grain contamination and market access, although a number of them can cause significant yield loss as well. Changes in farming practices or even climate change may increase or decrease the likelihood or incidence of a particular threat occurring in Australia. For example, the trend toward summer cropping with

maize (*Zea mays*), French millet (*Panicum miliaceum*), Japanese millet (*Echinochloa esculenta*), or sorghum, the adoption of no-tillage, the lack of resistant barley varieties, and prolonged wet weather during flowering increase the risk of the *Fusarium* spp. complex developing. These fungi can produce toxins that can contaminate affected grain and render it unsuitable for marketing and consumption.

The main exotic threats that are present in different states in Australia include the *Fusarium* spp. complex, European snails (*Helix pisana*), and corynetoxin contamination. A range of exotic threats including Khapra beetle (*Trogoderma granarium*), Russian wheat aphid (*Diuraphis noxia*), wheat stem sawfly (*Cephus cinctus*), European wheat stem sawfly (*Cephus pygmeus*), barley stripe rust (*Puccinia striiformis* f. sp. *hordei*), and barley stem gall midge (*Mayetiola hordei*), however, are not currently present in Australia, and we aim to keep Australia free from those threats.

TILLAGE

Until the 1970s, cultivation was a necessary part of Australian farming systems. The introduction of new herbicide options such as the nonselective, nonresidual knockdown herbicide Spray Seed® and the selective, residual herbicide trifluralin, along with the increasing use of tined seeders, saw the beginning of the direct drill or minimum tillage revolution (Reithmuller 2000). It was not until the early 1990s, however, did the use of narrow tined tungsten carbide points lead to one-pass seeding with minimum disturbance otherwise known as no-tillage. This system allowed farmers to sow earlier on less rainfall, and with engineering improvements to seeders such as disturbance below the seed, there has been a rapid adoption of no-tillage. Recent changes to the no-tillage system include greater retention of straw (stubble) and the sowing of crops on wider rows (i.e., widening from 18 to 22–36 cm).

In 2004, an estimated 72% of Australian farmers direct drilled their crop (Hodges and Goesch 2006), up from 26% in southeastern Australia and 58% in Western Australia in 1998 (Knopke et al. 2000). In 2008, it is estimated that nearly 70% of South Australian farmers and 88% of Western Australian farmers would be using no-tillage principles to sow their crop (D'Emden and Llewellyn 2006).

The study of D'Emden and Llewellyn (2006) suggests that the predominant reason for adopting no-tillage in Australia was soil conservation rather than weed control. Most growers believed that while weed emergence would be lower under no-tillage, there would be an increase in reliance on herbicides. No-tillage systems, however, increase the need for herbicides due to the reduction in cultural weed control. In order for no-tillage systems to be successful, growers have had to adopt integrated weed management systems, especially with the increase in resistance in annual ryegrass (*Lolium rigidum* Gaud.) to postemergent, selective herbicides and the risk of developing resistance to glyphosate (Llewellyn and Powles 2001; Neve et al. 2003). Some of the integrated weed management techniques adopted include manuring (green or brown), double-knock techniques presowing and rotating between paraquat and glyphosate presowing to minimize weed competition, and weed seed set (Walsh and Powles 2007).

On many soils with surface layers texturing from sandy to loamy sand (common in Western Australia), machinery traffic can cause compaction of the soil layer 10–20 cm below the surface (Hamza and Anderson 2003). This is because these soils have a particle size that, when moist, can be compacted. Barley roots can often penetrate this compacted layer, but they do not go all the way through, resulting in less grain yield and higher screenings. As most no-tillage operations do not penetrate this layer, deep ripping of the soil to 40 cm can improve productivity (yield and grain quality) by increasing rooting depth and, in some situations, by reducing the incidence of rhizoctonia bare patch (Ellington 1986; Jarvis and Brennan 1986; Reithmuller 2000). The impact of deep ripping can often be seen in subsequent seasons. In some situations (particularly where the soil has a clay texture), the use of gypsum can maintain the effect for longer or can improve the

effect of deep ripping (Hamza and Anderson 2003). Deep ripping is therefore a recommended practice for barley growers when cropping compaction-susceptible soils. In eastern Australia, where wheel track compaction on clay soils can also restrict water movement and root growth, controlled traffic systems in which all tractor and harvester wheels are restricted to permanent tracks are increasing in use.

One of the consequences of this swing to no-tillage and retention of straw has been an increase in the risk of stubbleborne diseases like the net form of net blotch (*P. teres* f. *teres*) and, in north-eastern Australia, common root rot (*Bipolaris sorokiniana*). To reduce this risk, Australian barley growers are advised to ensure that there is at least a 2-year break between barley crops in the same paddock as well as to avoid sowing susceptible cultivars on the downwind side of infected stubble.

No-tillage systems can also lead to more rhizoctonia bare patch problems (MacNish 1985; Macleod et al. 2008). With no-tillage, plants can become affected by the pathogen soon after germination. Treating the seed with a seed dressing containing the active ingredients difenoconazole and metalaxyl-M can suppress root infection from both rhizoctonia and pythium root rot (*Pythium* spp.). In problem areas, growers are advised to sow oats in preference to wheat or barley. Soil disturbance to at least 10 cm remains as the only effective method of reducing damage caused by the patch-forming strains of *R. solani*. Cultivation does not destroy the fungus but reduces its impact on barley.

The widespread adoption of no-till farming systems in the last 20 years, along with the associated increase in row spacing, has also probably played a role in reducing losses from cereal cyst nematode (Vanstone et al. 2008).

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Chapter 10
Abiotic Stresses in Barley: Problems and Solutions

Luigi Cattivelli, Salvatore Ceccarelli, Ignacio Romagosa, and Michele Stanca

INTRODUCTION

The optimum development of barley (*Hordeum vulgare* subsp. *vulgare* L.) over its life cycle depends on a number of environmental abiotic stress factors that can prevent the plant from expressing its maximum genetic potential. Severe grain losses are often caused by high or low temperatures, drought, anaerobiosis, and such soil anomalies as excess salt. The plant responses elicited by these stresses, when not lethal, include alterations in transpiration, photosynthesis, respiration, and hormonal regulation through the development of genetically controlled adaptive defense mechanisms. The duration of the stress and the plant's growth stage at the former's onset, in turn, affect yield. Different stress reactions and mechanisms are known leading from susceptibility to resistance/tolerance. Thus, genetic variability plays a primary role in determining positive adaptation to environmental abiotic stresses and, hence, in supporting the spread of various barley genotypes to extreme climatic conditions.

Plants can experience abiotic stresses resulting from the shortage of an essential resource or from the excess of a toxic substance or from climatic extremes. Occurrence, severity, timing, and duration of stresses vary from location to location and in the same location from year to year. Furthermore, an abiotic stress seldom occurs alone; the plants often face growing conditions characterized by a combination of different physical stresses (Cattivelli et al. 2002b).

Drought, defined as water availability below that required for maximum crop yield, is the main factor limiting crop production worldwide. It represents a permanent constraint to agricultural production in many developing countries, although drought also causes great yield losses in developed agricultures. Drought events are often associated with high temperatures, which imposed an additional level of stress to plants. The recent evidence from the Fourth Assessment Report Climate Change of the Intergovernmental Panel on Climate Change (Parry et al. 2007) indicates that the warming of the climate system is unequivocal, as it is now evident from observations of increases in global average temperatures, widespread melting of snow and ice, and rising global average sea level. As a consequence, there are expectations of increase in the frequency and severity of extreme temperature events as well as of droughts, and by 2050, droughts are expected to cause water shortages for about two-thirds of the future world population (Ceccarelli et al. 2004). Irrigation has often been seen as the way to alleviate drought, although it contributes to increased soil salinization. The presence of an excessive amount of soluble salts that hinder or affect the normal plant growth, with sodium chloride being often the dominant, represents an increasing emergence worldwide. The expected climatic changes will also modify the annual temperature profile (less frost during winter, more

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heat stress during summer) (Tubiello et al. 2000; McCarthy et al. 2001), which might imply, in the long term, a consequent variation in the sowing date, growth habit, or/and heading time.

The limitation and/or competition for water and energy resources, the need to avoid further environmental degradation (i.e., soil salinization), and the impossibility to modify some environmental factors (i.e., temperatures and soil toxicity) suggest that fitting crops to the environment is a more sustainable strategy than modifying the environment to fit the crops. Therefore, the central questions are whether crops, and varieties within crops, differ genetically in yield when stress factors are present and whether we can take advantage of these differences.

ASSESSMENT OF ADAPTATION OF GENOTYPES TO STRESS

Breeding for stress tolerance/resistance requires assessment of the differential sensibility of relevant genotypes. It is only when the response of a genotype to a given stress is known that more detail analyses of the underlying physiological and/or genetic mechanisms of adaptation to stress can be undertaken. However, responses to abiotic stresses are more frequently quantitative than qualitative, and therefore, this task is not trivial. Stress characterization is often addressed as a particular case of genotype-by-environment (GE) interaction. GE is one of the statistical areas more extensively reviewed in plant breeding (Cooper and Hammer 1996; Annicchiarico 2002; Voltas et al. 2002; van Eeuwijk et al. 2005; Romagosa et al. 2009).

The traditional approach for determining the differential genotypic responses to stress conditions has been empirical, without much physiological insight into the nature of responses. Phenotype-based statistical analyses of two-way tables of means of genotypes across a sample of natural or artificially induced stressed environments have provided determination of the different phenotypic performances across a range of environmental conditions putatively associated with stress. More recent statistical approaches aim at integrating external environmental and additional genetic information into a series of models in order to identify key environmental variables to explain differential phenotypic responses and to estimate the genotypic sensitivities to them.

Phenotype-based statistical analysis of adaptation

The most widely used method in breeding programs has been the regression-on-the-mean analysis made popular by Finlay and Wilkinson (1963) (FW). By regressing individual phenotypic responses on the average site performance across the full set of environments, differential responses are summarized by the slopes of the different genotypes. The rationale behind FW is that in the absence of any direct stress characterization, an acceptable approximation of the level of stress could be given by the average performance of all genotypes in that environment. However, this approach can only be acceptable in the rare events in which environmental differences are driven by a single major stress.

FW belongs to a much wider class of models named linear-bilinear, which describe genotypic sensitivities to one or more environmental characterizations that are linear functions of the phenotypic data themselves (van Eeuwijk 1995; van Eeuwijk et al. 1995; Denis and Gower 1996; Gabriel 1998). More powerful examples of the linear-bilinear model are the additive main effects and multiplicative interaction (AMMI) model (Gauch 1988) and the genotype plus genotype by environment (GGE) interaction model (Yan et al. 2000; Yan and Kang 2003). These models generate for every genotype and environment a series of scores, which may characterize the differential sensibility of the genotypes to the prevalent stresses. The environmental and genotypic estimates for the AMMI and GGE models can be visualized by means of a biplot (Fox et al. 1997; Yan and Kang 2003; Romagosa et al. 2009). Genotypes placed in the biplot close to each other behave similarly with regard to their stress tolerance patterns driven by the selected environments. Similarly, environments that are located

close to each other reflect similar interaction patterns.

These environmental and genetic scores are simple statistical estimates without any direct physiological meaning. However, the empirically determined sensibilities can be putatively associated with physiological processes by using the regression of the environmental scores on explicit environmental measurements (Vargas et al. 1999; Voltas et al. 1999a,b, 2002; Romagosa et al. 2009).

Integrated models incorporating environmental and genotypic information

Identification of tolerant and susceptible genotypes can also be based on physiological measures directly related to stress response. Several indices have been proposed to describe the yield performance of a given genotype under stress and non-stress conditions or in comparison with the average yield or the yield of a superior genotype (Fischer and Maurer 1978; Lin and Binn 1988; Yadav and Bhatnagar 2001). In a study on common wheat (*Triticum aestivum*) and faba bean (*Vicia faba*), water potential index (WPI) was suggested as a measure of the total water stress experienced by a crop in a given environment for a specific time interval (Karamanos and Papatheohari 1999). Other approaches aim to quantify the degree of drought based on specific environmental factors (such as weather and soil water availability). Araus et al. (2003) found that yield was well correlated with water input under different water stress conditions. Motzo et al. (2001) proposed a seasonal water stress index (WSI) based on soil–plant–atmosphere interaction, where stress was quantified as 1 − (fraction of transpirable soil water). Rizza et al. (2004) proposed an integrated WSI, based on a simple soil–water balance and the integrated reduction of plant transpiration relative to potential transpiration. They proposed that the yield potential and adaptability of cultivars is related to water stress by means of the intercept and slope of a linear regression of yield versus WSI. The analysis of yield as a function of an environmental index enables the comparison of genotype performances under different degrees of water limitation, allowing to select for a genotype that combine the highest yield (highest intercept) with the lowest sensitivity to water stress (lowest slope).

Factorial regression also belongs, as FW, AMMI, and GGE, to the class of linear-bilinear models, the statistical technique best suited to incorporate explicit environmental information into a single model (Denis 1988; van Eeuwijk et al. 1996). Rather than regressing phenotypic variables on the mean site grain yield, as FW does, any explicit agroecological variable recorded at each environment could be used as an independent variable of the GE tables of means. For example, describing the interaction as linearly driven by soil pH allows the estimation of slopes, which directly assess the genotypic sensitivities to changes in soil pH (Royo et al. 1993). Extension to multiple environmental covariables and complex response curves are conceptually simple and easy to compute using standard statistical packages.

As continuous recording of environmental variables increases, the challenge in developing factorial regression models lies in the choice of covariables to summarize the most relevant features of the stress environment. Furthermore, yield arises from an integration of growth processes over the entire crop cycle and, thus, the order of inclusion of environmental variables may reflect the sequence of growth stages. In this context, an ecophysiological understanding of genotypes and stress environments under study should dominate over pure statistical criteria. Examples where the factorial regression is used with physiological information to describe the environmental adaptation of barley genotypes are reported by Voltas et al. (1999a,b) and Romagosa et al. (2009).

Factorial regressions models can incorporate genetic covariables, such as molecular markers, to partition the genotype and the GE terms into quantitative trait locus (QTL) main effects and QTL × E interaction terms. Furthermore, the QTL and QTL × E estimates can be also regressed on any environmental covariable to identify differential QTL expression putatively associated with stress factors (Malosetti et al. 2004; van Eeuwijk et al. 2005; Boer et al. 2007;

Romagosa et al. 2009). Malosetti et al. (2004) analyzed the "Steptoe × Morex" doubled-haploid population yield data from the North American Barley Genome Project (now called the U.S. Barley Genome Project) incorporating a number of environmental covariables in 10 sites. A QTL allele substitution at chromosome 2H increased or decreased gain yield in 0.11 t/ha for every degree Celsius that the temperature range increased. Similarly, Romagosa et al. (2009) identified in a collection of 60 modern barley varieties grown at 12 environments a molecular marker in the short arm of chromosome 1H that significantly interacted with the temperature range during jointing. This marker increased yield in 0.25 t/ha for every degree Celsius that the temperature range increased.

BARLEY DIVERSITY FOR ADAPTATION TO ENVIRONMENT

Environmental factors have driven the evolution, the distribution, and ecology of the genus *Hordeum*, whose species are widespread in temperate, subtropical, and subarctic areas, from sea level to heights of more than 4500 m in the Andes and Himalayas (von Bothmer et al. 1995). Overall, the genus *Hordeum*, including annual and perennial species, shows a high degree of adaptation to different stressful environments, realized through morphological, physiological, and reproductive variants. Cultivated barley is grown either in the northern countries close to the polar circle or at the limits of the desert where the average rainfall is below 200 mm/year. Such a great diffusion, despite the differences in the climatic conditions, already suggests that the barley gene pool should contain characters for wide environmental adaptability and good stress resistance (Stanca et al. 2003).

Control of flowering time and frost tolerance

Genetic adaptation implies the shaping of population gene pools in response to the abiotic environmental challenges due to climate and soil. Genetic variability plays a primary role in determining positive adaptation to environmental stresses and, hence, in supporting the spread of various barley genotypes to extreme climatic conditions. Plant growth habit and heading date are the basic traits involved in barley adaptation to environments since they allow synchronizing the plant life cycle with seasonal changes. A number of well-defined loci are known to control the flowering time following the interaction with environmental signals. Three different genetic components are known: vernalization response (dependent on temperature), photoperiod response (dependent on day length), and "earliness per se" largely independent from both day length and low temperature.

Barley cultivars are separated into three agronomic classes: winter, spring, and facultative. Winter genotypes are sensitive to short days and require vernalization (from 1 to 8 weeks of cold temperature exposure) to induce reproductive development during a normal, annual growing season life cycle. Winter genotypes are therefore planted in the autumn and overwinter. Spring genotypes are inherently reproductively competent and do not require vernalization. The "facultative" class groups spring genotypes that do not require vernalization but are capable of overwintering (von Zitzewitz et al. 2005) or, alternatively, winter genotypes having a short yet distinct vernalization requirement (Braun and Saulescu 2002). Winter habit depends on the presence of the dominant allele at locus *Vrn-H2* and of the recessive alleles at the loci *Vrn-H1* and *Vrn-H3*. All other allele combinations are found in spring or facultative genotypes. The loci *Vrn-H2*, *Vrn-H1*, and *Vrn-H3* are located on the long arm of chromosomes 4H, 5H, and 1H, respectively (Laurie et al. 1995). The recent cloning of all *VRN* genes in wheat and barley (Yan et al. 2003, 2004b, 2006; von Zitzewitz et al. 2005) has allowed the understanding of the molecular mechanisms controlling the vernalization process. The genetic basis of vernalization in barley can be defined using the two-locus *Vrn-H1 Vrn-H2* model since no variations for *Vrn-H3* are known in cultivated germplasm (von Zitzewitz et al. 2005). *Vrn-H2* acts as the dominant repressor of flowering through the inhibition of *Vrn-H1* expression. In

winter genotypes, vernalization downregulates *Vrn-H2*, allowing the expression of *Vrn-H1*, which in turn promotes flowering. In spring genotypes, a loss of function at the *Vrn-H2* locus, as well as large deletions in the promoter of *Vrn-H1*, makes *Vrn-H1* expression independent from *Vrn-H2*, and therefore no vernalization is required to enhance *Vrn-1* expression and to promote flowering (Fu et al. 2005; von Zitzewitz et al. 2005).

Since winter genotypes are capable of surviving winter, winter hardiness is usually associated with the winter growth habit. Genetic investigations of winter hardiness have identified two major loci, Frost resistance-1 (*Fr-H1*) and Frost resistance-2 (*Fr-H2*) (Hayes et al. 1993; Vágújfalvi et al. 2000; Francia et al. 2004). *Fr-H1* and *Fr-H2* reside on chromosome 5H and are 20–50 cM apart, depending on the mapping population (Francia et al. 2004; Stockinger et al. 2007; Fig. 10.1). The *Fr-H2* locus was initially shown to be associated with natural allelic differences in frost tolerance and in the transcript accumulation of several cold-regulated (*Cor*) genes. Since these genes map to different chromosome locations than the *Fr-H2* locus, *Fr-H2* was suggested to control the regulation of *Cor* gene expressions (Vágújfalvi et al. 2003; Francia et al. 2004). In the Triticeae, the amount of *Cor* mRNAs correlate with freezing tolerance, and frost tolerant genotypes accumulate more *Cor* transcripts than frost-sensitive ones (Crosatti et al. 1996; Baldi et al. 1999; Giorni et al. 1999). A cluster of genes encoding C-repeat binding factors (*CBFs*) was found to cosegregate with *Fr-H2* (Vágújfalvi et al. 2003; Francia et al. 2004; Miller et al. 2006). Homologous *CBFs* in *Arabidopsis thaliana* play a key regulatory role in cold acclimatization and in the acquisition of freezing tolerance (Jaglo-Ottosen et al. 1998; Gilmour et al. 2004). The *CBFs* are transcription factors whose direct targets harbor the C-repeat (CRT)/drought responsive element (DRE) low-temperature *cis*-acting regulatory element present in multiple copies in the *Cor* genes of both *Arabidopsis* and cereals (Vazquez-Tello et al. 1998; Dal Bosco et al. 2003; Skinner et al. 2005). *In vitro* DNA binding analyses confirmed that monocot *CBFs* bind to CRT/DRE motifs in monocot *Cor* gene promoters (Xue 2003; Skinner et al. 2005). Collectively, these data suggest that different alleles at the *CBF* locus are responsible for differences in *Cor* gene expression and frost tolerance in barley as well as in wheat.

Fr-H1 cosegregates with *Vrn-H1* (Francia et al. 2004), suggesting a direct interaction between vernalization and frost tolerance. An interesting observation is that *Vrn-H1* transcript levels are negatively associated with the accumulation of *Cor* genes and with the degree of frost tolerance (Kobayashi et al. 2005; Limin and Fowler 2006; Stockinger et al. 2007). Frost tolerance increases during cold acclimation in young barley/wheat plants in the vegetative phase (Crosatti et al. 2008) but decreases after the transition between the vegetative and reproductive phases, at a time when *Vrn-H1* transcript levels increase. In a barley doubled-haploid population segregating for *Vrn-H1*, lines carrying the recessive *vrn-H1* allele showed higher *CBF* transcript levels than those carrying the dominant *Vrn-H1* allele (Stockinger et al. 2007). In addition, lines grown under short day (reduced *Vrn-H1* levels) showed higher *CBF* transcript levels than lines grown under long day. These data suggest that the presence of *Vrn-H1* in the leaves acts as a signal to downregulate the frost-tolerance regulatory network.

A further level of interaction between frost tolerance and flowering is determined by the loci controlling frost tolerance at the reproductive stage on chromosomes 2H and 5H (Reinheimer et al. 2004)

The genetics of earliness per se has been extensively studied in spring barley where flowering does not require vernalization. Traditional genetic linkage studies identified several major genes called *Ea* or *Eam* (early maturity) in barley (e.g., Gallagher et al. 1991) and *Eps* (earliness per se) in wheat (Worland 1996). Five *Eam* loci have been mapped by linkage analysis on chromosomes 1H, 2H, 3H, 4H, and 6H (Franckowiak 1997). Laurie et al. (1995) have found 13 genes (five major genes and the eight QTLs) regulating flowering time in a winter × spring barley cross, and among them, 9 (the *denso* dwarfing gene on chromosome 3H and eight QTLs) were not specifically dependent on photoperiod or vernalization. Photoperiod-responsive loci are known in wheat and barley as

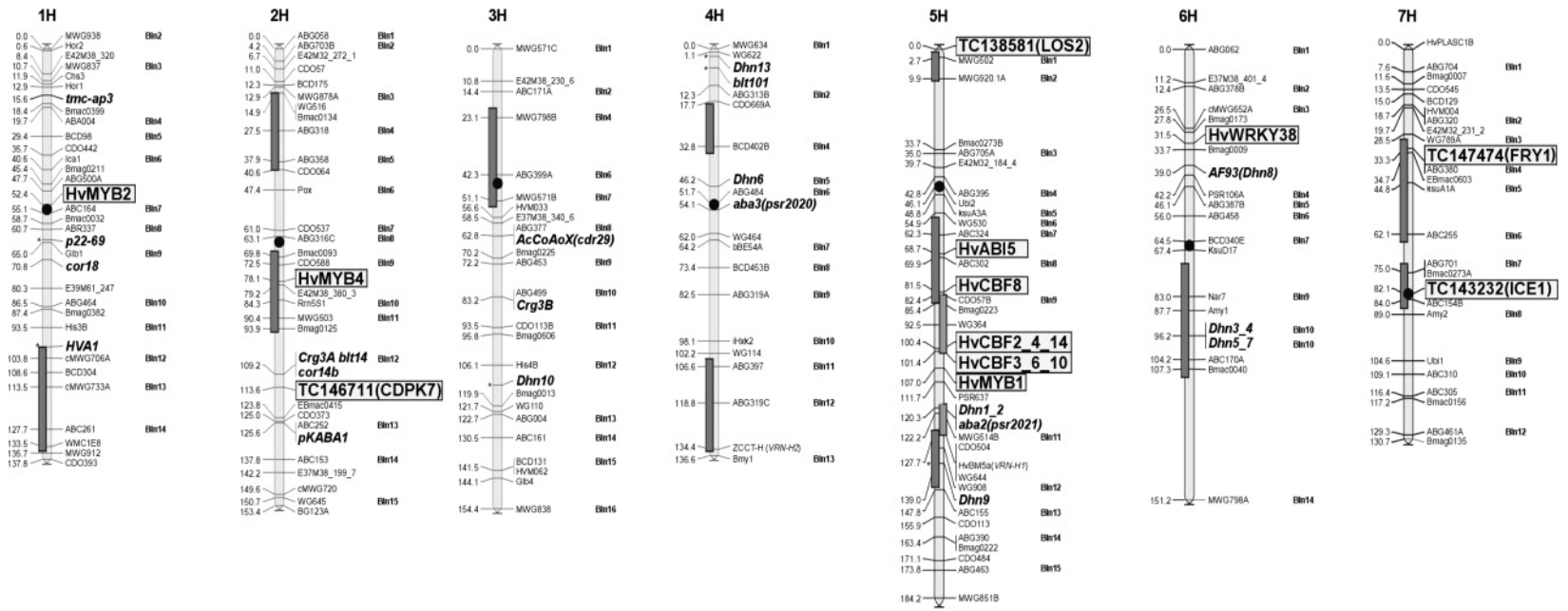

Fig. 10.1 Summary of the main QTLs mapped in barley related to frost tolerance in the vegetative phase (blue bars; data from Francia et al. 2004) and drought tolerance (brown bars; data from Teulat et al. 2001a, 2002, 2003; Diab et al. 2004). On the right side, known stress-related genes and transcription factors controlling stress-related gene expression are reported (data from Tondelli et al. 2006). For color details, please see color plate section.

Ppd. In barley, *Ppd-H1*, located on the short arm of chromosome 2H (Laurie et al. 1994), comaps with and most likely corresponds to the *Eam1* locus responsible for earliness per se (Laurie 1997). A second photoperiod-responsive gene, *Ppd-H2*, has been mapped onto barley chromosome 1H (Laurie et al. 1995). Variation in heading date is the primary cause for yield differences in different water regimes during grain filling (Passioura 1996; Richards 1996). Thus, the earliest cultivars generally perform better in rainfed low-yielding environments (van Oosterom et al. 1993; Abay and Cahalan 1995), escaping the harshest conditions at the end of the growing season.

Drought tolerance

Drought tolerance is a typical quantitative trait; however, single genes, such as those controlling flowering time, plant height, ear type, and osmotic adjustment (OA, an adaptive mechanism based on the maintenance of cell turgor by adjusting the osmotic pressure of cells), may have important roles in the adaptation to drought-prone environments. For instance, a single gene influencing OA in wheat was mapped on the short arm of chromosome 7A (*or* gene—Morgan and Tan 1996), and a breeding program for the *or* gene was shown to increase yield under reduced water supply conditions (Morgan 2000). During the last 10 years, the application of QTL analysis has provided unprecedented opportunities to identify chromosome regions controlling variations in almost all the physiological, morphological, and developmental changes observed during plant growth in water-limiting conditions. Particular attention has been paid to (i) genetic variation of OA (Teulat et al. 1998, 2001a; Robin et al. 2003); (ii) the ability of the roots to exploit deep soil moisture to meet evapotranspirational demand (Johnson et al. 2000; Nguyen et al. 2004); (iii) the limitation of water use by the reduction of leaf area and the shortening of growth period (Anyia and Herzog 2004); (iv) carbon isotope discrimination as estimator of water-use efficiency (Teulat et al. 2002; Saranga et al. 2004; Juenger et al. 2005); (v) the limitation of nonstomatal water loss from leaves, for example, through the cuticle (Lafitte and Courtois 2002); and (vi) the response of leaf elongation rate to soil moisture and evaporative demand (Reymond et al. 2003). With a limitation to the studies on barley, several dozen QTLs for different components of drought tolerance have been mapped (Teulat et al. 2001a, 2002, 2003; Diab et al. 2004; Tondelli et al. 2006; GrainGenes database <http://wheat.pw.usda.gov>). A summary of the main barley QTLs controlling drought tolerance is reported in Fig. 10.1.

Tolerance to soil toxicity and deficiency

Soil salinity and soil sodicity are common problems in arid and semiarid areas. Barley varieties grown in these marginal areas have to be tolerant to soil salinity. In plants, there is no specific carrier for Na^+ uptake; however, Na^+ enters by competition with other cations, particularly K^+. Regulation of cellular Na^+ is achieved by effluxing Na^+ through a Na^+/H^+ antiporter, driven by the electrochemical H^+ gradient across the plasmalemma. Intracellular compartmentalization can also occur due to the work of the vacuolar Na^+/H^+ antiporter driven by the electrochemical H^+ gradient across the tonoplast (Schachtman and Liu 1999). Salt tolerance in cereals is known to be associated with the control of shoot Na^+ content; tolerant lines have more efficient systems to exclude sodium from their cells (Dubcovsky et al. 1996). Loci involved in salt tolerance have been identified on chromosomes 4H and 5H of *H. vulgare* and $1H^{ch}$, $4H^{ch}$, and $5H^{ch}$ of *Hordeum chilense* (Forster et al. 1990). The gene pool of *H. vulgare* subsp. *spontaneum* may also represent an interesting source of new loci for salt tolerance, and several QTLs were detected on chromosomes 7H, 4H, 1H, and 6H (Ellis et al. 1997).

Barley, like other plant species, performs suboptimally when grown in soils with non-neutral pH conditions. Both alkaline and acid soils can produce unfavorable conditions. In some cases, a pure pH effect is present, related to hydrogen concentration, but mostly the effects are indirect and depend on the availability of other ions in the soil. When plants are grown in alkaline soils, manganese (Mn) deficiency may occur. Manganese,

although present in these soils, is usually in a complex form not available to plants. In barley, a genetic analysis of tolerance to Mn deficiency has revealed the presence of an Mn-efficiency locus (*Mel1*) located on chromosome 4HS (Pallotta et al. 2000). The interaction between Mn and nitrogen availability was studied using barley varieties with different tolerances to Mn deficiency. At high nitrate fertilization and low rates of Mn fertilization, the Mn-efficient genotype "Weeah" (tolerant to Mn deficiency) showed better growth of shoot and root than the Mn-inefficient "Galleon" genotype (sensitive to Mn deficiency) (Tong et al. 1997).

Acid soils are common in several areas worldwide. Lower soil pH affects soil structure, microflora, and the availability of mineral nutrients. In acid soils with pH values below 5.5, the presence of available aluminum (Al) probably represents the most important growth-limiting factor. Among winter cereals, barley is the most sensitive to the excess of soluble or exchangeable Al, and a limited genetic diversity for Al tolerance has been found (Minella and Sorrells 1992; Gallardo et al. 1999). The inheritance of Al tolerance in barley is reported to be controlled by a single major locus located on chromosome 4H (Raman et al. 2003).

Boron can also lead to phytotoxicity at high concentrations, a problem very common in many cropping soils in Australia. A major gene located on chromosome 4H controlling boron tolerance in barley has recently been cloned (Sutton et al. 2007), while additional loci have been mapped on chromosomes 2H, 3H, and 6H (Jefferies et al. 1999).

PHYSIOLOGICAL BASES SUSTAINING YIELD UNDER STRESS: THE CASE OF DROUGHT

Improving yield in drought-prone environments is by far the most challenging topic in the field of abiotic stress tolerance due to the complexity of the physiological and molecular mechanisms involved. The physiologically relevant integrators of drought effects are the water content and the water potential of plant tissues (Jones 2007). They in turn depend on the relative fluxes of water through the plant within the soil–plant–atmosphere continuum. Thus, apart from the resistances and water storage capacities of the plant, it is the gradient of water vapor pressure from leaf to air and the soil water content and potential that imposes conditions of drought on the plant. Once a drop in water potential develops, responses of a wide range of physiological processes are induced. Some of them are directly triggered by the changing water status of the tissues, while others are brought about by plant hormones (mainly abscisic acid [ABA]) that are signaling changes in water status (Chaves et al. 2003). Physiological traits relevant for the responses to water deficits and/or modified by water deficits span a wide range of vital processes (Table 10.1). As a consequence, it can be expected that there is no single response pattern that is highly correlated with yield under all drought environments.

The different crop developmental stages show different sensitivities to drought stress. In wheat, most of the floret primordia that reach the fertile floret stage become grains after anthesis. The number of fertile florets or grains per square meter, the most relevant component in ensuring high yield in drought conditions (Slafer and Whitechurch 2001), is determined during stem elongation, a few weeks before anthesis. An extended duration of the stem elongation phase without a change in the timing of anthesis has been proposed as a physiological determinant of an increase in the number of grains per square meter without altering the amount of water used by the crop (Slafer et al. 2005).

Loss of leaf water causes ABA production, which in turn promotes stomatal closure with a concomitant decrease in CO_2 availability in the leaves and hence in assimilate availability to the plant. Although the photosynthetic machinery has a range of photoprotective mechanisms to dissipate excess light, the continued exposure of leaves to excessive excitation energy can lead to photoreduction of oxygen and the generation of highly toxic reactive oxygen species (ROS) such as superoxides and peroxides (Niyogi 1999; Reddy et al. 2004). Maintenance of cell turgor can

Table 10.1 Physiological traits relevant for response to drought conditions (modified from Cattivelli et al. 2008)

Plant Traits	Effects Relevant for Yield	References
Stomatal conductance/ leaf temperature	More/less rapid water consumption; leaf temperature reflects the evaporation and hence is a function of stomatal conductance; stomatal resistance increases under stress	Jones 1999; Lawlor and Cornic 2002
Photosynthetic capacity	Modulation of concentration of Calvin cycle enzymes and elements of the light reactions, stress reduction photosynthetic activity	Lawlor and Cornic 2002
Timing of phenological phases	Early/late flowering; maturity and growth duration, synchrony of silk emergence and anthesis, reduced grain number; wheat and barley advanced flowering, rice delayed, maize asynchrony	Slafer et al. 2005; Richards 2006
Partitioning and stem reserve utilization	Lower/higher remobilization of reserves from stems for grain filling, effecting kernel weight; compensation of reduced current leaf photosynthesis by increased remobilization	Blum 1988; Slafer et al. 2005
Single plant leaf area	Plant size and related productivity; reduced under stress (wilting, senescence, and abscission)	Walter and Shurr 2005
Rooting depth	Higher/lower tapping of soil water resources under drought increased root/shoot ratio.	Hoad et al. 2001; Sharp et al. 2004
Cuticular resistance and surface roughness	Higher or lower water loss, modification of boundary layer and reflectance	Kerstiens 1996
Photosynthetic pathway	C_3/C_4/CAM, higher WUE, and greater heat tolerance of C_4 and CAM	Cushman 2001
Osmotic adjustment	Accumulation of solutes: ions, sugars, polysugars, amino acids, and glycinebetaine	Serraj and Sinclair 2002
Membrane composition	Increased membrane stability and changes in aquaporine function, regulation in response to water potential changes	Tyerman et al. 2002
Antioxidative defense	Protection against active oxygen species	Reddy et al. 2004

be achieved by adjusting the osmotic pressure of cells. Osmolytes such as proline, glycine-betaine, mannitol, fructans, and trehalose can be synthesized to readjust cellular osmotic potential (Serraj and Sinclair 2002). These osmolytes are also active in scavenging ROS, especially if they are targeted to the chloroplast (Shen et al. 1997). Other specialized organic molecules can be used to protect cellular membranes against physical damage, and proteins against unfolding (Hoekstra et al. 2001).

OA is a key mechanism enabling plants under drought to maintain water absorption and cell turgor pressure, thus contributing to sustained higher photosynthetic rate and expansion growth. A general analysis of OA in wheat under several drought stress conditions has shown that osmoregulation can be an effective selection criterion for drought tolerance and that it has a role in reducing drought-dependent yield loss especially when water deficit occurs during the reproductive growth stage (Moinuddin et al. 2005). Nevertheless, a number of contrasting reports on the role of OA have been published. For instance, the recent study of Turner et al. (2007) concluded that differences in OA were not associated with yield benefits in a population of chickpea (*Cicer arietinum*) advanced breeding lines developed from a cross between cultivars with high and low OAs. A comparative analysis of many studies dedicated to OA has suggested that OA cannot be considered equally useful in all crops and/or drought conditions, but that a general positive association between yield and OA can be found under severe water stress where yields tend to be low (Serraj and Sinclair 2002).

Besides OA, several other studies have addressed yield under drought stress as a function of single physiological traits. Fischer et al. (1998) studied representative semidwarf spring wheat cultivars for changes in stomatal conductance associated with selection progress. They found that stomatal conductance and maximal rates of photosynthesis were positively correlated with increased yields of advanced cultivars, while leaf temperatures were negatively correlated. Looking at the relationships between conductance and leaf water potential in modern wheat cultivars,

Siddique et al. (1990) suggested that wheat varieties may be "opportunistic" in relation to available water, having high rates of leaf conductance when soil moisture was favorable and a markedly reduced leaf conductance when soil moisture was limiting. Old cultivars, on the other hand, were characterized by a "conservative strategy" with lower leaf conductance even at high soil moisture. An effect of developmental plasticity affecting yield response under drought stress was shown by the fact that modern cultivars used less water in the preanthesis period and had more water available in the postanthesis period (Siddique et al. 1990).

A further example of a successful breeding program for dry environments with mild winters and stored moisture, based on a physiological trait, was reported by Rebetzke et al. (2002). They used carbon isotope discrimination (Δ) as a surrogate for water-use efficiency to select wheat lines with high water-use efficiency in drought-prone environments. During photosynthesis, plants discriminate against the heavy isotope of carbon (^{13}C) and, as a result, in several C_3 species, Δ is positively correlated with the ratio of internal leaf CO_2 concentration to ambient CO_2 concentration (C_i/C_a) and is negatively associated with transpiration efficiency. Thus, a high C_i/C_a leads to higher Δ and lower transpiration efficiency (Farquhar and Richards 1984). In wheat, when employing divergent selection, the resulting low Δ lines had increased aboveground biomass and kernel weight. Yield was increased by about 2% under mild stress conditions and up to about 10% under the driest conditions (Rebetzke et al. 2002).

When grain yield and grain carbon isotope discrimination were measured in yield trials with barley at three sites varying in expected seasonal rainfall (from the United Kingdom to Syria), discrimination was least (water-use efficiency was greatest) at the lowest-rainfall sites (Craufurd et al. 1991). The overall results indicated that, for barley grown in water-limited Mediterranean environments, a large discrimination against ^{13}C may be a useful indication of good yield. This positive association may not be consistent in extremely poor environments (Araus et al. 2002).

BREEDING PROGRESS IN STRESSED ENVIRONMENTS

Increases in yield potential achieved by plant breeding during the last century have been well documented for numerous crops. Frequently, genetic gain has been studied by comparing in the same field trial the yield of cultivars characterized by different years of release. For most crops, a linear relation between yield and year of release was found, the slope of which gives an estimate of the genetic improvement. Comparison of the different cultivars then enabled identification of the main morphophysiological traits modified during selection in association with yield improvement. For instance, studies carried out on barley and wheat genotypes commonly grown in the last century showed that the increase in grain yield was directly correlated to an increase of the harvest index from about 30% up to 55% (reviewed by Cattivelli et al. 1994; Slafer et al. 1994), while total biomass accumulation remained almost unchanged over the years due to a concomitant decrease in plant height, a process associated with the introgression in the elite germplasm of a few key genes affecting plant height. In general, a genetic gain from 10 to 50 kg/ha/year has been recorded for barley over the last century in all countries, including those characterized by vast drought-prone regions.

Information on genetic gain obtained by comparing cultivars with different years of release is not absolute due to the influence of the environment (mainly water availability) on the performance of the varieties. When grain yield values were estimated in environments with different levels of water availability, yield progress attributed to genetic improvement was, in absolute terms, higher in the environments characterized by a low level of water stress (Slafer et al. 1994). However, when yield increases were expressed as a percentage, no differences were recorded for environments with different degrees of water stress (Araus et al. 2002). This suggests that some of the traits selected to improve yield potential also led to yield increases in dry environments.

A number of physiological studies have identified some traits for which presence/expression is associated with plant adaptability to drought-prone environments. Among them, traits such as small plant size, reduced leaf area, early maturity, and prolonged stomatal closure lead to a reduced total seasonal evapotranspiration and a reduced yield potential (Fischer and Wood 1979; Karamanos and Papatheohari 1999). Depending on the stress conditions (timing and intensity) of the target environments, some adaptive traits can be considered for yield improvement under drought if they enable plants to cope with a stress event that tends to occur every year at the same growth stage. For instance, a good level of earliness is an effective breeding strategy for enhancing yield stability in Mediterranean environments where wheat and barley are exposed to terminal drought stress nearly every year. In this condition, shortening crop duration, a typical escape strategy, can be useful in synchronizing the crop cycle with the most favorable environmental conditions. However, extreme earliness might also lead to yield penalty, with earliness not being correlated to grain yield in Mediterranean environments nor in fertile conditions. In environments characterized by terminal drought, an improvement of the grain filling capacity can also be achieved by increasing the mobilization of the vegetative reserves from stems to ears (Blum 1988).

A more general "xerophytic" breeding strategy to improve plant survival through the limitation of the evapotranspiration can be applied in extremely harsh environments. Nevertheless, every breeding strategy for drought-prone environments also has to consider that the timing and intensity of the stress events vary significantly from year to year, and plants designed to cope with a specific type of drought may underperform when the stress conditions are different or absent.

In moderate drought conditions characterized by a grain yield between 2 and 5 Mg/ha, selection for high yield potential has frequently led to some yield improvements under drought conditions (Araus et al. 2002). In these cases, the breeders selected plants characterized by high yield potential and high yield stability, with the latter being attributed to a minimal GE interaction. This implies that traits maximizing productivity normally expressed in the absence of stress can still sustain a significant yield improvement under mild to moderate drought (Slafer et al. 2005; Tambussi et al. 2005). This can be explained by considering that the main targets of selection (high harvest index, resistance to insects and diseases, and nitrogen use efficiency) are equally beneficial under dry and wet conditions, and often, the best performances for these traits were overriding the differences in drought adaptability. An example is the success of wheat and rice (*Oryza sativa*) varieties bred at Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) and International Rice Research Institute (IRRI), where selection under stress-free environments identified genotypes with high yield in a wide range of conditions including regions with a low yield potential (Trethowan et al. 2002). The rational of this breeding strategy is also supported by several retrospective studies where the yield of large sets of cultivars was evaluated in parallel fields under different water regimes, thus enabling a direct comparison of the performance of the same cultivars in drought and nondrought (usually irrigated) conditions (Rizza et al. 2004). Old varieties were generally characterized by low yield in rainfed conditions and by a minimal ability to improve yield when water became available. On the other hand, modern cultivars showed a higher yield in rainfed conditions and strong yield increases in response to irrigation, suggesting that, in a typical Mediterranean environment, selection based on the absolute performance of the genotypes across environments was more successful than selection for the minimum yield decrease under stress with respect to favorable conditions (Rizza et al. 2004).

A different breeding approach was developed to improve yield in more harsh environments. In many predesertic regions, barley is the main crop where rainfall is below 300 mm/year and grain yield is generally below 2.5 t/ha. In these environments, the assumption that an increased yield potential has a carry-over effect when the improved cultivars are grown also in harsh conditions is not always confirmed. In a large, multienvironment trial involving 188 barley landraces, old and modern varieties, grown in 28 moisture-contrasted

Mediterranean environments, average grain yields ranged from near crop failure to 6 t/ha (Pswarayi et al. 2008a). Modern genotypes clearly outyielded landraces and old cultivars in environments yielding above 3 t/ha, whereas local landraces were adapted to environments with average grain yields below 2 t/ha.

Alternatively, a direct selection for grain yield in the presence of drought within the target environment was adopted (Ceccarelli 1989, 1994), although the response to each cycle of selection is slow. This breeding strategy faces two main limitations: precision of selection and existence of several target environments. Considerable progress has been made in both experimental design and statistical analysis, which improve the estimate of experimental error (Kempton and Fox 1997), making more reliable the results of experiments carried out in stress environments.

The existence of a multitude of target environments characterized by different types of drought raises the issue of selection for broad and/or specific adaptation. Selection for yield potential in favorable environments aims to select genotypes with broad adaptation, over time (the performance of a cultivar in a given location over several years) and over space (the performance of a cultivar in several locations). On the contrary, the selection in drought environment has to take into account the role of specific adaptation to a given stress environment and therefore the requirement of a breeding program for each different drought environment.

In the last century, the grain yield of barley has increased, in poor as well as in favorable environments, mainly through selection for high yield potential achieved with traditional breeding processes. Further progress will depend on the introduction in high-yielding genotypes of traits able to improve drought tolerance without detrimental effects on yield potential, thus reducing the gap between yield potential and yield in drought-prone environments. This goal can be achieved via the identification of drought tolerance-related traits and the subsequent accumulation of the corresponding genes in advanced genotypes using marker-assisted selection (MAS) and/or gene transformation.

MOLECULAR MARKERS TO DISSECT TOLERANCE-RELATED TRAITS

While the genetic dissection of frost tolerance has led to a genetically simple system with two loci explaining most of the genetic diversity (Francia et al. 2004) making MAS a feasible strategy, yield performance under drought is particularly complex. Yield itself is a quantitative trait, and plants exhibit a diverse range of genetically complex mechanisms for drought resistance, including mechanisms of drought escape, drought avoidance, and drought tolerance. A comparative analysis of QTL results clearly shows that chromosomal regions determining variation in agronomic and physiological drought-related traits cover a large proportion of the whole genome (Fig. 10.1).

Many efforts have been dedicated to understanding the genetic basis of physiological traits conferring advantages in dry environments, while less attention has been given to understanding the high yield stability in dry and wet environments. A first general conclusion is that developmental genes, notably those involved in flowering time and plant stature, often have pleiotropic effects on abiotic stress resistance and ultimately determine yield potential (Teulat et al. 2001a,b; Baum et al. 2003; Forster et al. 2004; von Korff et al. 2008).

To reduce complexity, physiological traits that have influence on yield performance under drought were investigated in several studies on OA, relative water content, and carbon isotope discrimination conducted on field-grown barley (Teulat et al. 2002) and under controlled conditions (Teulat et al. 1997, 1998, 2001a). The association between variation in drought-related quantitative traits and, ultimately, the effects of these traits on yield in drought and favorable environments is the main goal for present and future research. Looking for the coincidence of loci for specific traits and loci for yield under drought stress and in stress-free environments, it is possible to test more precisely whether a specific trait is of significance in improving drought tolerance and yield potential. For example, in rice, QTLs for plant yield under drought were coincident with QTLs for root traits and OA

(Babu et al. 2003). Several major loci for yield under different environmental regimes were mapped along with QTLs for late senescence of the flag leaf in winter wheat (Verma et al. 2004). Similarly, Lanceras et al. (2004) found that favorable alleles for yield components were located in a region of rice chromosome 1 where QTLs for many drought-related traits (root dry weight, relative water content, and leaf rolling and leaf drying) were previously identified (Zhang et al. 2001). These results may suggest that selection for drought tolerance could become more efficient thanks to the availability of flanking markers tightly linked to loci for stress-related traits. Marker diagnostics of individual QTLs represent an important surrogate for physiological trait measurements and may ultimately improve breeding efficiency through MAS.

The genetic linkage between a specific DNA marker and a target locus allele, established by QTL studies, can be broken by genetic recombination, although a QTL/gene can be tagged by two flanking markers to reduce the recombination risk. Furthermore, an accurate QTL mapping, usually resulting in a small QTL interval, is also a prerequisite to improve MAS-QTL efficiency. These intrinsic difficulties, together with the polygenic nature of drought tolerance and the interaction with the environment, make MAS for drought-tolerant QTLs still a challenge due to the number of genes involved and the interactions among them (epistasis) and with the environment. The fact that numerous genes are involved in the expression of polygenic traits means that the individual genes may have small effects on the plant phenotype. This implies that several regions (i.e., QTLs) must be manipulated at the same time in order to obtain a significant impact and that the effect of individual regions is not readily identifiable. Replicated field tests are needed in order to accurately characterize the effects of QTLs and to evaluate their stability across environments. Although significant QTL effects can be detected across a range of environments, the evaluation of the QTL × E interactions remains a major constraint on the efficiency of MAS (Collins et al. 2008). Qualitatively, QTL × E interaction is particularly relevant in the presence of stress conditions. Changes in allele frequencies of marker loci linked to yield QTL were studied in a collection of barley landraces and old and modern barley cultivars grown in six locations representing contrasting Mediterranean moisture-driven environments (Pswarayi et al. 2008b). The number of QTLs and the magnitude of their effects were comparable for low- and high-input conditions. However, most QTLs were specific to either high- or low-yielding environments. More significant changes in allele frequencies of markers associated with QTLs in landraces and old and new cultivars were detected in high yield environments (67%: 8 out of 12) than in low yield environments (19%: 3 out of 16). Thus, these results suggest that modern breeding may have increased frequencies of marker alleles close to QTLs that favor production under high yield potential environments. Landraces adapted relatively better to very low yield environments, and some of the key genetic regions responsible for such an adaptation pattern may have been unintentionally ignored by modern breeding.

The contribution of genomics-assisted breeding to the development of drought-resistant cultivars has so far been marginal, and only few significant examples of MAS for traits associated with drought tolerance have been reported. Ribaut and Ragot (2007) used marker-assisted backcrossing in maize to introgress the favorable alleles at five QTLs and explained about 38% of the total phenotypic variance for the interval between anther and silk extrusion (anthesis-silking interval [ASI]—Bolanos and Edmeades 1996), a trait negatively associated with yield in drought conditions. The grain yield of the best maize hybrids selected with molecular markers for four generations was, on average, 50% higher than control hybrids under severe water stress conditions. It is worth noting that no yield penalty was observed under well-watered conditions. In rice, MAS was used to transfer several QTLs for deep roots from *japonica* upland cv. Azucena, adapted to rainfed conditions, to lowland *indica* cv. IR64. MAS selected lines showed a greater root mass and higher yield in drought-stressed trials (Courtois et al. 2003).

An important step toward the application of molecular markers in breeding for drought tolerance is the cloning of DNA sequences underlying QTLs (Tuberosa and Salvi 2006). To date, most plant QTLs have been cloned by the positional cloning approach, although alternative strategies based on candidate genes and linkage disequilibrium may represent an interesting shortcut to QTL cloning (Salvi and Tuberosa 2005). A candidate gene for drought tolerance usually refers to a sequence for which the expression profile or protein function can be associated with the stress response/adaptation process, and the position on the genome comaps with a QTL conferring drought tolerance. Candidate genes can be selected from literature data among known stress-responsive genes, or by using bioinformatic analysis to search all genes present in QTL-underlined genomic regions.

So far, no QTL for drought tolerance has been cloned in crop species, although a study in *Arabidopsis* has led to the cloning of the *ERECTA* gene, a sequence beyond a QTL for transpiration efficiency (Masle et al. 2005). In plants with large genomes, the generation of molecular linkage maps based on candidate genes (molecular function maps) is one way to identify the genetic determinants of QTLs, that is, functional markers (Chen et al. 2001; Causse et al. 2004), in spite of the time-consuming fine mapping. This candidate gene strategy shows promise to bridge the gap between quantitative genetic and molecular genetic approaches to study complex traits. For example, it has been applied to find genes potentially involved in barley and rice drought tolerance (Zheng et al. 2003; Diab et al. 2004; Nguyen et al. 2004; Tondelli et al. 2006). The identification of the QTL-corresponding genes will also provide the best markers for MAS, those designed to directly tag the different alleles of the drought-related genes (quantitative trait nucleotide [QTN]).

In most QTL studies, the work has not been extended beyond their detection for a given trait under drought. The development of consensus QTL maps generated from a number of crosses is an important step toward the identification of regions commonly associated with drought tolerance. A major challenge remaining is to confirm that QTLs discovered in a given mapping population will improve drought tolerance when introduced into high-yielding elite genotypes. This is particularly difficult when the traits are governed by "context dependent" gene effects (i.e., interaction with other genes and/or environments). In these cases, the value of the QTL alleles can differ depending on the genetic structure of the current germplasm set in the breeding program (Wade 2002). Under these conditions, the value of a given QTL allele can change during selection due to changes in the background effects at any given time in the breeding process. As a consequence, when the background effects are important, the stacking of desirable alleles by MAS becomes inadequate because the initial target combination of alleles may no longer be the best target, or even a relevant target, for increasing trait performance in subsequent breeding cycles. The "mapping-as-you-go" strategy (Podlich et al. 2004) involves repeated reestimation and validation of the QTL effects throughout the breeding process to ensure that they remain relevant throughout. This method results in substantial increases in MAS efficiency compared to standard approaches based on the evaluation of the QTL effects only at the beginning of the breeding program, particularly when epistasis and/or GE interactions play a significant role.

GENES AND METABOLITES CONFERRING TOLERANCE

Adaptation of plants to low temperature and osmotic and drought stress induces an active plant molecular response. It is becoming increasingly clear that plants can efficiently sense stress and can mobilize the appropriate defense responses through a molecular mechanism that can be simplified in three main steps (Fig. 10.2): (i) perception of external changes, (ii) transduction of the signal to the nucleus, and (iii) gene expression and accumulation of the protective molecules. This response significantly improves tolerance to negative constraints and it is to a great extent under transcriptional control.

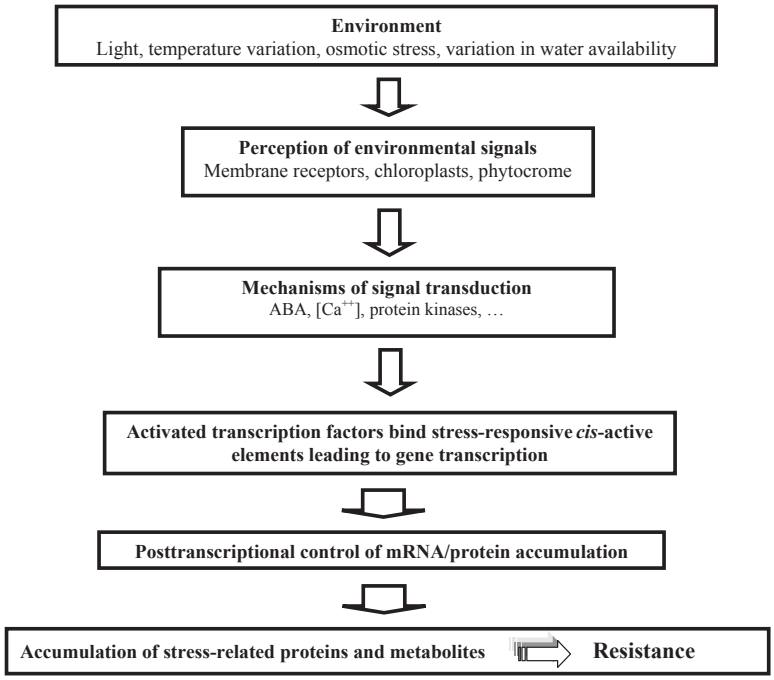

Fig. 10.2 Schematic representation of the mechanisms controlling plant response to abiotic stresses.

Many stress-related genes have been isolated and characterized in the last two decades in a variety of crop species (Cattivelli et al. 2002a; Ramanjulu and Bartels 2002); however, the complexity of the whole molecular response to cold and drought in crop plants has only recently been revealed by large transcriptome analyses (Kollipara et al. 2002; Buchanan et al. 2005; Hazen et al. 2005; Svensson et al. 2006). Molecular analysis in *Arabidopsis* has sketched the complex network constituting cell communication during cold/osmotic/drought response (Yamaguchi-Shinozaki and Shinozaki 2006; Mazzucotelli et al. 2008). From model plants, genetic information is being moved to crops exploiting genome synteny, taking advantage of conserved molecular pathways, including those controlling stress tolerance. Following this approach, the regulatory components of the drought response are being searched and identified in crop plants.

Transgenic plants have been developed either to upregulate the general stress response or to reproduce specific metabolic or physiological processes previously shown to be related to drought tolerance by classical physiological studies (Vinocur and Altman 2005). Transcription factors as well as components of the signal transduction pathways that coordinate expression of downstream regulons are thought to be optimal targets for engineering of complex traits such as stress tolerance. Successful examples are transgenic crops engineered with genes encoding the *DREBs/CBFs* transcription factors (i.e., rice [Dubouzet et al. 2003; Ito et al. 2006] and wheat [Pellegrineschi et al. 2004]). The transgenic plants showed increased stress tolerance as well as the overinduction of downstream stress-related genes and/or higher levels of soluble sugars and proline. A recent report has shown that rice plants overexpressing the stress-responsive NAC1 (SNAC1) transcription factor showed improved drought tolerance and yield potential under field conditions. The leaves of SNAC1-overexpressing plants lost water more slowly, showing an increased stomatal closure and ABA sensitivity (Hu et al. 2006). Ectopic expression of a stress-induced rice gene encoding a calcium-dependent protein kinase (*OsCDPK7*) also resulted in

enhanced levels of stress-responsive genes that contribute to improved salt and drought tolerance (Saijo et al. 2000).

The accumulation of several metabolites (fructan, mannitol, and sorbitol) or amino acids (proline, γ-aminobutyric acid (GABA), betaine-aldehyde (Serraj and Sinclair 2002; Mazzucotelli et al. 2006) acting as osmolites or as protectors of proteins and membranes has often been associated with stress tolerance. Metabolic engineering for increasing osmolyte contents was successful in several plants subjected to stress (Wang et al. 2003), although the real advantages of such a strategy are always a subject of debate (Serraj and Sinclair 2002). Given that the target compounds did not achieve levels sufficient to sustain a role in OA, chaperone-like activity and scavenging of ROS were proposed as alternative functions in plant protection during stress exposure. A classical example of metabolic engineering for drought tolerance is the overproduction of proline in rice (Zhu et al. 1998), resulting in an enhanced biomass under stress conditions. Garg et al. (2002) developed drought-tolerant transgenic rice lines showing a tissue- or stress-inducible accumulation of trehalose, which accounted for higher soluble carbohydrate levels, a higher capacity for photosynthesis, and a concomitant decrease in photo-oxidative damage, and more favorable mineral balance under both stress and nonstress conditions, without negative effects. A significant improvement of wheat tolerance to water deficit was also achieved by Abebe et al. (2003) through the ectopic expression of the mannitol-1-phosphate dehydrogenase (*mtlD*) gene that caused a small increase in the level of mannitol.

Since an earlier-than-normal stomatal closure is considered a positive trait to improve water-use efficiency in drought environments (Sinclair and Muchow 2001), developing transgenic plants with a drought-avoidance phenotype represents a possible strategy for crop improvement. A "stay green-like" phenotype was obtained when the *Arabidopsis* gene *GF14λ* encoding a 14-3-3 protein was introduced into cotton (*Gossypium* sp.) plants. The transgenic plants showed improved water stress tolerance and a higher photosynthesis rate due to an altered stomatal conductance under water deficit conditions (Yan et al. 2004a). The implication of abscissic acid hormone as a molecular signal in drought-activated pathways and in the control of stomatal closure makes ABA synthesis and response a possible target for improving drought tolerance. When a farnesyl-transferase acting as a negative regulator of ABA sensing was downregulated in a drought-inducible manner in rapeseed (*Brassica napus*), the transgenic plants showed enhanced ABA sensitivity, as well as a significant reduction in stomatal conductance and transpiration under drought stress conditions. Transgenic plants were more resistant to water deficit-induced seed abortion during flowering (Wang et al. 2005).

A more robust root system enables plants to take up greater amounts of water during water deficit stress, resulting in a more favorable plant water status and less injury. Although this consideration has been obvious for many years, only recent studies have found the way to increase root size through single-gene transformation. The gene coding for the vacuolar H^+-pyrophosphatase (H^+-PPase), *AVP1*, plays an important role in root development through the facilitation of auxin fluxes. Overexpression of *AVP1* in *Arabidopsis* and tomato (*Solanum lycopersicum*) resulted in a more pyrophosphate-driven cation transport into root vacuolar fractions, an increased root biomass, and an enhanced recovery of plants from an episode of soil water deficit stress (Gaxiola et al. 2001; Park et al. 2005).

In the last decade, many differently engineered plants have been proposed and tested for improved performance under drought. Nevertheless, in many reports, the desiccation and salt stresses applied are shock treatments, while for most crops, drought tends to develop slowly as the soil dries. The evaluation of the drought tolerance of transgenic plants has often been based on survival capacity, with very limited analyses of the transgene effects on yield potential. Few reports have been published on transgenic plants tested for drought tolerance in field trials, although more trials are presently carried out. Transgenic wheat plants from six independent transgenic events overexpressing the barley *HVA1* gene were tested under irrigated and

rainfed conditions over six seasons (Bahieldin et al. 2005). Although the effect of the transgene was changing from year to year, the field trials showed that the *HVA1* gene has the potential to confer drought stress protection in field conditions. Encouraging field trial data have also been published for alfalfa (*Medicago sativa*) plants overexpressing the superoxide dismutase gene (McKersie et al. 1996) and for rice overexpressing the SNAC1 transcription factor (Hu et al. 2006) as well as the stress-responsive *OsLEA3* gene (Xiao et al. 2007). The report showed that the higher yield under drought conditions achieved by transgenic *OsLEA3* rice was due to increased spikelet fertility. These phenotypic data as well as the fact that the *OsLEA3* gene is located within the interval of QTL controlling spikelet fertility under drought conditions support the key role of ovary fertility in drought tolerance (Xiao et al. 2007).

FUTURE DIRECTIONS

The integration of molecular genetics with physiology is leading to the identification of the most relevant QTLs/genes controlling abiotic stress tolerance and abiotic stress-related traits. Routine cloning of the genes underlying the QTLs is still a long way off, but ultimately, it will provide simple markers for effective MAS. Nevertheless, MAS for stress tolerance, particularly drought, will not be an easy task because dozens of QTLs for stress-related traits have been identified. Selecting which QTLs/traits to follow with MAS is now crucial. The improvement of drought tolerance should not be achieved with a parallel limitation of yield potential. Hence, stress tolerance traits should be tested in both stressed and nonstressed environments before being introduced in a MAS breeding program. QTLs for stress-related traits coincident with QTLs for yield potential should be considered as priority targets for MAS.

Due to the multigenic nature of abiotic stress tolerance, the introduction of a single gene or QTL into an elite germplasm may result in a subtle phenotypic effect or yield increase. Capacity for precise phenotyping under reliable conditions probably represents the most limiting factor for the progress of genomic studies, particularly on drought tolerance. Often, field experiments designed to evaluate genetic differences in drought tolerance are faced with contrasting requirements. There is a need for precision because the differences may be small and subtle, and detailed physiological measurements (i.e., evaluation of the photosynthetic activity) are difficult when large numbers of genotypes are involved. QTL studies, for instance, are based on segregating populations with hundreds of progenies per trial.

The success of any selection process relies on the availability of superior alleles for the target trait. Most QTLs for stress tolerance, rather than being chosen for their overall agronomic value, have been identified in segregating populations derived from parental lines chosen to maximize the differences in target traits. Typically, a segregating population from a cross between modern and old varieties allows the identification of many QTLs; nevertheless, a majority of the positive QTL alleles might derive from the modern parental line and therefore are already present in the best-performing cultivars. A chance to find new useful alleles is represented by the exploitation of wild germplasm (Pswarayi et al. 2008b; Comadran et al. 2009). During the domestication process, wild plants carrying promising traits were cultivated, leading to locally adapted landraces. These lost many undesirable alleles and useful alleles became enriched in the cultivated gene pool (Tanksley and McCouch 1997). Many studies have demonstrated the value of alleles originating from noncultivar germplasm (Tanksley and Nelson 1996; Tanksley and McCouch 1997), showing that centuries of selective breeding have thrown away useful alleles in addition to many useless ones. The effect of a given QTL/gene can also be influenced by the genetic background of the genotypes used in the breeding program; epistatic interactions among QTLs, for example, might hamper the development of an efficient MAS program.

Transgenic breeding will also have a role in the future, and the possibility of cloning stress-related

QTLs will enable the simultaneous engineering of multiple genes governing quantitative traits. However, the scarcity of field trials for stress-tolerant transgenic plants does not allow for final conclusions. New transgenic plants where the gene is introduced into elite genotypes have to be tested under optimal as well as drought conditions to evaluate the impact of the transgenes on yield potential and stress tolerance.

Breeders now have new perspectives for plant improvement; the so-called breeding by design strategy (Peleman and Van der Voort 2003) will lead to new, high-yielding cultivars able to improve performance in both high- and low-yielding environments by accumulating randomly dispersed QTLs and/or transgenes conferring stress tolerance into elite genotypes. A significant example showing how the integration of stress physiology and genomics can lead to an integrated view of plant breeding is represented by studies on transpiration efficiency. During drought stress, plants generally coordinate photosynthesis and transpiration, although significant genetic variation in transpiration efficiency has been identified both among and within species (Rebetzke et al. 2002). After the demonstration that carbon isotope discrimination is a reliable and sensitive marker negatively related to variation in transpiration efficiency, several selection programs based on this parameter were carried out (Rebetzke et al. 2002; Juenger et al. 2005) and new wheat cultivars with improved water-use efficiency, Drysdale and Rees, have been released in Australia. The isolation of a gene, *ERECTA*, which regulates transpiration efficiency in *Arabidopsis* (Masle et al. 2005), and the transcriptional analysis of wheat genotypes with contrasting transpiration efficiency (Xue et al. 2006) are providing molecular bases of the isotopic discrimination parameter. Hence, in the near future, an integrated approach for transpiration efficiency could involve the use of the physiological test, the markers for QTLs controlling carbon isotope discrimination, and the *ERECTA* gene as well as of other genes either to search for allelic variation in germplasm or as a tool for plant transformation. Every day, it becomes more evident that successful breeding for stable high yield under abiotic stress conditions will only be possible when a true integration of traditional breeding with physiology and genomics is achieved.

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Chapter 11
Biotic Stress in Barley: Disease Problems and Solutions

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INTRODUCTION

Barley (*Hordeum vulgare* L.) is cultivated over a wider geographic range than almost any other major crop species. It can be found growing from the tropics to the high latitudes and from the seacoast to the highest arable mountaintops. On marginal lands where alkaline soils, drought, or cold summer temperatures occur, barley can outproduce most other cereal crops (Mathre 1997). Over such a wide range of growing environments, it is not surprising that barley will encounter different plant pathogens and succumb to various diseases. Considering the hundreds of thousands of potential plant pathogenic microorganisms that exist in the world, resistance is the rule and susceptibility the exception in the plant world. Such is also the case with barley as just over 125 pathogens have been reported on the crop or its products in the United States (Farr et al. 1989). Mathre (1997) lists about 80 different diseases caused by infectious agents in his *Compendium of Barley Diseases*, but of this number, only a handful consistently cause widespread economic loss on an annual basis.

There are four major groups of plant pathogens: fungi, viruses, nematodes, and bacteria. Fungi and funguslike organisms (oomycetes or stramenopiles) are by far the most common plant pathogens. They are heterotrophic and must obtain fixed carbon from plants or organic matter. Some fungi, such as rusts and powdery mildews, are obligate parasites or biotrophs that can only grow and reproduce on living plants. Most other fungi are facultative parasites, functioning as saprophytes until they encounter a plant and switching to a parasitic mode. Once inside the plant, they secrete degradative enzymes and toxins that break down cell walls and other complex plant cell components so the smaller molecules can be absorbed by the fungus. Fungi form filamentous hyphae and mycelium and reproduce by forming spores, which are disseminated primarily by wind and rain. They enter plants directly or through natural openings such as stomata. Most of the fungal pathogens of barley attack the foliage; however, some infect belowground plant parts such as roots, planted seeds, and lower stems (crowns) and spend most of their lives in the soil.

Many root and foliar pathogens function as necrotrophs, killing the tissue in advance of the growing hyphae. Some foliar pathogens, such as *Pyrenophora teres*, are hemibiotrophs, initially growing in living cells and then spreading to dead tissue. Soilborne fungi can survive during periods without the host or during unfavorable environmental conditions by forming thick-walled, melanized, resistant spores such as chlamydospores or sclerotia. During the succeeding growing season, these resting structures will germinate to initiate new infections on the root. When a plant root or planted seed comes in close proximity to these dormant spores in the soil environment, the spores are stimulated to germinate by plant exudates and chemotactically grow toward the roots. Oomycetes and some primitive fungi can also form motile swimming spores called zoospores

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that exhibit chemotaxis, movement toward the root along nutrient gradients.

In the case of foliar pathogens, many survive in crop residue as mycelium and fruiting bodies, such as perithecia or cleistothecia, or wash into the soil as resistant spores, such as teliospores or conidia. Infection occurs by the movement of spores to the leaf surface by wind and rain. These can be conidia, urediniospores (in the case of rusts), or sexual spores such as ascospores.

Viruses are the simplest of the barley pathogens, consisting only of a nucleic acid and protein. They are submicroscopic and can only be viewed with an electron microscope. Viruses are obligate parasites and reproduce by subverting the metabolic machinery of living host cells to produce more virus particles. Most viruses are spherical or rod shaped and are spread through insect vectors, pollen, seed, and mechanical contact. They survive from season to season in their insect vectors or their plant hosts.

Nematodes are microscopic invertebrate wormlike organisms. They have a complex nervous, digestive, and reproductive system. Nematodes move through the soil in water films, and water-filled pores and can feed on the outside of roots (ectoparasites) or inside the root (endoparasites). Some become sedentary after invading the root and set up a feeding site by modifying a plant cell to function for nutrient transfer. Nematodes have a mouthpart called a stylet, a hollow needlelike feeding structure that can penetrate root cells, secrete substances into the cell, and suck up plant nutrients. Most nematodes spend significant parts of their life cycle in the soil, but some can migrate into foliar plant parts, including seeds.

Bacteria are prokaryotic organisms whose genetic material is not organized within a membrane-bound nucleus. They contain a distinct cell wall, are single-celled, and are mostly rod shaped. Bacteria reproduce by fission at an extremely rapid rate. Dissemination of bacteria occurs primarily through insect vectors, rain splash, and mechanical means (i.e., machinery). They gain entry into plants via natural openings or wounds. Bacteria can overseason in infected plant debris, seeds, and on volunteer or weedy hosts.

There have been several works written specifically on barley diseases, most notably, the *Compendium of Barley Diseases* by Mathre (1997) and the chapter by Kiesling (1985) in the American Society of Agronomy Series book *Barley*. Additionally, concise information about many barley diseases can be found in more general field crop disease publications such as *Diseases of Field Crops in Canada* by Bailey et al. (2003) and *Cereal Leaf and Stem Diseases* by Wallwork (1992). There are also monographs available that are dedicated to specific cereal diseases such as *Barley Yellow Dwarf: 40 Years of Progress* (D'Arcy and Burnett 1995) and *Fusarium Head Blight of Wheat and Barley* (Leonard and Bushnell 2003). The purpose of this chapter is to provide a brief summary of some of the major diseases impacting barley around the world, with an emphasis on North America. This updated overview highlights the general importance of the diseases, symptomatology, epidemiology, pathogen biology, and disease management strategies based primarily on cultural and genetic approaches. The chapter is divided into the sections of foliage and root and crown diseases of barley with subcategories of diseases caused by the different pathogen groups. In the section on foliar pathogens, management techniques will be discussed within each disease. However, for root and crown pathogens, the management strategies are common for a number of diseases and will be covered at the end of the section.

FOLIAGE DISEASES OF BARLEY

Fusarium head blight (FHB)

FHB or scab is a devastating and insidious disease of barley. It is endemic to eastern Asia but has reemerged in the 1990s as a serious problem of barley in North America and in several other regions of the world including South America and Europe (Steffenson 2003). The epidemics that occurred in the Red River Valley region of the United States and Canada caused economic losses that were unparalleled in the history of barley production in the region (Steffenson 1998;

Windels 2000). Consequently, the area planted to barley dropped significantly and has not recovered. FHB can cause yield reductions exceeding 40% (Perkowski et al. 1995). Malting quality parameters of kernel plumpness, malt extract, and germinative energy also can be adversely affected by FHB infection (Schwarz 2003). Moreover, *Fusarium*-infected malt may contribute to off-flavors and gushing in beer. Perhaps the most insidious part of this disease is that many of the *Fusarium* species causing FHB also produce various mycotoxins that can be carried in the grain. These mycotoxins are harmful to both human and animals, especially swine. Humans ingesting mycotoxin-contaminated grain (e.g., with deoxynivalenol) can experience acute toxicosis symptoms (Steffenson 2003), while swine often show vomiting and hyperestrogenism (Joffe 1986). Since mycotoxins cause so many problems for end users, mycotoxin assays have become a standard specification in grain purchasing. Plump, seemingly healthy grain with even low concentrations (e.g., 1.0 ppm) of mycotoxins can be severely discounted at the elevator. Due to the severe economic losses, grain processing problems for producers and end users alike, and also food/feed safety concerns, FHB has forced many barley breeders and pathologists in affected areas to completely redirect their research efforts into ameliorating the impact of this disease above all other biotic constraints (Steffenson 1998).

Barley spikes can be infected with *Fusarium* species as soon as they emerge from the flag leaf sheath. Generally, infected kernels are discrete and scattered across the spike (Tekauz et al. 2000; Fig. 11.1). This is in contrast to wheat where one-quarter or more of a contiguous section of the spike may show symptomatic infection. The pathogen enters the florets through the overlapping crevices between the lemma and palea, but also the apex opening of the floret (Skadsen and Hohn 2004; Lewandowski et al. 2006). Thereafter, the caryopsis can be infected. The first symptoms usually observed are tan to dark brown lesions at the base of the kernels (Fig. 11.2). These infections can spread up the entire kernel within a few days under warm, moist conditions. Early infections result in complete sterility of florets, where spikes appear thin and compressed. Yield losses from early infections are often very high. In contrast, late infections may only reduce yield slightly; yet, if the grain is contaminated with mycotoxins, the discounts on such crops may still be high. In addition to symptoms, pathogen signs are also very diagnostic for the disease. Pink- to salmon-colored masses of *Fusarium* mycelium and conidia can often be observed on kernels as can blackish perithecia of the *Gibberella zeae* sexual stage (Fig. 11.3). Under high moisture conditions, *Fusarium* mycelium can be extensive, infecting kernels from the outside as it spreads across the spike (Fig. 11.4).

Fig. 11.1. Severe Fusarium head blight infection on two-rowed barley in the field. For color details, please see color plate section.

The primary pathogens causing FHB in many parts of the world include *Fusarium graminearum* (telomorph: *G. zeae*), *Fusarium culmorum*, *Fusarium avenaceum* (telomorph: *Gibberella avenacea*), *Fusarium sporotrichioides*, *Fusarium poae*, and *Fusarium crookwellense* (Koizumi et al. 1991; Parry et al. 1995; Salas et al. 1999; Tekauz et al. 2000; Xue et al. 2006). These *Fusarium* species may exhibit different levels of pathogenicity on barley, with *F. graminearum*, *F. culmorum*, and *F. crookwellense* causing the highest disease severity in controlled greenhouse inoculations (Salas et al.

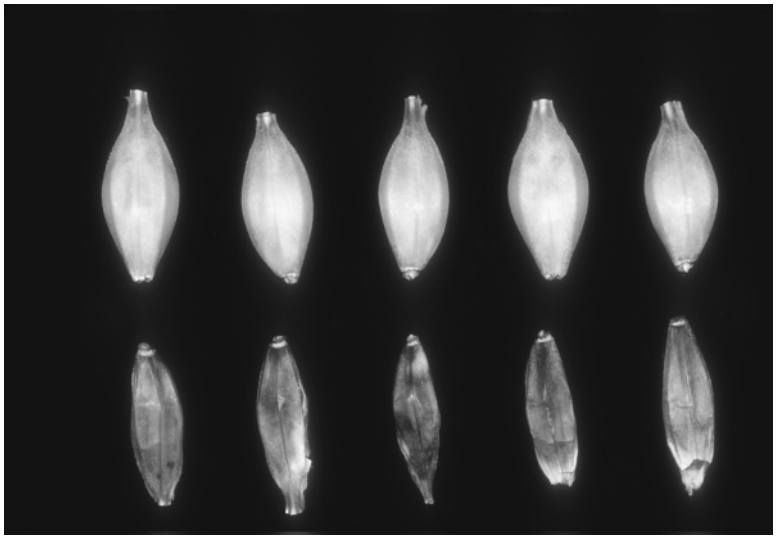

Fig. 11.2. Fusarium head blight-infected barley kernels (bottom row) in comparison to healthy, non-infected kernels (top row) from inoculation tests conducted in the greenhouse with *F. graminearum*. Note the dark lesions on infected kernels and their much smaller size. For color details, please see color plate section.

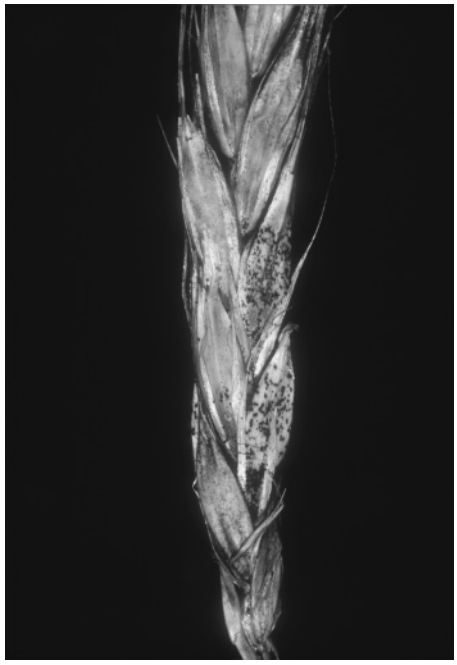

Fig. 11.3. Barley spike heavily infected with Fusarium head blight collected from the field. The base of the third kernel on the left from the bottom shows salmon to pinkish masses of *Fusarium* mycelium and conidia, whereas the lower right kernels show the black perithecia of the *Gibberella zeae* sexual stage. For color details, please see color plate section.

Fig. 11.4. Barley spike heavily infected with *Fusarium* mycelium in the field. For color details, please see color plate section.

1999; Xue et al. 2006). *F. graminearum* is the most common FHB pathogen in production areas where maize is cultivated. It readily produces the *G. zeae* perfect stage in nature and also in culture. FHB pathogens produce an array of various mycotoxins from deoxynivalenol (DON) and nivalenol to HT-2 and T-2 toxins (Joffe 1986). The most common mycotoxin associated with *F. graminearum* is deoxynivalenol; consequently, this compound is assayed by grain elevators before the crop is purchased from growers. Isolates of *F. graminearum* comprising different chemotypes (i.e., producing different arrays of mycotoxins) have been described (Goswami and Kistler 2004). Although differences in virulence have been reported within some of the FHB pathogens, the distinct pathogen isolate × host genotype interactions found with obligate parasites such as rusts have not been reported (Takeda and Kanatani 1991). In addition to small grain cereal crops and maize, *F. graminearum* can also attack many grass species (Shaner 2003).

F. graminearum and other *Fusarium* species survive as saprophytes on crop residue. Because maize stalks are large and more resistant to decay, they provide the largest substrate and hence source of inoculum for FHB infection. This was the basis of the old adage of avoiding planting small grain cereals after maize. In the northern latitudes, ascospores produced from perithecia are the most important source of primary inoculum, although conidia produced from overwintering mycelium on host residue may also contribute to early infections. Once spikes are infected, secondary spread by conidia can occur but is thought to be of little consequence in overall disease development (Shaner 2003).

The key factors contributing to the recent FHB epidemics in North America were the widespread cultivation of susceptible cultivars, abundance of virulent pathogen inoculum, and favorable weather conditions for disease development (Tekauz et al. 2000; Steffenson 2003). Since FHB had not been a serious problem for many years, barley cultivars had not been selected for resistance and were therefore very susceptible. Second, with the wide adoption of conservation tillage practices, there were ample substrates present in the field for the FHB pathogens to survive on and reproduce (Dill-Macky and Jones 2000). The final key element contributing to the FHB epidemics was the weather. Moist weather at heading is perhaps the most critical factor determining whether FHB epidemics will occur. In the semi-arid regions of the western United States, FHB pathogens are common and susceptible barley cultivars are widely grown; however, epidemics are extremely rare. The only reports of severe FHB epidemics occurring in this region were in sprinkler-irrigated fields of barley and wheat during seasons of frequent rainfall coinciding with the heading period. Fields receiving rill irrigation were not infected. Thus, overhead sprinkler irrigation plus frequent natural rainfall was required in order to provide sufficient wetness for FHB infection (Mihuta-Grimm and Forster 1989).

While infection can advance faster at higher temperatures (>25°C), FHB can still cause epidemics under cool weather conditions.

FHB is an extremely difficult disease to manage. Even at low disease levels, severe economic losses can occur due to discounts for the presence of mycotoxins (Steffenson 2003). An integrated management strategy is therefore required to reduce FHB and the contamination of grain with mycotoxins. This includes cultural practices, fungicide application, and host resistance. Cultural practices that can reduce pathogen inoculum and therefore FHB include plowing under crop residue and crop rotation with nonsusceptible hosts (Dill-Macky and Jones 2000). Given that moist weather conditions are critical for FHB infection, the cultivation of barley in more arid areas is another cultural practice that can reduce the incidence of the disease. Indeed, after the FHB epidemics of the 1990s in the north-central region of the United States, much of the barley production moved to the more arid regions of the west. Lodging exacerbates FHB infection (Steffenson 2003; Nakajima et al. 2008); thus, any practice that reduces crop lodging (i.e., avoiding excessive nitrogen fertilization) will also reduce FHB infection. Fungicides can reduce the severity of FHB and also mycotoxin levels in grain; however, they are not always consistent in their

effects (Jones 2000; Horsley et al. 2006a). Still, they remain an important part of an integrated management strategy. The deployment of resistant cultivars is the most cost-effective means of reducing FHB, but unfortunately, highly resistant barley accessions have not been identified. In wheat, two main types of FHB resistance have been described. Resistance to initial infection (referred to as type I resistance) is measured by spray inoculating spikes with inoculum and assessing the level of infection, whereas resistance to spread (referred to as type II resistance) is measured by needle injecting a single floret with inoculum and assessing the level of spread from the initial infection focus. In wheat lacking the latter type of resistance, single-floret inoculations can kill the entire spikes. Barley possesses an inherent level of resistance to spread as single-floret inoculations will not spread vertically to kill the entire spike. Lateral spread across kernels at a rachis node in six-rowed barleys frequently occurs, but vertical spread is very limited (Tekauz et al. 2000; Steffenson and Dahl 2004). Still, variation for resistance to vertical spread has been reported in barley (Zhu et al. 1999). Many large-scale evaluations have been undertaken to identify FHB resistance in barley (Steffenson 2003). Both the frequency and level of resistance are low in *Hordeum*. However, partial resistance to FHB has been identified in a number of diverse accessions of barley and also wild barley (*H. vulgare* subsp. *spontaneum*). In general, six-rowed barleys are more susceptible to FHB than two-rowed barleys, but it is not certain if this is due to true genetic susceptibility or possibly the spike architecture that allows for more frequent lateral spread to kernels at a node in the former. Growers in some regions of Japan and China have stopped growing six-rowed barley in favor of two-rowed types due to greater FHB damage in the former (Steffenson 2003; Choo 2009).

Many studies have been done to elucidate the genetics of FHB resistance in various sources and have revealed a complex, quantitative nature for the trait. Molecular mapping studies have corroborated the classical genetic studies showing that resistance to FHB and the accumulation of DON is a quantitative trait controlled, in most cases, by a number of genes with relatively small effects that are scattered across the barley genome (de la Pena et al. 1999; Zhu et al. 1999; Ma et al. 2000; Horsley et al. 2006b). A wide range of heritability values for FHB resistance (from 0.31 to 0.81) have been reported. Agronomic (e.g., heading date and plant height) and spike architecture (e.g., row type, kernel density, and spike angle) traits may affect the level of FHB infection on barley (Steffenson 2003; Yoshida et al. 2005). This complicates the identification of quantitative trait loci (QTLs) that truly are involved in conferring resistance to FHB.

Almost every facet of screening for FHB resistance and low DON concentration is time-consuming, labor-intensive, and expensive. Therefore, marker-assisted selection is being used to transfer resistance QTLs into breeding lines. To achieve the highest levels of FHB resistance possible, multiple-resistance QTLs are being introgressed in cultivars. Several barley cultivars with partial FHB resistance have been released in China, Japan, Ecuador, and, more recently, in the United States and Canada (Steffenson 1998, 2003; Tekauz et al. 2000). The full benefit of using these resistant cultivars can only be realized when used in conjunction with other management strategies.

Transgenic approaches (e.g., transformation of barley with antifungal proteins) are also being explored to ameliorate FHB of barley (Dahleen et al. 2001; Muehlbauer and Bushnell 2003). If a particular transgene proves to be consistent in its ability to reduce FHB and DON, it could ultimately find its way to the farm field if the proper regulatory and marketplace hurdles are cleared.

Stem rust

Stem rust is one of the most serious diseases of small grain cereals, and its effects on crops have been known since at least the time of the Romans (Peterson 2001; Leonard and Szabo 2005). A large body of literature has been written on wheat stem rust due to the crop's worldwide importance as a food crop and greater vulnerability to attack. Comparatively little has been written about barley stem rust, even though the disease can cause

severe damage in some years. Barley stem rust can be an important problem in the Great Plains region of the United States and Canada and in parts of South America, northeast Africa, Russia, and Australia. The disease has been controlled in North America through the deployment of cultivars with the durable resistance gene *Rpg1*. Races (e.g., QCCJ) with virulence for this resistance gene have been reported periodically, and in the early 1990s, they caused some losses in late-planted barley fields (Steffenson 1992). In northeast Australia, severe stem rust epidemics occurred in the early 1980s due to a buildup of inoculum on wheat and triticale and the widespread planting of susceptible barley cultivars (Dill-Macky et al. 1991). Although stem rust can be found on barley in other regions of the world, it often infects the crop late in the season, and therefore, losses are minimal. A new stem rust threat to barley worldwide is race TTKSK (isolate synonym of Ug99). This race of the wheat stem rust pathogen (*Puccinia graminis* f. sp. *tritici)* was first found in Uganda in 1999 and is capable of attacking over 70% of the world's wheat and barley cultivars (Singh et al. 2008; Steffenson et al. 2009; B. Steffenson, unpublished data). Since its first discovery, race TTKSK has spread to other countries in eastern and southern Africa and is now present in the Middle East (Nazari et al. 2009). It will likely spread to other major cereal production regions in the near future. Stem rust has the potential to cause complete crop failure. In Australia and Canada, yield losses over 50% were found in cultivars susceptible to stem rust (Dill-Macky et al. 1991; Harder and Legge 2000). Yield losses are due to a reduction in kernel size and weight and are related to the onset and severity of stem rust infection.

Stem rust primarily attacks the stems, leaf sheaths, and peduncles of plants; however, infection also may occur on leaf blades, glumes, and awns. Like most rust pathogens, infections occur only through stomata. Stem rust is one of the most easily recognizable diseases of barley due to the conspicuous brick-red uredinia (pustules) of the pathogen that break through the epidermal layer (Fig. 11.5). White frayed fragments of the epidermis are usually visible at the edges of uredinia. Stem rust is also known as "black rust" because as infected barley plants begin to senesce, uredinia are converted to black telia.

Fig. 11.5. Stem rust infection on barley in the field. For color details, please see color plate section.

Barley stem rust is caused by two different pathogens: *P. graminis* f. sp. *tritici* (the wheat stem rust pathogen) and *P. graminis* f. sp. *secalis* (the rye stem rust pathogen). Although these *formae speciales* (f. sp.) of *P. graminis* were first described from wheat and rye, respectively, they are also virulent on barley as well as on a number of different wild gramineous species. Historically, wheat stem rust has been more important than rye stem rust on barley (Roelfs 1985). In Australia, a putative hybrid between *P. graminis* f. sp. *tritici* and *P. graminis* f. sp. *secalis* was found and was reported to attack only barley and a few noncereal grass species (Luig 1985). *P. graminis* is a heteroecious, macrocyclic rust and therefore has five spore stages. The uredinial and telial spore stages occur on the grass hosts, whereas the pycnial and aecial spore stages occur on the alternate hosts—primarily *Berberis* (barberry) and *Mahonia* (Roelfs

1985). In North America, many of the stem rust cultures collected from *Berberis* in recent years are *P. graminis* f. sp. *secalis*. It is on the alternate hosts that *P. graminis* can undergo sexual hybridization, leading to new virulence combinations in the pathogen population. Like the other rusts and also powdery mildew, *P. graminis* is functionally a biotrophic fungus and therefore needs a living host to grow and reproduce. However, the fungus has been grown on artificial media under very stringent conditions (Leonard and Szabo 2005).

The alternate hosts of *Berberis* and *Mahonia* can provide a source of primary inoculum in the form of aeciospores, although this spore is generally disseminated over short distances (Roelfs et al. 1992b). In most regions, urediniospores are the primary inoculum for initiating stem rust epidemics. The stem rust fungus can overseason either as a urediomycelium or uredinia on various gramineous hosts. Urediniospores produced from these hosts can be easily wind disseminated and carried across hundreds or even thousands of miles. In the Great Plains of North America, urediniospores move from the overwintering areas of the southern United States to infect crops in the north along the "*Puccinia* pathway" (Roelfs et al. 1992b). Heavy dews lasting into the morning are ideal for infection by urediniospores, but drying and windy conditions are best for spore dispersal. Germination and infection by *P. graminis* urediniospores can occur over a wide range of temperatures, but 15–20°C is optimum (Roelfs 1985; Roelfs et al. 1992b). Once barley is initially infected, several successive urediniospore generations can be produced before the crop matures.

If the alternate host is growing in proximity to a barley crop, it should be eradicated if there is a danger of it becoming infected with basidiospores developing from germinating telia on overwintered straw. This action will eliminate an early and local source of inoculum and will also reduce the chance of new virulent hybrids arising in the pathogen population. The national program aimed at eliminating *Berberis* was largely successful in the north-central region of the United States in the last century, although this alternate host may be reemerging again in some areas where cereal crops are grown. Urediniospores, sometimes carried over very long distances, are the most important source of stem rust inoculum. In mild climates, urediniospores of *P. graminis* can overseason on barley, wheat, rye, volunteer plants, and a number of different gramineous species (Leonard and Szabo 2005). Elimination of these live "green" host plants and the planting of stem rust-resistant cereal crops will reduce this source of inoculum (Roelfs 1985). There are few cultural control strategies that one can take against stem rust, but one effective measure is planting early. Since stem rust often arrives late in the growing season, it is important to plant early to avoid damage from multiple urediniospore generations. Chemical control is another option for reducing the impact of stem rust on barley. There are several compounds that are very effective in controlling the disease, and they can be used in an emergency situation (e.g., in the case of race TTKSK) before resistant cultivars become available.

Breeding cereal crops for resistance to stem rust has been ongoing for over a century. In North America, several spectacular epidemics have occurred on wheat after the release of cultivars protected by one or just a few genes. These "boom and bust" cycles of resistance breeding were finally halted through the strategy of combining multiple genes into a single cultivar. A different situation occurred in barley as a single gene (*Rpg1*) provided effective protection against stem rust losses for more than 60 years (Steffenson 1992). Although races of the pathogen with virulence for this gene have been identified from time to time, it is still important to include *Rpg1* in cultivars because it is widely effective against many common races. Race TTKSK threatens both wheat and barley worldwide and is spreading rapidly (Singh et al. 2008; Nazari et al. 2009; Steffenson et al. 2009). Additionally, variants within this race's lineage have emerged that possess additional virulences for resistance genes in wheat and possibly in barley (Singh et al. 2008; Jin et al. 2009; Steffenson et al. 2009). It is clear that more broad-based resistance will have to be incorporated into barley to protect against this new stem rust threat. In barley, at least eight major genes for stem rust resistance have been

described (Steffenson et al. 2009), but it is not known how broadly effective they are against different races of the pathogen, particularly at the adult plant stage in the field. As a diploid plant species, barley is genetically vulnerable to many diseases and stem rust is no exception. From extensive evaluations of *Hordeum* germplasm, only a few accessions were found to be resistant to race QCCJ (Jin et al. 1994). Fortunately, in the recent case of race TTKSK, evaluations of *Hordeum* germplasm have identified several sources of resistance in various cultivars, landraces, and wild barley accessions (Steffenson et al. 2009). An association mapping approach is being used to identify and position stem rust resistance genes in both wild and cultivated barley germplasm (Steffenson et al. 2007; B.J. Steffenson et al., unpublished data). The stem rust resistance genes *Rpg1*, *rpg4*, and *Rpg5* in barley have been cloned and a number of molecular studies have been advanced in an effort to understand the basis of their resistance (Kleinhofs et al. 2009). In the near future, it may be possible to strategically transfer into adapted cultivars a suite of chimeric resistance genes, generated by *in vitro* sequence exchange, with the potential to confer a broader spectrum of resistance against stem rust. There is precedence for this as new motifs for two flax rust resistance genes were generated and transformed into the host, resulting in different specificities to the rust pathogen (Dodds et al., 2001; Howles et al., 2005).

Leaf rust

Leaf rust is a common disease of barley and has been reported in most production regions across the world. The pathogen will seldom cause severe epidemics over a wide area on an annual basis. Instead, epidemics often occur infrequently and late in the season when the crop is approaching maturity (Clifford 1985). Still, significant yield losses can occur in susceptible cultivars when the inoculum arrives early and levels are high. Under experimental conditions, yield losses over 60% were reported in highly susceptible barley cultivars, but losses about half that level are more common (Clifford 1985; Cotterill et al. 1992; Das et al. 2007; Ochoa and Parlevliet 2007). Leaf rust is often a more serious problem in barley under intensive cultivation.

Infection of plants occurs primarily by urediniospores, which invade via stomata. Initial infections are manifested by small chlorotic areas on the leaf or the leaf sheath. The pathogen then breaks through the epidermis to form orange to orange-brown uredinia (Fig. 11.6) that may darken over time. In some cases, the glumes and awns can be infected, especially late in the season (Clifford 1985). Leaf rust uredinia can be distinguished from stem rust uredinia by their generally smaller size, lighter orange color, and a more rounded to oval shape. Crown rust, another rust of barley, can be distinguished from leaf rust by its distinct light orange and linear uredinia, bordered by chlorosis (Mathre 1997; Jin and Steffenson 1999). As infected barley plants mature, leaf rust uredinia are converted into blackish brown telia. This is the basis for the moniker of "brown rust," the other common name for the disease.

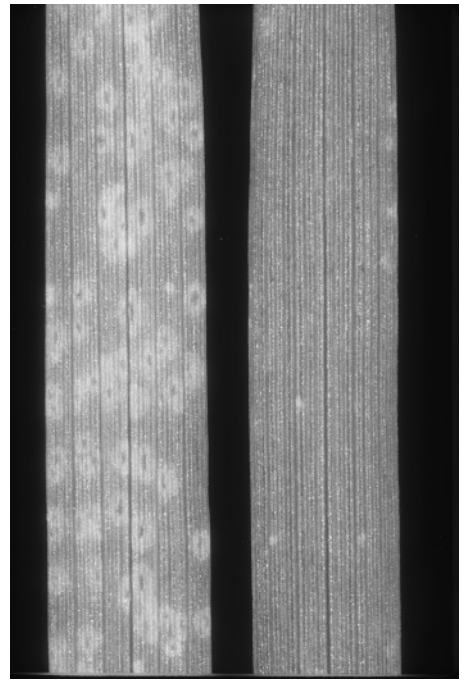

Fig. 11.6. Leaf rust infection on seedling leaves of a susceptible (left) and resistant (right) barley cultivar from the greenhouse. For color details, please see color plate section.

Leaf rust on barley is caused by *Puccinia hordei*, a heteroecious, macrocyclic rust. The uredinial and telial spore stages of the rust occur on barley and various wild *Hordeum* species, whereas the pycnial and aecial spore stages occur on alternate hosts in several genera in the Liliaceae, including *Ornithogalum*, *Leopoldia*, and *Dipcadi* (Clifford 1985). Leaf rust isolates infecting the alternate hosts can undergo sexual hybridization, resulting in new virulence types. Indeed, sexually reproducing populations arising from *Ornithogalum* may exhibit a higher level of pathotype diversity (Manisterski 1989) than asexually reproducing populations (e.g., Cromey and Viljanen Rollinson 1995; van Niekerk 2001; Woldeab et al. 2006) where just a few pathotypes dominate. However, asexual populations of *P. hordei* in Ecuador proved to be as diverse as sexual populations from Israel (Brodny and Rivadeneira 1996). Many pathotypes of *P. hordei* have been described from different parts of the world (e.g., Clifford 1985; Park 2003; Woldeab et al. 2006). It appears that the frequency of mutation in asexually reproducing populations occurs often enough to overcome important resistance genes in barley (Dreiseitl 1990; Steffenson et al. 1993; Cromey and Viljanen Rollinson 1995).

The star-of-Bethlehem lily (*Ornithogalum umbellatum*) is one of the most important alternate hosts for *P. hordei* and is distributed in many parts of the world. In the Mediterranean Basin and South Australia, *O. umbellatum* plays an important role in the survival of the pathogen and in the epidemiology of the disease because it often harbors aeciospores that can infect barley (Manisterski 1989; Wallwork et al. 1992). In areas where the alternate host is absent or does not play a role in the life cycle of the pathogen, urediniospores perpetuate the disease, surviving on volunteer barley plants and autumn-sown barley, or wild *Hordeum* species (Clifford 1985). Heavy dews and temperatures ranging from 10 to 20°C are optimal for leaf rust infection. Like other cereal rusts, *P. hordei* can be disseminated over long distances to infect barley crops.

O. umbellatum is important in the survival of *P. hordei*, in the evolution of new pathogen virulence combinations, and in the early infection of *Hordeum* in some regions of the world (Clifford 1985); however, its eradication is impractical. Urediniospores provide the primary inoculum for initiating epidemics in most production regions. In addition to planting resistant barley cultivars, this source of inoculum can be reduced by eliminating infected volunteer plants and populations of wild *Hordeum* species. Host resistance has been the primary means of controlling leaf rust. Extensive research has led to the identification of more than 20 major resistance genes in cultivated and wild barley (Jin and Steffenson 1994; Franckowiak et al. 1996; Pickering et al. 1998; Park and Karakousis 2002). Unfortunately, many of these genes are ineffective against the worldwide spectra of *P. hordei* pathotypes. To develop cultivars with more durable leaf rust resistance, researchers are utilizing partial or slow-rusting resistance. Several of the early, comprehensive studies on quantitative rust resistance in cereals were done with barley leaf rust and slow-rusting cv. Vada (Parlevliet 1976). The genetically complex nature of this resistance was confirmed in later investigations using molecular mapping as multiple QTLs were found to contribute to partial resistance (Qi et al. 1998). One critical question regarding partial leaf rust resistance is whether it can provide uniform resistance against all pathotypes and therefore provide durable resistance. Analysis of individual QTLs derived from Vada revealed that some QTLs are pathotype specific (Marcel et al. 2008). Thus, it remains to be seen whether such sources of partial resistance prove more durable in cultivars that are widely grown. Given the variable nature of *P. hordei*, it is best to breed cultivars with broad-based resistance using multiple major resistance genes and/or partial resistance. Two to three component mixtures of barley cultivars have been shown to reduce leaf rust severity in comparison to the individual cultivars grown in pure stands. Additionally, the cultivar mixtures also had higher and more stable yields (Gacek and Nadziak 2000). Fungicides are another effective measure for controlling barley leaf rust. A number of different antifungal compounds have shown good activity against this disease and are used routinely to not only control leaf rust but other foliar fungal

pathogens as well. Forecasting models have been developed for barley leaf rust to help growers make decisions about applying fungicides for control of the disease (Clifford 1985). The only practical cultural method that can be used for leaf rust management is early planting. Leaf rust often arrives late in the season; thus, early planting will help minimize crop damage.

Stripe rust

Stripe rust or yellow rust is a disease that has plagued barley for a long time in southern Asia, eastern Africa, Western Europe, and the Middle East. In 1975, the *forma specialis* of this disease that affects primarily barley was found for the first time on barley in the Western Hemisphere, near Bogota, Colombia (Dubin and Stubbs 1986). Since then, the pathogen has spread throughout many South American countries and was found in Mexico in 1987. It continued its northward spread and was detected in the United States in 1991 (Roelfs et al. 1992a). Now, the disease is found across the western part of the United States and Canada (Brown et al. 2001; Line 2002; McCallum et al. 2007). In some western states of the United States, stripe rust has become the most important disease of barley (Line 2002). The disease has the potential to cause complete crop failure in some countries such as Nepal (Stubbs 1985). Under experimental conditions, yield losses ranging from 20% to 72% were reported, depending on the level of susceptibility present in cultivars (Marshall and Sutton 1995). Stripe rust development is favored by cool weather conditions, and extended dew periods are needed for pathogen infection. A recent study suggests that this pathogen can become adapted to warmer, more arid environments because the disease has become a recurring problem of wheat in the central Great Plains of the United States—a region not previously considered congenial to stripe rust (Milus et al. 2009).

Since stripe rust is a cool season rust, the symptoms and signs will appear earlier than for other rust diseases (Mathre 1997). Low temperatures of about 11–15°C during the evening and free moisture (mediated by rainfall or dew) are optimal for infection (Stubbs 1985; Roelfs et al. 1992b). Initial symptoms include chlorotic flecks at the site of infection. The subsequent signs of the pathogen are distinct from other rusts of barley because the uredinia are yellow and occur in prominent stripes along the leaf veins (Fig. 11.7) and glumes. Stripe rust is unique among the cereal rusts because individual uredinia spread in a line from the site of initial infection (Roelfs et al. 1992b). Black telia readily develop from uredinia as infected barley plants approach maturity.

Fig. 11.7. Stripe rust infection on barley in the field. For color details, please see color plate section.

Puccinia striiformis f. sp. *hordei* is the causal agent of stripe rust on barley, although isolates of *P. striiformis* f. sp. *tritici* can sometimes infect the crop. The specialized *forma specialis* infecting barley apparently arose in Europe and attacks only a few of the most susceptible wheats (Stubbs 1985; Roelfs et al. 1992b). The uredinial and telial spore stages of *P. striiformis* f. sp. *hordei* occur on barley and various *Hordeum* species (Marshall and Sutton 1995). The alternate host of *P. striiformis*

f. sp. *tritici* was recently found to be *Berberis* (Jin et al. 2010); thus, it is likely that *P. striiformis* f. sp. *hordei* also will cycle on this host. Using randomly amplified polymorphic DNA markers, Chen et al. (1995) differentiated pathotypes of *P. striiformis* f. sp. *hordei* from *P. striiformis* f. sp. *tritici*. However, a strict generic level separation was not found with respect to host range as some isolates of the former could attack wheat and some isolates of the latter could attack barley. The original isolate of *P. striiformis* f. sp. *hordei* first collected in South America was typed as race 24, the same common pathotype found in Europe (Dubin and Stubbs 1986). Subsequent studies of virulence phenotypes in *P. striiformis* f. sp. *hordei* from the United States revealed that barley stripe rust entered as a mixture of races and genetic lineages. By 1998, 52 different races of *P. striiformis* f. sp. *hordei* were identified on 11 barley differentials in the United States (Line 2002). Thus, the pathogen has the ability to readily change and generate new virulence types. A variant of *P. striiformis* capable of attacking barley and wild *Hordeum* species was discovered in Australia in 1998 (Wellings et al. 2000). It is different in its virulence pattern on wheat and barley and may be a new *forma specialis* of *P. striiformis*.

The epidemiology of stripe rust depends on the specific production area, whether winter or spring crops are planted, and also the environment. For example, near the equatorial zones, year-around plantings of spring cereals across a range of elevations can foster the continual cycling of stripe rust inoculum. At higher latitudes, stripe rust may cycle over a longer period of time moving from spring cereals in the low elevation plains to grasses growing in the foothills and mountains and then back again (Roelfs et al. 1992b). In winter barley regions or in areas where spring barley is sown in the autumn, stripe rust can infect crops in autumn or winter. Depending on the severity of winter and, in some regions, the amount of snow cover, the stripe rust pathogen can survive in crops as urediniomycelium. As warmer temperatures return in the spring, the urediniomycelium will sporulate to initiate epidemics. Volunteer plants, autumn-sown barley crops, and wild *Hordeum* species can serve as inoculum reservoirs for barley stripe rust (Dubin and Stubbs 1986; Marshall and Sutton 1995; Line 2002). Stripe rust urediniospores are capable of being disseminated by wind over long distances and initiate new infection foci; however, the efficiency of this process is thought to be lower than for other cereal rusts due to the greater susceptibility of *P. striiformis* urediniospores to ultraviolet radiation (Stubbs 1985). Still, it is possible that the primary inoculum of this pathogen can be reintroduced into an area on an annual basis (Brown et al. 2001; Marshall and Sutton 1995).

Brown et al. (2001) advocated an integrated approach for stripe rust control in barley that includes disease resistance, cultural strategies, fungicides, and disease monitoring. As with most foliar fungal diseases, host resistance is the preferred means of controlling stripe rust. When stripe rust of barley was reported in the United States, a large screening effort was undertaken to identify sources of resistance. Over 40,000 barley accessions from the United States Department of Agriculture (USDA) National Small Grains Collection were evaluated for stripe rust reaction at the adult plant stage, and a number of promising resistance sources were identified (Brown et al. 2001). An analysis of these data revealed a center of concentration for stripe rust resistance in the high-altitude regions of Ethiopia (Bonman et al. 2005). Many genes exist for seedling stripe rust resistance in barley (Chen and Line 1999; Brown et al. 2001; Line 2002). In a study of 18 barley lines by Chen and Line (2003), at least 26 different resistance genes were identified. Surprisingly, many stripe rust resistance genes are recessive (Chen and Line 1999, 2003), which is unusual given that most rust resistance genes in cereals have dominant gene action. With the high level of variation for virulence types reported in the pathogen, it is likely that these seedling resistance genes would provide only ephemeral protection against the disease. Adult plant resistance offers more promise for achieving longer-lasting resistance and is governed by both single major effect genes (Yan and Chen 2008) and also multiple QTLs (Castro et al. 2003). Richardson et al. (2006) introgressed into susceptible barley line combinations of one, two, and three QTLs

and found lines carrying multiple-resistance QTLs had higher levels of resistance as measured by infection efficiency, lesion size, and pustule density. Thus, the strategy of combining multiple genetic resistance sources, be they major effect genes or QTLs, is a sound approach for achieving a more stable resistance and can be achieved through marker-assisted selection (Brown et al. 2001).

There are few cultural control strategies one can deploy to limit cereal rust development. In the western United States, stripe rust is often most severe on late-planted crops; thus, early planting can reduce the severity of the disease (Brown et al. 2001). With irrigated barley, it is advised to schedule watering so as to reduce the length of time the plant canopy stays moist as this will limit infection by *P. striiformis* f. sp. *hordei*. The elimination of volunteer barley plants and also a temporal break in the "green bridge" of winter and spring crop phenology can reduce stripe rust inoculum and the impact of the disease.

Foliar fungicides are effective against barley stripe rust, but in areas where the disease is severe, two applications may be required, a practice that is prohibitively expensive for most growers. Scouting fields for disease is an important aspect for chemical control of stripe rust because early detection will allow for fungicides to be applied in a timely manner, thereby averting losses and reducing pathogen inoculum. Brown et al. (2001) indicated that systemic fungicides applied as seed treatments may be useful for growers because of their ease of application and lower cost. For the best control of the disease, these seed treatments should be used in combination with foliar fungicides when the disease severity mandates their use. An expert system for barley diseases, including stripe rust, was developed for growers in the Pacific Northwest (PNW) region of the United States (Line 2002). This system, called MoreCrop (described at: http://pnw-ag.wsu.edu/MoreCrop/), integrates many facets about the biology of the pathogen, disease epidemiology, and cropping systems to assist growers in making informed management decisions on disease control.

Powdery mildew

Powdery mildew is a widely distributed disease of barley throughout the world but is most common and damaging in northern Europe, Japan, and, in some years, the eastern and southern production regions of the United States (Kiesling 1985; Mathre 1997). In general, the disease is favored by cool and humid weather but can also occur in warmer, semiarid environments (Mathre 1997). Both winter and spring barley crops can be affected by powdery mildew, resulting in losses typically ranging from 1% to 14% (Mathre 1997). Losses exceeding 14% can occur when disease onset is early and inoculum pressure is high. Aside from yield loss, powdery mildew infection can also reduce kernel weight, numbers of tillers and spikes, and root growth (Mathre 1997).

The powdery mildew fungus is a biotrophic pathogen. Germ tubes from both conidia and ascospores can penetrate the host cuticle directly. Haustoria with distinct fingerlike like appendages are subsequently formed within epidermal cells giving rise to superficial sporulating colonies. The pathogen chiefly attacks the adaxial part of the leaves (Fig. 11.8), although all aboveground parts of the plant may be infected. The most diagnostic features of the disease are the pathogen signs. They initially appear as fuzzy, whitish tufts of fungal mycelium. Later, powdery or fluffy white pustules of conidial chains develop from the mycelium (Fig. 11.9). Mycelium and conidia may turn gray or even slightly brownish in color with age (Kiesling 1985). Since the pathogen only infects the epidermal cells, conidia and mycelium can be readily scraped away from the surface of the leaves. Late in the growing season, the black, globose-shaped cleistothecia (i.e., the structure containing the sexual spores) of the fungus will form within the cottony masses of mycelium and conidia. The symptoms observed with powdery mildew infection include light green to yellow spots on the abaxial leaf position from the adaxial infection site of the fungus. As the pustules on the adaxial side mature, a yellow to brown or even reddish brown ring of necrotic tissue will appear. Under severe epidemics, the entire spikes of plants can be infected with powdery mildew in

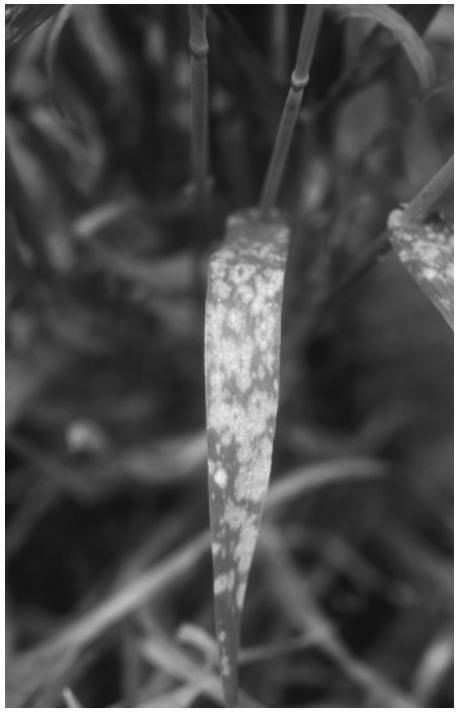

Fig. 11.8. Powdery mildew infection on barley in the field. For color details, please see color plate section.

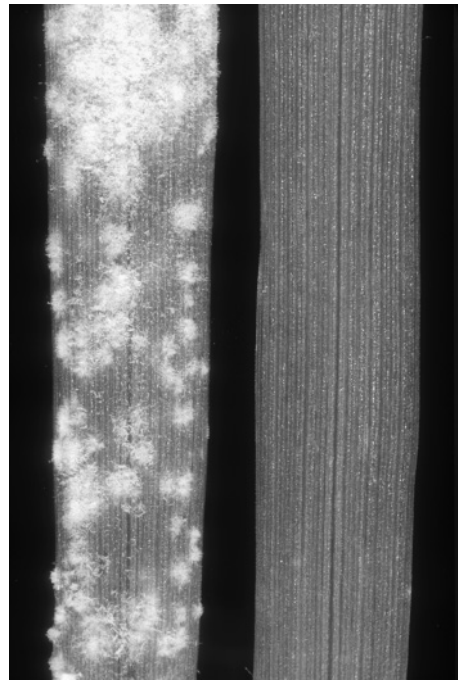

Fig. 11.9. Powdery mildew infection on seedling leaves of a susceptible (left) and highly resistant (right) barley cultivar from the greenhouse. For color details, please see color plate section.

addition to the leaves and leaf sheaths. Barley plants with resistance to powdery mildew will exhibit light to dark brown necrotic lesions on leaves that can, in some cases, form concentric rings.

Powdery mildew is caused by the ascomycetous fungus *Blumeria graminis* f. sp. *hordei* (formerly *Erysiphe graminis* f. sp. *hordei*), which has *Oidium monilioides* as the anamorph stage (Mathre 1997; Braun et al. 2002). The pathogen is unique in that it can infect barley without the presence of free moisture. That is why it is a scourge for researchers growing barley in the greenhouse. *B. graminis* has been divided into several *formae speciales* based on host range. While *B. graminis* f. sp. *hordei* primarily attacks cultivated and wild barley (Mathre 1997), other grass species in different genera may also be infected. Thus, the strict host range separations of *formae speciales* may not always apply. The barley powdery mildew pathogen is composed of many races and their population dynamics can rapidly change to overcome resistance genes in the host (Brown 1994; Wolfe and McDermott 1994). Mutation and genetic recombination via the sexual stage are the main factors contributing to new virulence types of the pathogen.

In the intensive barley production regions of northern Europe where both spring and winter barley are cultivated, mycelium and conidia of *B. graminis* f. sp. *hordei* can survive year round on green hosts (Wolfe and McDermott 1994). Conidia produced on these hosts can be easily disseminated by wind to infect newly sown crops or volunteer barley. In regions that are semiarid or have severe winters, the drought- and cold-tolerant cleistothecia are important in the epidemiology of the disease. Cleistothecia serve as a survival stage for the pathogen and produce sexually derived ascospores, which are discharged from asci to infect the barley host. Once infection

is initiated on barley foliage, secondary cycles of infection occur by conidia. Conidia may be produced in massive numbers from heavily infected crops. Although they are more sensitive to environmental conditions than ascospores, conidia can be disseminated over hundreds of miles to infect crops and are the most important propagule in the epidemiology of the disease (Wolfe and McDermott 1994).

For many years, fungicides have been used to control powdery mildew of barley in Europe and in other regions. Several effective compounds are known, but sometimes the development of fungicide-resistant isolates of *B. graminis* f. sp. *hordei* (Brown 2002) can negate the effectiveness of this strategy. Cultural control practices of crop rotation, destruction of crop residue harboring cleistothecia, and elimination of green bridge hosts, be they volunteer plants or off-season crops, can reduce the incidence and severity of powdery mildew. However, if significant exogenous inoculum arrives, these cultural control strategies will not halt the development of epidemics.

The use of resistant cultivars can be an effective strategy for controlling powdery mildew. In this regard, many different alleles for powdery mildew resistance have been identified in both cultivated (Jørgensen 1994) and wild barley (Dreiseitl and Dinoor 2004). However, the effectiveness of many resistance alleles deployed in agriculture has been short-lived due to the appearance of new virulence types. A notable exception has been the recessive allele of *mlo*, which has been bred into many barley cultivars and has remained effective for over 20 years to virulence types of *B. graminis* f. sp. *hordei* in Europe (Collins et al. 2002). Cultivar mixtures have been used as an alternative strategy to the deployment of pure line cultivars carrying one or a few major resistance genes. This diversification scheme has proven to be effective in reducing powdery mildew epidemics in some regions of Europe (Wolfe and McDermott 1994).

Net blotch

Net blotch is an important and common disease in nearly every barley production region of the world. The disease is named for the characteristic cross-hatch pattern of dark brown striations that develop within lesions (Shipton et al. 1973). A disease with more elliptical lesions and no netting pattern was reported to be widespread in Denmark in the late 1960s. The causal agent of this disease was morphologically identical to the net blotch pathogen *P. teres* but consistently caused this different symptom type. Smedegård-Petersen (1976) therefore designated two forms of *P. teres* based on symptom type: *P. teres* f. *teres*, the causal organism of net-form (or net-type) net blotch with the classical net-type lesions and *P. teres* f. *maculata*, the causal agent of spot-form (or spot-type) net blotch with the elliptical lesions. Both diseases have increased in many areas due to the common practice of minimum or no-till cropping, which leaves more inoculum available for initiating epidemics from crop residue. Generally, only one of the two forms of net blotch will predominate in a given area, and this is due to the cultivars grown and also perhaps due to management practices and environment. In Australia, Canada, France, and South Africa, spot-form net blotch has become the predominant net blotch disease (McLean et al. 2009). In Australia, yield losses up to 44% were reported due to spot-form net blotch with the quality factors of kernel plumpness and weight being markedly reduced (McLean et al. 2009). For net-form net blotch, losses up to 40% have been reported, but occasionally, complete crop failures can occur with susceptible cultivars under severe epidemics (Mathre 1997). Kernel plumpness and malt extract are two important malting quality traits that can be adversely affected by this disease.

P. teres is considered a hemibiotrophic fungus, growing initially in living cells and later ramifying through dead tissue. With successful infections, a primary vesicle develops within an epidermal cell. Hyphae breach the epidermal cell wall and then ramify intercellularly in the mesophyll cells (Keon and Hargreaves 1983). Net blotch is chiefly a disease of the leaves of barley plants, although leaf sheaths, stems, and kernels can also be infected (Mathre 1997; McLean et al. 2009). Initial symptoms of net-form net blotch on adult plants begin as small pinpoint to elliptical brown spots, which

Fig. 11.10. Net-form net blotch infection on barley in the field. For color details, please see color plate section.

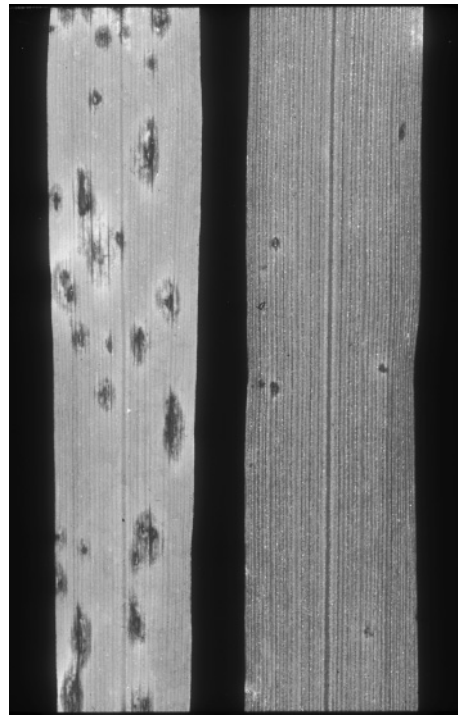

Fig. 11.11. Spot-form net blotch lesions on seedling leaves of a susceptible (left) and resistant (right) barley cultivar from the greenhouse. For color details, please see color plate section.

expand to form narrow, tan to light brown lesions, often delimited by leaf veins. Within these lesions, dark brown longitudinal and transverse striations appear, forming a netlike pattern (Fig. 11.10). Chlorosis may surround the necrotic lesions in susceptible genotypes. On infected seedlings and occasionally on adult plants in the field, initial symptoms may appear as greenish gray, water-soaked areas. Thereafter, these lesions will turn brown and netted striations will appear (Mathre 1997).

The initial symptoms of spot-form net blotch are very similar to those of net-form net blotch (McLean et al. 2009). On susceptible genotypes, advanced spot-form net blotch symptoms consist of dark brown, circular to elliptical lesions surrounded by bands of chlorosis. Similar symptoms may appear on seedling plants that have been artificially inoculated in the greenhouse (Fig. 11.11). These spot-form lesions can be readily distinguished from the classical netted lesions of net-type net blotch but may resemble those produced by the spot blotch pathogen *Cochliobolus sativus* (Fig. 11.12). Conidia of the two respective anamorph genera of *Drechslera* and *Bipolaris* are distinctly different in their morphology; thus, to confirm the identity of the causal pathogen, one must plate out the infected tissue and observe the characteristics of resulting conidia.

The symptoms produced by both forms of *P. teres* can vary greatly depending on the isolate of the pathogen, genotype and growth stage of the host, and environment (Kiesling 1985; McLean et al. 2009). In particular, reticulate lesions may not always be produced by *P. teres* f. *teres*. Instead, lesions may be uniformly brown without cross-hatched striations. Additionally, on resistant barley genotypes, *P. teres* may produce smaller, more restricted lesions with little or no chlorosis. When infection levels are very high, *P. teres*

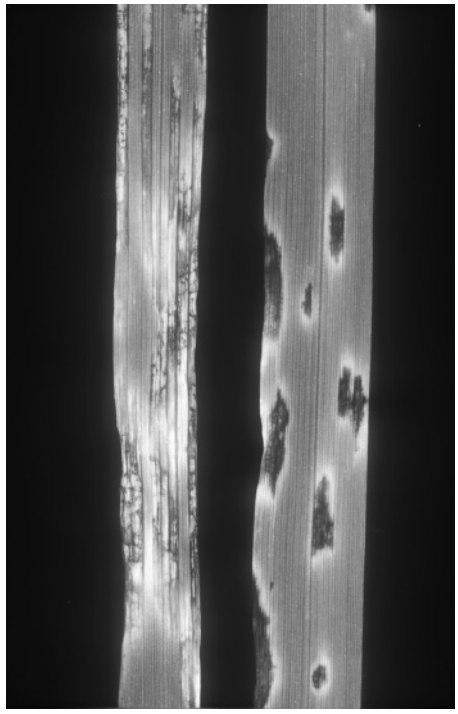

Fig. 11.12. Comparison of net-form net blotch (left) and spot blotch (right) lesions on barley seedling leaves from the greenhouse. Net-form net blotch has lesions with dark brown longitudinal and transverse striations, whereas spot blotch has uniform chocolate brown lesions. For color details, please see color plate section.

lesions will coalesce and kill the entire leaf. On kernels, *P. teres* f. *teres* produces dark to diffuse pale lesions (Shipton et al. 1973), although occasionally, the classic reticulate lesions may be produced (Mathre 1997).

Net blotch is caused by the ascomycetous fungus *P. teres*, which has the asexual stage of *Drechslera teres*. As mentioned above, two morphologically indistinguishable forms of the pathogen have been described (Smedegård-Petersen 1976): *P. teres* f. *teres*, the causal agent of net-form net blotch, and *P. teres* f. *maculata*, the causal agent of spot-form net blotch. The pathogens can be distinguished based on a particular primer set using the polymerase chain reaction (PCR) (Williams et al. 2001) and also the symptoms they cause on barley. The two forms of *P. teres* will hybridize in the laboratory upon pairing of isolates with compatible mating types, and symptom expression is governed by two independently segregating genes (Smedegård-Petersen 1976). Sequence analysis of mating-type genes in populations of *P. teres* f. *teres* and *P. teres* f. *maculata* in Sardinia revealed strong divergence and long genetic separation of the two forms. Rau et al. (2007) suggested that hybridization was therefore rare or absent between *P. teres* f. *teres*, and *P. teres* f. *maculata* in the field. In contrast, Campbell et al. (2002) studied genetic variation based on random amplified polymorphic DNA (RAPD) markers in populations of *P. teres* f. *teres*, and *P. teres* f. *maculata* in South Africa and also in hybrid progeny produced in the laboratory. The detection of an isolate from the *P. teres* f. *teres* population that carried a DNA band unique to *P. teres* f. *maculata* isolates and the closer clustering of this isolate to hybrid progeny suggested the possible hybridization of the two forms in the field. Additional investigations should be made to determine whether hybrids between the two forms are present in different regions.

In studies of different populations of *P. teres* f. *teres* and *P. teres* f. *maculata*, variation for virulence and also sensitivity to fungicides has been described (Steffenson and Webster 1992; Campbell and Crous 2002; Arabi et al. 2003; Wu et al. 2003). Virulence in the pathogen is governed, in some cases, by simple Mendelian inheritance and may operate on a gene-for-gene basis with resistance genes in barley (Weiland et al. 1999; Beattie et al. 2007; Lai et al. 2007). Toxins can be important virulence factors in many plant pathogenic fungi. The necrotic and chlorotic symptoms of both *P. teres* forms are caused by proteinaceous compounds and phytotoxic low-molecular-weight compounds, respectively (Sarpeleh et al. 2007).

Net-form net blotch epidemics can be initiated through seedborne inoculum of *P. teres* f. *teres* (Jordan 1981). It is not known whether *P. teres* f. *maculata* can also survive on seed to infect the crop in the next season (McLean et al. 2009), but there is no compelling reason why it could not given its close biological relationship to *P. teres* f. *teres*. Infection of seedlings from seedborne inoculum occurs most often under cool weather conditions and is a means for introducing the disease

into new areas (Mathre 1997). However, the most important source of primary inoculum for net blotch comes from infected host residue. The fungus can overseason as mycelium and pseudothecia on host stubble and then produce conidia and/or ascospores that can infect the next season's crop. In regions where the environment is not conducive to the production of the *Pyrenophora* perfect stage, conidia are the most important source of primary inoculum for initiating epidemics. Moreover, infected volunteer plants of barley or wild *Hordeum* species, which occasionally survive between cropping seasons, may also serve as sources of primary inoculum for newly sown crops. The net-form net blotch pathogen can attack a number of different grasses (Shipton et al. 1973; Brown et al. 1993), but wild *Hordeum* species are the most congenial to infection and therefore are most likely to play a role in the epidemiology of the disease (Brown et al. 1993; Khasanov et al. 1993). Little is known about the host range of the spot-form net blotch pathogen and which hosts might be important in the epidemiology. Once infection is initiated in a crop, conidia provide secondary inoculum for repeating infections during the season. Infection by *P. teres* conidia is favored by moist periods lasting 10 or more hours and temperatures in the range of 15–25°C.

P. teres can survive in the seed to infect young plants in the next season, although this occurs more frequently in cool, moist environments (Jordan 1981). To eliminate this possible inoculum source, it is important to use pathogen-free or fungicide-treated seed. These measures will also prevent the inadvertent introduction of *P. teres* isolates into areas where they do not exist. *P. teres* is not a soil inhabitant; therefore, it survives mostly in crop debris—sometimes up to two seasons after cropping (Duczek et al. 1999). Conventional tillage, burning, and crop rotation with nonhost crops can reduce the amount of viable inoculum surviving in host debris (Jordan and Allen 1984); however, the effectiveness of the treatments may vary across regions and environments (Turkington et al. 2006). Different classes of fungicides can effectively control net blotch and are used routinely with success in some regions (Jayasena et al. 2002). Caution must be taken regarding the overuse of fungicides since resistance to several compounds has been reported in the pathogen (Campbell and Crous 2002).

Resistant cultivars are the most cost-effective and environmentally benign means of controlling net blotch. Many sources of resistance have been reported from both cultivated and wild barley germplasm (Bockelman et al. 1988; Pickering 1992; Fetch et al. 2003; Bonman et al. 2005). Genetic studies have revealed both qualitative and quantitative types of resistance in barley (Mathre 1997). Major effect genes and QTLs conferring resistance to *P. teres* f. *teres* and also *P. teres* f. *maculata* have been mapped, and closely linked molecular markers have been identified for use in marker-assisted selection (Steffenson et al. 1996; Cakir et al. 2003; Emebiri et al. 2005; Manninen et al. 2006; Lehmensiek et al. 2007; Grewal et al. 2008). Barley cultivars should be bred with diverse sources of resistance because the evolutionary potential of the fungus is high given its ability to undergo sexual recombination in some regions. Cultivar mixtures have been utilized experimentally for the control of leaf diseases. Mixtures of moderately resistant and susceptible lines markedly reduced the severity of several leaf diseases, including net blotch (Mundt et al. 1994; Gacek and Nadziak 2000). This means of diversification could be used where the specifications on uniformity of barley cultivars is not as rigid.

Spot blotch

Spot blotch is a serious foliar disease and occurs in most barley production regions of the world. Epidemics have been reported in North America (United States and Canada), Australia (Queensland), South America (Uruguay and Brazil), Africa (Tanzania), Asia (India and Thailand), Middle East (Syria), and Europe (Poland, Scotland, and Sweden). The disease can be particularly severe in the warm, humid environments of the subtropics where barley is now being cultivated. Yield losses ranging from 10% to 30% are common in susceptible barley cultivars (Fetch and Steffenson 1994) but can greatly

exceed the 30% level under highly favorable environments. In addition to yield loss, kernel size and weight can be reduced by spot blotch infection (Mathre 1997).

C. sativus is a hemibiotrophic pathogen. It displays an early biotrophic phase when the hyphae penetrate the cuticle and cell wall and begin to grow inside living epidermal cells. Subsequently, hyphae will ramify in dead epidermal and mesophyll cells as the pathogen assumes its necrotrophic phase (Kumar et al. 2002). Spot blotch occurs primarily on the leaves and leaf sheaths of barley. As with many diseases, symptoms may vary due to the pathogen isolate, host genotype and ontogenetic stage, and environment. Fetch and Steffenson (1999) described in detail the range of spot blotch symptoms occurring on both seedling and adult plants with different levels of resistance as part of an effort to develop rating scales for the disease. On adult plants, typical early symptoms occurring on susceptible host genotypes include brown pinpoint lesions surrounded by faint chlorosis. Later, oblong or fusiform brown to dark brown lesions that are often bordered by chlorosis will form (Fig. 11.13). Spot blotch lesions tend to be somewhat restricted in their width by the leaf veins; however, they can enlarge width-wise and merge with others to form large blotches across the leaf. When leaves are heavily infected, they will desiccate rapidly and die. Pathogen signs of conidia and conidiophores can sometimes be observed in older lesions and appear as minute "dusty specks" visible to the naked eye. Early symptoms on susceptible seedling plants are very similar to those found on adult plants; however, older lesions are usually more rounded in shape and are chocolate brown in color (Fig. 11.12, right side). Sometimes the necrotic lesions may have a tan instead of a chocolate brown center, be diffuse instead of solid, or be surrounded by a distinct chlorotic halo (Fetch and Steffenson 1999). If moist conditions prevail late into the season, infections can occur on the kernels. This phase of the disease is known as black point or kernel blight (Mathre 1997). Typical symptoms of black point include dark brown lesions that occur chiefly on the basal end of the kernels.

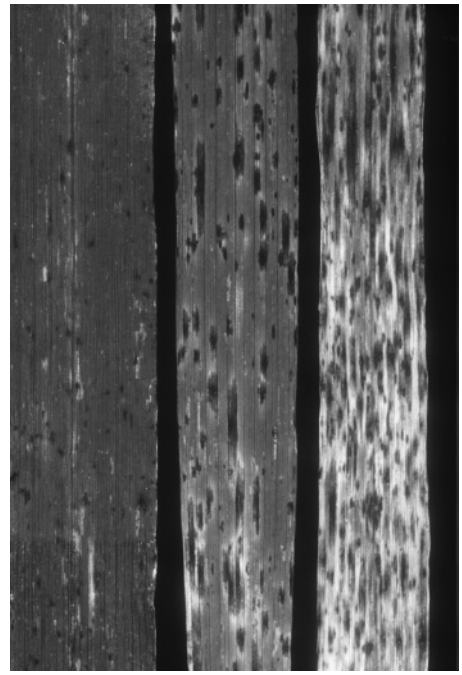

Fig. 11.13. Spot blotch lesions on a resistant, moderately resistant, and susceptible barley line collected from the field. For color details, please see color plate section.

Spot blotch is caused by the ascomycetous fungus *C. sativus*, which is the perfect stage of the anamorph *Bipolaris sorokiniana*. It is one of four barley diseases caused by *C. sativus*, the other three being common root rot/crown rot (see section on soilborne pathogens and root diseases), seedling blight, and black point or kernel blight (Tinline 1988; Mathre 1997). The *Cochliobolus* perfect stage is extremely rare in nature, yet in the laboratory, it can be readily produced when isolates with compatible mating types are paired (Kiesling 1985; Valjavec-Gratian and Steffenson 1997; Kumar et al. 2002). *C. sativus* is capable of surviving not only in seed and host debris but also in soil. Its ability to live in the soil as thick-walled conidia is unique among several other common foliar pathogens attacking barley. Sesquiterpenoid toxins produced by *C. sativus* have been implicated in pathogenesis on barley (Kumar et al. 2002). Isolates of *C. sativus* can

vary greatly for many different characters (Tinline 1988). With regard to virulence, some isolates exhibit distinct differential infection responses on barley (Fetch and Steffenson 1994) and may operate on a gene-for-gene basis with certain resistance genes (Valjavec-Gratian and Steffenson 1997). However, Ghazvini and Tekauz (2007, 2008) have shown that pathogen isolate–host genotype interactions can be better characterized on a quantitative basis as isolates may vary continuously for their disease-causing ability or aggressiveness. Karyotyping of *C. sativus* isolates using contour-clamped homogeneous electric field electrophoresis revealed a high level of chromosome-length polymorphisms, with chromosomal rearrangements detected in several isolates (Zhong and Steffenson 2007).

The spot blotch pathogen can survive in seed, host debris, and also the soil as conidia, chlamydospores, and/or mycelium (Kiesling 1985). Seedborne inoculum can initiate early epidemics and the movement of isolates into new regions. Soilborne inoculum is very important for common root rot and crown rot infection. If the infected portion of the crown emerges above the soil surface, the fungus can sporulate, providing inoculum for initiating spot blotch infections. Because *C. sativus* can live in the soil, there can be a long-lived reservoir of inoculum not only for root and crown infection but also foliar infections as well. Viable conidia of *C. sativus* may remain dormant in the soil until they are stimulated to germinate through exogenous substances from plants (Tinline 1988). Inoculum residing in infected host debris is most important for initiating spot blotch epidemics. Conidia of the pathogen forming on host debris can be readily dispersed by wind to infect barley and other hosts. The spot blotch pathogen has an extremely wide host range that even includes dicotyledenous hosts (Tinline 1988). These alternative hosts also may provide a source of primary inoculum. Once infection is initiated on the foliage of barley, multiple cycles of secondary infection occur via conidia.

To effectively manage spot blotch, inoculum residing in seed, alternative hosts, soil, and infected plant debris must be reduced or eliminated. Seedborne inoculum can be eliminated through clean seed production or by treatment with fungicides. There are many cultivated and weedy alternative hosts for *C. sativus*. If they reside close to barley fields and contribute significantly to epidemics, they should be eliminated if possible. The *C. sativus* inoculum residing in soil is difficult to eliminate, but it may not be as important for initiating spot blotch epidemics as infected host debris. Crop rotation with nonsusceptible hosts can reduce this latter source of primary inoculum as can various tillage practices that either bury or facilitate the breakdown of the residue.

The application of fungicides can effectively control spot blotch, but in some cases, two sprays may be required. This is a significant input cost for growers and may not be economically practical. Host resistance is the best strategy for controlling spot blotch in barley. Various sources of spot blotch resistance have been described in both cultivated and wild barley (Fetch et al. 2003, 2008; Arabi 2005; Bilgic et al. 2006), but the one that has provided widely effective resistance for many years in commercial six-rowed malting cultivars is line NDB112 (Steffenson et al. 1996). The NDB112 resistance has protected six-rowed malting barley cultivars in the United States and Canada from spot blotch damage since the mid-1960s. The durable resistance contributed by NDB112 is controlled chiefly by a major effect QTL on chromosome 1H (Steffenson et al. 1996). The expression of this and other QTLs can vary markedly depending on the genetic background of segregating populations (Bilgic et al. 2005). Isolates of *C. sativus* with virulence for cultivars carrying the NDB112 resistance were recently described from Canada (Ghazvini and Tekauz 2007). Moreover, the resistance in these cultivars is not effective against pathogen isolates under the environmental conditions of some subtropical regions (Gilchrist et al. 1995). Thus, it is important that new sources of resistance be utilized to protect barley against these and other new virulence types in the pathogen population. Several investigations have been made to assess the relationship between the reaction to spot blotch and the reaction to common root rot in barley. In some studies, a strong correlation was found for

reaction to the two diseases, and in other cases, no strong link was found (Gustafsson 1993; Almgren et al. 1999; Arabi et al. 2006).

Ramularia leaf spot (RLS)

RLS has become an important disease problem of barley in central and northern Europe since the 1980s. It was first described as a disease of barley more than a century ago but was not recognized as a serious production problem until the early 1980s, initially in Austria (Walters et al. 2008). Within Europe, the disease has been reported on barley from Scotland, Britain, Denmark, and Lithuania in the north down through Switzerland, Austria, and Hungary in the south (Frei and Gindrat 2000; Leistrumaite and Liatukas 2006; Manninger et al. 2008; Walters et al. 2008). It also was reported in New Zealand (Harvey 2002), Uruguay, and Argentina (Huss 2004). The reasons for RLS suddenly becoming an important disease of barley in many areas is not understood. Yield losses ranging from 20% to 35% have been reported in barley due to RLS infection (Pinnschmidt and Jørgensen 2009). Even low disease levels can result in a disproportionately high loss of yield (Walters et al. 2008). RLS can be difficult to diagnose because it resembles abiotic stress symptoms and also symptoms of several other diseases (Sachs et al. 1998; Pinnschmidt and Hovmøller 2004; Walters et al. 2008). Additionally, the fungus is difficult to isolate (Walters et al. 2008). For these reasons, RLS may well exist in other production regions but has not been diagnosed.

RLS infection occurs mostly on the leaves and leaf sheaths, although the stems and awns also can be infected. Like most other fungal pathogens, conidia of *Ramularia collo-cygni* require moisture for germination and infection. Penetration of the host is through stomata (Stabentheiner et al. 2009) and occurs rapidly—within 1 day. Thereafter, the fungus grows intercellularly and colonizes the mesophyll tissue (Sutton and Waller 1988). The initial symptoms will appear 1–2 weeks after infection and are manifested as small brown to dark brown spots delimited by the leaf veins (Fig. 11.14). The lesions are usually dark brown in the center and are more pale brown toward the edges. Chlorotic halos often appear around lesions. With time and under heavy infection conditions, the lesions can coalesce and produce larger areas of discoloration, sometimes progressing over the entire lamina. Spot blotch and spot-form net blotch lesions can be distinguished from RLS lesions by their more spindle to ellipsoid shape (Pinnschmidt and Hovmøller 2004). Lesions caused by RLS are most likely to be confused with physiological leaf spots. RLS is generally considered a late-season disease even though symptoms can be found early in the season. The typical late onset of symptoms after heading may be triggered by the transition of barley to the reproductive phase or perhaps even light intensity (Walters et al. 2008). Recently, Schützendübel et al. (2008) studied physiological alterations across different growth stages of barley in relation to the onset of RLS. They found that neither inoculum nor environmental factors were

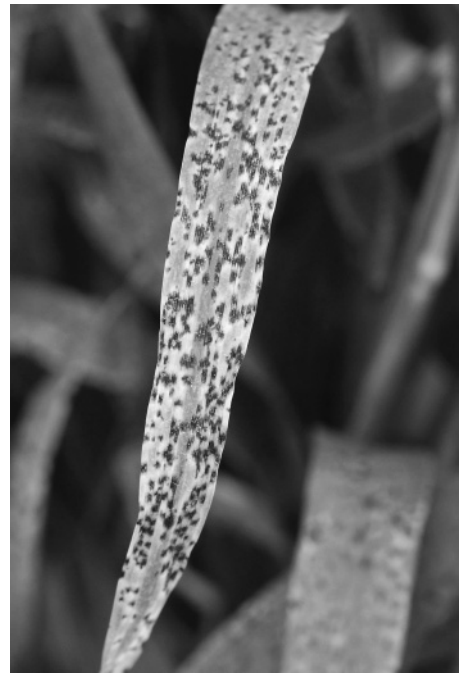

Fig. 11.14. Ramularia leaf spot on barley in the field (courtesy of Hans Pinnschmidt). For color details, please see color plate section.

limiting factors for the disease. Instead, they concluded that the typical late onset of RLS was due to the degradation of the antioxidative protection systems in barley leaves, which occurs at the onset of ripening.

R. collo-cygni is the causal agent of RLS. This fungus is very unique among *Ramularia* species because it has a curled conidiophore, much like the neck of a swan (Walters et al. 2008). The perfect stage of *R. collo-cygni* is thought to be a species in the genus *Mycosphaerella* based on molecular phylogenetic studies and the existence of *Asteromella*-like structures on barley leaves associated with *R. collo-cygni* (Salamati and Reitan 2006). The fungus sporulates only in necrotic tissue and mostly on the abaxial leaf surface. *R. collo-cygni* produces several anthraquinoid toxins called rubellins (Heiser et al. 2003). While not host specific (Miethbauer et al. 2003), these compounds may act as pathogenicity factors, according to Heiser et al. (2004).

R. collo-cygni is reported to survive as a saprophyte on dead leaves in the lower canopy and also possibly as a systemic invading pathogen (Salamati and Reitan 2006). The systemic growth of the pathogen was confirmed by a PCR-based detection system (Havis et al. 2006). The pathogen also may be transmitted in the seed. This contention has been corroborated from the PCR-based detection of *R. collo-cygni* in barley seed (Havis et al. 2006; Walters et al. 2008). Through infected seed, RLS could be introduced into many new barley production areas. In Switzerland, Frei et al. (2007) demonstrated that winter barley and volunteer plants can be an important source of inoculum for spring barley crops. Additionally, the primary inoculum of *R. collo-cygni* may also come from other infected cereal crops (such as oat, wheat, and rye) and the common weed of couch or quack grass (*Elytrigia repens*) and *Hordeum murinum* (Frei and Gindrat 2000; Huss 2004; Salamati and Reitan 2006).

An integrated strategy is the best option for managing RLS; however, one of the most important initial steps in combating the disease is getting an accurate diagnosis. This is especially critical since RLS lesions mimic other abiotic and biotic symptoms, and the causal agent *R. collo-cygni* is difficult to isolate. There are several actions that can be taken to reduce the amount of primary inoculum available for infecting barley. First, it is essential to obtain disease-free barley seed if this source of inoculum is significant in initiating epidemics (Walters et al. 2008). Second, since *R. collo-cygni* can survive as a saprophyte, every effort must be made to destroy the infected host debris of volunteer barley plants, other cereal crops, and weed species. This can be accomplished through crop rotation or plowing under infected residue. Management strategies also should include breaking the green bridge of congenial live hosts that contribute to the infection of spring barley, for example, winter barley or perennial weeds like *E. repens*. Several classes of fungicides have proven effective against RLS, but they must be applied at the correct time to be effective for this late-onset disease. Genetic diversity for reaction to RLS has been reported in both winter and spring barley cultivars (Pinnschmidt and Hovmøller 2004; Leistrumaite and Liatukas 2006; Manninger et al. 2008; Pinnschmidt and Sindberg 2009). Resistant cultivars are being recommended for growers in RLS-prone areas. Additionally, research is being advanced to breed cultivars with higher levels of resistance to RLS.

Scald

Scald is a common barley disease and can be found in most production areas of the world. It is particularly severe in the cool, moist areas of North America, Oceania, North Africa, Far East, Middle East, and South America. Scald is a serious problem in winter barley and in autumn-sown spring barley. Yield losses due to scald can be severe (up to 40%) and depend on the susceptibility of the host, the time of disease onset, and amount of inoculum (Shipton et al. 1974). However, losses under 10% are more common in barley. The yield component most affected by scald is kernel weight, but the number of kernels per spike and the number of spikes per plant also can be reduced by infection.

As with other foliar diseases, scald affects primarily the leaves and leaf sheaths of barley plants. Conidia landing on the leaf surface germinate to

form germ tubes and later appressoria. The pathogen then penetrates the cuticle of epidermal cells via a penetration peg. *Rhynchosporium secalis* can infect, ramify, and sporulate in leaves without showing visible symptoms (Zhan et al. 2008). The first symptoms that are manifested are water-soaked areas that are greenish gray to bluish gray in color. Then, the central portion of the lesion turns fully necrotic, changing color from greenish gray to steel gray and finally light tan or white (Fig. 11.15). Lesions are oval to oblong or fusiform in shape with distinct dark brown margins. In some cases, chlorotic areas may be present at the margins of the necrotic lesions. Lesions will expand across the leaf veins and coalesce under high infection levels. Infections near the auricles can stop the flow of nutrients, leading to the rapid death of entire leaves. When the weather conditions are highly conducive, the scald pathogen also can infect the floral bracts, awns, and pericarp. Lesions similar to those described on leaves can form on the upper part of glumes at the point of awn attachment, although in some cases, infected seed may be symptomless (Lee et al. 2001)

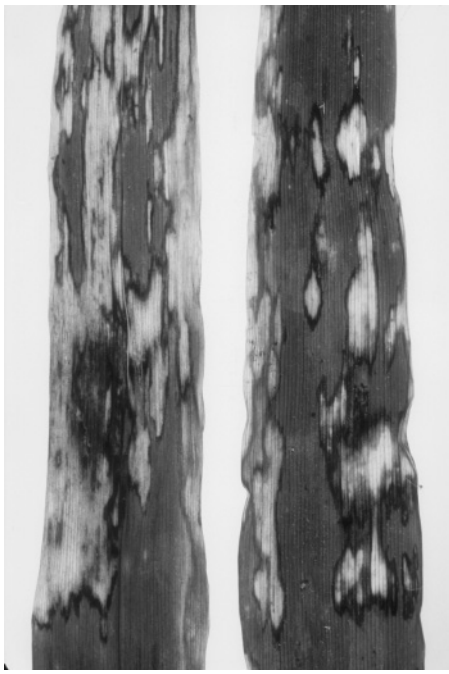

Fig. 11.15. Leaf scald infection on barley leaves collected from the field. For color details, please see color plate section.

Scald is caused by the fungus *R. secalis*. The perfect stage of the organism has not been found but is thought to exist based on the gametic equilibrium of many alleles at loci in *R. secalis* populations (Linde et al. 2003; Zaffarano et al. 2006; Zhan et al. 2008). After infection, the mycelium of *R. secalis* forms a stroma under the cuticle of leaves. Then, hyaline, "comma-shaped" conidia with two cells are formed on the stroma. *R. secalis* should be considered a hemibiotrophic pathogen given the fact that it grows for a time and also produces conidia on leaves before necrotic symptoms are visible (Zhan et al. 2008). The fungus sporulates only after wetting from rain or heavy dew (Mathre 1997). Rain episodes are essential for dissemination of conidia. *R. secalis* is a dynamic pathogen, and its populations can change rapidly in response to deployed resistance genes in the host and also exposure to different fungicides (Zhan et al. 2008). With regard to virulence diversity, many races of *R. secalis* have been described from various production regions of the world (Jackson and Webster 1976; Xi et al. 2003; Bouajila et al. 2007).

The scald pathogen can survive on infected host debris, seed, volunteer barley plants, and alternate grass hosts. Conidia produced from stroma on the surface of host debris are the most important source of primary inoculum for initiating epidemics (Shipton et al. 1974). The stroma, but apparently not conidia, are capable of surviving the cold winter extremes of temperate climates as well as the high summer temperatures of Mediterranean climates. Optimal conidia production occurs with free moisture and temperatures of 10–20°C (Shipton et al. 1974). The wind alone will not disperse conidia of *R. secalis* as is the case with a number of other common fungal pathogens of barley (e.g., *C. sativus*, *P. teres*, and *Puccinia* species). Instead, splashing rain, together with wind, is needed to effectively disseminate conidia beyond initial infection foci (Steffenson 1988). Small, scattered infection foci are commonly observed in fields during the first onset of scald due to the short dispersal distance of conidia. The

disease also can be spread when wetted conidia on infected plants contact adjacent healthy plants. The secondary spread of inoculum is also by conidia. Sporulation of *R. secalis* and subsequent disease spread is suppressed at temperatures above ~27°C. Infected seed also can serve as a source of primary inoculum, especially when cool, moist weather conditions prevail after planting. Hyphae present in the pericarp or hull of seeds can infect coleoptiles as they emerge from the embryo. The transmission rate from seedborne infections can be fairly high; thus, this source of inoculum can be particularly important in the absence of infected host residue (Mathre 1997). Infection and symptom development are markedly affected by the host genotype, pathogen isolate, and environment. Consequently, the importance of various alternative hosts in the epidemiology of scald is open for debate. Many grass hosts have been found naturally infected with *R. secalis* in the field, but there is evidence for isolate specialization to specific hosts based on cross-inoculation studies (Shipton et al. 1974). Nevertheless, various *Hordeum* species may be among the most important hosts for harboring *R. secalis* under natural field conditions.

Reducing infected host debris is the most important factor for lowering inoculum and disease levels in barley. This can be achieved through rotation with nonhost crops, plowing under debris, and burning debris. In areas where volunteer barley plants or alternative hosts play a role in the epidemiology of the disease, efforts should be made to eliminate them, especially in nearby headlands, given the limited dispersal distance of *R. secalis* conidia. Clean or fungicide-treated seed also should be used if infection rates are high in seedlots and the conditions are conducive for transmission. Foliar fungicide applications have been used with success against scald, but the increase of *R. secalis* isolates with resistance to some compounds has limited the effectiveness of this strategy (Locke and Phillips 1995; Zhan et al. 2008). Mixing fungicides with different modes of action may reduce selection of resistant *R. secalis* isolates. Host resistance is the most commonly used control strategy for scald. Many major effect genes and also QTLs for scald resistance have been reported in both cultivated and wild barley accessions (Shipton et al. 1974; Abbott et al. 1992; Garvin et al. 1997; Jensen et al. 2002; Cheong et al. 2006; Zhan et al. 2008). As mentioned previously, *R. secalis* is composed of many different virulence types that can rapidly put asunder cultivars carrying single resistance genes. The pyramiding of two or more scald resistance genes in cultivars may provide higher and more durable resistance (Brown et al. 1996). In many but not all studies, the deployment of cultivar mixtures reduced scald severity and increased yield in barley (Zhan et al. 2008). It is clear from all of the summarized results that the management of scald in barley can be best achieved using an integrated approach.

Septoria speckled leaf blotch (SSLB)

SSLB caused by *Septoria passerinii* can be found in many areas of the world where barley is grown including North America, North Africa, Europe, and Australia (Mathre 1997). Due to the pathogen's specific requirements of rain and extended moist periods for dispersal and subsequent infection, respectively, epidemics can be sporadic (Green and Dickson 1957; Toubia-Rahme and Steffenson 2004). Severe epidemics of SSLB occurred during the 1950s in the north-central region of the United States and in the Prairie Provinces of Canada (Mathre 1997), causing an estimated 20% yield reduction (Green and Bendelow 1961). The disease was of little consequence again until 1993 when it reemerged in epidemic proportions due to cool, wet weather conditions and a buildup of inoculum (Toubia-Rahme and Steffenson 2004). In more recent experimental trials, yield losses up to 38% were reported in barley due to *S. passerinii* infection in North Dakota (Toubia-Rahme and Steffenson 1999). SSLB can adversely affect the malting quality traits of kernel plumpness and malt extract (Green and Bendelow 1961). In addition to *S. passerinii*, *Stagonospora avenae* f. sp. *triticea* also can cause SSLB on barley, although it is a more important pathogen of wheat (Cunfer 2000).

SSLB pathogens primarily infect the leaves and leaf sheaths of plants. Early symptoms caused

by *S. passerinii* on seedlings include faint, yellowish areas that first turn greenish gray and then straw colored with time, forming a distinct demarcation next to healthy green tissue. Interspersed black pycnidia of the pathogen will often form inside mature lesions. The two SSLB pathogens produce different lesion types on adult plants in the field. *S. passerinii* generally produces long, rectangular lesions that are delimited by the leaf veins. These lesions first appear grayish in color and then turn tan or straw colored (Fig. 11.16). Heavily infected leaf sheaths often turn grayish. In contrast, *S. avenae* f. sp. *triticea* produces yellow brown lesions that traverse the leaf veins and are more shield or lens shaped. Under high infection levels, the lesions produced by both pathogens will coalesce and can kill the entire leaf or leaf sheath. Numerous pycnidia will develop in senescent leaf and leaf sheath tissue (Cunfer 2000) (Fig. 11.17). Severe SSLB infection will greatly weaken the plant leading to straw breakage, a common phenomenon associated with the disease. Infection by both SSLB pathogens is often greater at the necrotic sites surrounding rust uredinia.

Fig. 11.17. Pycnidia of *Septoria passerinii* forming in lesions of an infected leaf in the field. For color details, please see color plate section.

Fig. 11.16. Severe Septoria speckled leaf blotch infection caused by *Septoria passerinii* on barley in the field. For color details, please see color plate section.

S. passerinii and *S. avenae* f. sp. *triticea* (teleomorph: *Phaeosphaeria avenaria* f. sp. *triticea*) are the causal organisms of SSLB on barley. The perfect stage of *S. passerinii* is purported to be in the genus *Mycosphaerella* based on the DNA sequence similarity of the internal transcribed spacer region (Goodwin and Zismann 2001). In a subsequent study, Ware et al. (2007) coinoculated *S. passerinii* isolates of opposite mating types onto barley and subsequently trapped ascospores being discharged from the leaves. The ascospores proved to be putative hybrids of the isolates based on molecular marker analysis and were pathogenic on barley. Moreover, the ascospores resembled those of *Mycosphaerella*. Although well-preserved teleomorph material (i.e., ascoma)

could not be described, all evidence indicates that *Mycosphaerella* is the perfect stage of *S. passerinii*. Using amplified fragment length polymorphic (AFLP) markers, Lee and Neate (2007b) investigated the genetic structure of *S. passerinii* populations in North Dakota and Minnesota. The population structure found was consistent with a sexually reproducing fungus. Additional investigations should be made to identify the perfect stage of this fungus in this region. In North Dakota, both SSLB pathogens are present; however, *S. passerinii* is more commonly isolated from barley than *S. avenae* f. sp. *triticea* (Krupinsky and Steffenson 1999).

S. passerinii can survive for one or more seasons on infected host debris, at or just below the soil surface. Rain-splashed pycnidiospores provide the primary inoculum (Mathre 1997); thus, spore dispersal is mostly local (Cunfer 2000). The infection and incubation periods for *S. passerinii* are much longer than for other common foliar pathogens of barley (Green and Dickson 1957); nevertheless, under ideal weather conditions, SSLB can gain an early foothold and become the predominant disease infecting barley. In the north-central region of the United States and into Canada, pycnidia usually develop late in the season, so SSLB is essentially a single-cycle disease. The host range of *S. passerinii* is limited mostly to barley and a few wild *Hordeum* species such as *Hordeum jubatum*, but there is evidence that isolates from the latter may not be pathogenic on the former (Cunfer 2000). *S. avenae* f. sp. *triticea* has a wider host range than *S. passerinii*, attacking both barley and wheat as well as *H. jubatum* and other grasses. *H. jubatum* is considered an important source of inoculum for this pathogen and is very common in the north-central region of the United States and adjacent parts of Canada. In addition to pycnidiospores, ascospores are also a source of primary inoculum for *S. avenae* f. sp. *triticea* (Cunfer 2000).

The outbreaks of SSLB in North America in the 1990s occurred after a buildup of inoculum in crop residue due to the wide use of minimum tillage practices and also the onset of rainy weather. Thus, cultural practices that reduce infected crop residue (e.g., plowing and crop rotation) will lessen the severity of SSLB infection. Krupinsky et al. (2004) found that the risk for barley leaf spot diseases, including SSLB, was lower in rotations with wheat, crambe, canola, and dry pea than with barley after barley. Several fungicides have shown very good efficacy in controlling SSLB; however, host resistance is still the best means of controlling the disease. Sources of resistance to *S. passerinii* are legion in both cultivated and wild barley germplasm (Legge et al. 1996; Fetch et al. 2003; Toubia-Rahme et al. 2003; Toubia-Rahme and Steffenson 2004; Yun et al. 2005). A number of major genes from these resistance sources have been identified, mapped, and/or introgressed into breeding lines using marker-assisted selection (Rasmusson and Rogers 1963; Steffenson and Smith 2006; Zhong et al. 2006; Lee and Neate 2007a; St. Pierre et al. 2010). These genes have only been challenged against a limited number of *S. passerinii* isolates; thus, it is not known whether they are effective against the spectrum of virulence types that might exist in the pathogen population. This is an important factor to consider given that *S. passerinii* may have a functional sexual stage, capable of generating new virulence types (Lee and Neate 2007b). Moreover, no data are available on whether the genes conferring resistance to *S. passerinii* are also effective against *S. avenae* f. sp. *triticea*. Since *S. passerinii* and *S. avenae* f. sp. *triticea* are prevalent in some production areas (Krupinsky and Steffenson 1999), it is important to breed cultivars with resistance to both pathogens (Toubia-Rahme and Steffenson 1999). Cultivars with resistance to *S. passerinii* have been released for the north-central and mid-Atlantic regions of the United States as well as Canada.

Barley yellow dwarf

Barley yellow dwarf is the most widely distributed and important virus disease of barley and other small grain cereal crops worldwide. It is found in virtually every production area of the world and has caused epidemics in most of them. Losses to this disease can approach 100% if infection of the crop occurs early in the season (Mathre 1997). In the north-central region of the United

States, yield losses ranging from 8% to 38% were reported in three malting barley cultivars after artificial inoculation with barley yellow dwarf virus (BYDV) (Edwards et al. 2001). In addition to yield, the disease also reduced kernel weight, kernel plumpness, and malt extract.

BYDVs are obligately transmitted by several primary aphid vectors (see below). Aphids first acquire the viruses by feeding on infected plants. Acquisition access periods as short as 15 min are sufficient for the aphids to acquire the viruses; however, the efficiency of transmission is increased with longer access periods of 1–2 days. After the acquisition of the virus, a latent period extending from hours to days may be required before the aphid can transmit the virus to other plants. BYDVs can be transmitted to other plants by their aphid vectors with relatively short inoculation periods from 15 to 60 min (Power and Gray 1995; Mathre 1997). Aphid vectors introduce BYDVs into the phloem of plants via their saliva when feeding. Thereafter, the viruses move systemically in the plant. Barley yellow dwarf symptoms can vary due to factors of the host (e.g., genotype, age, and physiological condition), pathogen (strain and dose), and environment (light and temperature) (D'Arcy 1995). On barley, symptoms begin as uneven blotches of bright yellow discoloration on the tips and margins of older leaves. The yellow discoloration will then progress down toward the base, covering the entire leaf within a week or two (Fig. 11.18). Infected barley plants also will show dwarfing or stunting due to reduced internode elongation. When barley plants are severely infected at an early age, they may fail to head and remain in a rosette with leaves that are shortened and thickened with serrated margins. If spikes are produced from such plants, the florets are often completely sterile. Sometimes, the spikes of severely infected plants will turn black at physiological maturity due to colonization by saprophytic fungi.

The BYDVs are luteoviruses and consist of at least five different strains. These strains vary in their genome constitution and various biological properties. Initially, the designations given to BYDV strains were based on the efficiency of transmission by specific insect vectors. For example, the MAV strain was efficiently transmitted by *Macrosiphum* (=*Sitobion*) *avenae* (English grain aphid), RPV strain by *Rhopalosiphum padi* (bird-oat cherry aphid), SGV strain by *Schizaphis graminum* (greenbug), RMV strain by *Rhopalosiphum maidis* (corn leaf aphid), and PAV strain by *R. padi* and *S. avenae* (Lister and Ranieri 1995). It should be noted that not all BYDV strains follow these strict virus vector specificities. More recent taxonomic treatments placed the BYDVs into two subgroups based on nucleic acid sequences, serological properties, and cytopathology: subgroup 1 is composed of strains MAV, SGV, and PAV, and subgroup 2 of strains RPV and RMV (Mathre 1997). BYDVs are obligately transmitted by aphids in a persistent (circulative) manner but do not multiply in their vectors. They may persist in their aphid vectors for up to 3 weeks or possibly longer and also through successive aphid molts. However, viruliferous aphids cannot transmit BYDVs to their

Fig. 11.18. Barley yellow dwarf infection on barley in the field. For color details, please see color plate section.

progeny (Mathre 1997). BYDVs are limited to the phloem and systemically infect host plants via these conducting elements.

BYDVs have a very wide host range that includes more than 150 species in the Poaceae. This list includes all of the major cereal crops (i.e., barley, wheat, oat, rye, triticale, maize, and rice) as well as many lawn, pasture, range, and weedy grasses (D'Arcy 1995). Depending on the region, environment, and cropping practice, many different hosts can serve as reservoirs for the pathogens. BYDVs cannot be mechanically transmitted; thus, they depend entirely on their aphid vector for spread. In areas with mild winters, adult aphids can survive on winter cereals and various other hosts. If these aphids are viruliferous, they can immediately transmit the viruses to healthy plants. Aphids that are hatched from eggs must first acquire the pathogens by feeding on infected plants since BYDVs are not transovarially transmitted. These viruliferous aphids can, by their own flight, cause local epidemics or be carried by wind currents to initiate distant epidemics hundreds of miles away. The cool, moist weather that is favorable for barley growth is also conducive for aphid multiplication and migration. Once initial infections are established in a field, aphids can continue to spread BYDVs throughout the season (Mathre 1997).

As with most other diseases of barley, losses due to barley yellow dwarf can be minimized through an integrated management strategy. BYDVs have an extensive host range; therefore, to reduce the inoculum, grass hosts serving as a reservoir for the viruses must be eliminated. The date of sowing can have a marked affect on disease levels. For example, in the United Kingdom, autumn-sown crops should not be planted too early; otherwise, they will be exposed to the virus vector for a longer period of time and will sustain greater damage to barley yellow dwarf before the onset of winter. In the northern part of the United States and also in other countries where spring barley is grown, early spring sowing is recommended so that the crop can advance before viruliferous aphid migrants arrive and cause significant barley yellow dwarf damage. Systemic insecticides to control the aphid vector have been used with success in some regions. These pesticides are best applied early in crop development (i.e., prior to tillering) before virus infections can severely reduce yield. Host resistance and tolerance to barley yellow dwarf exists in barley (Burnett et al. 1995). Genetic studies have identified several genes for resistance/tolerance, but the one used most commonly in breeding is *Ryd2* or *Yd2*. This gene is not uniformly effective in all genetic backgrounds and shows differential effectiveness against various BYDV strains. Transformation of barley with the coat protein gene of BYDV is another promising strategy for the control of barley yellow dwarf (McGrath et al. 1997); however, no commercial cultivars have been developed using this technique as of this writing.

Bacterial blight

Bacterial blight can be found on barley in many parts of the world from the temperate zones with widely varying temperature and rainfall patterns to the subtropics where higher temperatures and rainfall prevail. The disease is especially severe on barley grown in high rainfall areas or under irrigation. Recent epidemics have occurred in the north-central region of the United States with some fields exhibiting up to 80% severity (B. Steffenson, unpublished data). Yield losses to bacterial blight have not been well-documented; however, based on the general severity of infection on flag leaves, grain weight losses of about 13% or more may occur (Shane et al. 1987).

Bacteria can enter plants via natural openings such as stomata and hydathodes, but also through wounds. Initial symptoms produced by bacterial blight on the leaves of susceptible barley plants include small, light to pale green spots, which later expand into linear streaks. These streaks will often extend the entire length of leaves and will lose their green color and appear translucent. Soon after, infected leaves will turn yellow and ultimately brown as cells die (Fig. 11.19). Under moist or high-humidity conditions, signs of the bacteria may be observed. They manifest themselves as viscous yellowish to cream-colored droplets, or if spread out and dried, as shiny,

Fig. 11.19. Bacterial blight infection on barley in the field. For color details, please see color plate section.

Fig. 11.20. Bacterial blight infection on barley in the field showing a droplet of bacterial exudate. For color details, please see color plate section.

shellacked exudate on the surface of infected leaves (Fig. 11.20). Bacterial blight occurs mostly on leaves, but glumes and awns may also be infected.

The nomenclature for the pathogens causing bacterial blight on small grain cereals has evolved over the past few decades with the addition of new molecular and biochemical data. In the past, the five pathovars causing the disease (e.g., pv. *cerealis*, *hordei*, *secalis*, *translucens*, and *undulosa*) have been classified together within the "translucens group" of *Xanthomonas campestris*, considered a single species (*Xanthomonas translucens*), or even a single pathovar (*X. translucens* pv. *translucens*) (Bragard et al. 1997). Vauterin et al. (1995) proposed the name of *X. translucens* and included in this taxon the pathovars of the "translucens group" as well as others attacking members of the Poaceae. In a study of 68 *X. translucens* sensu lato isolates derived from various members of the Poaceae, Bragard et al. (1997) identified four pathogenicity groups based on their disease-causing ability on barley and wheat. Those giving compatible reactions on wheat and barley were classified as type A, and those giving compatible reactions on barley only were classified as type B. The two types corresponded to *X. translucens* pv. *undulosa* and *X. translucens* pv. *translucens*, respectively. Other translucens group isolates are also capable of attacking barley. Thus, for many types of studies, it is important to identify the specific bacterial blight pathogen(s) attacking barley.

The bacterial blight pathogens can survive on the seed, residue, winter cereals (where present) as well as many alternative hosts in the Poaceae. They cannot survive, however, for a long time in the soil. Seedborne inoculum is probably the most important source of primary inoculum in many regions and can lead to the widespread distribution of the disease through experimental seed or grain exchange (Duveiller et al. 1997). Infected residue is also an important source of primary inoculum as the bacteria can survive on it for one or more seasons. The pathovars causing bacterial blight of barley have an extensive host range; thus, many hosts can serve as reservoirs of inoculum. Resident populations of bacterial blight pathogens may be present on the foliage

of cereals and other hosts before any symptoms are evident (Stromberg et al. 2000). This may be one reason why epidemics seem to appear suddenly across a wide region. Bacterial blight is often more severe in breeding or other experimental plots than in commercial fields (Duveiller et al. 1997). The most rapid and efficient spread of bacterial blight pathogens occurs during episodes of rain and windy weather. Plant-to-plant transfer by mechanical equipment or by humans and animals may also occur, especially when the host tissue is wet or water congested (Mathre 1997). Barley grown under sprinkler irrigation is particularly prone to bacterial blight due to splashing water and elevated humidity in the plant canopy.

The elimination of seedborne inoculum is a critical step for reducing bacterial blight of barley. This can be achieved by sowing the seed production crop in arid regions where either the pathogen does not exist or the environmental conditions are not conducive for infection. Seed treatments (both chemical and dry heat) can reduce inoculum in the seed, but they may not always be completely effective (Mathre 1997). Crop residue is another source of inoculum for the disease and can be reduced through rotations with nonhost crops and also plowing under or burning residue. Various grassy weeds can harbor the inoculum of *X. translucens* pathovars and should be eliminated from the headlands of barley crops if possible. Winter cereals, where present, also may serve as a reservoir for inoculum. Spring sown crops should therefore be separated spatially or temporally from infected winter cereals. Host resistance is another possible strategy for controlling bacterial blight in barley. Variation for reaction to bacterial blight has been observed in barley with some cultivars showing high levels of partial resistance (Alizadeh et al. 1994). Diallel analyses have identified lines with highly significant general and specific combining abilities for bacterial blight resistance. Moreover, QTLs conferring resistance to the disease also have been identified (El Attari et al. 1998). With a sustained effort, it should be possible to develop barley cultivars with good resistance to bacterial blight.

ROOT AND CROWN DISEASES OF BARLEY

Rhizoctonia root rot and bare patch

Barley is highly susceptible to rhizoctonia root rot and suffers more stunting and damage than wheat at similar inoculum levels. Aboveground symptoms include yellowing and purpling of the leaves and stunting, which can often occur in patches in the field, hence the names rhizoctonia patch, purple patch, bare patch, or barley stunt (Fig. 11.21). This disease has been reported in the United States (Weller et al. 1986), Australia (MacNish and Neate 1996), and Canada. Belowground, it causes distinct symptoms on the root that distinguish it from similar root rots such as *Pythium*. Root tips are brown, and crown root tips are rotted or tapered to a point, hence the name "spear tipping." (Fig. 11.22). Sections of the root cortex rot away, leaving the stele intact, and a constricted pinched appearance of the root. Under extreme conditions, where seedling growth is slowed by cool temperatures, *Rhizoctonia* can also cause damping-off.

A complex of *Rhizoctonia* species and groups can cause root rot. *Rhizoctonia solani* is a basidiomycete with a teleomorph (sexual stage) of *Thanatephorus cucumerinum*. *R. solani* is really a species complex that is divided into different groups called anastamosis groups (AGs), based on their ability to fuse hyphae or anastamose and exchange nuclei with the same group. *R. solani* AG-8 is most associated with bare patch and was discovered in the United States in the 1980s, but had been described in Australia in the early twentieth century. AG-4 and AG-5 have also been reported to cause rots. *R. solani* AG-8 has a wide host range, including cereals such as wheat and barley, but can also attack broadleaf (dicot) rotation crops such as pea (*Pisum sativum*), lentils (*Lens culinaris*), and chickpea (*Cicer arietinum*). *R. solani* forms characteristic hyphae that are fairly wide (>7 μm), multinucleate, with dolipore septa, and have a distinct right-angle branching with septation near the point of branching. *R. solani* AG-8 does not readily form microsclerotia but survives in dead roots as thick-walled melanized

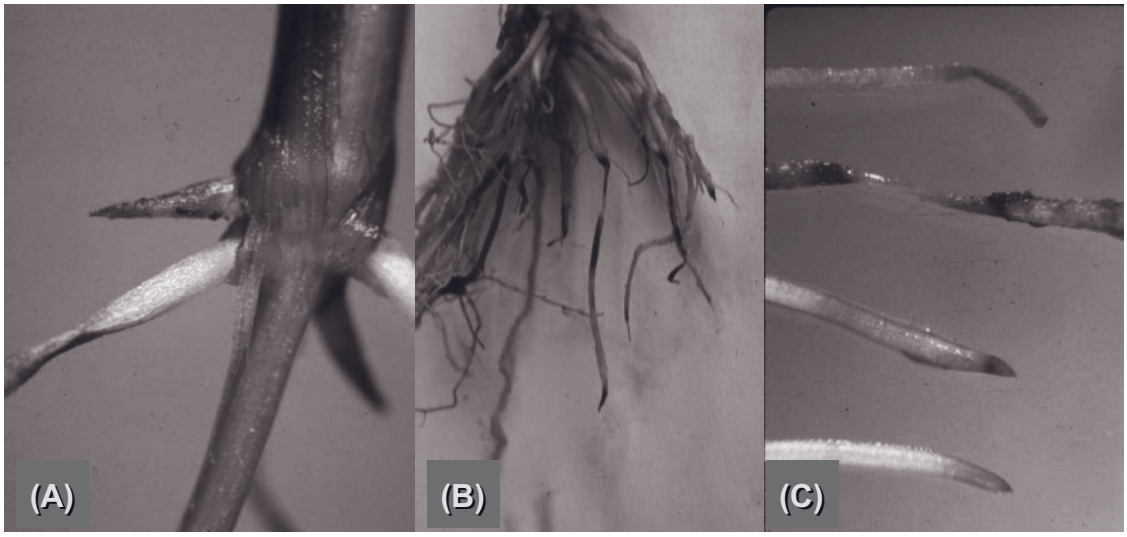

Fig. 11.21. Typical root symptoms of rhizoctonia root rot, including spear tipping, root lesions (A,B), and constriction of the root cortex (C) (A and B, courtesy of R. J. Cook and D. Weller; C, courtesy of R. Smiley). Reprinted from Bockus et al. 2010 with permission from the American Phytopathological Society. For color details, please see color plate section.

Fig. 11.22. Field symptoms of rhizoctonia root rot on spring barley. This field was direct seeded. Note uneven stand with varying heights of plants. For color details, please see color plate section.

monilioid hyphae. *R. solani* has the ability to grow saprophytically in the soil for a long distance from a food base, hence the ability to form patches in the field. Another characteristic of this disease is the increase in severity in no-till or conservation tillage systems. Tillage suppresses the development of patches, possibly by breaking up hyphal networks or by creating microbial conditions that restrict the growth of *Rhizoctonia*. *Rhizoctonia* can survive on living plants via a green bridge on grassy weeds or volunteers between crops (Smiley et al. 1992). If these weeds or volunteers are killed with herbicides immediately prior to planting, the necrotrophic pathogen can extensively colonize the root system of the dying weeds because of the breakdown of plant defense systems. This creates a large reservoir of inoculum that can cause severe disease on the succeeding crop. Rhizoctonia disease is more severe on spring barley than winter barley, possibly because of the cooler, wetter conditions in the spring and the fact that *R. solani* can cause damage at 12°C (Mazzola et al. 1996).

Recent work in the PNW has also identified *Rhizoctonia oryzae* as widely distributed and highly pathogenic to barley (Ogoshi et al. 1990; Mazzola et al. 1996; Paulitz et al. 2002b). This pathogen has also been reported in Alaska and in the United Kingdom (Burton et al. 1988; Leiner and Carling 1994). The teleomorph is *Waitea circinata* and probably exists as a number of varieties, including var. *zeae*, *oryzae*, *circinata*, and *agrostis*. The taxonomy of this group is still in flux. This fungus readily forms microsclerotia, which are salmon pink in color, 1–3 mm in diameter, and function in survival. The hyphae of *R. oryzae* branch at 30–50° and form a herringbone pattern in culture. *R. zeae* is a closely related species that is distinguished by different sizes and colors of sclerotia.

In addition to *R. solani* and *R. oryzae*, recent work in the PNW has identified binucleate *Rhizoctonia* species (teleomorph = *Ceratobasidium*) that can be pathogenic to barley (T.C. Paulitz, unpublished data). One group was isolated that is close to AG-I, based on molecular sequencing. This group is widespread, and can also be found in bare patches. Many of these binucleate *Rhizoctonia* species are also highly pathogenic to broadleaf rotation crops.

Rhizoctonia groups are difficult to quantify in the soil and are not amenable to dilution plating because of the relatively low density of propagules in the soil. A toothpick baiting technique was developed, which can quantify the hyphal activity of *R. solani* and *R. oryzae* in the soil (Paulitz and Schroeder 2005). Recently, techniques have been developed for the quantification of DNA from the soil using real-time PCR for specific groups of *Rhizoctonia*. For example, *R. solani* AG-8, along with eight other cereal pathogens (both fungi and nematodes), can be quantified (Ophel-Keller et al. 2008). Okubara et al. (2008) developed primers to quantify *R. solani* AG-8, 2-1 (a brassica pathogen), and AG-10, along with *Ceratobasidium* AG-I, and two groups of *R. oryzae*. The increased sensitivity and resolution of molecular identification techniques based on sequencing of the ribosomal internal transcribed spacer (ITS) DNA may lead to the discovery of other *Rhizoctonia* groups that may be pathogenic to barley.

Pythium root rot

In the older literature, this disease is referred to as browning root rot. Aboveground symptoms of *Pythium* are fairly nonspecific and under-recognized. In the PNW, the impact on wheat was not appreciated until the 1980s by the work of Cook and coworkers with metalaxyl, a *Pythium*-specific fungicide (Cook et al. 1980). Side-by-side comparisons with nontreated wheat in the field showed stunting, delayed crop development, and crop losses of 13%–36%, depending on the year (Cook et al. 1987). The symptoms on the root are less distinct than *Rhizoctonia*. In general, root tips, feeder roots, and root hairs are rotted away, and washed roots show less biomass and root length. But this is often only evident when compared to a healthy control, for example, in greenhouse experiments comparing natural to pasteurized soil. *Pythium* can attack germinating seeds and seedlings, attacking the embryo at an early stage. Sporangia of *Pythium* can germinate within a few hours of sensing root exudates and

can infect the embryo within 12–24 h (Fukui et al. 1994). In most cases, the cereal seedlings emerge, unless there is extremely high inoculum densities and cold, wet conditions. But the first leaf length is reduced, and the plant remains stunted for the rest of the growing season. *Pythium* species are pioneer colonizers and can rot the root before other secondary pathogens move into the root. Once the root is rotted, *Pythium* forms thick-walled sexual spores in the root called oospores, the result of the fusion of two gametangia, the antheridium and oogonium. Oospores can survive in the root or soil for many seasons and often have a dormancy factor. Pythium diseases tend to be most severe in cold, wet soils. Many species produce zoospores, motile swimming spores formed in a vesicle at the tip of a sporangium. These biflagellated spores swim toward the root, sensing a gradient of root exudates. They attach to the root, encyst by forming a cell wall, and infect the root cell via a germ tube. In some species, zoospores are not formed, but sporangia (hyphal swellings) germinate directly and infect the root.

Pythium species are classified as oomycetes or stramenopiles, distinct from true fungi, although their morphology is similar. Oomycetes are more closely related to brown algae and, unlike fungi, contain cellulose instead of chitin in their cell walls. Unlike most fungi, they also have a motile spore (zoospore). Like *Rhizoctonia*, *Pythium* species have fairly wide host ranges, attacking both cereals and broadleaf crops. Around 15 species have been reported from barley, primarily based on host indices in the United States (Farr et al. 1989) and the Canadian Plant Disease Survey, mostly done from 1950 to 1970. The most commonly cited species are *Pythium ultimum*, *Pythium debaryanum*, *Pythium graminicola*, and *Pythium arrhenomanes*, the latter two reported mostly from the United Kingdom and the Midwest United States. The *debaryanum* species designation is presently invalid and has not been used much in the last 30 years. Other species that have been reported include *Pythium aristosporum*, *Pythium irregulare*, *Pythium splendens*, and *Pythium rostratum*. *Pythium iwayami* and *okanoganense* are cold-tolerant snow mold species. Since about the turn of the twenty-first century, due to the ease of DNA sequencing and the development of large ITS sequence databases (Lévesque and de Cock 2004), accurate identification is possible, but the taxonomy of *Pythium* is in flux. Using a combination of both classical and molecular techniques, the wheat-based cropping system of eastern Washington was extensively surveyed. Spring barley is often used as a rotation crop in much of this region. Paulitz and Adams (2003) identified 13 species of *Pythium* in the soils and roots of wheat and barley. The most widely distributed were *P. irregulare* group IV (sensu Matsumoto) and a newly described species, *Pythium abappressorium* (Paulitz et al. 2003). Other species included *P. ultimum*, *P. rostratum* (later reclassified as *Pythium rostratifingens*), *Pythium intermedium* (later reclassified as *Pythium attrantheridium*), *Pythium heterothallicum*, *P. irregulare* group I, *Pythium sylvaticum*, *Pythium paroecandrum*, and *Pythium torulosum*. On wheat, the most pathogenic species were *P. ultimum*, *P. irregulare* group I, and *P. irregulare* group IV (Higginbotham et al. 2004a). Using species-specific primers developed from ITS sequences and real-time quantitative PCR (Schroeder et al. 2006), eastern Washington has been extensively surveyed in the last few years, and clear trends of biogeography are emerging. Surprisingly, *P. graminicola* and *P. arrhenomanes*, which are common in the Midwest United States and also attack maize, are not found in the dry summer cropping zones of PNW. *P. ultimum*, although the most virulent, is rarely found in dryland areas but is more common in irrigated areas. *P. irregulare* group I appears to be favored by cropping systems with legume rotations, especially in wheat–lentil rotations. The diversity of *Pythium* species is much less in areas with low rainfall and a wheat–fallow rotation compared to annual cropping areas. But other species such as *P. abappressorium* and *P. irregulare* group IV are widely distributed across all precipitation zones. This research also indicates that there may be a rotation crop/host preference for some species, but in general, most *Pythium* spp. have wide host ranges, at least based on inoculation experiments under controlled conditions.

Common root rot and crown rot

Common root rot is caused by *B. sorokiniana* (*C. sativus*), which is associated with rots in the Great Plains of the United States, the Prairie Provinces of Canada, and in Europe. In drier Mediterranean climates such as Australia and the PNW, crown rot is caused by both *B. sorokiniana* and by *Fusarium pseudograminearum* (formerly *F. graminearum* group I) and *F. culmorum*. Diseases caused by Fusarium are exacerbated by drought stress. *Fusarium* causes yield losses on wheat of up to 35%, average 9.5% (Smiley et al. 2005a). This section will focus on the common root rot caused by *B. sorokiniana*. This pathogen causes a seedling blight and root rot. Under severe conditions, seedlings can be killed and stand establishment is reduced. The pathogen forms brown lesions on the roots and especially on the subcrown internode. Under severe conditions, these lesions become almost black, and the brown color can extend into the crown and leaf sheaths. The same pathogen also causes spot blotch on the leaves and kernel blight or black point on seeds (see section on foliar diseases). Infected plants are stunted, with reduced tillering and reduced yield. White heads can be formed, and heads contain fewer kernels that are small and shrivelled.

B. sorokiniana was formerly classified as *Helminthosporium sativum*. This pathogen also attacks wheat, oat (*Avena sativa*), rye (*Secale cereale*), rice, and other grasses, although oat and rye are not as susceptible as wheat and barley. The pathogen forms characteristic 3–10 septate brown to olive brown colored conidia, 6–10 $\times$ 111–120 µm in size. The fungus sporulates readily on natural substrates such as straw or seed, which can be useful for diagnosis by putting infected plant tissues in a moist chamber for 1–2 days and examining the spores. *B. sorokiniana* survives in the soil via thick-walled conidia, which can persist in the soil for many years. In most areas, soilborne inoculum from conidia is the primary source of infection. Infection is initiated from spores (conidia) in the soil. Conidia germinate in the presence of a host and can infect the emerging coleoptile or primary roots. The fungus can produce phytotoxins, which aid in the pathogenesis and colonization of the root. Seedborne inoculum can be important in more humid areas. The pathogen can survive on roots of grassy weeds and some dicots or on host debris.

Take-all

Take-all is probably the most important soilborne disease on wheat worldwide and is found primarily on fall-sown wheat in areas of higher rainfall in Australia, North America, Japan, Europe, and South America. The causal agent is *Gaeumannomyces graminis* var. *tritici* (*Ggt*). Barley is susceptible to take-all but shows less damage and disease severity than wheat. Rye is even less susceptible, and oat is resistant to this fungus (although oats can be attacked by *G. graminis* var. *avenae*). Although *Ggt* shows specialization on wheat, it can also attack grassy weeds such as downy brome (*Bromus tectorum*) and quack grass (*E. repens*).

The primary symptom aboveground are plants that die around the time of grain filling, leaving white heads that contain no shrivelled seeds or a few shrivelled seeds, leading to severe yield losses. Plants are stunted with few tillers. With severe infection, roots near the base of the plant are covered by a dark black layer of external mycelium, and this blackening can move into the lower crown and leaf culms. This disease is most severe in areas with high soil moisture and alkaline soil conditions. *Ggt* survives by saprophytic growth on host debris or growth on grassy weeds and does not form long-lived survival structures. Perithecia can be formed on rotting crowns, but ascospores do not appear to play a major role in this disease. Microcondia can be formed by the *Phialophora* anamorph, but these do not play a major role in infection. Plants are infected by dark runner hyphae that form hyphopodia and penetrate roots from hyphae arising under the hyphopodia. These runner hyphae spread along the outside of the root and can move into the crown if infection occurs on young plants. The runner hyphae

can also spread from plant to plant. Take-all can increase when wheat and barley are grown in monoculture year after year. However, take-all can decline after a period of monoculture due to a buildup of antagonistic bacteria, *Pseudomonas* species, which suppress the disease by producing antifungal compounds. This phenomenon, called take-all decline, has been documented around the world (Weller et al. 2002).

Ggt is identified based on simple hyphopodia and the size of ascospores, although it is often difficult to distinguish groups without pathogenicity testing and molecular characterization. In addition, there are saprophytic *Phialophora* species (anamorph of *Gaeumannomyces*) that resemble *Ggt* in culture that can be isolated from soils.

Cereal cyst nematode

Cereal cyst nematode (*Heterodera avenae*) is a sedentary endoparasite that infects the roots of cereal members of the Poaceae (wheat, oat, barley, rye, and triticale [×*Triticosecale*]). Cereal cyst nematodes cause short branching and swelling (knots) on the roots of seedlings of wheat but do not cause distinctive root symptoms on barley, other than a bushier root system. Aboveground, the plants are severely stunted, usually with a patchy distribution, and show symptoms of nutrient deficiency. Juveniles gain entry to the root, and females set up a feeding site in the vascular system of the root. The females become swollen, produce eggs, and are transformed into cysts, which protrude through the roots. These cysts are white when young and then turn brown. The eggs are formed within the cyst, and the cysts can survive for long periods of time and overwinter. Eggs hatch out the following season. The nematode consists of distinctive pathotypes or races, and resistance genes have been identified (see section on management). *H. avenae* is widely distributed throughout the world, including Australia, the Mediterranean basin, Europe, and Oregon in the United States. However, recent surveys indicate that this nematode has spread in the Intermountain and PNW of the United States to Colorado, Utah, Montana, and Idaho (R. W. Smiley, unpublished data). In addition, a new species was detected in Oregon in 2008, *Heterodera filipjevi* (Smiley et al. 2008). Cysts can be spread by the movement of soil with wind, transplants, shoes, tubers, machinery, harvesters, and so on. The disease is reduced by rotation with a nonhost for 1–2 years, including controlling grassy weeds. In general, barley is more tolerant of cyst nematode than are wheat or oats.

Root lesion nematodes

Root lesion nematodes (*Pratylenchus* spp.) are migratory endoparasites that feed on a wide variety of hosts. In dryland cereal production, *Pratylenchus neglectus* and *Pratylenchus thornei* are the most well studied and are found in Australia and the PNW of the United States. In irrigated cropping systems, *Pratylenchus penetrans* is more important. Lesion nematodes cause similar symptoms to root rot pathogens such as *Rhizoctonia* and *Pythium*, including pruning of root tips and cortical rot, stunting, reduced tillering, and yellowing of leaves. Nematodes may have synergistic interactions with root infecting fungi by providing wounds for fungal entrance. Recent surveys in the PNW (Smiley et al. 2004) have shown that lesion nematodes are widespread across the dryland cereal production areas and are above the economic threshold. Using resistant/tolerant Australian varieties and applications of alidcarb (a nematicide), Smiley et al. (2005b,c) demonstrated that *P. neglectus* and *P. thornei* could cause significant yield reduction on spring wheat, but little work has been done on barley. Barley is more resistant than wheat and supports lower levels of reproduction of nematodes on the roots (Vanstone et al. 2008). Variety trials by R.W. Smiley et al. (unpublished data) showed that barley varieties are more tolerant than wheat (i.e., result in less yield reduction) under high nematode populations. Although root lesion nematodes have a wide host range, there are differences among different crops in their ability to support *P. neglectus* and *P. thornei*. Rye and safflower (*Carthamus tinctorius*) are resistant, and chickpea is highly susceptible. Oat and barley are susceptible to moderately resistant to *P. neglectus* but are

moderately resistant to resistant to *P. thornei* (Hollaway et al. 2000; Taylor et al. 2000; Vanstone et al. 2008). Because of the wide host range, crop rotation is not effective in reducing populations, but cropping intensity can affect nematode population, with lower populations in summer fallow systems compared with annual cropping systems (Smiley et al. 2004).

Management of Soilborne Diseases of Barley

Genetic resistance or tolerance

As with most soilborne pathogens of cereals, there is little tolerance or resistance to many of these diseases in adapted cultivars. Spring wheat, spring barley, and synthetic wheat hexaploids were screened in *R. solani* AG-8 inoculated greenhouse and field tests, but no resistance or tolerance was detected Smith et al. (2002a,b). However, *Dasypyrum villosum*, a wild grass of the Mediterranean that can be hybridized with wheat, showed resistance. Spring wheat cultivars were screened with *P. ultimum* and *P. irregulare* group IV (identified as *P. debaryanum* in this chapter) in a greenhouse assay. Cultivars differed in susceptibility but did not display true resistance or tolerance (Higginbotham et al. 2004b). Attempts have been made to use transgenic methods or induced mutagenesis to obtain tolerance to *Rhizoctonia*. The endochitinase gene from *Trichoderma harzianum* (Wu et al. 2006) was expressed in barley, but this technology has not yet been successful under field conditions (T. Paulitz, unpublished data). Spring wheat was mutagenized with ethane-methyl sulfonate (EMS) and screened for tolerance to *R. solani* AG-8. Tolerant mutants were identified and backcrossed to other spring wheat varieties (Okubara et al. 2009). These crosses show less root loss and stunting in the greenhouse but still need to be evaluated in the field. A similar approach is being taken with barley (S. Ullrich, unpublished data)

Resistance to strawbreaker foot rot, cephalosporium stripe, and snow molds have been incorporated into winter wheat (Bruehl et al. 1986; Allan and Roberts 1991; Gaudet 1994), but no resistance has been incorporated into winter barley.

Resistance to cereal cyst nematode has been found in wild relatives of wheat. Over 11 different single gene resistances have been identified in barley, which may confer race or pathotype-specific resistance. For example, "Ortolan" is resistant to Group Ha1 but susceptible to Groups Ha2 and Ha3. "Siri" is resistant to most biotypes of Ha1 and Ha2 but susceptible to Ha3, while "Morocco" is resistant to all three groups. Ha2 resistance was mapped to chromosome 2H of barley and was shown to be effective against the Australian pathotype (Kretschmer et al. 1997). QTLs and major gene resistance to the root lesion nematodes *P. neglectus* and *P. thornei* have been found in wheat (Zwart et al. 2006; Thompson 2008), but none have been identified in barley. Wheat genes have been found for both tolerance (reproduction takes place but no yield reduction) and resistance (reduces nematode reproduction).

Chemical control

Unfortunately, chemical control for most soilborne pathogens of barley is not effective nor economical, and no products are registered for soil application on barley in the United States. Fungicides applied to soil can be bound to soil particles, inactivated or metabolized by other microbes, or may not move through the soil profile. Even if the fungicide was mobile, large amounts would need to be applied to treat the soil profile, which may cause environmental problems by moving into the groundwater. However, growers use protective and systemic seed treatments for barley, especially to protect against smuts and bunts (Smiley et al. 1990a,b). The most commonly used are demethylation inhibitors such as tebuconazole (Raxil™), triticonazole (Charter™), and difenoconazole (Dividend™). Metalaxyl and mefanoxam (Ridomil™, Subdue™, and Allegience™) have specific activity against oomycetes such as *Pythium* spp. Fludioxonil (Maxim™) and imazalil have activity against *Rhizoctonia*. Most of these fungicides protect the

seed against seedborne pathogens or damping-off pathogens in the soil that attack the germinating seed. The systemic fungicides move into the developing shoot to protect the young seedling, but eventually the fungicide is diluted out by the growing plant. However, none of these fungicides are mobile in the phloem, that is, move into the developing root tips to protect the constantly expanding root system. As a result, they are not effective against root rots later in the season, and often seed treatments do not statistically increase yield of wheat (Smiley et al. 1990a,b) or may give slight yield increases (Cook et al. 2002b). However, we often see statistical improvements in the seedling health of wheat from treated seed early in the season when planted into soil with high levels of *Rhizoctonia* (Paulitz and Scott 2006).

Crop rotation

Crop rotation is effective against some barley pathogens that have a narrow host range, such as the take-all pathogen *G. graminis* var. *tritici*. A rotation of 1–2 years with a broadleaf crop, such as a cool season grain legume, alfalfa (*Medicago sativa*), or soybean, may reduce the pathogen below an economic threshold but not eliminate it. But grassy weeds must be controlled in the rotation. Many soilborne pathogens have a wide host range and can attack both grasses (such as wheat, which is often in rotation in barley) and dicots. For example, *R. solani* AG-8 has a wide host range, including wheat, barley, pea, lentil, and canola (*Brassica* spp.). *R. oryzae* can attack both cereals (Paulitz et al. 2002) and dicots (Paulitz 2002). Likewise, *Pythium* spp. attack both cereals and dicots and are generalized necrotrophs that rot the seeds and roots of most plants. However, recent work using real-time PCR in the PNW has shown that *Pythium* spp. may have preferences for certain host rotations (K.L. Schroeder, unpublished data). For example, *P. irregulare* group I is associated with lentil containing crop rotations.

For common root rot, the rotation effects are more complex. This disease can be reduced in rotations with canola, oat, or bromegrass (*Bromus* spp.) compared to 2 years of barley (Piening and Orr 1988). Flax (*Linum usitatissimum*) was not effective in reducing common root rot in one study (Bailey et al. 2001) but was effective in another (Conner et al. 1996). But in 2 years, back-to-barley after wheat, flax, or oats, the severity increased in barley and the rotational effects were lost. Increased diversity of crops can reduce the soil populations of *B. sorokiniana* (Bailey et al. 2001).

Crop rotation can reduce the cereal cyst nematode. *H. avenae* only attacks members of Poaceae, including wheat, barley, oats, and grassy weeds. When a nonhost is grown, the populations quickly decline because hatched juveniles die. But all the cysts do not hatch every year, so one year of a rotation with a nonhost will not eliminate the nematode. Work in Australia has compared different crops for their reproductive potential and susceptibility to lesion nematodes (Vanstone et al. 2008). Wheat and barley varieties range from susceptible to moderately resistant to *P. neglectus*, but barley is more resistant or tolerant to *P. thornei* than wheat (Taylor et al. 2000; Vanstone et al. 2008). Thus, barley could be used as a rotation crop to reduce *P. thornei* in subsequent wheat crops. Field pea and lentil are less favorable hosts than barley and could reduce both species in a following barley crop.

Tillage and residue management

Of all the soilborne diseases of barley, rhizoctonia bare patch and root rot is the most influenced by tillage. Tillage can reduce disease caused by *R. solani* AG-8, which is more severe under no-till conditions (MacNish 1985; Rovira 1986; Pumphrey et al. 1987). Tillage may break up hyphal networks of the fungus or may produce a flush of microbial activity that is suppressive to *Rhizoctonia*. Schroeder and Paulitz (2006) found that it took 2 years after tillage was stopped before *Rhizoctonia* became a serious problem in the third and fourth year. On the other hand, common root rot and take-all were more severe under conventional tillage (Bailey et al. 2000). Tillage also has an effect on crop residue. In reduced or no-till

operations, more crop debris is left on the soil surface. This residue can make the soil cooler and wetter in the spring by retarding evaporation and reflecting the sunlight, compared with dark exposed soil, which absorbs infared radiation. This can make conditions favorable for *R. solani* AG-8, which can cause damage at 12°C or less (Mazzola et al. 1996). Pythium diseases, likewise, are more favored by cold, wet conditions. For example, *P. ultimum* and *P. irregulare* are active at soil temperatures of 10 and 5°C, respectively (Ingram and Cook 1990). Some growers use burning, mowing, or harrowing to break up the straw or chaff and to incorporate straw spreaders on the back of combines. Excessive residue can favor pathogens that survive as inoculum in crop residue, resulting in more disease development, as is the case with fusarium crown rot (Summerell et al. 1989; Smiley et al. 1996). However, in the case of pathogens that survive in the roots, residue management may not have a significant effect. For example, burning and mechanical removal of the straw did not reduce *R. solani* AG-8 in a no-till rotation (Paulitz et al. 2009).

Greenbridge management

One of the most effective cultural techniques for reducing many soilborne pathogens of barley is greenbridge management. Most of these pathogens can survive and multiply on the living roots of volunteer crops and grassy weeds. For example, barley is often grown in rotation with wheat. In many areas, volunteer crops and weeds can overwinter. In the spring, growers will treat with nonspecific contact herbicides such as glyphosate prior to planting. However, when plants are dying from herbicides, necrotrophic pathogens such as *Rhizoctonia* and *Pythium* can quickly invade the dying tissue, creating a more fungal biomass and inoculum (Smiley et al. 1992; Paulitz et al. 2002). This is particularly a problem with glyphosate, which kills plants by interfering with the synthesis of aromatic amino acids, which are key for plant defense pathways. This has been termed "glyphosate synergy" (Lévesque and Rahe 1992). When growers plant into these dying weeds, the increased reservoir of inoculum can infect the new crop. It is recommended that growers wait at least 2 weeks after herbicide application before planting to allow time for the inoculum to decline (Mathre 1997). Recent work in spring barley has found that the optimal time of spraying out the greenbridge was 2–4 weeks before planting based on plant responses, and waiting longer did not result in additional plant growth (T. Paulitz, unpublished data). Spraying out in the fall provided even more reduction in rhizoctonia disease on spring barley (Paulitz and Reinertsen 2005) (see Fig. 11.23).

Fallow

Can soilborne pathogens of barley be reduced by a growing season without host plants? Fallow is often used to control weeds, by mechanical cultivation or herbicides, or used to conserve and accumulate water in the soil in areas with low rainfall. As with crop rotation, the effectiveness of this method depends on the ability of the pathogen to survive in the soil. In the absence of a host, nutrients in the inoculum will be depleted, and microflora and microfauna will attack and degrade propagules. A fallow period of 3–6 weeks in a direct-seed system in Australia reduced rhizoctonia disease (Roget et al. 1987). Reduced tillage mechanical fallow or chemical fallow reduced the activity of *R. solani* AG-8 in the year following tillage but did not affect *R. oryzae*, which produces microsclerotia, which can probably survive the fallow period (T. Paulitz, unpublished data).

The principle of fallow also operates in greenbridge management—the 2- to 3-week period of no weeds before planting allows for the inoculum to decline due to the natural degradation of the inoculum. A winter cereal–spring cereal rotation or spring cereal–spring cereal rotation provides a 6-month fallow period in a temperate climate, which may reduce diseases compared to back-to-back winter cereals.

Can fallow affect a pathogen that forms resistant survival structures such as oospores? Work with real-time PCR quantification examined the populations of nine different *Pythium* spp. in chemical and mechanical fallow, as well as other

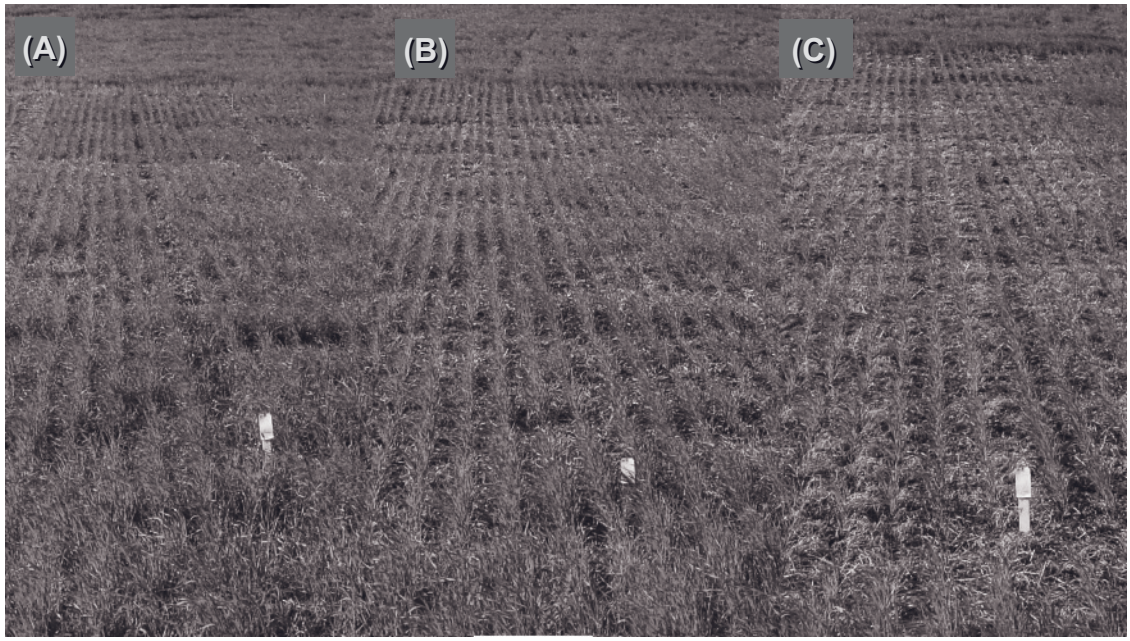

Fig. 11.23. Effect of greenbridge management on the rhizoctonia root rot of barley. All three plots of spring barley were in the same field, direct seeded at the same time. (A) Weeds and volunteers were killed with the herbicide glyphosate in the previous fall. (B) Weeds and volunteer sprayed out with glyphosate 3 weeks before planting. (C) Weeds and volunteer sprayed out with glyphosate 3 days before planting. For color details, please see color plate section.

cropping systems. Most *Pythium* species were reduced to below the level of detection in the fallow treatments. The exceptions were *P. abappressorium* and *P. irregulare* group IV, which were reduced in population in the fallow treatment but were not eliminated (K. L. Schroeder, unpublished data).

Plant nutrition

As with most plant diseases, plant nutrition plays an important role. In the case of soilborne pathogens, which reduce root biomass, especially feeder roots, the plant is compromised in its ability to take up nutrients, especially nutrients that are relatively immobile in the soil, such as phosphorus. Growers will often apply a starter fertilizer in the seed row, just under or to the side of the seed. These nutrients are immediately available to the seedling to compensate for reduction in uptake capacity. Application of Zn to Zn-deficient soils decreased disease severity of rhizoctonia bare patch in Australia (Thongbai et al. 1993; MacNish and Neate 1996). But similar Zn applications in the PNW did not have an effect as these soils were not deficient (Cook et al. 2002a). Take-all can be decreased by the addition of Mn in deficient soils (Wilhelm et al. 1988). Pathogens can also be influenced by the form of N applied. For example, take-all is more severe in alkaline soils. The application of ammonia forms of N decreases soil pH and take-all, whereas nitrate forms increase soil pH and take-all (Colbach et al. 1997).

Conclusions

Barley is susceptible to a number of soilborne fungal and nematode pathogens, many of which also attack wheat and other members of the Poaceae. These pathogens are difficult to manage because of the lack of distinctive symptoms

for identification and the lack of soil-applied fungicides or nematicides that are effective or economic on a relatively low-value crop such as barley. Genetic resistance or tolerance to most of these generalized wide host range root rotting pathogens is also lacking. Thus, growers must rely on a variety of cultural techniques to manage these diseases, especially when growing barley in rotation with wheat. However, there are a number of pathogens that barley is less susceptible to than wheat, and barley provides benefits as a rotation crop in wheat production.

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Chapter 12
Biotic Stress in Barley: Insect Problems and Solutions

Dolores W. Mornhinweg

Over 100 species of arthropod pests attack barley. The majority are occasional pests such as cutworms, armyworms, wireworms, grasshoppers, and other chewing insects that consume plant tissue and can cause economic damage in outbreak years when climate and cultural conditions are favorable (Starks and Webster 1985). Mites such as wheat curl mite (*Aceria tosichella* [Keifer]), can vector barley viruses, reducing yields in any given year. However, the most important widespread insect pests of barley are aphids (Starks and Burton 1977b), which suck plant sap and, depending upon the aphid, also inject toxins or vector viruses or both. Aphids, in general, prefer barley to other small grains (Starks and Webster 1985). Many aphids such as corn leaf aphid (*Rhopalosiphum madis* [Fitch]), English grain aphid (*Sitobion avenae* [Fabricius]), rice root aphid (*Rhopalosiphum rufiabdominalis* [Sasaki]), and western wheat aphid (*Metopolophium dirhodum* [Walker]) are occasional pests that damage barley mostly through transmission of viruses.

Three of the five most important barley pests worldwide are Russian wheat aphid (*Diuraphis noxia* [Kurdjumov]), greenbug (*Schizaphis graminum* [Rondani]), and bird cherry-oat aphid (*Rhopalosiphum padi* [L.]). Besides the removal of plant nutrients from the phloem, Russian wheat aphids and greenbugs inject toxins that cause visible injury, severe yield-limiting plant damage, and even death. Russian wheat aphid injury includes desistance (stunting and chlorosis) as well as deformation (misshapen spikes and pseudogalling), while greenbug injury involves desistance (necrosis) alone (Quisenberry and Ni 2007). Bird cherry-oat aphids cause no visible injury symptoms, and yield loss from aphid feeding is attributed solely to removal of plant nutrients. However, the bird cherry-oat aphid is a very efficient vector of the PAV strain of barley yellow dwarf virus (BYDV), the most yield-limiting small grain virus in the world. PAV is the most damaging and widespread serotype of BYDV (Lister and Ranieri 1995). PAV is less efficiently vectored by greenbugs, while Russian wheat aphid has yet to be shown to vector PAV. Aphids are notorious for producing biotypes, which are populations within an aphid species that have the ability to damage previously resistant plants (Puterka and Burton 1991). A disproportionate number of biotypes are found in Aphididae (Wilbert 1980). Their rapid rate of multiplication, potential for asexual and/or sexual reproduction, and vast host range contribute to selection of biotypes within aphids. Greenbugs and Russian wheat aphids have a history of biotype development.

The other two important pests of barley worldwide are Hessian fly (*Mayetiola destructor* [Say]) and cereal leaf beetle (*Oulema melanopus* [L.]). The Hessian fly is a sap-feeding insect. Only one individual Hessian fly larva per plant can have a severe negative impact on crop production (Starks and Webster 1985). The cereal leaf beetle is a chewing insect. Yield reductions of 50% have been reported (Brook and Dewar 1977) from leaf defoliation caused by larval feeding. According to the January 2005–March 2006 progress report of

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the Utilization of Intelligent Systems for Plant Protection Project, a joint survey conducted by the International Center for Agricultural Research in the Dry Areas (ICARDA), the International Crops Research Institute for the Semi-Arid Tropics (ICRISTAT), the Central Laboratory for Agricultural Expert Systems (CLAES), and the International Rice Research Institute (IRRI), Russian wheat aphid, greenbug, bird cherry-oat aphid, Hessian fly, and cereal leaf beetle are pests of barley in 23%, 24%, 35%, 26%, and 13% of the countries in the world, respectively (Figs. 12.1–12.5). *Hordeum* germplasm stocks worldwide (30,000 accessions in United States Department of Agriculture [USDA] National Small Grains Collection, 22,000 in ICARDA, and 9000 in International Maize and Wheat Improvement Center [CIMMYT]) have been a source of strong resistance to barley insect pests (Starks and Burton 1977b).

RUSSIAN WHEAT APHID

The russian wheat aphid, indigenous to east central Asia/southern countries of the former Union of Soviet Socialist Republics, Mediterranean Rim countries, Afghanistan, and Iran (Walters et al. 1980), was first reported as a pest of cereals in 1901 (Mokrzhetsky 1901). Although not a persistent pest in its area of origin, short-lived outbreaks have occurred (Basky et al. 2000). The Russian wheat aphid, first reported in Turkey in 1959 (Duran and Koyuncu 1974), became epidemic in 1962 when yield losses of 25%–60% were reported in Konya province (Duran and Koyuncu 1974; Altinayar 1981). The Russian wheat aphid was detected in South Africa in 1978 (Walters et al. 1980) and quickly became a major and persistent pest of small grains including barley. The Russian wheat aphid was next identified in Mexico in 1980/1981 (Gilchrist et al. 1984), in the southern United States in 1986, and as far north as Canada in 1988. By 1991, Russian wheat aphids were found in all states west of the one-hundredth meridian and in three western provinces in Canada (Pike and Allison 1991; Stern and Orloff 1991), and the Russian wheat aphid had replaced the greenbug as the major aphid pest of barley in the United States. The Russian wheat aphid was also on the move in Europe, reported in Yugoslavia (Starý 1999) and Hungary (Basky and Eastop 1991) in 1989, Serbia

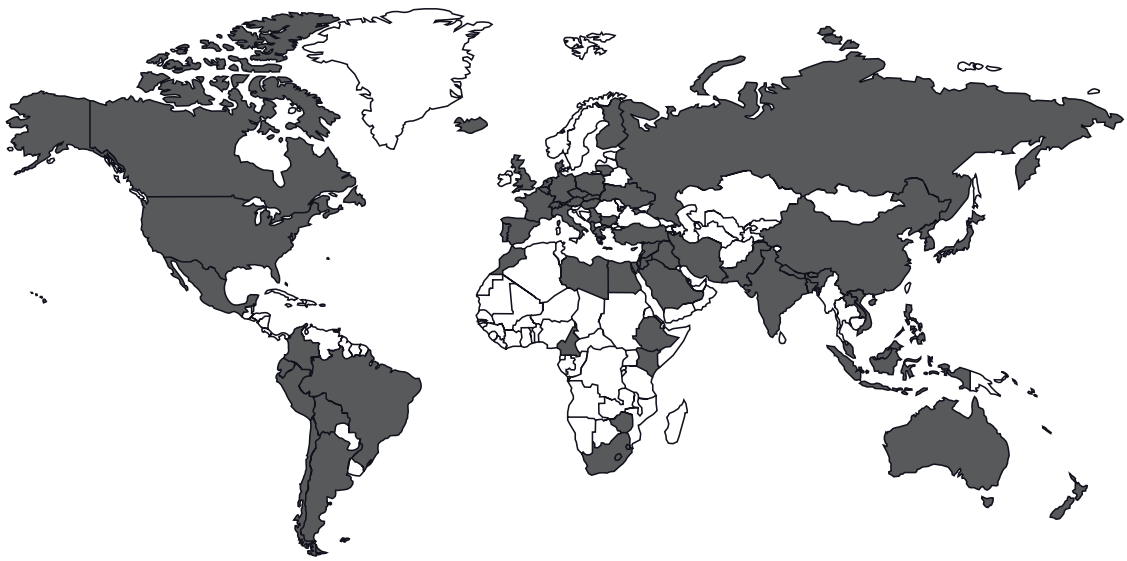

Fig. 12.1. Worldwide distribution of the five major insect pests of barley: bird cherry-oat aphid.

Fig. 12.2. Worldwide distribution of the five major insect pests of barley: cereal leaf beetle.

Fig. 12.3. Worldwide distribution of the five major insect pests of barley: greenbug.

Fig. 12.4 Worldwide distribution of the five major insect pests of barley: hessian fly.

Fig. 12.5 Worldwide distribution of the five major insect pests of barley: Russian wheat aphid.

in 1990, Czech Republic in 1993 (Starý 1999), and Poland in 1998 (Shliephake et al. 1998); in South America, it was reported in Chile in 1990 and in Argentina in 1992 (Ortega and Delfino 1994). Northeastern China and Australia are the only major small grain production areas in the world where the Russian wheat aphid has yet to be detected (Barta and Cagáň 2007). Small grain production in southern Australia is in a temperate zone with low rainfall, hot summers, and mild winters ideally suited to Russian wheat aphids (Jones et al. 1989; Starý 1999). RWA introduction may only be a matter of time.

Russian wheat aphids damage cereals by removing assimilates from the leaf vascular tissue and by injecting a phytotoxic substance during feeding (Fouché et al. 1984). Russian wheat aphid feeding on susceptible barley typically results in chlorotic spotting and longitudinal white or yellow streaking of emerging leaves, which do not unroll. Russian wheat aphid in the whorl can move down the leaf axis to younger cells and continue to move downward as the leaf extrudes from the leaf sheath (Miller and Porter 1997). Under early infestation, growth is often prostrate. As the crop matures, spikes become trapped in rolled leaves, resulting in deformed spikes with severely reduced fertility. Aphids feeding in rolled leaves are protected from contact insecticides, and chemical control of the Russian wheat aphid often requires repeated applications of economically and environmentally detrimental systemic insecticides. Russian wheat aphids can feed on the peduncle, developing seed and awns as plants mature.

Phloem sap is generally regarded as a poor diet for aphids due to its low concentration of essential amino acids or other sources of nitrogen (Slansky and Scriber 1985). Some substance in the saliva of Russian wheat aphid elicits a symptomatic response (Al-Mousawi et al. 1983; Fouché et al. 1984; Fereres et al. 1986) inducing senescence-like changes (chlorotic lesions) that allow aphids to take advantage of increased translocation from the breakdown of leaf proteins (Dorschner et al. 1987). The most striking change in the phloem from Russian wheat aphid feeding was increased concentrations (4×) of essential amino acids, which would be nutritionally advantageous for the aphids and diminish dependence on intercellular bacterial symbionts (*Buchnera*) for amino acid requirements (Sandström et al. 2000).

The Russian wheat aphid has no preferential probing or entry site. Stylets typically follow an intercellular route through the leaf mesophyll until phloem contact (Fouché et al. 1984; Girma et al. 1992). Stylets appear to secrete lipoprotein sheaths that surround them and may protect them from plant wound reactions (Miles 1990). Early symptom development indicates localized changes in the ultrastructure and photosynthetic function of chloroplasts adjacent to the stylet paths (Belefant-Miller et al. 1994). The chloroplast membrane and photosynthetic pigments are suggested as primary sites of action in the damage response of wheat (Fouché et al. 1984). The initial response to Russian wheat aphid feeding was the retraction and convolution of the plasmalemma followed closely by the distention of the chloroplast granal and stromal lamellae, followed by a rapid disassembly of the chloroplast lamellae, which resulted in a substantial increase in the volume of plastogobuli. Subsequent degeneration of the chloroplast envelope was followed by the disintegration of other cell organelle membranes, which culminated in cell bleaching. Russian wheat aphid feeding on susceptible barley resulted in a 48% decrease in total chlorophyll content due to a significant decrease in the capacity and efficiency of the primary photochemistry of photosystem II (Burd and Elliott 1996). Chlorophyll fluorescence induction kinetics indicated photoinhibitory damage was caused by a blockage in electron transport on the acceptor site of photosystem II. It was suggested that substantially restricted electron flow and possible irreversible photoinhibition could be arising at the D1 protein or QB binding sites and that antennal chlorophyll complexes become detached from photosystem II reaction centers, which could result in chlorophyll photooxidation or cell bleaching.

The Russian wheat aphid generally prefers drier geographical areas, poorly fertilized or neglected fields, and especially border areas where the plants are widely spaced or relatively weaker owing to less fertilization or more drought (Starý

1999). Russian wheat aphid densities are greater on drought-stressed barley (Oswald and Brewer 1997). Environmental stresses may enhance herbivore performance because of stress-induced changes in the plant tissue (Rhoades 1985). Russian wheat aphid losses increase under drought stress, resulting in devastating losses for marginal dryland producers (Stern and Orloff 1991).

The Russian wheat aphid causes drought stress symptoms in barley leaves even in the presence of adequate root moisture (Riedell 1989a). Longitudinal leaf rolling is a well-known drought stress response in cereals (Esau 1977). Further, Russian wheat aphids limit the ability of barley to adjust to drought stress. Loss of chloroplast function with Russian wheat aphid feeding limits the capacity of leaves to accumulate glycine betaine and proline in response to drought stress (Riedell 1989a). Decreased relative water potential of drought-stressed barley results in loss of turgor, wilting, stomatal closure (Bradford and Hsiao 1982), and leaf desiccation. Under drought conditions, in susceptible barley, the relative water content of Russian wheat aphid-infested leaves drops severely and steadily and leaves begin to desiccate immediately, whereas the relative water content of noninfested leaves is stable for several days followed by slow decline and slower leaf desiccation (Riedell 1989a). There is also a significant decrease in the survival of susceptible winter wheat to freezing when plants are infested with the Russian wheat aphid (Bravo et al. 1997).

Russian wheat aphid feeding on susceptible barley results in reduced grain yield (Stern and Orloff 1991; Robinson 1994; Mornhinweg et al. 2006b), decreased spike number (Calhoun et al. 1991a; Robinson 1993; Oswald and Brewer 1997; Mornhinweg et al. 2006b), decreased kernel weight (Stern and Orloff 1991; Robinson 1993; Bregitzer et al. 2003; Mornhinweg et al. 2006b), decreased straw weight (Robinson 1993), decreased root and tiller biomass (Stern and Orloff 1991), reduced kernel quality(Stern and Orloff 1991; Bregitzer et al. 2003), and livestock grazing losses (Stern and Orloff 1991). Yield losses of up to 70% have been reported in U.S. barley production areas (Meyerdirk 1989), from 41% to 85% in Ethiopia (Miller and Haile 1988; Adisu and Tadesse 1999), 59% in Mexico, and 25%–60% in Turkey (Duran and Koyuncu 1974; Altinayar 1981).

The Russian wheat aphid has been reported to vector BYDV to wheat in South Africa and Morocco (Araya 1990) but has yet been shown to do so in wheat or barley in the United States.

Losses occur every year in much of the barley-producing areas of the western United States (Porter et al. 1999) and southern Alberta, Canada (Aung 1991). Russian wheat aphids infested 3 million hectares of barley, 58% of the U.S. barley production area, in 1991(Aung 1991). From 1986 to 1999, Russian wheat aphids resulted in combined direct and indirect losses in excess of $1 billion in U.S. small grains (Vandenberg 1996; Porter et al. 1999; Bregitzer et al. 2003). The Russian wheat aphid is a persistent pest to U.S. barley growers in Colorado, western Nebraska, western Kansas, eastern Wyoming, and New Mexico, especially in hot dry years. Early heavy infestations can result in total crop loss. Barley is no longer grown in these areas as chemical control is economically prohibitive in these low-productivity dryland environments. In years of heavy infestation, especially in the spring, the Russian wheat aphid has resulted in total crop failure of barley in Ethiopia (Adisu et al. 2003).

Development of host plant resistance offers the most economical and least environmentally disruptive strategy for managing Russian wheat aphid. Greenhouse seedling screening revealed that all U.S. barley cultivars were susceptible to Russian wheat aphid (Webster et al. 1991). All available accessions (23,070 accessions) of *Hordeum vulgare* in the USDA Agricultural Research Service (ARS) National Small Grains Collection were screened as seedlings in the greenhouse for Russian wheat aphid resistance by Jim Webster, a research entomologist at USDA-ARS, Stillwater, Oklahoma, from 1990 through 1993. One hundred nine accessions were found with some level of resistance according to Webster's scale of 1–9, where seedlings rated 1–3 are resistant, 4–6 are moderately resistant to moderately susceptible, and 7–9 are susceptible (Pike and Allison 1991). Seedling resistance ratings

have been shown to accurately predict field performance (María Luz Salas et al. 1991; Robinson 1992; Brewer et al. 1998; Bregitzer et al. 2003; Mornhinweg et al. 2006b). Since 1991, seven more resistant sources have been identified among *H. vulgare* accessions newly available in the collection. Homozygous resistant unadapted germplasm lines were selected from each of the 116 heterogeneous accessions. Two Russian wheat aphid-resistant, six-rowed spring barley germplasm lines, STARS-9301B and STARS-9577B, were released by USDA-ARS in 1993 and 1995, respectively (Mornhinweg et al. 1995b, 1999). STARS-9301B rated 2, while STARS-9577B rated 3 on Webster's scale. A prebreeding program was initiated at USDA-ARS to transfer resistance from unadapted Russian wheat aphid-resistant germplasm lines into adapted backgrounds of every type of barley grown in the United States—winter, spring, feed, malt, two-rowed, and six-rowed barley. STARS-0501B–STARS-0507B, Russian wheat aphid-resistant, six-rowed, winter feed barley germplasm lines with seven different sources of resistance in a Schuyler background, were released in 2005 (Mornhinweg et al. 2006a). A total of 43 Russian wheat aphid-resistant germplasm lines were released in 2006 (Table 12.1): STARS-0601B–STARS-0619B, six-rowed, spring barley germplasm lines with 19 different sources of resistance in one of four six-rowed malting barley backgrounds(Mornhinweg et al. 2007a); STARS-0620B–STARS-0636B, two-rowed, spring barley germplasm lines with 17 different sources of resistance in one of four two-rowed malting barley backgrounds(Mornhinweg et al. 2007b); and STARS-0637–STARS-0643B, two-rowed, spring barley germplasm lines with seven different sources of resistance in one of four, two-rowed feed barley backgrounds(Mornhinweg et al. 2008). Thirty-six sources of resistance are represented in these 50 germplasm lines (Table 12.1). Tolerance, the main component of resistance in these lines, is considered preferential to antibiosis in that it should reduce selection pressure, which could promote biotype emergence.

"Burton," the first Russian wheat aphid-resistant barley cultivar in the United States, was released in 2003 (Bregitzer et al. 2005). Burton is a two-rowed, spring feed barley with resistance derived from STARS-9301B. "Stoneham" and "Sidney," drought hardy, two-rowed, spring feed barley cultivars with resistance from STARS-9577B and STARS-9301B, respectively, were released in 2006. "RWA1758," a two-rowed, spring feed barley with resistance derived from STARS-9577B, was released in 2007 (Bregitzer et al. 2008).

Barleys have been screened for Russian wheat aphid resistance in the field by CIMMYT/ICARDA since 1993. Three lines, "S8," "S12" (Gloria/Come), and "S13" (ASE/2CM//B.7.6.B.B.), were identified as the most resistant (Calhoun et al. 1991a,b; Robinson et al. 1991; Robinson 1992). Of the three, S8 had the highest resistance exhibiting antixenosis, antibiosis, and tolerance, while S13 was mostly tolerant with some antibiosis and S12 exhibited tolerance and antibiosis but less than that of S13 (Robinson et al. 1991). S13 is a widely tested, high-yielding, spring feed barley with multiple disease resistance. Screening of 76 barley genotypes in Iran identified 17 resistant barley lines (Castro et al. 1998). Shz.B-106 and Shz.B-108 were the most resistant.

Resistance to Russian wheat aphid has been identified in wild species of *Hordeum* (Butts 1989; Kindler and Springer 1991; Gianoli and Niemeyer 1998; Porter et al. 1999). *Hordeum chilense*, *Hordeum bogdanii*, *Hordeum brevisubulatum* subsp. *violaceum*, and *Hordeum bulbosum* have been reported to have resistance to Russian wheat aphid (Clement and Lester 1991; Clement et al. 1997). Beta-glucosides are hydroxamic acids in cereals that are enzymatically converted to aglycones when plants are damaged (Virtanen and Hietala 1960). 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and 2,4-dihdroxy-1,4-benzoxazin-3-one (DIBOA), the main aglycones in cereals, have been studied for their possible role in Russian wheat aphid resistance. Russian wheat aphid probed less before reaching the phloem phase and a lower percent of aphids achieved sustained phloem ingestion in wheat with differing levels of DIMBOA. However, there was no significant correlation between

Table 12.1 RWA-resistant barley germplasm lines released by USDA-ARS

Line	Pedigree	Source Accession	Seedling RWA Rating[a]	Row Type	Growth Habit
STARS 9301B	R027	PI 366450	2	6	Spring
STARS 9577B	R006	CIho 4165	3	6	Spring
STARS 0501B	"Schuyler"*4/R011	CIho 10687	2	6	Winter
STARS 0502B	"Schuyler"*4/R001	CIho 1412	2	6	Winter
STARS 0503B	"Schuyler"*4/R035	CIho 6347	3	6	Winter
STARS 0504B	"Schuyler"*4/R037	CIho 10684	3	6	Winter
STARS 0505B	"Schuyler"*4/R017	CIho 10721	2	6	Winter
STARS 0506B	"Schuyler"*4/R049	CIho 9990	3	6	Winter
STARS 0507B	"Schuyler"*4/R009	CIho 6925	2	6	Winter
STARS 0601B	"Excel"*4/STARS 9577B	CIho 4165	3	6	Spring
STARS 0602B	"Excel"*4/R010	CIho 10679	2	6	Spring
STARS 0603B	"Excel"*4/MR055	PI 564601	4	6	Spring
STARS 0604B	"Morex"*4/R001	CIho 1412	2	6	Spring
STARS 0605B	"Morex"*4/R007	CIho 4166	3	6	Spring
STARS 0606B	"Morex"*4/R009	CIho 6925	2	6	Spring
STARS 0607B	"Morex"*4/R011	CIho 10687	2	6	Spring
STARS 0608B	"Morex"*4/R016	CIho 10712	2	6	Spring
STARS 0609B	"Morex"*4/R018	CIho 12258	3	6	Spring
STARS 0610B	"Morex"*4/R019	CIho 13134	2	6	Spring
STARS 0611B	"Morex"*4/R028	PI 366453	2	6	Spring
STARS 0612B	"Morex"*4/R033	CIho 6316	3	6	Spring
STARS 0613B	"Morex"*4/R043	PI 556966	3	6	Spring
STARS 0614B	"Morex"*4/R052	CIho 6349	3	6	Spring
STARS 0615B	"Morex"*4/R053	CIho 13695	3	6	Spring
STARS 0616B	"Morex"*4/R054	CIho 14242	3	6	Spring
STARS 0617B	"Robust"*4/R026	PI 366449	3	6	Spring
STARS 0618B	"Robust"*4/R029	PI 366454	3	6	Spring
STARS 0619B	"Stander"*4/STARS 9301B	PI 366450	2	6	Spring
STARS 0620B	"B1202"*4/STARS 9301B	PI 366450	2	2	Spring
STARS 0621B	"B1202"*4/STARS 9577B	CIho 4165	3	2	Spring
STARS 0622B	"B1202"*4/R034	CIho 6322	3	2	Spring
STARS 0623B	"B1202"*4/R040	CIho 14679	3	2	Spring
STARS 0624B	"B1202"*4/R044	PI 564608	3	2	Spring
STARS 0625B	"Crest"*4/R021	PI 328692	3	2	Spring
STARS 0626B	"Crystal"*4/R004	CIho 4125	3	2	Spring
STARS 0627B	"Crystal"*4/R009	CIho 6925	2	2	Spring
STARS 0628B	"Crystal"*4/R023	PI 366444	2	2	Spring
STARS 0629B	"Crystal"*4/MR001	CIho 3694	4	2	Spring
STARS 0630B	"Crystal"*4/MR009	CIho 10587	4	2	Spring
STARS 0631B	"Crystal"*4/MR013	CIho 11958	4	2	Spring
STARS 0632B	"Crystal"*4/MR022	CIho 14259	4	2	Spring
STARS 0633B	"Harrington"*4/R002	CIho 2432	3	2	Spring
STARS 0634B	"Harrington"*4/R003	CIho 2436	3	2	Spring
STARS 0635B	"Harrington"*4/R029	PI 366454	2	2	Spring
STARS 0636B	"Harrington"*4/R043	PI 556966	3	2	Spring
STARS 0637B	"Bowman"*4/ STARS 9577B	CIho 4165	3	2	Spring
STARS 0638B	"Bowman"*4/R041	CIho 14806	3	2	Spring
STARS 0639B	"Bowman"*4/R043	PI 566966	3	2	Spring
STARS 0640B	"Hector"*4/ STARS 9301B	PI 366450	2	2	Spring
STARS 0641B	"Hector"*4/R003	CIho 2436	3	2	Spring
STARS 0642B	"Otis"*4/ STARS 9577B	CIho 4165	3	2	Spring
STARS 0643B	"Otis"*4/ STARS 9301B	PI 366450	2	2	Spring

[a]Rating based on Webster's scale of 1–3 (resistant), 4–6 (moderately resistant to moderately susceptible), and 7–9 (susceptible).

DIMBOA level and the total time of phloem feeding (Mayoral et al. 1996). It was concluded that DIMBOA was only part of the defenses of cereal plants to RWA. Neither DIMBOA nor DIBOA are found in cultivated barley. DIBOA has been reported in wild *Hordeum* species *H. brevisubulatum* subsp. *violaceum*, *H. bulbosum*, and *H. chilenese* and in high enough levels in *H. brevisubulatum* subsp. *violaceum* and *H. bulbosum* to be negatively correlated with Russian wheat aphid population size (Gianoli and Niemeyer 1998). *Neotyphodium*-like endophytes have been reported in accessions of perennial *Hordeum* with varying levels of antibiosis to Russian wheat aphid (Clement and Lester 1991; Kindler and Springer 1991). Endophyte-mediated resistance is the result of grass–fungus interaction, which produces metabolites such as alkaloids, which deter phytophagous insect feeding (Porter 1994). Clement et al. (1997) found that endophyte-associated resistance resulted from antibiosis or starvation. Endophyte infection of 98% for *H. brevisubulatum* subsp. *violaceum* and 62% for *H. bogdanii* has been reported (Clement et al. 1997). Resistance varies with the genotype or species of a crop relative and the *Neotyphodium*-like species or strain. *H. vulgare* does not cross well with wild species (Burd et al. 1993), but *H. vulgare* subsp. *spontaneum* does hybridize with *H. vulgare* subsp. *vulgare* and may be a bridge between wild *Hordeum* and cultivated barleys (Burd et al. 1993).

Worldwide, the Russian wheat aphid has high biotypic diversity. Both anholocyclic and holocyclic reproduction is known in the Russian wheat aphid (Kiriac et al. 1990; Puterka et al. 1992). Anholocycly, continuous parthenogenetic reproduction, enables rapid establishment of existing aphid genotypes (Puterka et al. 1993), while holocycly, parthenogenetic reproduction interrupted by an annual sexual generation, has the potential to produce new genotypes (Blackman 1985). Sexual reproduction is considered a factor in biotype development (Briggs 1965; Puterka and Peters 1989, 1990).

Biotypic variation has been documented within Russian wheat aphid populations worldwide (Puterka et al. 1992; Basky 2002; Smith et al. 2004). In 1991, Puterka et al. found unique virulence profiles for seven of eight world isolates (two isolates from France, two from the former Union of Soviet Socialist Republics, and one each from the United States, Turkey, Syria, and Jordan) based on leaf chlorosis of wheat and barley differentials. The isolates from Turkey and Syria did not differ significantly. USSR1, a Kyrgyzstan biotype, was the most virulent, and the U.S. isolate was most like the French isolate. PI366450 barley, the best performer against this world collection, was damaged only by USSR1. Random amplified polymorphic DNA-amplified by polymerase chain reaction (RAPD-PCR) cluster analysis has indicated strong similarities between the United States, South African, Mexican, French, and Turkish isolates (Puterka et al. 1993). A Hungarian isolate has been shown to differ from the original South African isolate (Basky and Jordaan 1997). From its introduction in 1986 until 2003, Russian wheat aphid was believed to be anholocyclic in the United States. The same was true for South Africa, where no new biotypes were detected 26 years after Russian wheat aphid introduction. Identification of a new biotype, RWA2, damaging to resistant wheat in Colorado in 2003 (Haley et al. 2004) and a new biotype of Russian wheat aphid in South Africa in 2007 (Tolmay et al. 2007) hinted at holocyclic reproduction in the United States and in South Africa. Holocyclic reproduction has since been confirmed in the United States (G. Puterka, pers. comm.). Three more biotypes were reported by Burd et al. (2006) and a system for naming biotypes in the United States was proposed, which designated the original Russian wheat aphid introduction as RWA1, the Colorado biotype that damages *Dn4*-resistant wheat as RWA2, and the new biotypes as RWA3, RWA4, and RWA5. RWA3 and RWA4 were collected on wheat in Texas, and RWA5 was collected on barley in Wyoming. Russian wheat aphid collections were made from 98 fields of wheat and barley east of the Rocky Mountains in Oklahoma, Texas, New Mexico, Colorado, Nebraska, and Wyoming in 2005. Only RWA1 and RWA2 were identified. RWA2 was the predominant biotype in all states but New Mexico, where RWA1 still comprised 78% of the biotype complex (Puterka et al. 2007). Three

Table 12.2 Summary of wheat and barley reactions to Russian wheat aphid biotypes

	Russian Wheat Aphid Biotypes							
Resistance Source	RWA 1[a]	RWA 2[b]	RWA 3[a]	RWA 4[a]	RWA 5[a]	RWA 6[c]	RWA 7[c]	RWA 8[c]
Dn1	S	S	S	S	S	MR	S	MR
Dn2	R	S	S	S	S	MR	MR	MR
dn3	R	S	S	S	S	S	S	MR
Dn4	R	S	S	R	R	R	MR	MR
Dn5	R	S	S	S	R	MR	MR	R
Dn6	R	S	S	R	R	R	R	R
Dn7	R	R	S	S	R	R	R	R
Dn8	S	MR	S	S	S	S	S	MR
Dn9	S	S	S	S	S	S	S	MR
Yuma	S	S	S	S	R	S	S	S
Custer	S	S	S	S	S	S	S	S
TAM 105/107	S	S	S	S	S	S	S	S
STARS 9301B[d]	R[e]	R[e]	R[e]	R[e]	R[e]	R	R	R
STARS 9577B[d]	R[e]	R[d]	R[e]	R[e]	R[e]	R	R	R

[a]Burd et al. (2006).
[b]Haley et al. (2004).
[c]Weiland et al. (2008).
[d]Barley.
[e]Puterka et al. (2006).
R, resistant; MR, moderately resistant; S, susceptible.

more biotypes, RWA6, RWA7, and RWA8, were identified by Weiland et al. (2008). RWA6 was collected on wheat in Colorado, and RWA7 and RWA8 were collected from noncultivated grasses within 4 m of a cultivated wheat field. Noncultivated grasses, which are alternate hosts for Russian wheat aphid between harvest and planting of small grains, may act as a reservoir for new aphid biotypes (Weiland et al. 2008). The differential response of wheat and barley to currently identified Russian wheat aphid biotypes in the United States is summarized in Table 12.2.

Barley resistance in STARS-9301B, STARS-9577B, STARS-0501B–STARS-0507B, and STARS-0601B–STARS-0643B has been shown to be quite durable as all lines developed for resistance to RWA1 are also resistant to RWA2–RWA5 (Puterka et al. 2006, 2007). Only STARS-9301B and STARS-9577B have been tested against RWA6–RWA8. STARS-9301B and STARS-9577B were resistant to RWA6–RWA8. STARS-9301B is resistant to many world biotypes as well.

A single dominant gene controls resistance in S13 (Robinson et al. 1992). Traditional inheritance studies indicate resistance in STARS-9301B is controlled by incompletely dominant *Rdn1* and dominant *Rdn2* with epistatic effects such that when *Rdn2* is homozygous recessive, *Rdn1* is expressed as a dominant gene, and when *Rdn1* is recessive, *Rdn2* has no effect (Mornhinweg et al. 1995a). Two major quantitative trait loci (QTLs), which explain 54% of total phenotypic variation, have been identified for Russian wheat aphid resistance in STARS-9301B (Mittal et al. 2008). One major QTL, mapped to the short arm of chromosome 1H, explained 26% of the variation, while the other mapped to chromosome 3H and explained 38% of the variation. A minor QTL was also identified, which explained 6% of the phenotypic variation and mapped to chromosome 2H. Combined analysis indicated that marker–QTL associations explained 59% of the phenotypic variation for Russian wheat aphid resistance in STARS-9301B. Lack of significant interaction between markers associated with QTL indicated no epistasis. The QTL on 1H showed dominance and could be *Rdn2*, while the QTL on 3H showed additive effects and is likely *Rdn1*.

A traditional inheritance study (Mornhinweg et al. 2002) indicated that dominant alleles at two

loci control Russian wheat aphid resistance in STARS-9577B. One was dominant and highly resistant, while the other had intermediate resistance, which was masked unless recessive alleles were present at the other locus. QTL analysis indicated two loci control of Russian wheat aphid resistance in STARS-9577B (Mittal et al. 2009). One QTL mapped to the short arm of chromosome 1H, showed additive gene action, and explained 19% of Russian wheat aphid phenotypic variation, while the other QTL showed partial dominance, explained 47% of the phenotypic variation, and mapped to the long arm of chromosome 3H. The QTL on chromosomes 1H and 3H are associated with the same molecular markers as STARS-9301B. The type of gene action at the 3H locus was similar for both lines, indicating both lines carry *Rdn1*. A difference in gene action at the 1H locus suggested different alleles for STARS-9577B and STARS-9301B. STARS-9577B also differed from STARS-9301B in that it did not have a QTL on chromosome 2H.

Transcript profiling with Affymetrix GeneChip Genome arrays showed 4086 genes differentially expressed in tolerant Sidney ("Otis"*4/STARS-9301B)and susceptible Otis barley (Gutsche et al. 2008). A total of 909 genes showed levels of change unique to Sidney, several of which are associated with plant defense and scavenging of reactive oxygen species (ROS). Fourteen genes were associated with oxidative stress, 27 with photosynthesis, 37 with signaling, 63 with defense, 24 with abiotic stress, 60 with cell maintenance, 12 with cell wall fortification, and 17 with development. Peroxidase genes were up- and downregulated in the tolerant plants, indicating peroxidases could be important in the tolerance process. Findings implied that the ability to elevate and sustain levels of ROS-scavenging enzymes along with other changes in plant metabolism could account for the survival of a tolerant genotype under high aphid pressure (Gutsche et al. 2008).

Chlorotic spotting is observed on both susceptible and resistant barley early in Russian wheat aphid infestation. Collapse of mesophyll and bundle sheath cells adjacent to Russian wheat aphid stylet sheaths is similar to hypersensitive cell death response typical of resistance to microbial pathogens. STARS-9301B resistant barley produced significantly more collapsed autofluorescent cells (CACs) than susceptible Morex (Belefant-Miller et al. 1994). CACs were observed as early as 1 day after infestation.

Calhoun et al. (1991a) and Robinson (1993) found that Russian wheat aphid feeding on Russian wheat aphid-resistant antibiotic barley genotypes decreased grain yield and spike number but less so for resistant than for susceptible barley. Even while supporting high Russian wheat aphid populations, leaves of tolerant Russian wheat aphid-resistant germplasm lines do not roll or streak, and therefore, yield reductions due to head trapping and chlorosis of susceptible cultivars do not occur (Mornhinweg et al. 2006b). Mornhinweg et al. (2006b) reported Russian wheat aphid-infested, highly resistant tolerant barley genotypes maintained or increased grain yield (5%) and yield components, while susceptible cultivars had a large reduction in grain yield (56%) and yield components. Moderately resistant genotypes were intermediate and continuous between resistant and susceptible genotypes with an average reduction in grain yield of 20%. Bregitzer et al. (2003) reported that Russian wheat aphid feeding had a devastating effect on grain yield and malting quality of susceptible barley if infestations occur early, whereas infestations around or after the boot stage resulted in a 10% reduction in grain yield, which would still be unacceptable to barley producers. STARS-9301B maintained its agronomic and malting quality characteristics even under high Russian wheat aphid populations that occurred early in crop development. Advanced breeding lines with high levels of resistance from STARS-9301B maintained agronomic and malting quality similar to susceptible recurrent parents under Russian wheat aphid infestation. Resistance transferred from STARS-9301B into adapted barley backgrounds protected agronomic performance and malting quality.

With no Russian wheat aphid resistance available in U.S. barley cultivars, chemical control was the only alternative immediately available to U.S. barley producers. Russian wheat aphid was susceptible to all common insecticides used for aphid

control, but because Russian wheat aphids are protected from contact insecticides by rolled leaves, only systemic insecticides were effective in the field (González et al. 1992). Imidacloprid systemic seed treatment (Gaucho) controlled Russian wheat aphid for 27–85 days after planting and significantly reduced damage and increased grain yield of infested barley (Pike et al. 1993). Gaucho (imidacloprid seed treatment) had acute aphid toxicity in early growth stages of barley (Pike et al. 1993), but control often required two or three insecticide applications per season (Stern and Orloff 1991). In wheat, the greatest yields were in plots treated six times with insecticides (Peairs 1990). As a seed treatment, imidacloprid with low mammalian toxicity (Elbert et al. 1990; Fluckiger et al. 1992) and innocuous to natural enemies (excluding host mortality) (Fluckiger et al. 1992; Mizell and Sconyers 1992; Baldson et al. 1993) offers environmentally safer Russian wheat aphid control than wide-spectrum aerial or in-furrow granular insecticides (Pike et al. 1993), but the cost of multiple applications is economically prohibitive especially for growers in marginal production areas where the Russian wheat aphid is often a persistent pest. Continued use of insecticides could destroy natural enemies, accelerate the development of insecticide resistance in Russian wheat aphid and other insect pests, and upset secondary pests with short-term consequences of induced pest outbreaks and long-term consequences of contamination of soil and water, wildlife losses, and with systemic insecticides, increased the risk of residual in barley end products.

Parasites and predators native to the western United States did not effectively control Russian wheat aphid (Feng et al. 1992; González et al. 1992). The diminished pest status of Russian wheat aphid in central Asia where Russian wheat aphid is endemic has been attributed in main to natural enemies (González et al. 1992). A national program was initiated to develop and implement strategies to manage Russian wheat aphid in the United States. The biocontrol component involved international, federal, and state cooperation in foreign exploration, quarantine, mass production, release, and recovery efforts (Hopper et al. 1998; Prokrym et al. 1998). Native natural enemies have adapted to parasitizing Russian wheat aphid (Kindler and Springer 1989), and exotic natural enemies have been released and recovered (Michels and Whitaker-Deerberg 1993). Nine species of parasitoids were released in 16 western states. *Diaeretiella rapae* McIntosh, both introduced and indigenous strains (Brewer et al. 1999), and *Aphelinus asychis* and *Aphelinus albipodus* Hayat and Fatima, which were introduced (Michels and Whitaker-Deerberg 1993; Brewer et al. 1999), have spread throughout the production region (Brewer et al. 2001). *A. albipodus* is fast becoming the predominant species in small grains (Brewer et al. 2001). However, the role of parasitoids in the control of Russian wheat aphids has been found to be limiting (Feng et al. 1992; Adisu et al. 2003). Host plant resistance combined with biocontrol could eliminate the need for chemical control (González et al. 1992).

Once resistant cultivars were available, the effect of plant resistance on the effectiveness of biocontrol came into question. Would plant response to aphid feeding (rolled or flat leaves) and aphid response to host plant resistance (abundance) affect the ability of parasitoids to utilize Russian wheat aphid (Reed et al. 1991; Bernal et al. 1994) *D. rapae*, a large parasitoid, was more effective parasitizing Russian wheat aphid on curled leaves of susceptible barley where Russian wheat aphid densities were high than on flat leaves of STARS-9301B where aphid densities were low (Brewer et al. 1998). *A. albipodus*, a smaller parasitoid, had the same parasitism on susceptible and resistant barley (Brewer et al. 1998). Parasitism of *D. rapae* and *A. albipodus* was correlated with seasonal abundance of Russian wheat aphid and similar on resistant and susceptible barley relative to Russian wheat aphid abundance (Brewer et al. 1999). Plant resistance and biocontrol were deemed compatible (Brewer et al. 1998, 1999).

Entomophthoralean fungi have been documented as natural enemies for Russian wheat aphid (Feng et al. 1991; Wraight et al. 1993). Specifically, Russian wheat aphid has been shown to be susceptible to several isolates of *Beauveria bassiana* (Balsamo) Vuillemin (Feng et al. 1990a)

and *Paecilomyces fumosoroseus* (Wize) Brown and Smith (Mesquita et al. 1996) at doses that are logistically and economically achievable in the field (Vandenberg 1996). These naturally occurring pathogens of aphids, including Russian wheat aphid, are commercially developed homopteran control agents (Feng et al. 1990b; Humber 1992). In Hungary, Russian wheat aphid seldom reaches pest status. High humidity and temperature in more densely planted fields (Hunkar Zemankovics 1991) are conditions favorable to the naturally occurring fungus, *Pandora neoaphidis*, which may be an important limiting factor for Russian wheat aphid population growth in Hungary (Basky and Hopper 2000). In Slovakia, *P. neoaphidis*, the predominant parasitic fungi on Russian wheat aphid, did not control Russian wheat aphid populations because the infection rate was low and did not build up on the host until high host densities had already been established (Barta and Cagán 2007). Combined biocontrol of parasitic wasp, *A. asychis*, and hyphomycete fungus, *P. fumosoroseus*, showed no detrimental effect of the fungus on the parasitoid. Although suboptimal in terms of adequate control, the wasp and fungi have potential for joint use in integrated control (Mesquita et al. 1997).

Alternate (oversummering) hosts are too numerous and too widely distributed to offer any hope in terms of Russian wheat aphid management (Peairs 1990). Several species of native and introduced grasses, including Bermuda grass (*Cynodon dactylon* [L.]), ripgut brome (*Bromus diandrus* [Roth]), rescuegrass (*Bromus catharticus* [Vahl]), and foxtail barley (*Hordeum jubatum* [L.]), will support Russian wheat aphid populations (Stern and Orloff 1991). The crested wheatgrass complex, principally *Agropyron cristatum* (L.) and *Agropyron desertorum* (Fischer ex Link) Shultes, consists of long-lived Eurasian perennials growing on 15–26 million acres in the western United States, including several million acres of idled cropland in the Conservation Reserve Program (Rogler and Lorenz 1983), a federal program that pays growers to take land out of production and plant to native grasses. Russian wheat aphid populations were low on these grasses compared to densities on spring barley, which, as volunteer in and near production fields, was a key noncultivated host of Russian wheat aphid before emergence of winter small grains in the fall (Brewer et al. 2000). Although wild grasses play a role as alternate hosts for Russian wheat aphid and their natural enemies, they may be more important for their potential role in biotype development.

GREENBUG

Greenbug is one of the world's most destructive cereal pests (Morgham et al. 1994). An important pest of cereals in North America since 1882 (Wadley 1931; Starks and Burton 1977b), greenbug was the most serious insect pest of barley in the United States until the introduction of the Russian wheat aphid in 1986 (Porter et al. 1999). Greenbug, most often found in Central, Northwest, and Southeast United States (Starks and Burton 1977b), is a chronic problem in the Southern Plains and the Pacific Northwest and in outbreak years can extend to southern Canada (Starks and Webster 1985). Greenbug was first reported in Canada in 1949 (Starks and Burton 1977b) and in Argentina in 1977 (Starks and Burton 1977a). Outbreaks in the United States occur when cool moist summers follow mild winters (Starks and Webster 1985).

Greenbug readily generates virulent biotypes colonizing new hosts and possessing enhanced resistance toward deployed control strategies (Puterka and Burton 1991). Historically, greenbug biotypes were differentiated by capability of injury and reproduction on different hosts or by pesticide resistance (Beregovoy and Starks 1986; Hesler et al. 2005). New biotypes are characterized mainly by their ability to damage host plants previously resistant to greenbug (Puterka et al. 1988). The first greenbug biotype in the United States, biotype "B," was identified in 1958 (Wood 1961). All greenbugs before 1958 are considered biotype "A" (Inayatullah et al. 1987a; Burd and Porter 2006). Biotype "B," virulent to greenbug-resistant DS 28A wheat, predominated in the Southern Great Plains (Tyler et al. 1985). Biotype "C," which attacked sorghum and small grains,

was discovered in 1968 (Harvey and Hackerott 1969). Biotype "D" was reported in 1975 (Teetes et al. 1975) and was based on pesticide resistance (Beregovoy and Starks 1986). Biotype "E" was discovered in 1979 (Porter et al. 1982). Biotypes B, C, and E, were morphologically distinct (Inayatullah et al. 1987b). Based on multivariate analysis of appendage measurements, B was more closely related to C than E. The large variability within E body measurements indicated a new biotype could evolve from E (Fargo et al. 1986; Inayatullah et al. 1987b). Biotype "F," reported in 1986 (Kindler and Spomer 1986), preferred sorghum over small grains, while E preferred small grains over sorghum (Puterka et al. 1988). Biotypes "G" and "H," whose host plant relationships were not consistent with previously described biotypes, lacked the mid-dorsal dark green stripe characteristic of B, C, and E and had the general appearance of F (Puterka et al. 1988). By 1986, biotypes A and D had not been found in the field for several years (Fargo et al. 1986). Biotype "I" was reported in 1991 (Harvey et al. 1991) and biotype "J" in 1994 (Beregovoy and Peters 1994a). Biotype J was reported to damage barley more than biotype E, whereas biotype E damaged wheat and rye more than barley (Beregovoy and Peters 1994b). Biotype J killed resistant Post barley (Beregovoy and Peters 1994a). Biotype "K" was reported in 1997 (Harvey et al. 1997).

Molecular phylogenetic analysis of biotypes A–K plus a probable isolate of A (NY), a new biotype collected from *Elymus canadensis* (L.) (CWR), and an isolate from Germany (EUR), revealed three clades. One contained the agricultural biotypes C, E, I, K, and J, found on small grains and sorghum; another contained F, G, and NY; and the third contained B, CWR, and EUR, which are rarely found on crops. H fell outside all three clades. It was suggested that greenbug biotypes are a mixture of genotypes belonging to three clades and that they may have diverged as host-adapted races on wild grasses (Shufran et al. 2000).

Michels (1986) confirmed 70 graminaceous species in 44 genera as greenbug hosts in North America. Wild grasses and volunteer wheat are vital to the maintenance of most greenbug biotypes especially in the summer when they are a bridge between wheat harvest and fall planting of the next years' crop (Daniels 1960). Eight grass species in five genera, which are grown on extensive acreage of rangeland, pasture, and roadsides in the Plains states, were all found to support greenbug and to sustain plant damage equal to or greater than cultivated wheat. Many of the grasses that host greenbugs are native grasses of the Graminae tribe, which contains the genera of wheat, barley, and sorghum. These grasses were growing and harboring greenbugs long before cultivated cereal crops were grown in the Great Plains (Kindler and Hayes 1999). Partitioning of populations by mtDNA haplotype and host suggests that the greenbug has evolved host-adapted races. Greater diversity among clones from noncultivated hosts suggests that noncultivated hosts may act as reservoirs for both genetic and biotypic diversity (Anstead et al. 2002). Noncultivated grasses are important during the sexual cycle as sexual morphs are produced and eggs are laid before emergence of fall-planted small grains (Anstead et al. 2003). Gene flow between races could allow new combinations of virulence genes (biotypes) to emerge and potentially affect cultivated crops (Anstead et al. 2002, 2003).

Genetic studies indicate that the greenbug species complex is made up of host-adapted races that have diverged on noncultivated grass species well before the advent of modern agriculture, and biotypes are composed of genetically diverse individuals among different host races that merely share virulence genes (Anstead et al. 2002). A biotype is a phenotypic expression of an infinite number of genotypes (Puterka and Peters 1990). Today, a greenbug isolate is considered a biotype when its plant response profile is unique using an established set of plant differentials including wheat, barley, rye, and sorghum (Burd and Porter 2006). In 2002, 112 greenbug isolates were collected from cultivated wheat and sorghum as well as noncultivated grasses along field margins from four states: Nebraska, Kansas, Oklahoma, and Texas. Biotypes A, C, F, and J were not detected, while B, D, E, G, H, and I were detected. Biotypes E and I were the most prevalent in all four states

(Burd and Porter 2006). Unique profiles were exhibited by 16 clones, 11 from noncultivated grasses, 3 from cultivated wheat, and 2 from cultivated sorghum. Thirteen new biotypes were identified. Both Post 90 and PI 426756 barleys were resistant to 11 of the 12 previously reported biotypes. Post 90 was susceptible to 10 of the 13 new biotypes, and PI 426756 was susceptible to 6 of the 13 new biotypes. The most virulent of the new biotypes were collected on noncultivated grasses. However, the new biotypes were not widely distributed and were not collected on more than one noncultivated host species. The wide dispersion of biotypes E and I is probably due to their wide noncultivated host range and ability to exploit cultivated wheat and sorghum. The differential response of wheat and barley to currently identified greenbug biotypes in the United States is summarized in Table 12.3.

Greenbug feeding can cause either seedling death or yield decrease depending on the intensity of infestation on susceptible cultivars (Giménez et al. 1997). Greenbugs suck sap and inject a toxin during feeding (Starks and Burton 1977a,b). A relatively small number of greenbugs can cause more damage than a much larger number of other aphids. Greenbugs induce chlorotic halos around necrotic spots usually of mature leaves (Al-Mousawi et al. 1983; Dorschner et al. 1987; Beregovoy and Peters 1994a). The effect of greenbugs is very rapid. Degenerative changes in vascular cells occur after 1 h of ingestion; 2 days after infestation, chloroplasts in mesophyll cells are altered (Al-Mousawi et al. 1983), resulting in macroscopic color changes of leaves 2–3 days after infestation (Sandström et al. 2000).

Injection of toxic saliva, not food uptake, is the primary cause of greenbug damage (Chatters and Schlehuber 1951; Morgham et al. 1994). Visible damage (chlorosis/necrosis) at the feeding site is biochemically associated with the feeding track of the aphid (Saxena and Chada 1971; Al-Mousawi et al. 1983; Morgham et al. 1994). Penetration of the greenbug stylet is intercellular, becoming intracellular when it reaches the vascular cells. Salivary pectinases may be injected by the greenbug to dissolve the middle lamellae as the aphids protrude their stylets (Morgham et al. 1994). Rapid severe organelle degeneration in phloem parenchyma cells and increase in the size and number of plastoglobuli in the chloroplast of mesophyll cells adjacent to the stylet path is similar to what occurs in senescence (Morgham et al. 1994). Glutamine, the major form of nitrogen translocated from senescent leaves to sink organs, is increased with greenbug feeding. Greenbugs induce senescence-like changes and take advantage of increased translocation from the breakdown of leaf proteins (Dorschner et al. 1987). Reductions in total chlorophyll, carbon assimilation rates, transpiration rates, and stomatal conductance (Gerloff and Ortman 1971; Ryan et al. 1987) are induced by greenbug feeding on wheat and barley. In wheat, sucrose translocation in the phloem was reduced 50%, but carbohydrate partitioning patterns to root and shoot were not altered. Inhibition of vein loading may result from a localized inactivation of the electrogenic pump system (Burd 2002). Greenbug feeding caused the amount of free amino acids in mature wheat leaves to significantly increase at the feeding site (Dorschner et al. 1987). In barley, infestation of the first leaves increased soluble amino acids in all leaves (Riedell 1989b). Greenbug saliva contains pectinases, celluloses, lipases, and proteolytic enzymes that disrupt chloroplasts and mitochondria and eventually degenerate all of the cytoplasmic contents of the cell (Al-Mousawi et al. 1983). Loss of chlorophyll and increased levels of soluble amino acids in leaves other than those infested could have resulted from injection and subsequent mobilization of the phytotoxic saliva enzymes in the vascular system of the infected plant (Riedell 1989b). Requirements of aphids are dependent in part on provisioning by bacterial symbionts (*Buchnera*), which provide some essential amino acids. The ability of aphids to increase amino acid levels in the ingested phloem sap should improve the quality of the susceptible plant as a food source (Morgham et al. 1994; Sandström et al. 2000) and affect the economy of the aphid/symbiont association, reducing the demands for symbiont provisioning (Sandström et al. 2000).

Greenbugs have specific effects on hosts that extend beyond feeding sites or sieve elements.

Table 12.3 Wheat and barley reactions to known greenbug biotypes and unique greenbug isolates collected from Kansas (KS), Oklahoma (OK), and Texas (TX)

	Greenbug Biotypes																									
Cereal Selection (R Gene)	A	B	C	E	F	G	H	I	J	K	CWR	WWG	NY	KS 1	TX 1	TX 2	TX 3	TX 4 TX 5	KS 2 TX 6	TX 7	TX 8	KS 3	OK 1	OK 2 TX 9	TX 10	OK 3
Custer	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S
DS 28 A (Gb1)	R	S	S	S	R	S	S	S	R	S	S	S	R	R	R	R	R	R	R	R	S	S	S	S	R	S
Amigo (Gb2)	—	R	R	S	S	S	S	S	R	S	R	S	R	R	S	S	S	S	S	S	S	S	R	S	R	S
CI 17882 (Gb5)	—	S	R	R	S	S	S	R	R	R	S	R	S	S	S	S	S	R	R	R	S	R	S	S	R	S
CI 17959 (Gb4)	—	S	R	R	S	S	S	R	R	R	S	R	S	S	S	S	S	R	R	R	S	R	S	S	R	R
Largo (Gb3)	—	S	R	R	S	S	R	R	R	R	S	S	S	S	S	S	S	R	R	R	S	R	S	S	R	R
GRS 1201 (Gb6)	—	R	R	R	S	R	S	R	R	R	S	R	S	S	R	R	R	R	R	R	R	R	R	S	R	S
Elbon	—	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S
Insave (Gb2, Gb6)	—	R	R	R	S	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	S
Wintermalt	—	S	S	S	S	R	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Post 90 (Rsg1)	—	R	R	R	R	R	S	R	R	R	R	R	R	R	S	S	S	S	S	S	S	R	R	S	S	S
PI 426756 (Rsg2)	—	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	S
TX 7000	—	—	S	S	—	S	—	S	—	S	S	—	S	R	S	S	S	S	S	S	S	S	S	R	R	S
TX 2737	—	—	R	S	—	S	—	S	—	S	R	—	S	R	S	R	S	S	S	S	R	S	S	S	R	S
TX 2783	—	S	R	R	S	S	—	S	—	S	S	—	S	S	S	S	S	S	R	S	S	R	S	S	R	S
PI 550607	—	R	R	R	S	R	R	R	—	S	R	—	R	R	S	R	R	S	R	R	R	R	R	R	R	R

Wintermalt, Post 90 (Rsg1), and PI 426756 (Rsg2) are barleys.

R, resistant; S, susceptible; —, indicates data not available; CWR, Canada wildrye isolate; NY, New York isolate; WWC, western wheatgrass isolate (Burd et al. 2006).

The action of greenbugs is systemic within a single leaf where they are feeding in the sieve element (Sandström et al. 2000). Greenbug feeding disturbed growth-regulating pathways. Two days after infestation, before expression of chlorosis, new leaf promordia on the main shoot apex were inhibited. This inhibition plus a shorter length of leaf lamina and sheaths reduced leaf area and total dry weight. Damage in the aerial biomass production of the seedling is a consequence of the greenbug inhibitory effect on the apical and intercalary meristems, thus altering the mechanisms that regulate growth (elongation/cell division). In the initial days of infestation, this action is independent of local leaf tissue damage, which includes destruction of photosynthetic leaf areas and results in chlorosis (Castro and Rumi 1987). Early inhibition in the seminal laterals and nodal axes occurred simultaneously with the inhibition of the main and secondary shoot apices. Greenbug feeding causes a decrease in total root biomass and density of root hairs (Gerloff and Ortman 1971; Castro et al. 1991). P-influx is reduced after 6 h of greenbug infestation and may be attributed to an alteration in the phosphate uptake system. Feeding on the leaves affects phosphate uptake by the roots (Giménez et al. 1997). Competition for metabolites between aerial parts and the root system might be one of the major restraining factors of early growth under greenbug infestation (Castro et al. 1988).

Since 1886, periodic outbreaks of greenbug have occurred in the United States, resulting in millions of dollars of damage (Hayes et al. 1999). In Texas and Oklahoma in 1942, $38 million in small grain losses were attributed to greenbugs, and $80 million in damage and control costs were reported in Oklahoma alone in 1976 (Starks and Burton 1977b). One hundred greenbugs per barley stem caused a greater than 50% reduction in the number of heads, number of kernels, and total kernel weight (Keickhefer and Kantack 1986). Infestation of 20–30 greenbugs per barley stem in the seedling stage caused significant yield loss (Keickhefer and Kantack 1986). Yield losses of 35%–60% have been reported for small grains (Riedell et al. 2007). A difference in reproductive rate of half an aphid a day may make the difference between small or mediocre yield and complete crop destruction (Painter 1958). Crop productivity in the U.S. Great Plains is often reduced by aphid infestations and aphid-vectored virus infection (BYDV) (Hoffman and Kolb 1997). Greenbugs can transmit SGV, RMV, and PAV. The greenbug is not an important vector in Europe (von Wechmar 1984) or Great Britain (Signoret 1990) but can be in South America (Araya 1990) and the United States.

Greenbug resistance in barley was first observed in the United States in 1945 (Atkins and Dahms 1945). A chance infestation of barley lines from China and Korea in the field in Texas and Oklahoma led to the screening of 577 germplasm lines from Asia. One hundred ninety-four accessions showed some level of resistance to greenbugs including the Korean landrace "Omugi" (CI5144) and "Dobaku" (CI5238) (Dahms et al. 1955). Genetic analysis indicated that a single dominant gene controlled resistance in Omugi. This gene, originally designated *Grb* (Gardenhire and Chada 1961), was later designated *Rsg1a* (Merkle et al. 1987) and has most recently been designated *Rsg1* (Porter et al. 2007). *Rsg1* was located on the centromere-bearing segment of chromosome 1 (7H) in a T1(7H)-6(H)a translocation (Gardenhire et al. 1973). Breeding for greenbug resistance resulted in the release of several resistant barley cultivars including Kearney, Kerr, Will, and, most recently in 1981, "Post 90" (Mornhinweg et al. 2004), all of which contained *Rsg1* resistance. A new source of resistance from Pakistan, PI426756, was reported in 1984 (Webster and Starks 1984). This resistance was controlled by a single dominant gene, *Rsg2b*, which was nonallelic and independent of *Rsg1* (Merkle et al. 1987). *Rsg2b* was later designated *Rsg2* (Porter et al. 2007). No cultivar has been released to date with *Rsg2* resistance. "Wintermalt" barley (Jensen et al. 1982) was reported to be resistant to greenbug biotype G but was susceptible to all other biotypes (Puterka et al. 1988; Ogecha et al. 1992). Wintermalt was later found to be resistant to biotype J as well. This differential response pattern varies from that of *Rsg1* and *Rsg2* and is an indication of a different gene for resistance in Wintermalt, which could prove

useful in the development of new greenbug-resistant barley cultivars (Porter and Mornhinweg 2004). While both *Rsg1* and *Rsg2* provide excellent protection from the major greenbug biotypes, they have a differential reaction to 13 new biotypes (Burd and Porter 2006). *Rsg2* provides broader protection than *Rsg1* (Table 12.2).

Insecticides, as well as cultural and biological control practices, can reduce or eliminate greenbug damage to infested small grains. Adverse weather conditions such as hard rains and blowing soil particles can dislodge greenbugs from plants (Starks and Webster 1985). Grazing of winter grains in the Southern Great Plains of the United States can reduce greenbug populations. Destruction of volunteer grains and wild grasses that support greenbugs between crop cycles can also reduce greenbug numbers (Starks and Burton 1977b). There are several species of natural enemies of greenbugs, such as lady beetles, parasitic wasps, and lacewings; however, these biological control agents often lag behind greenbugs in seasonal development and are too late to prevent economic loss (Starks and Burton 1977a,b; Starks and Webster 1985). Greenbugs can be controlled by low doses of organophosphate insecticide; however, these applications often kill beneficial insects as well (Starks and Webster 1985). Greenbugs have a history of developing tolerance to insecticides (biotype D on sorghum) (Starks and Burton 1977a), and greenbugs can reproduce at low temperatures where insecticides are seldom effective (Starks and Webster 1985). Considering the high economic and environmental cost of insecticides, greenbug-resistant cultivars and deployment of simply inherited resistance genes are considered the most economical solution to controlling greenbugs (Hayes et al. 1999). It is, however, a challenge for plant breeders to stay ahead of biotype changes in greenbug-resistant cultivar development.

BIRD CHERRY-OAT APHID

The bird cherry-oat aphid is often considered problematic only through its ability to vector BYDV, but feeding damage can cause a substantial yield loss up to 50% in the absence of BYDV (Stern 1971; Leather et al. 1989; Porter et al. 1999; Riedell et al. 1999). The bird cherry-oat aphid, first found in North America in 1855 (Simon and Hebert 1995), is considered one of the most serious pests of cereals in North America (Porter et al. 1999) and in Europe (Aspinall 1961; Kolbe 1969, 1970; Dewar et al. 1984; Weibull 1987; Porter et al. 1999) and is the most frequent species of cereal aphid in New Zealand (Farrell and Stufkens 1989). Although in Great Britain the bird cherry-oat aphid is a pest mainly because of its ability to vector BYDV, in Scandinavia, Poland, and Czech Republic, the bird cherry-oat aphid is a major pest in its own right (Leather et al. 1989). The bird cherry-oat aphid shows preference for barley compared to other cereals (Leather et al. 1989; Starý 1996).

The bird cherry-oat aphid feeds in phloem and nonphloem tissues such as mesophyll parenchyma (Casaretto and Corcuera 1998). Because bird cherry-oat aphid feeding is asymptomatic (no visible leaf damage symptoms) (Webster et al. 1987; Züst et al. 2008), it is believed that this aphid species does not inject toxic salivary substances during feeding (Riedell et al. 2007). Bird cherry-oat aphid infestations of barley can cause direct feeding damage as well as transmission of BYDV (Hsu and Robinson 1962; Mallott and Davy 1978). Subjecting barley to levels of bird cherry-oat aphid infestation higher than those normally found on cereals in the field resulted in considerable reduction in the number of leaves, number of tillers, living leaf area, dry weight, and mean relative growth rate. Decreased growth of barley (Mallott and Davy 1978; Ni and Quisenberry 2006) was due to cumulative assimilate loss from aphid feeding (Mallott and Davy 1978). Reductions also occur in roots in response to bird cherry-oat aphid feeding.

BYDV, the most important viral disease of small grain cereals in the world (Henry and Dedryver 1991), is a complex of viruses that differ in several respects including aphid transmission specificity (von Wechmar 1984). BYDV is transmitted persistently by more than 25 species of aphids (Smyrnioudis et al. 2001) and has nearly 100 host species among Gramineae

(Starks and Webster 1985). The bird cherry-oat aphid is the most effective aphid vector of BYDV (Starks and Webster 1985). Aphid biotypes interact with virus strains. Phloem contact is required for the acquisition of any luteovirus by an aphid (Scheller and Shukle 1986). Once the aphids have acquired BYDV from infected grasses, they may remain viruliferous for life (Rochow 1959). The probability of an epidemic developing depends upon many factors, including aphid transmission characteristics of the isolates present, the intensity of vector flight, and the proportion of viruliferous individuals (Halbert et al. 1992). The duration of access periods and the availability of virus in the source plants are two factors that influence the transmission of BYDV by its aphid vectors (Gray et al. 1991). Gray et al. (1991) reported that although 2.5% of bird cherry-oat aphids acquired PAV within a 15-min acquisition access period (AAP), 1- to 2- or 2- to 3-h AAP was required for 50% of bird cherry-oat aphids to transmit the PAV or RPV strain of BYDV, respectively. The transmission efficiency of various BYDV isolates is differentially influenced by several factors, including aphid vector, length of acquisition feeding period, and physiological age of source tissue. Virus titer as it is affected by age and infection stage of the source tissue can have a strong influence on acquisition and transmission efficiency (Gray et al. 1991). The rate at which the virus can be transported across the aphid hindgut membrane is also a limiting factor in virus acquisition. Acquisition efficiency may depend more on the length of time the aphid spends in the phloem acquiring the virus than on the amount of virus in the sap. A more effective disease control strategy than breeding for reduced virus titer, or one to be used in conjunction with reduced virus titer, would be to reduce the ability or the desire of the aphid to feed on the plant for extended periods of time. Reducing the acquisition access time would reduce the level of virus in the aphid vector and the probability that the aphid would transmit the virus to additional host plants (Gray et al. 1991). Low temperatures reduce transmission efficiency (Guo and Moreau 1996; Lowles et al. 1996).

The three mechanisms of resistance to insects as defined by Painter (Painter 1958) include (i) nonpreference, or antixenosis, in which insects avoid the plant; (ii) antibiosis, in which the plant biology adversely affects the insect biology; and (iii) tolerance, in which the plant is able to support insect populations that would damage a susceptible plant. A total of 474 barleys from the Canadian Genetic Stock of Barley were tested over a period of years for bird cherry-oat aphid resistance in the greenhouse. Promising lines were also tested in the field. No line exhibited antixenosis. Forty-three showed both antibiosis and tolerance in the greenhouse and in the field and were considered as possible source material for resistance breeding (Hsu and Robinson 1962, 1963). Resistance in these studies was measured as the effect of the plant on the aphid, not the aphid on the plant. Whether the resistance reported would translate to useful resistance in terms of grain yield has yet to be determined.

Due to asymptomatic bird cherry-oat aphid damage to seedlings, traditional seedling screening techniques with visual rating scales have not been successful. A greenhouse seedling screening technique and visual rating scale for bird cherry-oat aphid has been reported (Mornhinweg 2008). Although susceptible and resistant checks have been identified and differences in seedling survival have been shown, the rating scale has yet to be proven accurate to predict resistance in terms of grain yield in the field.

Infestation of barley by the bird cherry-oat aphid is inversely correlated with the amount of leaf surface wax (Tsumuki et al. 1989). There are qualitative and quantitative differences in wax composition between susceptible and resistant cultivars (Tsumuki et al. 1987).

Indole alkaloids in artificial diets have been reported to have deleterious effects on aphids (Corcuera 1984). Gramine, the only indole alkaloid in barley, has been reported to modify the feeding behavior of bird cherry-oat aphids on artificial diets (Zúñiga et al. 1988). Salivation and the number of probes increased and ingestion time decreased (Zuniga et al. 1988; Kawada and Lohar 1989). Gramine has been identified in the epidermis and parenchyma cells of resistant

barley leaves (Argandoña et al. 1987) and not in the vascular bundles. Since the bird cherry-oat aphid feeds in the mesophyll parenchyma as well as in the phloem, they suggested that the defensive role of gramine may be a result of its toxic and feeding deterrent properties, its location and concentration in the plant, and the feeding behavior of the bird cherry-oat aphid. Gramine appears to be a major factor in the resistance of some barley cultivars to aphids (Zuniga et al. 1988; Kawada and Lohar 1989; Rustamani et al. 1992).

Proteinase inhibitors, proteins that form complexes with proteases inhibiting their proteolytic activity, are found in plant seeds, embryos, roots, and leaves (Weiel and Hapner 1976). These proteins are wound inducible and have been shown to effectively decrease leaf consumption by chewing insects (Pearce et al. 1991; Suh et al. 1991). The existence of proteinase inhibitors in barley has been documented (Mikola and Suolinna 1969; Boisen 1983). These inhibitors act mainly against trypsin, chymotripsin, and some microbial proteases (Boisen et al. 1981). Infestation of barley with bird cherry-oat aphids resulted in accumulation of chymotripsin inhibitors, which decreased the survival of bird cherry-oat aphids reared on artificial diets (Casaretto and Corcuera 1998). The role of proteinase inhibitors in barley leaves as bird cherry-oat aphid resistance factors needs to be further explored.

Chitinase and B-1,3-glucanase, two families of pathogenesis-related (PR) proteins induced by fungal pathogens in barley, were also induced in barley by bird cherry-oat aphid infestation (Forslund et al. 2000). These inductions were stronger in resistant plants, indicating a possible role in defense against the bird cherry-oat aphid. The inductions were not due to wounding caused by penetration only. A mechanism for the function of chitinase and B-1,3-glucanase in defense against aphids remains to be shown. A possible role for B-1,3-glucanase action is the release of oligosaccharides from the plant cell wall, which are known to trigger other defense reactions in plants.

High levels of resistance to bird cherry-oat aphids have been reported in many wild *Hordeum* species (Weibull 1987). Resistance in *H. vulgare* subsp. *spontaneum* showed continuous segregation in the F2, indicating multiple gene control with possible additive effects (Weibull 1994). A screening of 27 *Hordeum* species and interspecific hybrids showed large interspecific variation for resistance to bird cherry-oat aphids. Wild barleys had low aphid numbers compared to *H. vulgare* (Weibull 1987). *H. chilense* has been reported resistant to bird cherry-oat aphid (Clement and Lester 1991). The highest resistance was in wild species most distantly related to *H. vulgare*, and crossing incompatibility can be expected (Weibull 1988). DIBOA occurs in wild barleys and its concentration correlated negatively with the fecundity of the bird cherry-oat aphid (Barria et al. 1992; Gianoli and Niemeyer 1998). DIMBOA, a hydroxamic acid, plays a major role in aphid resistance in wheat and maize. It is toxic, negatively correlated with growth of aphids and aphid populations, and alters feeding behavior on plants and in artificial diets (Barria et al. 1992). Hydroxamic acids have not been found in cultivated barleys (Zuniga et al. 1988), but DIBOA has been found in wild barleys. DIBOA is toxic to bird cherry-oat aphids in artificial diets, and DIBOA levels are negatively correlated with the performance of bird cherry-oat aphids on wild *Hordeum* species.

Weibull (1988) reported that resistance in wild barleys was related to nutritional factors such as concentration of free amino acids in the phloem. Fifty-five to eighty-five percent of the variation in aphid resistance among wild species could be explained by the composition of individual amino acids in the phloem sampled from cut aphid stylets. Resistant plants had more glutamic acid and aspartic acid and less arginine, phenylalanine, isoleucine, leucine, and lysine. They also found that free amino acids in exudates from cut leaves or petioles corresponded to pure sap sample from the cut aphid stylets and suggested this sampling would allow for a more rapid selection of plants for resistance.

Barley cultivars with resistance to bird cherry-oat aphids have not been developed in the United States. Breeding efforts have concentrated on BYDV resistance. Tolerance is available but is unstable because of the many viral strains that make up BYDV (Zwiener et al. 2005). As a result, environmentally and economically costly chemi-

cal control is the only option for U.S. barley producers. Bird cherry-oat aphids can feed on winter barley in the fall and spring. Because aviruliferous bird cherry-oat aphid feeding is asymptomatic, the effect of decreased plant and root growth on winter survival with heavy fall infestations is often not recognized. Bird cherry-oat aphids are generally not controlled unless BYDV is being transmitted by the aphids. The bird cherry-oat aphid is relatively easy to control with chemicals, but economic injury thresholds and management strategies vary between states and regions. Imidacloprid, a nitroguanidine with both contact and systemic properties, when applied as a seed treatment, can provide protection from sowing until well into the growing period. Unlike other aphidcides, which may cause viruliferous aphids to be restless and disperse to other plants by spreading BYDV, imidacloprid interrupted feeding and caused rapid paralysis of aphid vectors sufficient to significantly lower the incidence of disease (Gourmet et al. 1994; Gray et al. 1996). In Kenya, where yield losses to bird cherry-oat aphid and BYDV can be devastating, imidacloprid treatment alone increased barley yields 36%–43%, and the incidence of BYDV was reduced to 25% compared to 60%–70% with spraying and 80% when untreated (Wangai et al. 2000).

Semiochemicals play a significant role in the behavioral ecology of bird cherry-oat aphids (Ninkovic et al. 2003). Antifeedants, which interfere with the process of host plant selection and feeding behavior, have been suggested for aphid control. Antifeedants such as azadirachtin triterpenoid, which reduced bird cherry-oat aphid probing activity on topically and systemically treated barley seedlings (West and Mordue (Luntz) 1992), may have important implications for reducing the spread of plant viruses as well. Semiochemical methyl salicylate, which is produced by the winter host of bird cherry-oat aphid as a plant response to aphid feeding and plays a role in spring migration, acting as a take-off stimulus from *Prunus padus*, and three density-related substances (DRSs), volatile compounds released at the feeding site when a certain density threshold is exceeded, applied to barley plants in the field caused a significant delay in aphid establishment in the crop and reduced average infestation from 25% to 50% compared to untreated controls (Ninkovic et al. 2003).

The bird cherry-oat aphid has a variety of parasites and predators as well as entomopathogenic fungi that act as biological control agents and that can play a significant role in maintaining low aphid populations. In one laboratory study, fewer barley plants exposed to the parasitiod *Aphidius rhopalosiphi* were infected with BYDV than those exposed to the predator *Coccinella septempunctata* Linneaus (Smyrnioudis et al. 2001). The effect of biocontrol of bird cherry-oat aphids on virus spread in the field needs to be studied. In Sweden, ground-living natural enemies reduced the abundance of the bird cherry-oat aphid and increased barley yields by 23% in 10 commercial farms, equivalent to yield increases from insecticide applications (Quiroz et al. 1997).

Cultural practices can have an effect on bird cherry-oat aphid infestation severity. There has been a trend toward conservation tillage in the United States over the past 20 years. Bird cherry-oat aphids typically infest the stems of small grains (Wiktelius 1987). Conservation tillage leads to greater infestations of spring small grains as increased surface residue provides a favorable microclimate for bird cherry-oat aphids (Hesler and Berg 2003).

HESSIAN FLY

Hessian fly (HF), *M. destructor* (Say), has existed in Europe for many centuries and is widely distributed throughout cereal-growing regions of Europe, North Africa, Asia, New Zealand, and North America (Fig. 12.4). The Hessian fly is thought to be endemic to the southern Caucasus and Southwest Asia (Harlan and Zohary 1966) and to have dispersed to North Africa and Europe (Ratcliffe and Hatchett 1997). First noted in the United States on Long Island, New York, in 1779, it is generally believed that Hessian fly was introduced to the United States in straw used for bedding of Hessian soldiers fighting in the Revolutionary War. The possibility of additional

introductions during the colonization of North America by Europeans, specifically the Spanish in California, has been suggested (Johnson et al. 2004). By 1871, the fly was reported as far west as Kansas. The Hessian fly occurs in all major cereal-growing regions of the United States where widespread outbreaks can occur periodically but local outbreaks can cause extensive crop losses in wheat almost every year (Ratcliffe 2007).

Barley is damaged by the Hessian fly in North America, but damage is generally not as severe and widespread as in wheat (Starks and Webster 1985). During a severe outbreak in Georgia in the 1989–1990 growing season, spring infestations of winter barley decreased grain yield when Hessian fly infestation exceeded one immature per culm. Yield loss was attributed to a reduction in spikelets per spike (Buntin and Raymer 1992). Extensive damage to barley has been reported in Northern Africa (Olembo et al. 1966). In New Zealand, barley cultivars and breeding lines were found to be less attractive to ovipositing females than wheat, but all barleys were good hosts for Hessian fly larvae and appeared to support growth and survival similar to that of larvae on wheat (Harris et al. 1996). Wheat is the preferred host of Hessian fly, but barley, rye, and triticale are also periodically infested. Ovipositional preferences exhibited by the female Hessian fly toward grasses may be explained by quantitative differences in the amounts of the active foliar chemicals (Foster and Harris 1992). Wild grasses may serve as hosts when cereal crops are not available (Jones 1936; Hill et al. 1952; Harris et al. 1996). Fifteen native wild species of *Hordeum* have been identified as hosts of the Hessian fly. Little barley, *Hordeum pusillum*, with susceptibility to Hessian fly, wide distribution throughout the cereal production areas of the United States, and life habits similar to winter wheat, is a host capable of carrying over Hessian fly populations, which will later infest fall cereals (Jones 1936).

Biotype formation is common in the Hessian fly. Deployment of wheat cultivars with single genes expressing high levels of antibiosis has exerted strong selection pressure on Hessian fly populations, leading to the selection of more virulent biotypes capable of surviving and reproducing on resistant wheat (Gallun et al. 1961; Gallun and Kush 1980). From the 1970s through the 1980s, 16 Hessian fly biotypes, "GP" (Great Plains) and "A"–"O" (Ratcliffe et al. 1994), were identified in the United States using differential responses to wheat genes *H3*, *H6*, *H7*, and *H8* (Ratcliffe 2007). GP was believed to have been the predominant biotype in Hessian fly populations prior to the release of resistant cultivars and the subsequent selection for virulence to resistance genes. The most virulent U.S. biotype, "L," is the predominant biotype in the Midwest and eastern United States (Ratcliffe 2007). More recent evaluations have used all available Hessian fly resistance genes in wheat (*H3–H32*) to evaluate biotype distribution. Biotypes able to overcome each resistant gene have been discovered in Kansas, Oklahoma, and Texas (Chen et al. 2009). The Syrian Hessian fly biotype has been found to be the most virulent worldwide (El Bouhssini et al. 2008).

Adults emerge in the fall from infested stubble or volunteer small grains. Although the majority of Hessian fly flights are short, moving from one plant to the next, they can take longer flights after a series of short ones to move beyond a localized area (Harris et al. 2003) or be carried 3–8 km by the wind (McColloch 1923). Female Hessian flies lay 200–300 eggs on the upper leaf surface of young leaves and die within a few days of emergence. Eggs hatch within 3–10 days, and larvae crawl behind leaf sheaths and migrate to the crown. Only the first instar is mobile. If the plant is not suitable, the larvae cannot move to another plant. In a compatible interaction, larvae inject salivary substances into epidermal cells and epidermal and mesophyll cells become nutritive. Nutritive tissues become sinks benefiting the growth of larvae by importing photoassimilates (Harris et al. 2006). The Hessian fly is classified as a gall inducer because of similarities between nutritive tissue at the feeding sites and nutritive tissues inside macroscopic galls (Harris et al. 2003). The Hessian fly causes little injury to host plants, making only two small holes with their mouthparts (Hatchett et al. 1990; Harris et al. 1996), yet host plants become permanently and irreversibly stunted after 4 days of feeding by a

single first instar larvae (Byers and Gallun 1972). Feeding occurs during two instars for a total of 10–14 days with the greatest intake occurring on the fifth day before larvae molt to the second instar. When the second instar completes its growth, the outer skin hardens and forms a protective puparium (flaxseed stage) in which the third instar and pupa subsequently develop. Hessian flies overwinter as third-instar larvae. Pupae complete development in the spring and adults emerge and infest cereals at the jointing stage. Spring generation larvae, found just above the nodes under the leaf sheath, oversummer in puparia in dry stubble. In northern U.S. cereal production areas, there are usually two generations of Hessian fly per year, one in the spring and one in the fall. In southern areas, more broods may develop either before or after the main fall or spring generations. As many as five broods have been reported in Kansas in a 12-month period (Starks and Webster 1985). Many biotic and abiotic factors affect the abundance and destructiveness of the Hessian fly.

Feeding injury is caused entirely by the larval stage of the Hessian fly. Only one individual Hessian fly per plant can have a severe negative impact on crop production (Starks and Webster 1985). On young plants, larvae feed beneath the leaf sheath at the base of the plant. Successful establishment of larvae on susceptible plants is associated with a wide range of plant developmental changes, including increases in leaf sheath cell permeability (Shukle et al. 1992), formation of nutritive tissue around the feeding sites (Harris et al. 2003), plant stunting (Cartwright et al. 1959), and accumulation of chloroplasts (Robinson et al. 1960). The salivary glands of Hessian fly larvae are specialized tissues for synthesis of proteins for host injection (Chen et al. 2008). The Hessian fly has highly specialized mandibles that inject salivary fluids into the plants (Hatchett et al. 1990). These secretions are believed to contain enzymes that inhibit plant growth and increase cell wall permeability, which allow larvae to suck juices from the plant. It has been suggested that Hessian fly larvae may take advantage of wheat defense protein Hfr-2 and use it against the plant to obtain ions and water, and may possibly cause plant cell lysis to spill all cellular contents, making them available to developing larvae (Puthoof et al. 2005). Hessian fly feeding reduces the number of tillers and leaves as well as the weight of leaves and roots (Ratcliffe 2007). Leaves of infested plants are more erect, shorter, and darker green than leaves of noninfested plants. In fall-planted winter barley, larvae can kill tillers and young plants or severely stunt growth (Starks and Webster 1985). When infestations are severe, stunting occurs early and young plants can die after larvae have matured. At lower infestation levels, seedlings can develop new tillers that allow the plant to survive despite the loss of the main stem (Ratcliffe 2007). Larval feeding in the fall on winter barley can reduce winter hardiness (Starks and Webster 1985). In the spring, when plants are jointing, larvae feed just above the internode between the leaf sheath and stem. Feeding injury prevents normal elongation of internodes and transport of nutrients to the developing spike, reducing grain quantity and quality. Grain yield is further reduced when infested culms break at weakened nodes prior to harvest.

Hessian fly resistance in wheat, expressed as larval antibiosis, is controlled by dominant alleles at one or two loci, while the virulence of the Hessian fly, expressed as the ability of the larvae to survive on and stunt plants, is controlled by recessive alleles at a single loci in a gene-for-gene relationship with resistance (Hatchett and Gallun 1970; Gallun 1978; Formusoh et al. 1996; Zantoko and Shukle 1997). Resistant wheat limited the ability of avirulent larvae to elicit generalized changes in cell permeability necessary for larvae feeding (Shukle et al. 1992). Avirulent larvae elicited localized cell necrosis similar to that associated with hypersensitivity to pathogens. If hypersensitivity is the phenotype of resistance in the Hessian fly, genes for resistance in this insect–plant interaction should be genes for recognition, and DNA rearrangements at avirulence loci in the insect would be associated with biotype change from avirulence to virulence on wheat cultivars containing genes for resistance (Shukle et al. 1992). Protein encoded by the Hfr-3 gene of wheat has been implicated in Hessian fly resistance targeting the peritrophic matrix (PM) of the larval

midgut and contributing to avirulent larval mortality by starvation (Giovanni et al. 2007). The resistance mechanism is likely a network of inducible responses believed to be initiated by R gene-mediated recognition of larval salivary elicitors delivered to the plant during the early stages of the interaction (Giovanni et al. 2007).

In 1916, "Tennessee Winter" and "Michigan Winter" were the first barley varieties identified as resistant to the Hessian fly (McColloch and Salmon 1918). Fewer eggs were laid on them than wheat, but puparia developed on both. Over 5000 barley accessions in the National Small Grains Collection were screened for Hessian fly resistance over a period from 1945 to 1950 (Hill et al. 1952). Seven accessions were identified as resistant and 26 as moderately resistant. Nearly all the resistant varieties came from North Africa, with the greatest number from Egypt. Genetic analysis of three highly resistant barley cultivars, Delta, Nile, and Abusir, indicated two independent dominant genes, *Hf1* and *Hf2*, control resistance to the Hessian fly in barley (Olembo et al. 1966). These three barleys were resistant to all four biotypes of the Hessian fly identified on wheat at that time. Resistance was due to antibiosis, which killed the first instars. Cultivars with this resistance have been developed (Buntin and Raymer 1992) but have not been released to the barley industry because of undesirable agronomic traits (Starks and Webster 1985). Ovipositional nonpreference was suggested to play a role in Hessian fly resistance in "Anson" barley (Buntin and Raymer 1992). Both low egg counts on tillering plants and very low or nonexistent larval populations were reported on "Baronesse" spring feed barley (Clement et al. 2003). The genetic basis for this resistance is unknown. A total of 128 barleys from the 1997 Western Spring Barley Nursery, the 1997 Mississippi Valley Barley Nursery, and the 1997 Advanced Two-Rowed Barley Yield Trials of USDA-ARS were screened for resistance to Hessian fly biotype GP. Ten lines, including Baronesse, had 100% resistance, indicating that one or more genes for the Hessian fly are present in these selections (R.H. Ratcliffe, pers. comm.). Three New Zealand barley cultivars, "Gwylan," "WBPS 316/80," and "Fleet," have been reported to have resistance to biotype L and heterogeneous populations of the Moroccan Hessian fly. Resistance was mainly antibiotic, resulting in first instar mortality, but small numbers of larvae developed normally on resistant plants, indicating tolerance may also be involved (Lhaloui et al. 1996).

Neotyphodium (Clavicipitaceae: Balansieae) fungal endophyte in wild barley *H. brevisubulatum* subsp. *violaceum* and *H. bogdanii* conferred resistance to the Hessian fly (Clement et al. 2005). Its potential use for resistance in cultivated cereals is limited due to the potential for accumulation in seeds of metabolites toxic to humans and or livestock and due to potential incompatibility between modern cereal cultivars and natural or genetically modified endophyte strains (Clement et al. 1994).

Because economic infestations depend upon many unpredictable factors, preventive measures such as resistant cultivars, delayed planting of winter cereals after the fly-free date, and destruction of volunteer are preferred for Hessian fly control. Systemic insecticides applied at planting can provide some control. Systemic granular insecticides applied in-furrow at planting can effectively control autumn infestation of the Hessian fly in susceptible wheat (Brown 1960; Buntin 1990). In-furrow injection of liquid insecticides using a microtube injection system is more environmentally desirable and also effective for fall infestations (Buntin 1992). Insecticides are not effective against spring infestations (Buntin and Chapin 1990; Wilde et al. 2001). Environmentally and economically costly insecticides are only applied when heavy infestations are likely.

All three preventative measures are recommended for Hessian fly control in the northern Great Plains. Increased damage on wheat in eastern Washington and northern Idaho has been associated with increased acreage of spring cereals and increased adoption of conservation tillage (Ratcliffe et al. 2000). Delayed planting is less effective in the Southern Plains where adult oviposition and larval development can occur through mild winters (Buntin and Chapin 1990). Destruction of volunteer grains and tillage to

bury stubble are the most effective cultural controls in the Southern Plains.

Hymenopterous parasitoids of the Hessian fly have been documented as early as 1817 (Gahan 1933) and parasitism has long been suggested as a potential means of regulating Hessian fly populations (Packard 1928). The failure of Hessian fly larvae to elicit an indirect defensive response (volatiles) from their host plants may help explain why natural enemies, which often rely on induced volatile cues, fail to inflict significant mortality on Hessian fly populations in the field (Tooker and de Moraes 2007). Natural enemies do not appear to be significant sources of mortality in their introduced range of North America (Tooker and de Moraes 2007). Because parasitism is often at the end of the cropping season, parasites can contribute to reduction of populations for the following season but offer no protection for the current crop (Ratcliffe 2007).

CEREAL LEAF BEETLE

The cereal leaf beetle, *O. melanopus* (L.), most likely of Eurasian origin, is a chrysomelid pest of small grains throughout Europe, Asia, Canada, and the United States (Fig. 12.2). Cereal leaf beetles feed only on cereals and related grasses with spring barley, oats, and wheat as preferred hosts (Herbert et al. 2007). Damaging populations can also occur in early spring on fall-planted wheat, barley, and rye. Spring barley, spring wheat, and winter wheat were shown to provide the same nutritive quality to cereal leaf beetle (Hennecke 1987). A recent introduction in the United States, the cereal leaf beetle was first detected in southwestern Michigan in 1962 and by the mid-1980s was a potential pest of most barley-growing regions east of the Mississippi River. In 1984, the first major infestations west of the Mississippi occurred on irrigated spring barley fields in Utah (Starks and Webster 1985). The cereal leaf beetle has been a common pest in Montana since 1989 (Tharp et al. 2000) and has also been reported in Idaho, Washington, and Wyoming (Herbert et al. 2007). The cereal leaf beetle was first discovered in Ontario, Canada, in 1965 and spread northeast to Prince Edward Island by 1994. Cereal leaf beetles are expected to spread slowly by natural means throughout Canada (LeSage et al. 2007).

The cereal leaf beetle, which has only one generation per year, overwinters as an adult in small grain stubble, along fence rows, in leaf sheaths and ears of standing corn, and under the loose bark of trees. Adults become active during warm days (10°C) in the spring (March through mid-April in the east and April through June in the west) and are found on grasses and winter grains. Winter barley often escapes heavy damage because of abundant vegetative growth by the time the beetles become active in the spring (Webster and Smith 1983). The natural spread of cereal leaf beetles occurs in the adult stage as beetles are winged. Cereal leaf beetles colonize spring small grains and after feeding and mating, they oviposit on these hosts. Eggs hatch in about 5 days and larvae develop in 10–12 days under ideal temperatures. Peak larval populations occur in mid-April to May in the east and June in the west. Larvae are usually covered with a globule of mucus and fecal matter. At the end of the fourth instar, larvae pupate in the soil at a depth of 2–5 cm in the cells of soil and mucuslike substances. Pupae become adults in 20–25 days, and summer adults emerge in late May and June in the east and July in the west. Newly emerged beetles feed on native grasses, corn, and late-planted spring cereals. After about 3 weeks of feeding, they seek overwintering sites.

In barley, larvae cause most of the damage by feeding on the leaf epidermis. Larvae, which can reach high numbers in small grains, eat long strips of green tissue from between leaf veins and may skeletonize entire leaves, leaving only the transparent lower leaf surface tissue (Herbert et al. 2007). Heavily infested fields resemble frost-damaged fields because leaves are almost translucent. The time of peak larval feeding occurs during April and May in the eastern United States and late May through June in the west. Although adults will feed on young small grain plants, eating narrow channels through the entire leaf (Starks and Webster 1985), their feeding

seldom affects crop performance (Herbert et al. 2007). Small larvae eat a small amount, but large larvae have voracious appetites. When larvae are large and temperatures are warm, severe damage can occur in as little as 5 days. Leaf feeding limits reproductive growth and reduces the plant's ability to photoassimilate and fill kernels especially if the upper leaves are destroyed. Yield reductions of 45% have been reported when defoliation occurs early in the heading period (Herbert et al. 2007). Damage later in grain filling does not have as great an impact.

Grain yield losses of 10%–20% are typical in the eastern United States (Herbert et al. 2007), with losses of 38%–56% (Webster and Smith 1979) and 50% (Brook and Dewar 1977) also reported in spring barley in the eastern and western United States, respectively.

In 1963, 172 barley cultivars were field evaluated for resistance to cereal leaf beetle adult and larvae. Only 6% showed some resistance (Hsu and Robinson 1962). Screening of 8634 barley accessions from the National Small Grains Collection (NSGC) identified only 1% as resistant to larval feeding damage (Gallun et al. 1964). Resistance to the cereal leaf beetle was reported in barley accessions CI6469 and CI6671 (Schillinger 1966). Resistance in these accessions, based on larval counts and feeding damage scores, was reported to be recessive and complex (Hahn 1968; Lee 1970). The mechanism of resistance was nonpreference for both oviposition and larval feeding (Hahn 1968). Nonpreference for oviposition as one component of cereal leaf beetle resistance in spring barley has also been reported by Lee (1970). Transgressive segregation was found in a cross of CI6469 and CI6671, which indicated the possibility of obtaining a higher level of resistance (Hahn 1968). These accessions were not adapted to U.S. barley production systems and thus were used as parents to produce two improved, six-rowed, cereal leaf beetle-resistant spring barley germplasms, CI15820 and CI15821, with more desirable agronomic traits (Smith et al. 1984). These germplasm lines, jointly released by USDA-ARS and the Michigan Agricultural Experiment Station in 1984, had considerably lower egg and larval counts and larval damage ratings than a susceptible check. No cereal leaf beetle-resistant cultivars have been made available to producers (Porter et al. 1999).

In the United States, throughout much of its eastern range, a complex of three larval parasitiod species (*Tetrastichus julis*, *Diaparsis temporalis*, and *Lemophagus curtus*) and one egg parasitoid species (*Anaphes flavipes*) introduced from Europe (Dysart et al. 1973) and established in 1973 (Haynes and Gage 1981) control the cereal leaf beetle. In some western, southeastern, and mid-Atlantic states, damaging populations of cereal leaf beetle still occur where these parasites have not been successfully established or where cultural practices are not favorable to the parasites. Reduced tillage may aid cereal leaf beetle management by enhancing parasite survival.

In the west, intense rainfall and sprinkle irrigation during egg hatch can reduce the larval populations such that further control is unnecessary (Herbert et al. 2007). Late-planted, thinly sown fields are more attractive to cereal leaf beetle for egg oviposition in the spring. Following good agronomic practices for high yield small grain production reduces the likelihood and impact of cereal leaf beetles.

A timely, single application of several insecticides registered for use on small grains can effectively control cereal leaf beetles. The most effective treatments are timed to coincide with the presence of eggs and small larvae (commonly when one larva per flag leaf is observed on maturing plants or a combination of three eggs or larvae on less developed plants) (Starks and Webster 1985). Imidacloprid applications in spring barley resulted in 40% mortality of cereal leaf beetle larvae when applied as a seed treatment and greater than 90% mortality when applied as a foliar spray (Tharp et al. 2000).

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Chapter 13
The Development, Structure, and Composition of the Barley Grain

Sabine Gubatz and Peter R. Shewry

INTRODUCTION

The Food and Agriculture Organization (FAO) figures for 2007 show that over 136 million tons of barley (*Hordeum vulgare* subsp. *vulgare* L.) were produced worldwide, being grown on about 56.6 million hectares with a mean yield of about 2.4t/ha (http://faostat.fao.org/default.aspx). Although this places it only fourth in world cereal production, well below wheat, rice, and maize, it is the major staple crop and a significant source of food for the populations of many countries, and is widely used for livestock feed and for malting, brewing, and distilling in other countries.

The quality of the grain for these three major end uses, as food, feed, and raw material for processing, is determined by the structure and composition of the mature grain. Consequently, many accounts of barley focus on the mature grain and within this on the major groups of components that determine end use properties: starch, proteins, and cell wall polysaccharides. However, it is also necessary to understand the structure of the grain and how it develops if we are to understand its composition and properties and wish to manipulate these in the future.

Barley breeding programs in developed countries have traditionally focused on two end uses, feed and malting, which differ significantly in their requirements. These are discussed in more detail in other chapters in this volume, with the present account describing the development, structure, and composition of a "typical" barley grain.

Barley: Production, Improvement, and Uses. Edited by Steven E. Ullrich

DEVELOPMENT AND STRUCTURE

Prefertilization development

General remarks

The barley grain is a dry, nondehiscent fruit with the pericarp surrounding a single seed in the anatropous ovule. At ripeness, the outermost layer of the seed, the seedcoat, is fused with the pericarp. This defines the fruit of barley as a caryopsis. The ripe caryopsis, which is tightly enclosed by the lemma and palea, represents the diaspore (von Bothmer et al. 1991).

Pre- and postfertilization events similarly determine the structure and appearance of the ripe barley grain. Some fundamental morphological features are established before the embryo and endosperm originate. However, the developing grain changes continuously, and any description of this dynamic system is only valid in its known temporal context. Tissues are generated or die, acquire or cease specialized functions, accumulate reserves or deplete them, adapt to growth, or collapse. Events between the initiation of the inflorescence and the ripening of the grain will be the subject of the following sections.

The inflorescence

The indeterminate inflorescence of barley is a distichous spike, a raceme with alternate sessile spikelets sitting in groups of three at the joints of

the rachis (Bonnett 1966). The fertile lateral spikelets are sessile to pedicellate and are almost as well developed as the central spikelet (von Bothmer et al. 1991).

The barley floret consists of a carpel with a single ovary, two styles with dry plumose stigmata (Heslop-Harrison and Heslop-Harrison 1980), three stamens, and two lodicules representing modified petals. The lodicules are considered to be perianth appendages (Guédès and Dupuy 1976), and B-class flower genes are involved in lodicule development in the Poaceae (Erbar 2007; Rancělis and Vaitkūnienė 2007). Each floret is enclosed and subtended by two bracts, the upper inner palea and the lower outer lemma. Their adherence is controlled by the *Nudnud* gene, and they either adhere to the ripe grain, forming the husk in hulled barleys (*Nud-*), or are readily removed during threshing in naked or hulless types (*nudnud*) (Taketa et al. 2004). The top region of the lemma in barley develops into the awn. Mutants affecting lemma development include *Hooded* and *leafy lemma* (Roig et al. 2004). The homology of the lemma and palea to leaves (Pozzi et al. 2000), bracts (Whipple and Schmidt 2006), or perianths (Erbar 2007) in monocots or eudicots is not clear (Kellogg 2001; Zanis 2007).

The single-flowered spikelets comprise the floret and two subtending glumes. In two-rowed barley, the activity of a homeobox gene (*vrs1*) renders the two lateral spikelets sterile and reduced (Komatsuda et al. 2007).

The barley inflorescence is classically regarded as a spike with spikelets attached directly to the inflorescence axis (rachis); that is, the spikelet is interpreted as a flower at the end of a branch. An alternate interpretation of grass inflorescence and floret organization considers the spikelet as a morphologically contracted flower-bearing branch, showing some similarities to a flower but being unique in evolutionary terms (Malcomber et al. 2006).

Using descriptions of morphological mutations of barley, Forster et al. (2007) proposed a revised phytomer model, suggesting a repeated paired–single paired–single sequence for vegetative and generative structures.

Ear and spikelet development

An early indication of the onset of spikelet initiation is the decline of ear growth, which reaches a minimum a few days before the last spikelets are initiated (Cottrell and Dale 1984). Double ridges on the shoot apex visually indicate the existence of alternate spikelet and subtending leaf (branch) primordia (for a comparison to leaf primordia development, see Dannenhoffer and Evert 1994). The timing of transition of the ear to floral development depends on the genotype and environment but usually occurs when 6–10 leaves have been initiated (Hay and Ellis 1998). *Apetala/fruitful*-like genes appear to be involved in the transition to flowering and in controlling meristem identity in spikelets of grasses (Preston and Kellogg 2007). The upper spikelet ridge differentiates into three distinct mounds for the median and lateral spikelets, while the lower ridge leaf is usually suppressed early in development (Pizzolato 1997). The primordia for the glumes, lemma, palea, stamen, and pistil appear consecutively. In the ovary, the pistil primordium grows up, over, and around the differentiating ovule, thereby forming the style and stylar canal. After the maximum number of primordia is generated, the growth rate of the ear increases and rapid elongation of the rachis starts (Kirby 1977; Cottrell et al. 1985). This coincides with the establishment of vascular connections to the spikelet primordia (Kirby and Rymer 1974). The lemma and palea elongate after the head emerges from the boot and finally enclose the floret. The sequence of visible events leading to fertile florets has been described in detail by Bonnett (1966), while a scale of spike and pistil development in barley was established by Waddington et al. (1983).

The vascular system of the ear starts to develop at the triple mount stage with the initiation of procambium between spikelet primordia 6 and 10. Differentiation proceeds basipetally and acropetally from here, and connections to the peduncle and then the vascular system of the stem are established. As mentioned above, a procambium generates the vascular connection between spikelet and rachis at the beginning of the ear

elongation phase (Kirby and Rymer 1974). The procambial lateral and first central traces of the rachis differentiate originally as traces for the lower ridge leaves and not as traces related to the spikelets. The procambial glume traces differentiate basipetally and join the rachis traces, thereby linking the spikelet and rachis (Pizzolato 1997).

Critical factors for spike and grain development

Temperature and long day length are critical factors for the initiation of flowering in barley. Winter varieties and the wild progenitor of barley (*H. vulgare* subsp. *spontaneum*) require vernalization (i.e., cold treatment) and usually long-day conditions, while spring barleys do not require vernalization and their dependence on long-day conditions varies (Turner et al. 2005). The role of several interacting genes (*HvVRN1-1*, *HvVRN2*, and *Photoperiod-H1*) has been investigated in doubled-haploid lines (Hemming et al. 2008). Genes controlling flowering in barley are reviewed by Cockram et al. (2007).

Differences in grain size along the ear are established at an early stage. A positive correlation exists between the width of the spikelet primordium at the double ridge stage and the final weight of the grain. Differences in carpel dry weight at anthesis are maintained during development (Scott et al. 1983). Spikelets in the middle of the ear are more developed than spikelets at the base, followed by those at the top (Bonnett 1966). The spikelet primordia closer to the base of the spike can be twice as wide as those further up, and they also grow over a longer period (Cottrell and Dale 1984).

As mentioned above, vascular development in the ear starts between spikelets 6 and 10 (Kirby and Rymer 1974). Pizzolato (1997) notes that the distal spikelets tend to be linked to the lateral vascular system of the rachis (providing continuity with spikelets on both sides), while the proximal spikelets are linked with the central system (providing continuity with spikelets on the same side).

Sufficient radiation between early development and ear emergence is critical for final grain size in barley (Bingham et al. 2007), but high temperature during booting and ear emergence reduces grain weight (Ugarte et al. 2007). The effects of drought and heat stress on reproductive development in cereals have been reviewed by Barnabás et al. (2008). Shading experiments show that the amount of assimilates that reaches the spike early in development affects the number of fertile florets (Arisnabarreta and Miralles 2008b). The critical time for grain number determination is 30 days preceding heading in six-rowed barleys (Arisnabarreta and Miralles 2008a). Pre- and postanthesis factors influencing grain weight are reviewed by Coventry et al. (2003).

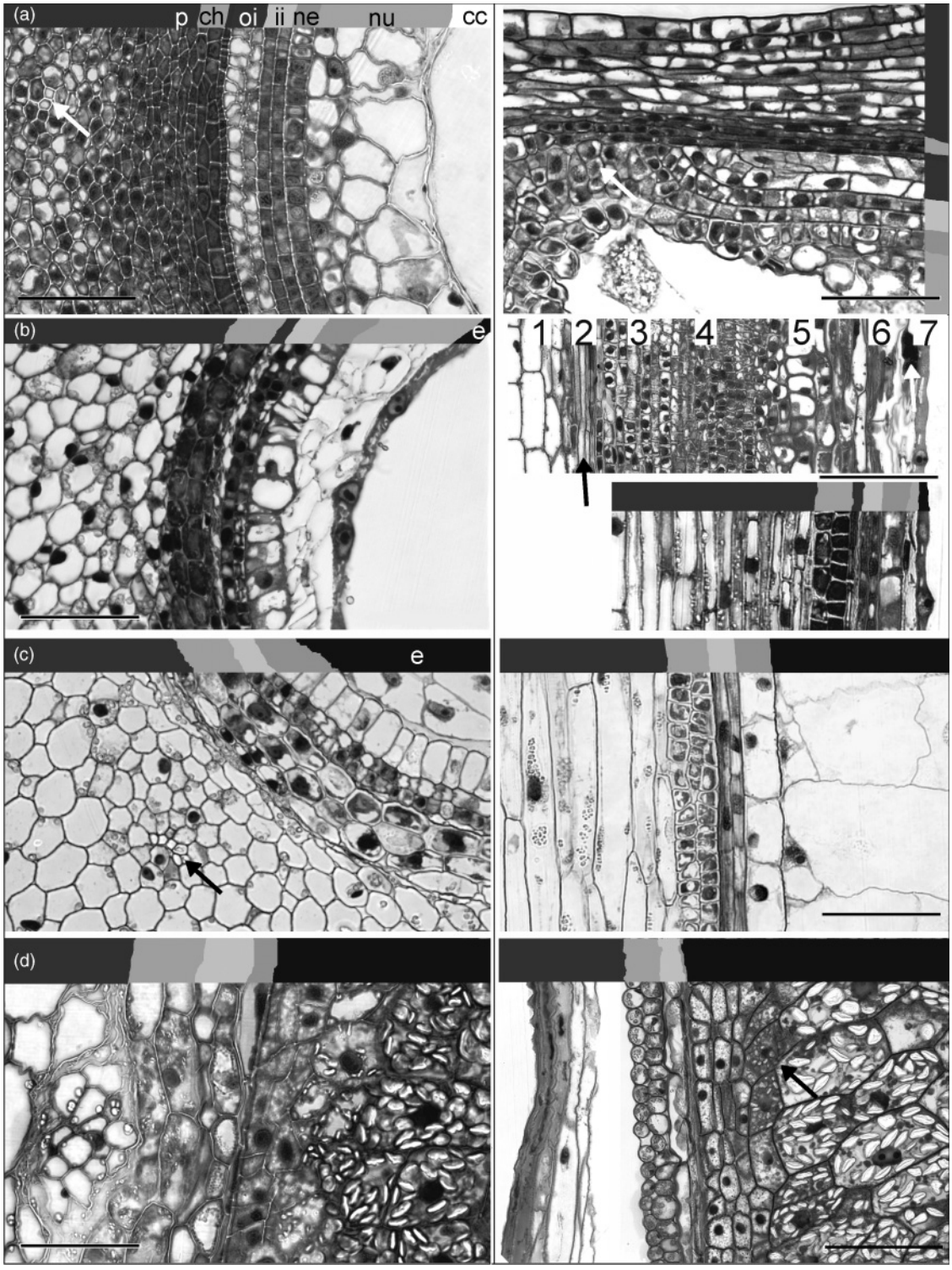
Development of the gynoecium

Ovule development

The gynoecium in the Poaceae usually consists of a single carpel containing a single ovule located adaxially (Rudall et al. 2005), but the monocarpellary character of the grass gynoecium is not undisputed (Philipson 1985). While only a single gynoecial primordium exists, the existence of two or three styles and the organization of the vascular system indicate the possibility of an underlying tricarpellary structure. In the barley gynoeceum, a smaller lateral vascular bundle supplies each style. In addition, one or two sieve elements run in the dorsal pericarp up to the height of the upper third of the ovule (see Figs. 13.1a and 13.2d).

The ovule is sitting or afunicular (Shamrov 1998) with a placento-chalazal zone without clear borders defining the contact zone to the ovary wall (see Figs. 13.1b [3 and 4] and 13.2a). Unequal intercalar growth under the chalaza turns the ovule (Krauß 1933; Savchenko and Petrova 1963; Korchagina 2002) from its initial upright position to an angle of about 90° during early megaspore development (Göbel 1923; Klaus 1966) and to an angle of about 130° at the four-nucleate stage of the megagametophyte (Savchenko and Petrova 1963). At the time of pollination, the endostomic microplyle faces away from the style.

The outer and inner integuments, both comprising two cell layers, originate on both sides of the placento-chalazal zone. When fully grown, the integuments leave only the micropyle and the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contact zone free (see Fig. 13.1a). The main vascular bundle runs parallel to the contact zone in the adaxial pericarp, indicating the importance of this region for transport (as discussed in the section below on supply of nutrients).

The nucellus, which is the homolog of the megasporangium, develops out of apical zones of the ovule primordium (Bouman 1984; Rudall 1997; Shamrov 1998; Nikitcheva 2002) and in barley persists up to several days after fertilization (DAF) (see Fig. 13.1a,b). The archesporial cell is located in the hypodermal layer and acts directly as the megaspore mother cell. The classification of nucelli has been based on archespore position and development, nucellar morphology, or other features (Bouman 1984; Shamrov 1998; Nikitcheva 2002). This has led to descriptions of the barley ovule as crassinucellar (Luxová 1967), intermediate between those with massive and delicate nucelli (Norstog 1974), as nearly tenuinucellate at anthesis (Engell 1989), tenuinucellate (Bennett et al. 1973), or as medionucellate, syndermal, and multilayered (Shamrov 1998).

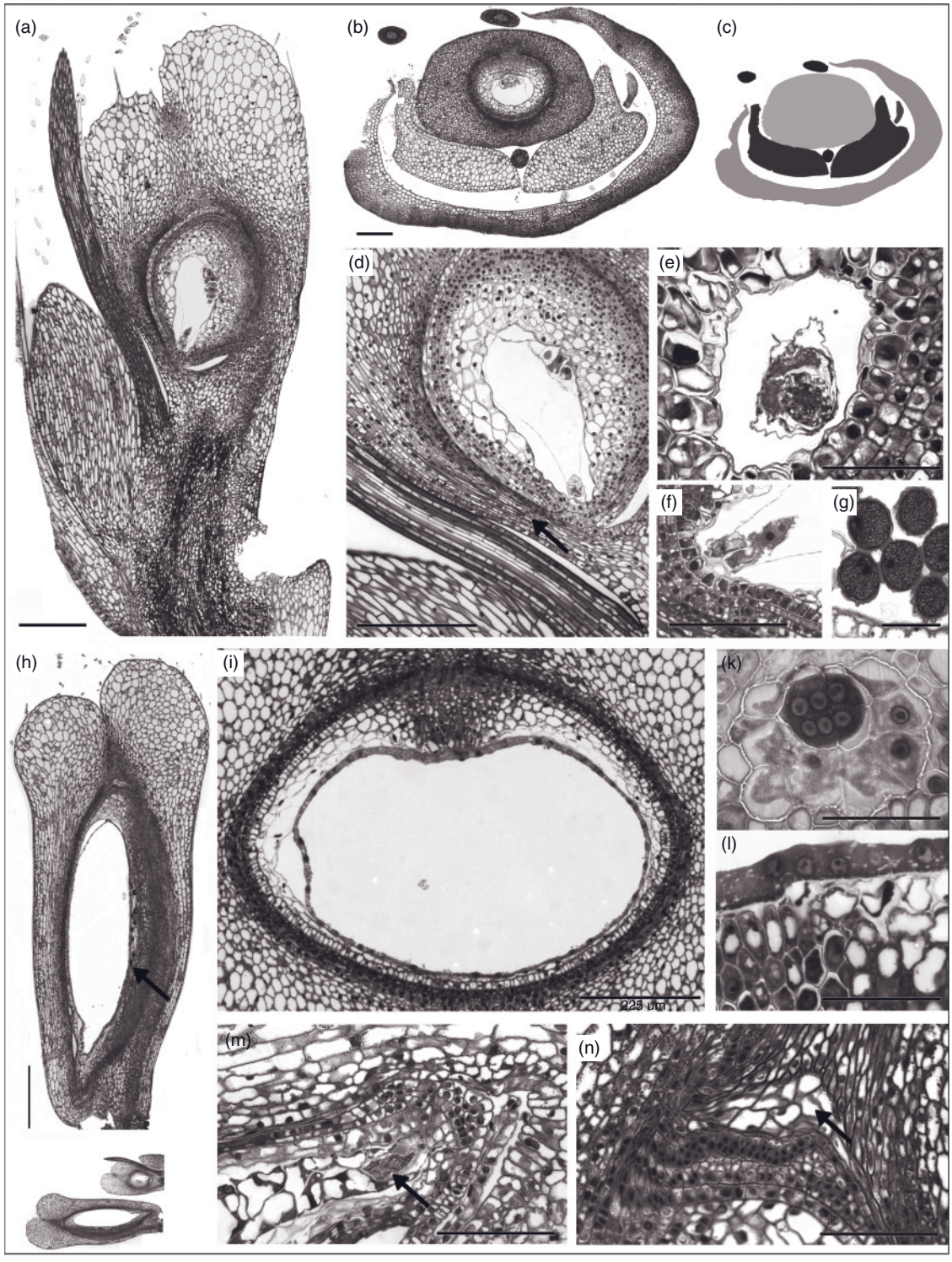
Megasporogenesis

At female meiosis, the integuments do not yet cover the nucellar dome (Savchenko and Petrova 1963; Klaus 1966; Bennett et al. 1973; Mouritzen and Holm 1995). The embryo sac mother cell wall is surrounded by a thick layer of callose ((1-3)-β-D-glucan). At 20°C, female meiosis lasting about 40 h occurred synchronous with male meiosis in the anthers of the floret (Bennett et al. 1973). Megasporogenesis leads to a linear or T-shaped (Bennett et al. 1973) tetrad, but development is monosporic and only the chalazal megaspore survives. Most plastids and the nucleus are localized in the upper part of this cell, while the lower part of the megaspore is vacuolated (Mouritzen and Holm 1995). The functional megaspore undergoes three mitotic divisions.

Development of the embryo sac

Megagametophyte development in the Poaceae follows the *Polygonum* type, but a higher number of antipodal cells are often present (Anton and

Fig. 13.1 Changes during barley caryopsis development. Light micrographs of sections of developing barley caryopses (cv. Optic) at anthesis (panel a) and before (panel b) and after (panel c) cellularization of the endosperm and during starch accumulation (panel d) shown in cross sections (left panels) and longitudinal sections (right panels). Color bars at the margins indicate the positions of the pericarp (p), chlorenchyma (ch), inner epidermis of the pericarp (red), outer (oi) and inner (ii) integuments, nucellar epidermis (ne), nucellus (nu), central cell (cc), and endosperm (e). At anthesis (panel a), the nucellus (nu) is prominent. The nucellar epidermis (ne) consists of small cells. Inner (ii) and outer (oi) integuments are clearly visible. The narrow inner epidermis of the pericarp (indicated by red color) is best observed at this stage. Cells in the pericarp are small; a frontal vascular trace in the cross section is indicated by an arrow. The longitudinal section shows the inner integument coming from the left, right, and behind (arrow) at the micropyle close to the egg cell. Before cellularization of the endosperm (panel b), the nucellus is already reduced and a thin layer of endosperm with free nuclei is shown. The nucellar epidermis and chlorenchyma have increased in size, while the outer integument and the inner epidermis of the pericarp are already degenerating. Note the starch in the pericarp cells. Cells in the pericarp, nucellar epidermis, and integument appear different in the longitudinal section due to their orientation in the grain. The top longitudinal view in the right-hand panel shows a ventral section through the crease area; the bottom image shows a dorsal section. (Compare with Figs. 13.2 and 13.3 for different aspects of the projection.) After cellularization (panel c), only the two cell layers of the inner integument remain. The pericarp shows some degeneration close to the chlorenchyma. The vascular tissue is indicated by an arrow. During grain filling (panel d), starch is accumulated in the endosperm cells. The aleurone and subaleurone (cells indicated by the arrow) are differentiated and the pericarp cells appear depleted and detached in the longitudinal view. The inner layer of the inner integument accumulating secondary plant products appears more prominent in the cross section. All sections are cut at about 3 µm with glass knives and are stained with toluidene blue. Bars: 55 µm for cross sections in panels a–d and for longitudinal sections in panels a and c. Bar: 110 µm for the longitudinal section in panel d. The bar between the two longitudinal sections in panel b indicates 110 µm for the upper image and 55 µm for the lower image. 1, pericarp; 2, vascular tissue, vessel indicated by arrow; 3, parenchyma cells; 4, chalazal zone at the base of the projection; 5 and 6, nucellar projection cells; 7, endosperm layer close to degenerating antipodal cell (arrow) in the nucellus. For color details, please see color plate section.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225 µm

Cocucci 1984). According to Bennett et al. (1973), callose persists during the meiotic divisions, leading to the functional megaspore (see Megasporogenesis), and accordingly, Cass et al. (1985) reported that remnants of callose have disappeared at the binucleate stage of the megagametophyte. Larger central and chalazal vacuoles are already visible at the four-nucleate stage. Wall formation at the eight-nucleate stage establishes the central cell with two polar nuclei, the egg cell, two synergids, and three antipodal cells (Cass et al. 1985). The cellularization of megagametophytes involves nonconventional cytokinesis between nonsister nuclei (Otegui and Staehelin 2000b; Brown and Lemmon 2001b).

The vacuole dominating the central cell probably originates from the expansion of the vacuole present in the early megagametophyte (Cass et al. 1985). The cytoplasm of the central cell is closely pressed to the lateral walls of the embryo sac (Bennett et al. 1975), covering the parts of the antipodal cell walls that face the central cell and surrounding the chalazal part of the egg apparatus. The two adjacent polar nuclei (see Fig. 13.2f) lie close to the egg apparatus in a massive cytoplasmatic strand that crosses the central cell from the egg apparatus to the antipodal cells (Cass and Jensen 1970). The wall between the central cell and the chalazal pole of the egg cell disappears during ripening of the egg apparatus (Zhukova 2002)

Additional mitoses of the three original antipodal cells (Mogensen 1982) result in a group of cells that can occupy a considerable part of the volume of the embryo sac (Bennett et al. 1975). Cellular (and nuclear) proliferations of antipodal cells have been reported in the Poaceae (Holloway and Friedman 2008). The antipodal cells initially lie in the chalazal region of the embryo sac, but after the growth and expansion of the nucellus and gametophyte, they are positioned adjacent to the placental region in the upper part of the embryo sac (see Fig. 13.2a,d) (Engell 1994). The maximum cell number is reached several days before anthesis, with reported values varying between an average of 25 and a maximum of between 50 and 100 (Cass and Jensen 1970; Bennett et al. 1975; Engell 1989, 1994). The volume of the antipodal complex increases after fertilization, while the single cells remain uninucleate. On average, the nuclei can more than double their volumes (Engell 1994; Shestopal and Blankovs'ka 2006). The ultrastructure of polytene chromosomes in the antipodal cells of barley, which result from endoreduplication, has been described by Pushkina et al. (1989). Pulse-chase experiments carried out at 1 and 2 days after pollination (DAP) indicate that RNA synthesis occurs in the antipodal nuclei (Bosnes and Olsen 1992).

The egg apparatus lies behind a single layer of nucellar epidermis. The egg cell is characterized by a more central lying nucleus with vacuoles and starch in the cytoplasm and lies chalazally of the pyriform synergids (see Figs. 13.1a and 13.2e). The basal walls of the synergids facing toward the micropyle are modified into the filiform apparatus, a wall ingrowth that has the characteristics of

Fig. 13.2 Early grain development in barley cv. Optic. Light micrographs of sections taken at anthesis (panels a–g) and during early coenocytic endosperm development (panels h–n). Cross section (b) lies at the height of the antipodal cells. Scheme of cross section (c) indicates the position of filaments (dark blue), lodicules (violet), lemma (light blue), and remnants of the palea (red). Longitudinal sections (a,d) show the egg cell and several antipodal cells in the embryo sac. The arrow in (d) indicates a vascular strand. Panels e–g are detailed views of a cross section (e) through the egg apparatus, a longitudinal section (f) of both polar nuclei and part of the egg apparatus, and a section (g) of pollen with vegetative and sperm nuclei. A cross section of caryopsis (i) at medium height shows a thin layer of developing endosperm, thicker on the projection side. For details of the endosperm over the nucellar projection, see (l). A corresponding longitudinal section (h) shows remnants of antipodal cells (arrows). The embryo in cross sections (k) and longitudinal sections (m) comprises only a few cells at this stage. A longitudinal section (n) shows part of the nucellar cap that is providing pollen tube guidance. The scheme in (h) indicates the relative sizes of the developing grain at anthesis and before cellularization of the endosperm. All sections are cut at about 3 µm with glass knives and are stained with toluidene blue. Bars: (a) 250 µm, (b), 225 µm, (d) 225 µm, (e) 55 µm, (f) 110 µm, (g) 55 µm, (h) 450 µm, (i) 225 µm, (k) 55 µm, (l) 55 µm, (m) 110 µm, (n) 110 µm. For color details, please see color plate section.

transfer cell walls and may play a role in the secretion of molecules that act as signals for pollen tube attraction (Fan et al. 2008). The wall between the degenerate synergid and the central cell is degraded, facilitating fertilization (Cass et al. 1986; Engell 1989). The persistent and degenerate synergid can be distinguished visually even before pollination (Engell 1989).

At anthesis, the ovule is pear shaped, bitegmic, and almost anatropous. The embryo sac embedded in the nucellus is characterized by a huge central vacuole, a large block of antipodal cells, and the egg apparatus. Two polar nuclei are located, often close to each other (Luxová 1967; Engell 1989), at the chalazal end of the egg apparatus (see Fig. 13.2a,b,d,f).

Pollen development, pollination, and fertilization

Microsporogenesis and pollen characteristics

The structure of the anthers (Młodzianowski and Idzikowska 1978) and pollen grain development in barley resembles those known in other members of the Poaceae (Charzyńska and Lenart 1989). The duration of meiosis in the barley variety Sultan grown at 20°C was about 48 h between leptotene and the release of microspores from the tetrads (Bennett and Finch 1971). Various meiotic stages occur simultaneously within a spike (Ekberg and Eriksson 1965). The pollen grains are almost circular and uniporate with a scabrate (rough) exine. Both pollen size and pore size are influenced by the ploidy level (Rajendra et al. 1978).

Pollen viability in Poaceae is short (Hammer 1977), ranging between several minutes and a few hours. Barley pollen kept at 24°C and at 50%–53% relative humidity survived for 5–10 min (Kison 1979), while Pande et al. (1972) reported a survival time of 3 h. In autogamous barley, the pollen reaches the stigma shortly after anthesis. A lower temperature and humidity around 50% are recommended for manual pollination to maximize pollen viability. Ceccarelli and Catena (1973) achieved seed setting rates of about 70% by removing parts of the glumes, dipping the receptor spike in 30%–40% ethanol, and provoking filament elongation and anthesis in the donor spike.

Transgenic flow from pollen was suppressed by spraying with jasmonic acid, which inhibits anther extrusion (Honda et al. 2006).

Among the environmental factors that influence pollen development are heat, mineral deficiency, and salt stress. They represent possible causes of pollen sterility. Empty anthers, pollen without cytoplasm, or pollen with reduced starch content are observed after 5 days of temperature stress during microsporogenesis. The extent of damage depends on the developmental stage affected, the most sensitive stage being premeiosis of the pollen mother cells (Sakata et al. 2000).

High-temperature damage leads to transcriptional inhibition of several typically abundant genes (Abiko et al. 2005), while stress-related genes are upregulated and several key genes are prematurely activated, causing premature progression to meiosis (Oshino et al. 2007).

Copper deficiency may cause degeneration of sporogenous tissue and the fusion of tapetal cells, the tapetum showing an invasive character. Applying indole-3-acetic acid (IAA) to anthers mimics some of the effects of copper deficiency, but not all of the anthers in all of the florets of copper-deficient plants are affected (Jewell et al. 1988). A study of 39 barley varieties grown under salt stress showed that they developed small sterile pollen that lacked cytoplasm (Rehman et al. 2004a). In barley, the male gametophyte is involved in the selection of salt tolerance genes, which occurs mainly as the pollen grains compete during tube germination on the stigma (Koval 2000).

Gene expression analysis of barley anthers showed that a high number of transcripts were specifically upregulated (Druka et al. 2006). Similarly, 1350 transcripts were temporally regulated in whole wheat anthers during the early stages of meiosis (Crismani et al. 2006). Maraschin et al. (2006) used microarray analysis to compare normal zygotic development to stress-induced microspore development, while Muñoz-Amatriaín et al. (2006) analyzed the transcriptome of barley anthers after mannitol treatment.

Floret opening and the role of potassium

Barley florets normally open during flowering, but chasmogamy is not essential as barley is self-pollinating (Honda et al. 2005). Cultivated barley is mainly inbreeding (von Bothmer and Jacobsen 1985). During flowering, potassium acts as an osmoticum evoking a rapid influx of water and thereby the swelling of the lodicules. This pushes the lemma of the grass floret outward (see Fig. 13.2c) (Heslop-Harrison and Heslop-Harrison 1996). In cleistogamic accessions of barley, the morphology and auxin response of the lodicules are changed (Honda et al. 2005). Genes controlling cleistogamy have been identified and mapped (Turuspekov et al. 2004).

The opening of the florets is synchronized with anther dehiscence and filament elongation (Honda et al. 2005), and roles for potassium in dehiscence (Matsui et al. 2000a,b), pollen imbibition (Rehman et al. 2004b, 2005) and hydration of the stylar papillae in barley (Rehman and Yun 2006) have been suggested.

Path of the pollen tube

The style of barley is dry and plumose (Heslop-Harrison and Shivanna 1977). Starch has not been detected in stigmatic hairs or stylar branches (Heslop-Harrison and Shivanna 1977) or in the path of pollen tube growth along the ovary wall (Luxová 1967), but carbohydrate reserves in the tube itself could support growth (Cass and Peteya 1979).

The pollen is trinucleate at anthesis (see Fig. 13.2g) and adheres to the stigmatic hair surface by mucosubstances after the first contact. The pollen germinates rapidly through the single aperture, the tube growing first along the hair surface then penetrating into the hairs (Cass and Peteya 1979). During tube growth, the two sperm cells are closely associated (Mogensen and Wagner 1987).

Conducting or transmitting tissue lies under the epidermis along the median inner surface of the stylar branches. It leads down to a cone-shaped projection (see Fig. 13.2n) comprising cells of the outer integument (Pope 1946), which Savchenko and Petrova (1963) described as an integumental obturator. This projection guides the pollen tube to the dorsal side of the ovule where it grows between the outer integument and the inner epidermis of the pericarp (i.e., along the inner ovary wall) (see Figs. 13.1a and 13.2a), reaching the micropyle between 20 and 60 min after pollination (Luxová 1967, 1968; Bennett et al. 1975; Mogensen 1982; Engell 1989).

The tube penetrates between the nucellar cells and enters the degenerating synergid through the filiform apparatus (Cass and Jensen 1970; Mogensen 1982; Engell 1989). The maize *Zea mays egg apparatus 1* gene, expressed in egg cell and synergids, is involved in pollen tube attraction (Márton et al. 2005), and a related gene is present in barley (McCormick and Yang 2005). The synergids have a similar appearance 2 days before anthesis (Engell 1989), but which synergid will degenerate is determined before fertilization (Cass 1981). Engell (1989) also reported synergid degeneration independent of pollination. Membrane contact is possible between the synergid and egg cell and between the egg cell and the central cell due to wall modifications during megagametophyte development (Cass et al. 1986; Engell 1989). The persistent synergid survives for about 1 day, and remnants can still be found 50 h after pollination (Engell 1989). According to Mogensen, the degeneration of both synergids is equally possible (Mogensen 1984).

Double fertilization

The contents of the pollen tube are discharged onto the egg cell (Engell 1989) in the space between the central cell, the egg cell, and the degenerating synergid (Mogensen 1982). This area has been interpreted as being part of the apoplastic system of the embryo sac (Yang 2001). One sperm cell makes contact with the egg, the nucleus enters the egg cytoplasm, and the nuclei fuse. A spike of calcium ions originating from the egg cell may be a signal of fertilization (Fan et al. 2008). Exclusion of the sperm cytoplasm occurs at this point, resulting in maternal inheritance of plastids (Mogensen 1988). Most of the paternal mitochondrial DNA is excluded during pollen development (Sodmergen et al. 2002).

The second male nucleus enters the central cell after membrane contact and reaches either the

single (Luxová 1967; Engell 1989) or the already partially fused polar nuclei (Batygina and Bragina 2006) in the cytoplasm close to the egg apparatus. The nuclei then migrate up to the antipodal cells and a triple fusion generates the primary endosperm nucleus (Erdelská 1967; Mogensen 1982). Varying values for the duration of pollination and fertilization events have been reported, which may be due to the effects of the environment and genotype (Luxová 1968; Cass and Jensen 1970; Mogensen 1982; Engell 1989). Migration of the polar and sperm nuclei to the antipodal cells was observed after 2 h 15 min and triple fusion 13 h 30 min after pollination by Engell (1989). Erdelská (1967) reported that, independent of fertilization, the polar nuclei migrated to the antipodal cells in 25 of 50 embryo sacs at 3–5 days after presumed anthesis.

Postfertilization development

General remarks

Based on developmental and biochemical indicators, barley seed development can be regarded as a sequence of three stages, with morphogenesis and cell divisions occurring during the first stage followed by reserve compound accumulation (often called grain filling) and finally desiccation (or maturation) (Wobus et al. 2005). At a given point in time, grain development more closely resembles a network of interrelated synchronous events than a sequence, with the development of individual tissues often being interdependent. Polarity and spatial and temporal gradients exist across and along the grain, and individual areas of the developing caryopsis can differ (Gubatz et al. 2007). A single tissue such as the endosperm is not necessarily uniform during development (DeMason 1997) or even at ripeness, as discussed below and by Chandra et al. (1999).

Endosperm and embryo development are fundamental steps after fertilization. At the same time, the tissues of the ovary and ovule establish and maintain an effective flow of nutrients in the filial tissues to support growth and storage compound accumulation. Protective layers are also generated to protect the developing caryopsis during seed dormancy and germination.

Endosperm development

Bosnes et al. (1992) and Olsen et al. (1992) defined four stages of barley endosperm development: the syncytial, cellularization, differentiation, and maturation stages. A general survey of endosperm development in cereals can be found in Brown and Lemmon (2007).

The syncytial endosperm

The primary endosperm nucleus divides repeatedly and at first synchronously without cell wall formation and generates a coenocytic ("nuclear type") endosperm in the cytoplasm between the central cell wall and the central cell vacuole (Olsen et al. 1999). In a study of 17 barley varieties, divisions were nearly synchronous up to the 32-nuclei stage and then synchrony was less pronounced (Forster and Dale 1983).

Although this endosperm develops by multiple divisions of the triploid endosperm nucleus and not by the fusion of a number of cells, it is also referred to as a syncytium (Dumas and Rogowsky 2008). The syncytial layer is thicker on the ventral side (see Figs. 13.1b and 13.2h,i,l) over the degrading antipodal cells and the nucellar projection than on the dorsal side of the central cell (Bosnes et al. 1992). During the free nuclear divisions, the mitotic apparatus is typical of plant cell division, but phragmoplasts exist only for a short time and a cell plate is not formed (Brown et al. 1994). The mitoses in barley can stop for up to 2 days when the final number of nuclei is reached (Brown et al. 1994). Bennett et al. (1975) determined a mean number of approximately 2300 endosperm nuclei on the day of cellularization. Pulse labeling experiments demonstrated that RNA is synthesized in the coenocyte and that synthetic activity increases to about sixfold the initial value (Bosnes and Olsen 1992), while *in situ* hyridization experiments using *endosperm1* cDNA showed an asymmetric expression of RNA in the coenocyte (Doan et al. 1996; Drea et al. 2005).

Cellularization

A special type of cell plate is associated with the cellularization of the endosperm (Otegui and Staehelin 2000a,b; Otegui et al. 2001; Otegui 2007). Radial microtubule systems (RMS)

originating from evenly spaced nuclei organize the syncytium into nuclear cytoplasmic domains (NCD) (Brown et al. 1994) and prior to cell wall deposition, these domains change into columns of cytoplasm with their nuclei lying closer to the central cell vacuole. The domains are outlined by vacuoles and only small bridges of cytoplasm remain between them. Waves of synchronized polarization and then divisions start in the region close to the nucellar projection, proceed on both sides, and spread around to the dorsal side (Brown et al. 1996).

The deposition of the first anticlinal and periclinal walls has been described in detail by Brown et al. (1994, 1996). Anticlinal walls are deposited first by adventitious phragmoplasts that are generated between neighboring NCDs, the resulting open structures being termed alveoli (Olsen et al. 1995; Brown and Lemmon 2007). The radial microtubuli reorganize at this point and extend more in the direction of the central cell vacuole, while adventitious phragmoplasts continue to increase the height of the alveoli. The nucleus is anchored on the central cell wall, divides synchronously with all other nuclei, and the periclinal wall is finally generated by a functional interzonal phragmoplast. A new alveolus is built accordingly around the free nucleus and cytoplasm, and cellularization progresses further into the space of the central cell vacuole (Olsen 2004).

A study using monoclonal antibodies against key compounds of the barley endosperm cell wall showed that callose ((1 → 3)-β-D-glucan) and cellulose ((1 → 4)-β-D-glucan) are present in the central cell wall before and during the first 2 days after the onset of cellularization (Brown et al. 1994; Wilson et al. 2006). Cellularization follows the same pattern of progression described above for the synchronized division (Bosnes et al. 1992).

Differentiation

The endosperm differentiates into cells surrounding the embryo (see Fig. 13.3c,f), the starchy endosperm cells (see Figs. 13.1c,d and 13.3a,b), and peripheral cells (see Fig. 13.3d (tr) and 13.3o). The peripheral endosperm cell types comprise the aleurone layer covering the surface of the starchy endosperm, the modified one cell-layered aleurone over the embryo (germ aleurone), and the transfer cell layer over the starchy endosperm that faces the nucellar projection. Transfer cells can be regarded as a modified epithelium generated after the first centripetal periclinal division during endosperm cellularization and differentiated in the area where maternal supply reaches the filial tissue (Royo et al. 2007).

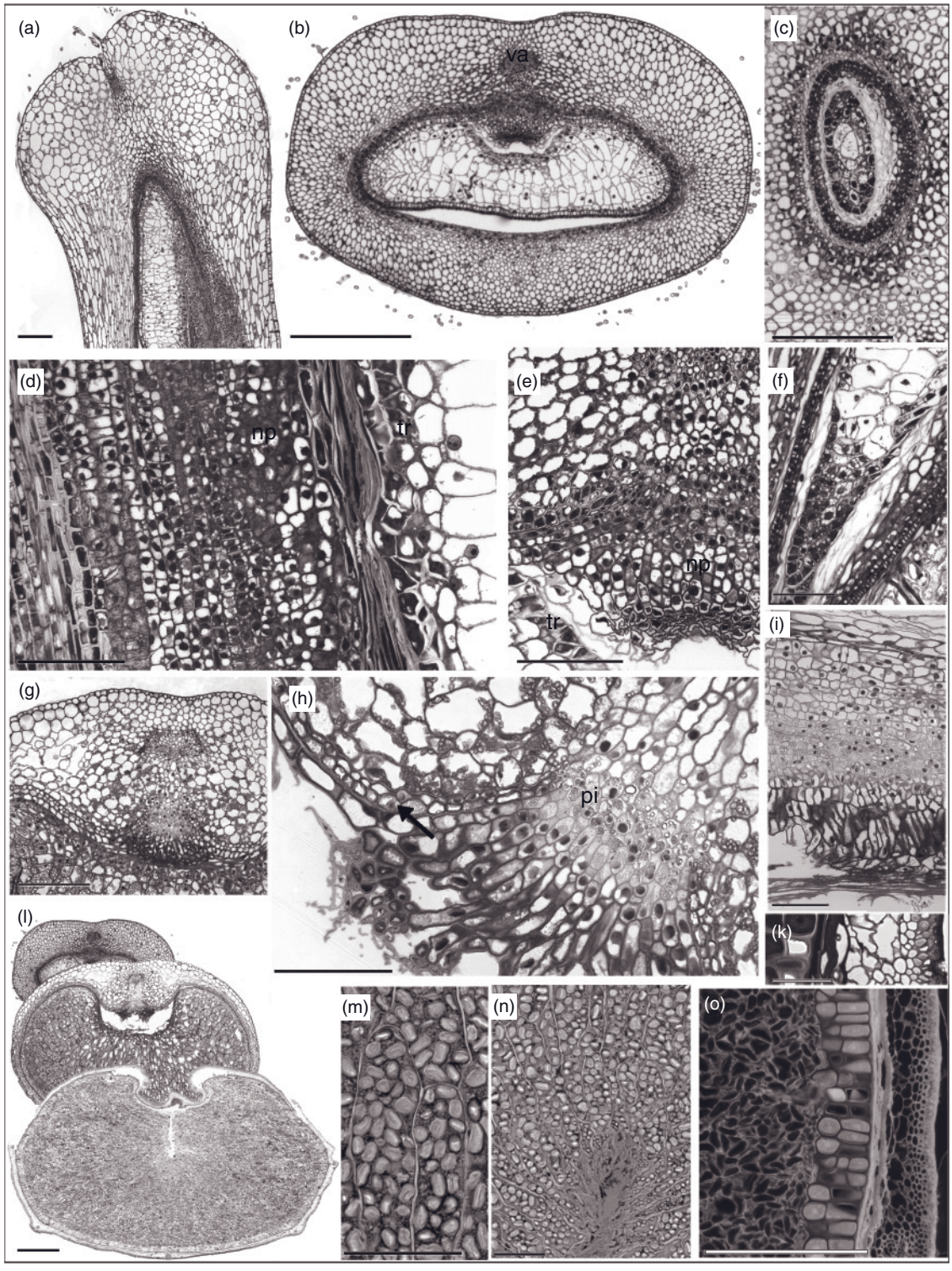
Positional signaling seems to be involved in cell fate specification in the endosperm of cereals (Becraft and Asuncion-Crabb 2000; Olsen 2001). Free endosperm nuclei localized in the syncytial cytoplasm over the nucellar projection express END1, a transcript that is present after cellularization in transfer cells and ventral starchy endosperm cells (Doan et al. 1996).

The embryo surrounding region (ESR)

Engell (1989) and Forster and Dale (1983) noted that cellularization of the endosperm occurs much earlier around and above the zygote and embryo in barley. Cells in this region are visibly different from typical young, starchy endosperm cells in cross section (see Figs. 13.2k and 13.3c,f). Modified endosperm near the developing embryo has been described for wheat and barley (Smart and O'Brien 1983) and may share characteristics with the ESR of maize and the micropylar endosperm (MCE) of *Arabidopsis* (Cosségal et al. 2007).

The starchy endosperm

As cellularization progresses centripetally, cells from opposing sides meet, and for a short time after closure, the cell walls form a visible line in that zone. Further cell divisions are more randomly orientated (Brown et al. 1994), starting in the center of the wings leading to irregular cells (see Fig. 13.3b), while meristematic cells close to the nucellar projection form prismatic cells (Bosnes et al. 1992). This concept is supported by the analysis of a barley mutant that builds endosperm in the wings but lacks the prismatic cells that spread out from the area of the nucellar projection. A range of mutants (Felker et al. 1985; Bosnes et al. 1987; Djarot and Peterson 1991; Schulman et al. 1994) may help to follow cell lineage in barley endosperm (Olsen et al. 1992).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Cells of the starchy endosperm develop cortical microtubules but no preprophase bands (Brown et al. 1994). Divisions in the endosperm continue until the final number of cells is reached.

Cells in the starchy endosperm expand considerably, which is facilitated by the low content of cellulose in their walls (Otegui 2007). Environmental factors may also influence the composition and morphology of endosperm cell walls (Lazaridou et al. 2008).

The nuclei in the central endosperm cells undergo endoreduplication, which may be required to support the higher transcriptional capacity of the cell, as suggested for maize (Larkins et al. 2001), or may maintain a balance between the size of the nucleus and the whole cell. In maize, endoreduplication is initiated when the cells start to expand rapidly (DeMason 1997). A review of endoreduplication in the cereal endosperm with a focus on maize can be found in Nguyen et al. (2007). Autoradiography indicates that ongoing DNA synthesis continues in starchy endosperm cells of barley cv. Bomi up to 25 days after anthesis (DAA), with the DNA content of whole grains increasing 10-fold between 5 and 25 DAA (Giese 1991).

Starch accumulation usually begins in the wings and proceeds from there. The accumulation of hordein storage proteins occurs at the same time as starch accumulation, with deposition occurring in protein bodies originating either from trafficking via the Golgi apparatus to the vacuole or from direct accumulation within the lumen of the endoplasmic reticulum (Cameron-Mills and von Wettstein 1980; Miflin et al. 1981). These two pathways have been described in more detail in wheat (Rubin et al. 1992; Loussert et al. 2008; Tosi et al. 2009).

The grain weight and size are influenced by the number of endosperm cells generated and by the duration (Renwick and Duffus 1987) and rate of grain filling (Coventry et al. 2003). A positive correlation between total number of nuclei in the endosperm and grain weight was found in cv. Bomi and the derived Risø mutants (Klemsdal et al. 1986). The duration of cell division and final cell numbers also differ between cultivars with about 170,000 endosperm cells being counted in mid-grain cross sections after the onset of cell expansion at about 14 days after flowering in cv. Bomi (Cochrane and Duffus 1981, 1983; Evers et al. 1981).

Aleurone layer

Barley caryopses usually have three layers of aleurone cells (see Figs. 13.1d and 13.3o) that remain alive throughout grain maturation. Their walls are about 10–30 µm thick with the primary and secondary walls being separated by a middle lamella (Pomeranz 1972). Plasmodesmata interconnect the aleurone cells and connect them to starchy endosperm cells (Gram 1982). Reserve materials in the form of numerous aleurone grains (also called protein storage vacuoles or protein bodies by some authors) of 1- to 5-µm diameter and lipid-containing spherosomes are also present in mature aleurone cells (Jones 1969). Aleurone grains contain phytin–globoids and protein–carbohydrate bodies (crystalloids) in a proteinaceous matrix (Buttrose 1971; Jacobsen et al. 1971) that consists mainly of 8S storage globulins (discussed in the section below on storage

Fig. 13.3 Grain development in barley (cv. Optic) after cellularization of the endosperm. Panels a–f show the developing caryopsis at the onset of starch accumulation. (a) Longitudinal section through the upper part. (b) Median cross section with vascular tissue (va) indicated. (d) Longitudinal and (e) cross section views of the nucellar projection (np) and endospermal transfer cells (tr) at the border of the endosperm. (c) Cross and (f) longitudinal sections showing both the endosperm close to the embryo and the (white) cells of the starchy endosperm. Panels g–i show the developing caryopsis during starch accumulation. The cross sections in panels g and h and the longitudinal section in panel i show the pigment strand (pi) in the chalazal zone behind the nucellar projection. Panels k, m, n, and o are sections of ripe grain showing the endosperm (m), the crease region (n), and the organization of the outer wall layers (k) and aleurone (o). The scheme in (l) indicates the relative sizes of the three stages shown in Fig. 13.3. All sections are cut at about 3 µm with glass knives and are stained with toluidene blue. All images are light micrographs and panel (o) is color inverted. Bars: (a) 220 µm, (b,l) 450 µm, (c,g,o) 225 µm, (d,e,f,h,i,m) 110 µm, (k) 55 µm, (n) 80 µm. For color details, please see color plate section.

globulins) (Yupsanis et al. 1990; Bethke et al. 1998). The developing globoids in vacuoles and the spherosomes in the cytoplasm of the aleurone cells are clearly associated with membranes (Gautam et al. 1993). Lonsdale et al. (1999) used high-pressure freezing followed by freeze substitution to achieve improved structure preservation because the preparation of material using chemical fixation and solvent extraction results in artifacts due to partial extraction of the aleurone grains.

Aleurone cell development has been reviewed by Becraft (2007). Signs of aleurone differentiation are visible at about 10 DAF, in the denser cytoplasm and vacuolarization of the cells (Bosnes et al. 1992). Regulation of the differentiation is determined by the position relative to other grain tissues (Royo et al. 2007). This is demonstrated by a barley mutant lacking the prismatic endosperm cells fanning out from the nucellar projection. This mutant forms aleurone cells only over the two lobes of endosperm, indicating that the differentiation of the aleurone depends on the underlying endosperm (Bosnes et al. 1992) and not on the position of the cells in the grain. Transcripts encoding oleosins and a dormancy-related protein can be used as markers for aleurone differentiation but are also expressed in the embryo (Aalen et al. 1994).

The *Dek1*, *Cr4*, and *Sal1* genes control aleurone identity in *Arabidopsis* and rice and orthologs of these genes have been identified in barley. The *defective seed5* (*des5*) mutant of barley has less aleurone cells and shows changes in the anticlinal walls of the aleurone layer. Embryo development is also affected and microarray analysis indicates downregulation of *HvCr4* (*H. vulgare crinkly4*) (Olsen et al. 2008).

Aleurone cells continue to divide for longer than the starchy endosperm cells (Kvaale and Olsen 1986) and develop hooplike cortical microtubules and preprophase bands typical of histogenesis (Brown and Lemmon 2001a). The mechanism of posttranscriptional control of barley aleurone transcripts late in maturation appears to involve destabilizing mRNAs (Aalen et al. 2001).

The inheritance of the thickness and number of aleurone layers has been investigated in the progeny of two lines differing in these characters (Jestin et al. 2008), showing that they were strongly correlated. The aleurone cells may undergo only a single round of endoreduplication unlike the starchy endosperm cells (Keown et al. 1977). Based on a scanning electron microscopy (SEM) analysis of freeze-dried caryopses at 20 DAF, Adler (1990) suggested that cell divisions in the aleurone layer continue into the late phase of seed maturation.

Germ aleurone

The single layer of germ aleurone cells in barley contains very few phytin or protein deposits (Cochrane 1994a). These cells are thick walled, smaller than normal aleurone cells, and are irregular in shape with a large nucleus and dark cytoplasm. They also share some characteristics with the aleurone cells covering the starchy endosperm (Pogson et al. 1989) including the presence of phenolic acids in their cell walls (indicated by autofluorescence). Phenol oxidase and peroxidase activities present in the layer at maturity may have protective functions during and after germination (Cochrane 1994b).

Maturation

Maturation of the starchy endosperm involves massive accumulation of the reserve substances, starch (see Fig. 13.3l–n), and hordein protein, while the aleurone cells accumulate oil, 8S globulin protein, and minerals (including phytin). During maturation, the cells undergo programmed cell death (PCD) showing the typical degradation pattern of nuclear DNA (Young and Gallie 2000), while the cells in the aleurone layer persist and only show PCD during germination and follow a different pathway (Fath et al. 2000). The mature endosperm cells in the wings of wheat reach about 70–140 μm in length and 70–120 μm in width (Evers and Millar 2002).

Embryo development

Embryos of grass seeds are highly differentiated showing root and shoot meristems and several leaf

initials at maturity. The scutellum, a modified haustorial organ (Malcomber et al. 2006), is unique to the grasses and may represent a modified cotyledon (Guignard and Mestre 1970; Rudall et al. 2005). However, the homology of embryonal structures with organs in other taxonomic groups has been long disputed (Brown 1960, 1965; MacLeod and Palmer 1966; Natesh and Rau 1984).

When cultured barley embryos are supplied with kinetin, the scutellum and coleoptile develop hairs and synthesize chlorophyll (Norstog 1969), supporting the suggestion that the scutellum is a modified cotyledon or leaf (Natesh and Rau 1984; Chandler 2008). Embryos can also be readily induced from callus formed from immature scutella (Oka et al. 1995).

MacLeod and Palmer (1966) recognize four structurally distinct regions in the embryo: the root covered by the coleorhiza with subsidary root initials, the acrospire comprising the cylindrical coleoptile, the stem apex and leaf primordia, the nodal region between root and shoot, and the scutellum.

The zygote in *H. vulgare* is slightly larger than the egg cell, with dimensions of 80 μm × 38 μm being reported by Engell (1989). It contains 14 chromosomes at metaphase and the nuclear cell content of the proembryo is 4C (Bennett and Smith 1976; Mogensen and Holm 1995). The zygote is attached to nucellar cells at the micropylar end as is the suspensor of the young embryo. When the embryo has grown to 20 cells, it lies free in the endosperm (Norstog 1972).

The embryo does not increase in size initially because the first division of the zygote results in smaller cells (Engell 1989). Ten to fifteen cells are generated until the third day (see Fig. 13.2k,m) after fertilization (Merry 1941; Engell 1989; Kunert and Kunert 1990), with a club-shaped undifferentiated embryo being present by about 6 days (Merry 1941). Early embryo development has been described in detail by Engell (1989) and Norstog (1972), and authors ascribe the embryo development to the Poa variation of the Onagrad type. Batygina (1969) defined a graminad type of embryo development for *Triticum* based on the oblique first division plane, the T-shaped form of the four-celled embryo, early scutellum differentiation, and dorsiventral symmetry.

The first visible differentiation observed at about 7 DAF is a small bulge of the outermost cell layer in the upper third of the embryo resulting from mainly periclinal divisions in the subprotodermal layer. Further differentiation of the stem meristem generates three leaf primorida (Merry 1941; Klaus 1966). The developing coleoptile initially appears as a ridge above the stem meristem and finally grows around and over the shoot apex and leaf primordia. The primary root primordium in grasses is of endogenous origin (Natesh and Rau 1984). In barley, primary root development starts about 10 DAF below the scutellar node, with the primary seminal root of the mature embryo being about 850 μm long. During germination, the primary root penetrates the coleorhiza and forms the first root of the new plant. Several pairs of secondary seminal root primorida are visible after 3 weeks of embryo development (Luxová 1986). The embryo length increases continuously until about 20 DAF (Kunert and Kunert 1990).

The scutellum is initiated in the apical zone of the young embryo. It extends upward, growing considerably more in length than thickness and builds the typical shieldlike structure. Rapid growth and expansion of the scutellum pushes the apical meristem of the embryo into a lateral position (Norstog 1969), and the ventral surface of the scutellum generates a ridge of cells called ventral scale (Merry 1941). The abaxial surface of the scutellum covering the endosperm differentiates as an epithelium with small elongated cells (MacLeod and Palmer 1966) about 30–40 μm long and 5–8 μm wide in the ripe nongerminated grain (Gram 1982) and 80 μm long at the period of active absorption (Negbi 1984). The cells of the epithelium and parenchyma are rich in lipid reserves and protein bodies, while starch is present in the basal third of the scutellum (Smart and O'Brien 1979a). The presence of ferulic acid, the major phenolic compound in barley scutella and grain, results in autofluorescence (Smart and O'Brien 1979b). The cells of the aleurone layer and the scutellum in grasses show similarities in

their histology, responsiveness to hormones, and transcript profiles (Aalen et al. 1994).

Early supply of nutrients to the embryo may be provided or influenced by the ESR (Cosségal et al. 2007) or by the suspensor, but only a more vacuolated cytoplasm distinguishes the suspensor cells from other embryo cells. Ultrastructural analysis (Norstog 1972) indicates that the embryo is isolated from the nucellus tissue at an early stage. An early supply by cells of the developing endosperm is considered unlikely (Raghavan 2006), and plasmodesmata connecting embryo cells to endosperm cells were not observed by Norstog (1972). Radiolabeled photoassimilates from the green pericarp layer reached the embryo via chalaza and micropyle (Patrick et al. 1991).

In *in vitro* culture, the embryos of barley are able to accumulate sucrose by active transport, and depletion of the endosperm layers close to the scutellum (intermediate layer) was observed, indicating that endosperm nutrients support the embryo later in development (Cameron-Mills and Duffus 1979). Biochemical changes in the embryo of barley (protein, sugar, DNA, and RNA) are consistent with a rapid accumulation of carbohydrate and protein (Duffus and Rosie 1975).

The mature grass embryo is small in relation to the endosperm and positioned dorsally at the base of the grain (Natesh and Rau 1984). It lies between the intermediate layer (comprising depleted endosperm cells and up to 30 μm thick) and the germ aleurone layer (MacLeod and Palmer 1966). Desiccation and developmental arrest occur in the last phase of barley embryo development (Bartels et al. 1988).

Coordination of embryo and endosperm development

Little is known about the coordination of development of the embryo and the endosperm (Nowack et al. 2006; Dumas and Rogowsky 2008), although signals from the fertilized egg are known to influence endosperm development in other angiosperms (Berger et al. 2006; Nowack et al. 2006). In barley, the *des5* mutation affects cellularization of the endosperm and development of the aleurone and embryo (Olsen et al. 2008). Coculture with feeder cells or ovules is also generally required after *in vitro* fertilization to maintain the growth of zygotes and endosperms (Kranz and Scholten 2008) indicating the requirement for factors secreted by these tissues. Furthermore, the *Retarded growth of embryo1* gene in *Arabidopsis* controls the development of the heart stage embryo but is actually expressed in the endosperm (Kondou et al. 2008). Ungru et al. (2008) proposed a model with four different signal transduction pathways between the embryo and endosperm.

Development of the endosperm precedes embryo development in barley (Engell 1989). The mean cycle time for embryo cells (10.8 h) was about three times longer than that of nuclei in the endosperm (3.5 h) in barley cv. Sultan. The mean cell doubling times of embryos of 17 varieties were between 9.2 and 12.9 h, with a possible correlation between the growth rate of the embryo and the endosperm cell number (Forster and Dale 1983). This difference in cycle time is partly attributed to the lower demand on biosynthetic activity (e.g., cell wall biosynthesis) in the coenocyte.

Observations of simultaneous events in the embryo, endosperm, and various other tissues have been used to relate visible outer changes with internal events in caryopsis development. The developmental stages in the embryo and endosperm have, therefore, been related to the size of caryopsis and lemma at 13 time points up to 33 DAF (Xi and Ye 1997).

Supply of nutrients and water in the developing grain

General remarks

The effective transport of water and nutrients into and within the developing barley grain involves the coordinated participation of specialized tissues. The vascular systems of the ear and spikelet supply the ovary, especially its main ventral vascular bundle, with assimilates from source tissues (Biscoe et al. 1975) such as leaf, stem (Daniels et al. 1982; Bonnett and Incoll 1992), tillers (Lauer and Simmons 1988), and awn

(Jiang et al. 2006). The smaller lateral and frontal bundles of the ovary are stretched and torn during grain elongation and are obliterated about 2 weeks after fertilization (Lingle and Chevalier 1985). Further possible sources of nutrient supply at early stages of development are from the degrading nucellus, antipodal cells (indicated by their position and ultrastructure) (Engell 1994), starch in the parenchymatic pericarp cells, and (depending on stage) photoassimilates from green pericarp cells (Morrison 1976; Nutbeam and Duffus 1978; Tambussi et al. 2007).

Vascular tissues

The early development of the vascular system of the ear and spikelet primordium has been described above. Even before anthesis, a direct vascular connection is established between glumes and pistil (Pizzolato 1998).

Two to three days before the emergence of the ear from the leaf sheath, cross sections of the rachis show four to six median (or central) vascular bundles, arranged in the form of an ellipse. One main lateral bundle and two smaller outer lateral (or wing) bundles lie on each side of the ellipse. The two main lateral bundles and four outer lateral bundles join the traces of each spikelet (Pizzolato 1997) as do the central bundles on the same side of the rachis as the spikelet (Kirby and Rymer 1974; Kirby and Rymer 1975). The bundles are no longer discernible between the branching zones below the insertion of the glumes, and cells in this area of discontinuity show characteristics of transfer cells (Kirby and Rymer 1974), as described for the respective zone in the wheat ear by Zee and O'Brien (1970a) and may control the influx of substances or may be involved in nutrient recapture (Patrick and Offler 2001). Tracer substances known to be confined to the apoplast failed to cross the xylem discontinuity in wheat (Zee and O'Brien 1970a).

The branches that supply the lateral spikelets, glumes, lemma, rachilla, palea, lodicules, and stamens separate at different positions of the spikelet axis before the remaining vascular bundles finally reach the ovary where they separate into an abaxial bundle, two lateral bundles, and one adaxial strand (Kirby and Rymer 1975).

At anthesis, the lateral bundles with sieve tubes extend into the stigmatic branches. The anterior strand shows only a few elements and extends upward in the anterior pericarp (see Figs. 13.1a and 13.2d). The larger ventral vascular bundle in the crease region with xylem and phloem elements is provascular at anthesis but differentiates and adapts after fertilization (in about 6 days), while the caryopsis elongates. It represents the main vascular supply of the grain. The number of sieve tubes and xylem elements is highest at the base (see Figs. 13.2a and 13.3b (va), e) and decreases toward the tip of the ovary (Lingle and Chevalier 1985).

Transfer cells

Transfer cells are ubiquitous in plants and are characterized by secondary wall ingrowths and enhanced transport or secretory capacities. They are important components of nutrient transport pathways (Gunning and Pate 1969; Offler et al. 2002) and are involved in grain filling in cereals including barley (Cochrane and Duffus 1980) and other cereals. Transfer cells often lie at the borders of tissues and at apoplastic/symplastic interfaces (Offler et al. 2002). In barley, they line both sides of the endospermal cavity; on the maternal side, they are the outer cells of the nucellar projection, while on the filial side, the cells of the endosperm transfer layer show massive wall ingrowth (see Fig. 13.3d,e,h,i). Their specialized structure in wheat has been described by Wang et al. (1994a, 1995b). The plasma membrane in transfer cells follows the wall ingrowth, leading to a considerable amplification of surface (Pate and Gunning 1972).

Pathway from the vascular tissue to the endosperm

Solutes and water are unloaded from the phloem in the ventral vascular tissue of the ovary. Sucrose uptake into the developing grain is the basis for starch production in the endosperm (Lingle and Chevalier 1984). In addition to assimilates, the phloem sap may also contain proteins (among them probably isoforms of a sucrose transporter) and mRNAs (Gaupels et al. 2008).

The symplastic postphloem pathway (Zhang et al. 2007) passes through a strand of parenchyma cells that lead to the integument-free contact zone between ovule and ovary wall (often refered to as chalaza or chalazal zone) and continues into the nucellar projection up to the endospermal cavity (see Fig. 13.3d,e,g). In wheat, abundant plasmodesmata connect the parenchyma cells to the cells in the chalazal region (Wang et al. 1995a). At the maternal filial border, substances are released from the cells of the nucellar projection into the apoplastic space. The transfer cells of the endosperm regulate the uptake and further symplastic passage of nutrients into the starchy endosperm (Wang et al. 1994b, 1995b). Movement within the endosperm occurs radially and experiments suggested a symplastic route. No indication has been found of longitudinal transfer of assimilates (Patrick et al. 1991). Uptake from the phloem occurs along the length of the grain (Sakri and Shannon 1975).

Fluorescent dyes have been used as symplastic and apoplastic tracers in wheat (Wang and Fisher 1994; Wang et al. 1994b) and barley (Cook and Oparka 1983) to study the pathway of assimilates. This was supported by anatomical studies.

The *seg1* mutant in barley leads to shrunken grains with reduced grain filling instead of normal assimilate supply (Felker et al. 1983). In this mutant, early degeneration of chalaza interrupts normal transport into the filial tissues, probably due to high cytoplasmic concentrations of tannins and collapse of the projection (Felker et al. 1984).

Nucellar projection

The nucellar projection extends between the chalaza (the zone between the origins of the integuments) and the endospermal cavity. It is a dynamic structure with the appearance of the projection cells changing depending on their position and developmental stage (see Figs 13.2i and 13.3d,e,h). Modifications in the tissues leading to the projection also influence its functionality.

At 10–15 DAF, the cells in the core of the projection are isodiammetric and thin walled with large nuclei and few vacuoles. Thick-walled cells with a transfer cell character (see below) border the endospermal cavity, while cells at the base of the projection are less differentiated. The projection cells in the core elongate during development and the nucellar projection divides into lobes (see Fig. 13.3e,l). With further development, the transfer cells are located more in the center of the projection and the cells closer to the cavity degenerate (Cochrane and Duffus 1980). A comparable structure of the developing nucellar projection with four stages and zones of projection cell development has been described by Linnestad et al. (1998).

Wang et al. (1994a) defined four morphological stages in the cells of the nucellar projection in wheat, with increasing degrees of differentiation and wall ingrowth. He estimated that the plasma membrane surface area was increased about 22-fold to provide sufficient sucrose transfer capacity. *In situ* hybridization (Sturaro et al. 1998) and transcriptional analysis of excised projection tissue (Thiel et al. 2008) confirmed the presence of extensins, cell wall proteins that may be involved in defense (Wei and Shirsat 2006) or wall stability.

Callose ((1-3)-β-D-glucan) was identified in the projection cells using immunohistology and histochemistry, notably at 10–20 DAA (Asthir et al. 2001).

Endospermal transfer cell layer

After cellularization, specialized transfer cells ("crease aleurone") differentiate on the endosperm surface facing the endospermal cavity and nucellar projection (see transfer cells [tr] in Fig. 13.3d,e). The regulation of this coordinated development in barley may involve the availability of solutes for transport as shown for other systems (Offler et al. 2002).

These cells enhance transport capacity into the starchy endosperm (Wang et al. 1994a, 1995a) and thereby improve sink strength. Invertases, hexose transporters, and sucrose transporters are expressed in barley endospermal transfer cells and regulate the uptake of hexoses and sucrose into the endosperm (Weschke et al. 2000, 2003), while upregulated expression of an amino acid permease (Thiel et al. 2008) indicates a role in amino acid uptake from the endospermal cavity. Thirty-two transfer cell-specific transcripts were

characterized in wheat including invertase, pectin methylesterase inhibitors, and proteins involved in cell wall biosynthesis (Drea et al. 2005). The presence of specific small, hydrophilic proteins in cereal transfer cells may be related to defense and the prevention of pathogens from entering via the chalaza (Royo et al. 2007).

Transfer cell-specfic promoters from rice were successfully tested in barley, offering the opportunity to modify gene expression in this important tissue (Li et al. 2008).

Developmental changes in transport tissues

Morphological changes in the crease region include modification of cells walls and accumulation of pigmented secondary products in cells. Developmental changes in the vascular tissue, chalazal zone, and nucellar projection have been suggested to result in the reduction and termination of grain filling (Zee and O'Brien 1970b; Lingle and Chevalier 1985).

The chalazal cell walls are not thickened early in development, but with increasing age, the cells accumulate polyphenolic substances (tannins) in vacuoles (Cochrane 1983). About 12–14 DAF, these substances appear as small particles (see Fig. 13.3g–i) and later form massive rings that fill the cells (Cochrane and Duffus 1980). The release of tannin from the vacuoles and the degeneration of cytoplasm were observed in chalazal cells after grain filling had ceased. Cochrane (1983) suggests that these events may initiate the dehydration phase of grain development and that the cells of the xylem and parenchymatic cells leading to the projection may be involved in the process (Cochrane et al. 2000).

The chalazal cell walls are lignified and a layer of suberin is built on the secondary wall (Cochrane 1983), beginning at about 18 DAF in cv. Halcyon (Cochrane et al. 2000). The deposition of lignin and suberin increasingly isolates the apoplast and symplast, closing the apoplastic pathway through the chalaza, but the extent to which this influences the water content of the grain is not clear (Cochrane et al. 2000). However, high resistance to apoplastic transfer was found in the pigment strand (see pi in Fig. 13.3h) of midstage wheat (Wang et al. 1994b). The deposition of lignin and suberin involves peroxidases and a peak of peroxidase activity was observed in crease extracts of barley around 10 and 40 DAF (Cochrane et al. 2000). Diaminoxidase activity was also found in the chalazal cells, and this may be the source of the H_2O_2 required for peroxidase activity (Asthir et al. 2002). Comparable secondary modifications also occur in the cells in the nucellar projection close to the chalaza. The main components of the extracted and hydrolyzed tannins from chalaza and nucellar projection in barley cv. Betzes were delphinidin and cyanidin (see section below on phenolic compounds). The tannins were localized in vacuoles that increased in size during maturation (Felker et al. 1984).

Thiel et al. (2008) isolated nucellar projection cells at 8 DAF by laser dissection and determined their transcriptome profiles. This confirmed the upregulation of genes related to transport, amino acid metabolism, and secondary plant metabolism.

Effective transport is maintained despite rapid changes in size and organization of ear and grain from the initiation of the procambium in the ovule primordium to the end of the grain filling. The final collapse of cells in the phloem and chalaza does not precede the end of grain filling but follows later (Lingle and Chevalier 1985; Cochrane et al. 2000).

Development of the protective layers of the grain

The protective layer of the ripe barley grain comprises remnants of transient tissues and persistent layers.

The outermost of these is the husk comprising the fused palea and lemma, which adhere to the pericarp by a lipidic cementing substance (Gaines et al. 1985; Taketa et al. 2008). In cultivated naked barleys, the husks do not adhere and fall off during threshing (Taketa et al. 2004). Below the husk is the thickened epidermis of the pericarp with its outer cuticule, remnants of the hypodermis and walls of crushed parenchyma cells (see Fig. 13.3k,o). Cross cells, oriented at right angles to the longitudinal axis of the grain, are remnants of the two to three

chlorophyll-containing cell layers lining the inner epidermis of the pericarp (see Fig. 13.1), while scattered tube cells originate from the small cells of the inner pericarp epidermis (see Fig. 13.1a) (Krauß 1933; Tharp 1935).

The seed coat itself represents the remaining cells and cell walls of the integuments and includes the chalazal zone on the ventral side of the grain (Werker 1997). In barley, it is formed from the inner layer of the inner integument (see Fig. 13.1a), from the compressed walls of the outer layer and from associated cuticulae. In principle, the cuticulae can cover the outer and inner surfaces of each integument and, additionally, the outer nucellar epidermis (Werker 1997). In barley, the cuticular layers lie on both sides of the inner integument (Krauß 1933). In barley and wheat, the layer on the inside originates from the nucellar epidermis and later adheres to the inner side of the seed coat (Morrison 1975; Cochrane and Duffus 1979; Freeman and Palmer 1984; Duffus and Cochrane 1993).

The nucellar epidermis collapses at the time of aleurone differentiation (see Fig. 13.1d). Only crushed cell walls remain (Tharp 1935) and form the hyaline layer, which adds to the protective layer around the endosperm in the ripe grain (Duffus and Cochrane 1993)

At anthesis, the inner and the outer integuments, each comprising two cell layers, constitute the outer border of the ovule. The two integuments differ in orientation and their cells differ in structure (compare the cross section and longitudinal section in Fig. 13.1a). The cells of the outer integument have thinner radial walls, and this layer degrades during the massive elongation of the ovule and is mostly lost a few days after fertilization. In contrast, the inner integument persists longer, adapting to ovule growth by cell divisions and, in later stages, by extension of the cells (Krauß 1933; Tharp 1935).

The cells of the inner layer of the inner integument accumulate proanthocyanidins in vacuoles (see Figs. 13.1d and 13.3h, arrow). These phenolic compounds may confer resistance to pathogens and also make the cells impermeable (Freeman and Palmer 1984; Quinde-Axtell and Baik 2006). Microarray analysis of transcripts from seed coats of wild-type barley and a mutant deficient in anthocyanin/proanthocyanidin synthesis allowed the identification of transcripts involved in proanthocyanin synthesis (Pang et al. 2004).

In *Arabidopsis*, the endosperm and seed coat interact to coordinate the size of the endosperm and integument. Restriction of cell elongation in the seed coat restricts endosperm growth and seed size, while endosperm growth modulates seed coat cell elongation (Garcia et al. 2005; Ingouff et al. 2006). Extra cell divisions in the outer integument of a transgenic line of *Arabidopsis* overexpressing the *KNAT1* gene were compensated by smaller cell sizes, again indicating a control mechanism (Truernit and Haseloff 2008). A survey of genes expressed in the *Arabidopsis* seed coat is reported by Moïse et al. (2005).

Seed coat-specific promoters have been identified in barley, offering the possibility to express antifungal agents and the modification of cell wall composition and structure (Wu et al. 2000).

The permeability of the protective layers of the grain to water is mainly determined by the testa and nucellar epidermis and their cuticular layers and by the pigment strand (Briggs and MacDonald 1983), the pericarp and husk being readily permeable.

Cell death during grain development

Various cells and tissues are depleted or degraded as an integral part of caryopsis development (see Fig. 13.1), among them the nucellus, antipodal cells, outer integument, inner epidermis of the pericarp, pericarp parenchyma, outer layer of the inner integument, nucellar epidermis, starchy endosperm and cells of nucellar projection and chalaza. During monosporic megasporogenesis, three meiotic products are regularly discarded, and synergid degeneration is necessary during fertilization (An and You 2004; Rogers 2005).

PCD is a common phenomenon in development with various mechanisms being reported in plants (Beligni et al. 2002). These include apoptotic-type cell death involving capsaselike activity, DNA cleavage to generate nucleosomal ladders, and the release of mitochondrial proteins

(Reape and McCabe 2008). Cysteine proteases (Solomon et al. 1999) and aspartic proteinases such as nucellin and phytepsin (Simões and Faro 2004) are also involved in forms of plant cell death.

In cereals, PCD occurs in the starchy endosperm (Young and Gallie 2000) and in aleurone cells, but at different times and using different pathways (Fath et al. 2000). In barley, a capsase-like proteolytic activity can be detected in randomly distributed cells of the starchy endosperm as early as 9 DAF and is present throughout the endosperm at about 20 DAF. The enzyme activity colocalizes with autophagosomes. The starchy endosperm cells do not show nuclear DNA fragmentation before about 19–25 DAF (Borén et al. 2006). The initiation of cell death corresponds to the mid- to late postfertilization stage of wheat development, and the whole starchy endosperm is affected by 30 DAF (Young and Gallie 1999).

Cell death in the barley aleurone after germination is controlled by giberellic acid (GA) and abscisic acid (ABA) and may result from autolysis (Cejudo et al. 2002; Domínguez et al. 2004; Bethke et al. 2008). Reactive oxygen is involved in this process (Fath et al. 2001) and cell death can be delayed by nitric oxide treatment (Beligni et al. 2002).

The degeneration and lysis of the nucellus cells begin shortly before fertilization and take place over several days (Engell 1994; Norstog 1974). Engell (1994) described the presence of nucellar lysate close to the antipodal cells (see Fig. 13.2h). Nucellin (an aspartic protease) may be related to nucellus degradation and is strongly expressed at 3–4 DAF in all cells of the barley nucellus. Before fertilization, nucellin activity is restricted to a group of cells close to the embryo sac in the chalazal area (Chen and Foolad 1997) where nucellar degradation starts. When expressed in rice and barley, the rice ortholog of barley nucellin was located in the nucellus and the embryo (Bi et al. 2005). A three-dimensional visualization (for more information, see http://3d-barley.ipk-gatersleben.de) of the expression pattern of nucellin at anthesis in barley is demonstrated by Gubatz et al. (2007). DNA fragmentation was reported in the nucellus of barley at 4 DAA (Borén et al. 2006).

Nucellain, a vacuolar processing protease, was immunolocalized in walls of autolysing nucellus, nucellar epidermis, and nucellar projection of wheat (Linnestad et al. 1998), which is similar in structure to barley.

Most of the cells in the nucellar projection at 13 DAA and the cells near the pigment strand at 18 DAA tested positive in an assay for internucleosomal DNA fragmentation. The cells close to the endosperm cavity were already totally degraded at this point. Electron microscopy showed features of typical cell death but not typical of the (animal) apoptotic pathway (Domínguez et al. 2001). A transcript encoding a small protein associated with cell death and/or differentiation has also been reported in the nucellar projection (Radchuk et al. 2006).

Phytepsin, a vacuolar protease, has also been related to DNA fragmentation in the scutellum of barley during germination (Lindholm et al. 2000).

Degradation of the nucellus and antipodal cells and depletion of the pericarp are correlated to changes in the supply of nutrients to the filial tissues at the respective stages of development.

Staining for viability showed that cell death was already occurring at 4 DAA in the pericarp, while internucleosomal fragmentation was observed at 5 DAA (Domínguez et al. 2001). Gene expression analysis suggests the involvement in this tissue of a specific class of proteases under the control of jasmonic acid and ethylene (Sreenivasulu et al. 2006).

COMPOSITION OF THE MATURE GRAIN

Distribution of storage components in the mature barley grain

Aleurone layer

The aleurone layer of the mature barley grain comprises two or three layers of thick-walled cells that are rich in storage reserves: lipid, protein, and minerals. The lipids are present as oil droplets and the expression of oleosin transcripts during grain development (Aalen et al. 1994; Aalen 1995) indicates that these are likely to be stabilized by an outer layer of oleosins

and phospholipids as in other seed tissues (Napier et al. 2001).

The aleurone layer also contains "aleurone grains," storage bodies that contain two types of inclusions called globoids and crystalloids (Buttrose 1971; Jacobsen et al. 1971). The globoids are composed of phytin (see section below on minerals) while the crystalloids stain for protein and carbohydrate. The major component in the crystalloids is probably the 8S globulin storage protein (see section below on storage globulins), but the nature of the components responsible for reaction with carbohydrate stains has not been identified. The 8S globulin has been purified and characterized from preparations enriched in aleurone cells (Yupsanis et al. 1990; Banciu et al. 2007), and its presence in wheat aleurone cells has been demonstrated by tissue printing (Wiley et al. 2007) and proteomic analysis (Laubin et al. 2008). Little or no starch is observed.

Embryo

The distribution of storage components in the embryo (germ) of barley has not been studied in detail. However, the embryo is known to be rich in lipids, accounting for over 20% of the dry weight (Bhatty 1982). About 90% of the lipid is triacylglycerols (Bhatty 1982), which are predominantly located in oil bodies in the scutellum. The expression of oleosin transcripts in the developing embryo suggests that these bodies are surrounded by oleosins, as discussed above for the aleurone. The embryo also contains 8S storage globulins (Heck et al. 1993), as in other cereal species (Kriz 1999).

Although starch is present in the embryo during development (Duffus and Cochrane 1993), it is digested and little or none is present at maturity.

Starchy endosperm

Although the starchy endosperm is often considered as a homogenous tissue comprising cells that are rich in starch, this is clearly not the case. Light microscopy of mature grain shows a clear gradient in cell size and composition from the subaleurone to central parts of the endosperm, with more details being provided by the use of specific stains and antibodies and by analyzing grain fractions.

The subaleurone layer is not clearly defined but generally comprises two or three cell layers immediately below the aleurone. These cells are rich in proteins, which form a continuous matrix, with few or no starch granules (Fig. 13.1d, arrow). Below this layer, the cells have a typical "starchy endosperm" appearance, being packed with starch granules but with low contents of proteins (see Figs. 13.1d and 13.3m).

In addition to this gradient in protein content, there are also gradients in composition. This has been demonstrated by the analysis of fractions removed sequentially from the outside of the grain by pearling (Millet et al. 1991; Shewry et al. 1996) and by immunocytochemical labeling of tissue sections (Darlington et al. 2000; Tesci et al. 2000). Such studies show that the subaleurone cells are rich in the S-rich and S-poor B, C, and γ-hordeins but lack D hordein. In contrast, D hordein is concentrated within the central cells of the starchy endosperm.

Studies of developing barley and wheat grains (Philippe et al. 2006; Wilson et al. 2006; Toole et al. 2007) indicate that gradients in cell wall composition may also exist in the starchy endosperm of the mature barley grain, but these have not so far been studied.

The existence of gradients of protein, starch, cell wall composition, and other components is clearly of interest in relation to the exploitation of the grain.

Carbohydrates

Total carbohydrates have been reported to account for 78%–83% of the mature barley grain (MacGregor and Fincher 1993) and starch for 50%–70% (see data in Table 13.1 compiled by Henry (1988). Most of the remaining carbohydrate is cell wall polysaccharides, including β-glucans, cellulose, and arabinoxylans (AX). Only small amounts (2%–35%) of sucrose and other sugars are present.

Table 13.1 Examples of reported carbohydrate compositions of barley grains

Carbohydrate	Fraction	% of Dry Weight
Monosaccharides	Glucose	0.03–0.6
Disaccharides	Fructose	0.03–0.16
	Sucrose	0.34–2.0
Oligosaccharides	Maltose	0.006–0.14
	Fructans	0.019–0.97
Polysaccharides	Raffinose	0.14–0.83
β-D-glucans	Starch	51.5–72.1
	Pentosan	4.4–7.8
	(arabinoxylan)	3.64–6.11
	β-glucans	1.44–5.0
	Cellulose	
Total carbohydrate		78.0–83.9

Taken with permission from Henry (1988).

Starch

Although starch is synthesized in the pericarp and embryo of developing cereal grains, its presence in these tissues is transitory, and little or no starch is present in any tissues of the mature grain except for the starchy endosperm where it is the major component. The details of starch biosynthesis in cereals have recently been reviewed by Tomlinson and Denyer (2003) and will not be discussed here. Similarly, many features of barley starch are common to starches from all cereals or all plants and are therefore not discussed in detail.

Composition

As in all species, barley starch is a mixture of two components, amylose and amylopectin, both comprising glucose units. Amylose consists of essentially linear chains of (1 → 4)-α-linked D glucose residues, with a low level of (1 → 6)-α-linkages leading to infrequent branching. Although the size of the amylose molecule varies, MacGregor and Fincher (1993) calculated an average degree of polymorphism (DP) (i.e., number of glucose units per molecule) of 1800. They also suggested that the degree of branching may resemble that of amylose from wheat, which has an average of five side chains per molecule (Takeda et al. 1984, 1987). These branches vary from about 4 to over 100 glucose units in length in amyloses from other cereals (Takeda et al. 1984, 1990).

In contrast, amylopectin is a highly branched molecule, with most linkages being (1 → 4)-α but with 1 in 20 being (1 → 6)-α. It has an average molecular weight of about $3.6–4.1 \times 10^8$ (MacGregor and Fincher 1993) and comprises several thousand glucose units. Amylopectin is the major component in "normal" barley starches, with reported proportions ranging from about 65% to 77% (reviewed by MacGregor and Fincher 1993). However, two types of mutation can result in changes in the proportions of amylose and amylopectin.

First, "waxy" lines typically have 90%–98% amylopectin (see MacGregor and Fincher 1993 for details of analyses) resulting from mutations in the granule-bound starch synthase I (GBSSI) enzyme responsible for amylose synthesis (Patron et al. 2002). However, Ishikawa et al. (1995) reported on two sodium azide-induced hulless waxy mutants from a nonwaxy hulless progenitor without amylose. Bhatty and Rossnagel (1997) described two zero amylose hulless barley lines resulting from a waxy/waxy cross. Waxy phenotypes occur in most other cereals (notably maize and rice) and find specific uses in food processing. Second, high-amylose (sometimes called amylose extender) types result from mutations affecting amylopectin synthesis. Banks et al. (1973) reported that two varieties of barley, Glacier CI9676 and Glacier AC38, had amylose contents of 28% and 45%, respectively, while Morell et al. (2003) described two ethylmethane sulfonate (EMS)-induced mutant lines of cv. Himalaya, which contained 62.5% (M342) and 70% (M292) amylose. Subsequent analysis showed that M292 and M342 had mutations in the gene encoding the starch synthase IIa enzyme, which catalyses amylopectin synthesis (Morell et al. 2003). The mutation also resulted in a decrease in the chain length distribution of the amylopectin and had pleiotropic effects on other enzymes of the starch biosynthetic pathway. Hence, the effects on starch composition and properties were more complex than a simple change in the amylase-to-amylopectin ratio. Burton et al. (2002) also described two induced mutants of barley, Risø 17 and Notch 2, which

had lesions in the isoamylase gene. This resulted in the accumulation of phytoglycogen, a soluble form of $(1 \rightarrow 4{:}1 \rightarrow 6)$-α-glucan in the grain.

High-amylose starch is resistant to digestion in the gastrointestinal tract and is therefore considered to have health benefits (Nugent 2005). Such benefits have been demonstrated by feeding Himalaya M292 to rats, pigs, and humans (Topping et al. 2003; Bird et al. 2004a,b, 2008).

Starch granules

Starch granules from all plants have a similar structure, with concentric rings of hard and soft materials, which may represent daily deposits of starch. The organization of the individual amylose and amylopectin molecules within these rings is still incompletely understood, but it is thought that the structure is based on parallel molecules of amylopectin, with both crystalline and non-crystalline regions. The reader is referred to reviews by Tomlinson and Denyer (2003), Kossmann and Lloyd (2000), and Gallant et al. (1997) for more detailed discussions.

Barley starch granules comprise two discrete size classes, large A granules, which are about 15–25 μm in diameter, and small B granules of less than 10 μm in diameter. Although the B granules vastly outnumber the A granules, accounting for 80%–90% of the total, they only account for about 10%–15% of the total weight of starch. This bimodal distribution is clearly seen in scanning electron micrographs of fractured cells (Fig. 13.4) and results from two phases of granule initiation, with B-type granules being initiated and accumulating later in grain development than A-type granules (McDonald et al. 1991). The large starch granules are lenticular (lenslike) in morphology, with the small granules being less regular in shape (see Fig. 13.4)

Several reports have shown that B-type granules have lower contents of amylose (MacGregor and Ballance 1980; MacGregor and Morgan 1984; Stark and Yin 1986) than A-type granules, by about 3%–4%, although no difference was reported by Evers et al. (1973).

Differences in the time course of synthesis of amylose and amylopectin result in increases in the proportions of amylose in both A- and B-type granules during grain development (McDonald et al. 1991).

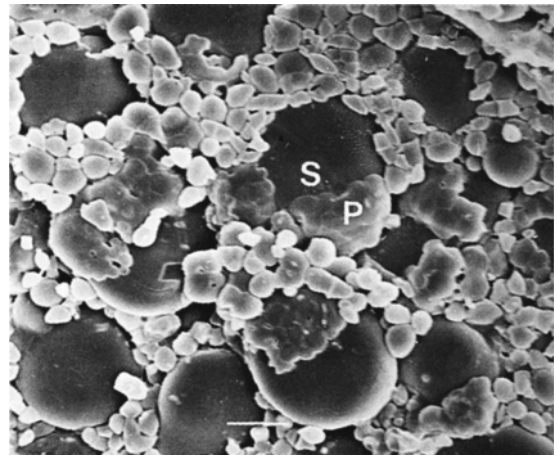

Fig. 13.4 SEM image of fractured endosperm of Chariot, a good malting, spring-sown barley cultivar, showing starch–protein binding. Taken with permission from Brennan et al. (1996). The bar represents 10 μm. S, large starch granule; P, protein.

However, granule morphology, and presumably also structure, may be affected by mutations in starch synthesis (Burgess et al. 1982; Tomlinson and Denyer 2003). Thus, the *Notch 2* mutation in the isoamylase gene results in small, irregular starch granules (Burgess et al. 1982; Tomlinson and Denyer 2003), while the *lys3a* gene in the high-lysine mutant Risø 1508 results in decreased total starch and in a reduction in the number of small starch granules (Burgess et al. 1982). Starch granule morphology is also modified in the high-amylose M292 and M342 mutant lines of Himalaya, with a shrunken irregular appearance compared to the wild-type granules. This is consistent with a role of amylopectin in the organization of the granule structure.

Cell wall components

Cell wall polysaccharides are the second most important group of carbohydrates in the grain. They comprise several polymers, which vary in their properties and distributions among tissues (Table 13.2).

Table 13.2 Approximate proportions of polymers in the cell walls of barley grain tissues

	Starchy Endosperm	Aleurone	Outer Layers
Arabinoxylan	20	71	—
β-Glucan	75	26	—
Callose	1	—	—
Cellulose	1–2	1–2	30[a]
Glucomannan	1–2	1–2	—
GAX	—	—	40–50[a]
AGP	0.02–0.03[b]	—	—

[a]Based on values for wheat.
[b]Assuming total cell walls are 10% of dry weight of grain.
—Indicates not determined or absent.

The two major cell wall polymers in the whole grain and its component tissues are (1 → 3, 1 → 4)-β-D-glucans (also called mixed linkage β-glucans or just β-glucans) and arabino-(1 → 4)-β-D-xylan (also called AX, heteroglucans, or pentosans), but the proportions of these vary among tissues. In the aleurone cells of the endosperm, the major component is AX (71%), with less β-glucan (26%) (Bacic and Stone 1981a,b). In contrast, the walls of the starchy endosperm cells comprise 75% β-glucan and 20% AX (Fincher 1975, 1976; Ballance and Manners 1978). Cellulose ((1 → 4)-β-D-glucan) and glucomannans ((1 → 4)-β-D-glucomannan polymers of approximately 30% β-glucopyranose and 70% β-D-mannopyranose) are minor components, accounting for 1%–2% of the total cell wall polysaccharides in all tissues (Henry 1988). Similarly, callose ((1 → 3)-β-D-glucan) accounts for about 1% of the total cell wall polysaccharides in the starchy endosperm, being present particularly in the subaleurone layer (Bacic and Stone 1981a; Stone and Clarke 1993). Finally, the cell walls of the starchy endosperm cells also contain an arabinogalactan peptide (AGP), which has not been quantified in barley but, based on wheat, may account for 0.2%–0.3% of the flour dry weight.

The outer layers of the mature grain (pericarp and testa) have not been analyzed in barley but have been studied in wheat and are rich in glucuronoarabinoxylans (GAX) (which contain glucuronic acid and galactose) (40%–50%) and cellulose (up to 30%) (reviewed by Saulnier et al. 2007).

Henry (1986) compared 17 barley cultivars grown at three locations in Australia and found variation from 3.44% to 5.68% dry weight in β-glucans and from 4.38% to 7.79% in pentosans. The ratio of pentosans to β-glucans varied from 2.26 to 1.05, and their combined amounts from 8.5% to 11.8% dry weight. Henry (1985) also showed that total β-glucans varied from 4.03% to 5.26% in 13 cultivars and 12 lines grown on two sites in Australia over 2 years.

β-Glucans

(1 → 3, 1 → 4)-β-D-Glucans are linear polymers of β-D-glucopyranosyl monomers, joined by (1 → 3) and (1 → 4) linkages. They comprise a mixture of molecules, which may differ in their size and ratio of linkages, and this may be reflected in differences in solubility and viscosity of solutions. In general, there are about twice as many (1 → 4) linkages as (1 → 3) linkages with single (1 → 3) linkages being interspersed between two or more (1 → 4) linkages. In contrast, adjacent (1 → 3) linkages are rarely, if ever, present. MacGregor and Fincher (1993) reviewed the extensive literature on barley β-glucan fractions, which are soluble at 40 and 65°C. The fraction that was soluble at 40°C contained a slightly higher proportion of (1 → 4) linkages (72% compared with 69%), and this was reflected in the presence of a greater number of blocks of three or more adjacent (1 → 4) linkages.

Stone (1996) suggested that, whereas most of the β-glucans in the cell walls of the starchy endosperm are soluble in water at 40°C, dilute alkali is required to extract the β-glucans from the aleurone cell walls.

Rimsten et al. (2003) showed that β-glucan fractions extracted from a naked barley variety had average molecular weights of 164×10^4 (hot water extract), 173×10^4 (sodium hydroxide extract), and 162×10^4 (sodium carbonate extract), while Andersson et al. (2008) reported average molecular weights ranging from 152×10^4 to 184×10^4 for hot water extracts of 10 barley lines grown on the same Hungarian site.

β-Glucans are considered to have an extended "snakelike" conformation in solution, with the (1 → 3) linkages disrupting cellulose-like stretches of (1 → 4) linkages (Stone 1996). Such solutions also have high viscosity, reported as 18–24.7 dL/g in water (Aastrup 1979).

Mixed linkage β-glucan is a significant source of dietary fiber with the β-glucans from barley and oats having recognized health benefits in attenuating postprandial glycemic and insulinemic responses, these benefits having been recognized by the U.S. Food and Drug Admnistration (FDA), which has accepted health claims for products containing barley β-glucan (Anonymous 2008). These benefits and their physiological basis are discussed in several recent review articles (Lazaridou and Biliaderis 2007; Topping 2007; Wood 2007; Baik and Ullrich 2008).

A key characteristic determining the health benefits of β-glucans appears to be their viscosity in solution (as discussed by Wood 2007). Viscosity is also partly responsible for the negative effects of β-glucans in brewing, resulting in slow filtration. β-Glucans may also limit modification of the endosperm during malting and contribute to the formation of hazes and precipitates in beer. However, they may also contribute positively to quality, affecting "body" (mouth feel). These effects on malting and brewing are discussed in detail by MacGregor and Fincher (1993).

AXs

AXs consist of a chain of (1→4) linked β-D-xylopyranose residues with some of the xylose residues carrying an L-arabinofuranose residue. These arabinose residues are usually linked to the *O*-3 position but less frequently to the *O*-2 position, or both positions. In husk fractions, the AX polymers are also substituted with glucuronopyranosyl residues, which account for about 4% of the total sugars. These GAXs account for about 40%–50% of the cell walls in the husk (see Table 13.2) but do not occur in aleurone or starchy endosperm cell walls (MacGregor and Fincher 1993). The ratio of arabinose to xylose varies significantly, being about 1:9 in the husk but much lower in the starchy endosperm.

Phenolic acids are also linked to AX in the endosperm, aleurone, and husk, with ferulic acid being the main component followed by *p*-coumaric acid. Phenolic acids have been reported to account for about 0.05% of the cell walls in the starchy endosperm and 1.2% in the aleurone (MacGregor and Fincher 1993). However, a recent study indicated that significant variation may occur between cultivars. Andersson et al. (2008) determined the amounts and compositions of bound (i.e., mainly cell wall-associated) phenolic acids in 10 barley cultivars. The total amounts varied from 133 to 523 μg/g with ferulic acid and *p*-coumaric acid accounting for 54%–81% and 2%–24% of the total, respectively. Several isomeric dehydrodimers of ferulic acid also occur in barley. These are mainly present as cross-links between AX molecules but probably also form cross-links with lignin in the outer layers of the grain. The formation of these cross-links occurs by oxidative coupling of ferulate after its attachment to the AX molecule and could be catalyzed by an enzyme such as an oxidase or peroxidase. Hernanz et al. (2001) reported detailed studies of hydroxycinnamic acids (caffeic, ferulic, and *p*-coumaric) and dehydrodimers of ferulate in a range of barley varieties and grain fractions isolated by mechanical dissection. The total *p*-coumaric acid and ferulic acid contents in 11 cultivars ranged from 79 to 260 μg/g and from 359 to 624 μg/g, respectively, and the total contents of ferulic acid dehydrodimers from about 135 to 233 μg/g. Analysis of three fractions corresponding to the outer layers, endosperm, and an "intermediate fraction" showed that the former (which corresponded to 47.5% of the grain) contained about 78% of the *p*-coumaric and ferulic acid and 81% to 86% of the dehydrodimers of ferulic acid.

The solubility of AX polymers varies and may depend on differences in both the A : X ratio and in phenolic acid cross-linking. Estimates of the proportions of water-soluble and total AXs in "bran" and "flour" fractions of 10 barley cultivars were reported by Andersson et al. (2008), although yields of flour and bran varied widely from 18% to 36% and from 3% to 63%, respectively. Total and water-extractable AXs in the bran fractions

varied from 5.81% to 9.03% dry weight and from 0.15% to 0.35% dry weight, respectively, and in flour from 1.40% to 2.24% dry weight and from 0.15% to 0.38% dry weight, respectively.

Although interest in the nutritional properties of AXs in barley is limited compared to that in β-glucans, they may share at least some of the health benefits (Topping 2007).

AGP

Early studies of wheat flours showed the presence of a water-soluble component, which had an M_r of about 22,000 and comprised about 92% polysaccharide, with a ratio of about 1.5 galactose : 1 arabinose (Fincher and Stone 1974; Fincher et al. 1974). This AGP has since been shown to have a mass of 22,700 and to comprise 15 amino acid residues including three hydroxyprolines (glycosylation sites) (van den Bulck et al. 2002). It accounts for about 0.24%–0.33% of white flour and from 0.29% to 0.38% of milling fractions (Loosveld et al. 1997, 1998).

Van den Bulck et al. (2005) described a related component in barley flour, comprising 16 amino acids (including three conserved hydroxyprolines as in the wheat AGP) and with a mass of 24,700. The yield was low, only 0.01%, but the authors noted that severe losses occurred during purification. It is therefore possible that similar amounts of AGP occur in barley as in wheat.

Other sugars

Little work has been carried out in recent years on other sugars in barley, with the earlier work being reviewed by Henry (1988) and MacGregor and Fincher (1993). The only three sugars that are present in significant amounts are sucrose, raffinose, and fructans, with MacGregor and Fincher (1993) quoting values of 0.74%–1.9% dry weight, 0.16%–0.56% dry weight, and 0.35–0.78% dry weight for these, respectively.

Fructans are oligosaccharides of fructosyl residues with components comprising up to 10 fructosyl units being present in barley (MacLeod 1953). Wider variation in fructan content (0.7%–2.9% dry weight) has been reported in wheat (Huynh et al. 2008), where they are concentrated in the bran and shorts (a fine bran-rich fraction from milling) and comprise up to 19 fructosyl units (Haskå et al. 2008). Fructans are of current interest as their fermentation in the colon may increase the absorption of minerals (Gregor 1999; Niness 1999; Coudray et al. 2006).

Proteins

Proteins comprise about 8%–15% of the total dry weight of the mature barley grain, the total amount depending primarily on the availability of nitrogen and to a lesser extent on genetic differences between lines. Barley grain proteins have been studied for over 200 years (Einhof 1806) and an extensive literature has developed. Much of the earlier work has been reviewed by Shewry (1993), so the present account will focus on the current picture.

How many proteins are present in the grain?

Gene expression studies using the Affymetrix Barley1 GeneChip showed that 13,782 of the 21,439 genes on the arrays (i.e., about 64%) were expressed in caryopses at 5 DAA, falling to 11,453 in caryopses at 16 DAA and 10,345 in endosperms at 22 DAA (Druka et al. 2006). However, the number of expressed genes determined by this study may be an underestimate as the GeneChip provides incomplete coverage of the barley genome. Furthermore, many of the proteins encoded by these genes are also likely to be present at low levels and/or only transiently, and the total protein complement of the mature dry grain is not known. Nevertheless, it is likely to amount to thousands rather than the hundreds of proteins that can currently be separated by proteomic studies. For example, Ostergaard et al. (2004) detected about 1200 spots on 2D gels of water-soluble proteins from mature barley seeds when a sensitive silver staining method was used, but only about 600 when using less sensitive Coomassie Brilliant Blue as a stain. Similar studies using silver staining by Finnie and Svensson (2003) showed about 850 spots in aqueous extracts of whole seeds and 575, 850, and 1000 spots in similar extracts of starchy endosperm, aleurone,

and embryo fractions, respectively. Combining the spots mapped in the three fractions gave an increase of about 15% in the total number compared to the whole seed extracts. Finnie et al. (2006) reported that currently, some 450 individual spots on 2D gel separations had been analyzed leading to the identification of 260 different proteins.

Similarly, detailed mapping of the starch granule proteins was reported by Borén et al. (2004). Starch granule preparations from mature grain typically showed about 150 spots on 2D gels, of which 74 spots were identified by mass spectrometry. Forty-nine of the spots were found to correspond to forms of GBSSI, while others corresponded to prolamin storage proteins (hordeins), which were presumably bound to the outside of the granules.

Barley seed proteins, in common with those of other cereal grains, are traditionally classified on the basis of their extraction in a series of solvents, a concept developed by Osborne (1895, 1924). These "Osborne fractions" include proteins soluble in water (albumins), dilute salt solutions (globulins), aqueous alcohols (prolamins), and dilute acid or alkali (glutelins). This classification has proved to be remarkably durable, although with extensive modifications, mainly because it allows a simple separation of the prolamins (hordeins) from the other grain proteins. The latter include other types of storage protein, a range of enzyme inhibitors and enzymes (all mainly present in the albumin and globulin fractions), as well as structural proteins present in cell structures and cell walls (mainly present in the glutelin fraction).

It is not possible to discuss the whole protein complement of the grain here and we will therefore focus on those components that are present in the mature grain in sufficient amounts to affect the overall composition and properties. These groups are summarized in Fig. 13.5 in which they are classified into Osborne fractions (based on solubility) and on the basis of function.

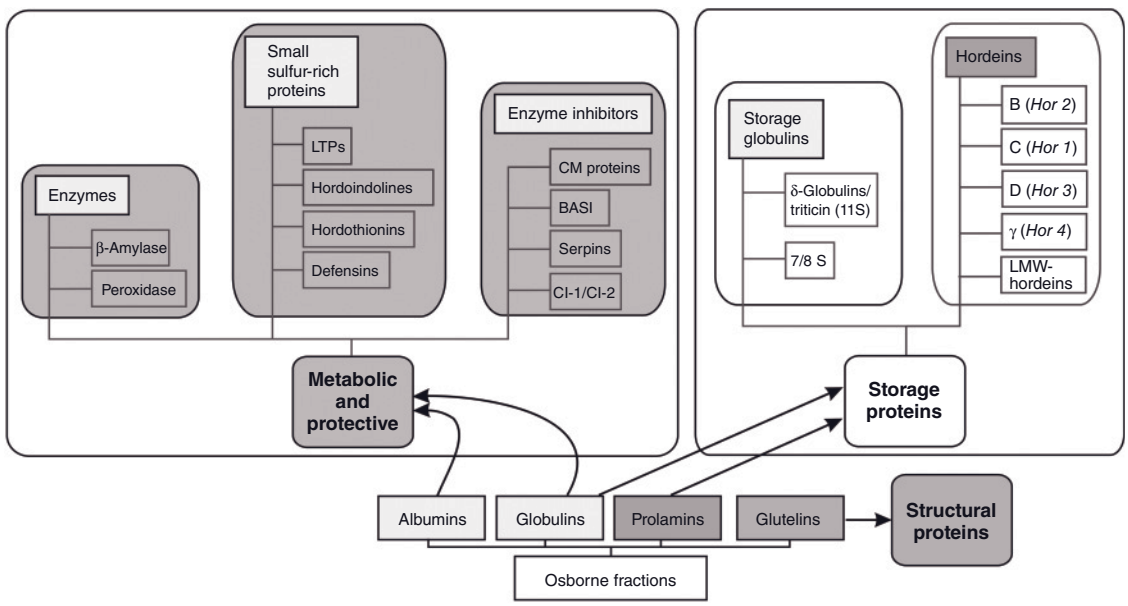

Fig. 13.5 Summary of the groups of ripe grain proteins that are described in the text. For convenience, they are classified in Osborne fractions (based on solubility) and functional groups, with members of the prolamin superfamily outlined in red boxes.

Storage proteins

Hordeins

The hordeins are the major group of storage proteins in barley grain, being located in the starchy endosperm cells where they are deposited in discrete protein bodies. They account for between about 35% and 50% of the total grain nitrogen, depending on the grain protein content, which is itself determined by nitrogen availability to the plant (Fig. 13.6) (Kirkman et al. 1982).

Total hordeins can be prepared in an essentially pure form by extracting milled grain with aqueous alcohol (usually 50% [v/v] propan-1-ol) containing a reducing agent (dithiothreitol or 2-mercaptoethanol), the latter being required to reduce the disufide bonds, which stabilize polymeric forms (see Shewry et al. 1986 for a discussion).

Hordeins are readily separated by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE), which shows a number of components varying in apparent molecular mass from about 100,000 to 35,000. There is also a high level of polymorphism among the patterns of hordein components among different genotypes, which is sometimes used to confirm varietal identity and sample homogeneity. A typical example of hordein fractions from six cultivars grown in the 1980s is shown in Fig. 13.7 (taken from Bunce et al. 1986). This figure also shows the classification of the hordein components into four groups, which are encoded by separate loci on barley chromosome 1H(5) (see Table 13.3). The major groups are the B and C hordeins, which are encoded by the *Hor2* and *Hor1* loci, respectively, and which account for about 70%–80% and 10%–20% of the total fraction, respectively.

γ-Hordeins are relatively minor components (<5% of the total) and have similar mobilities on SDS-PAGE to the B hordeins. They were initially identified in Risø mutant 56, a line in which deletion of the *Hor2* (B hordein) locus has occurred allowing the γ-hordeins to be readily purified and characterized (Shewry et al. 1985). Analysis of this mutant also demonstrated that the γ-hordeins are encoded by a separate locus to the B hordeins, and this was subsequently mapped and designated as *Hor5* (also called *HrdF*) (Shewry and Parmar 1987). A minor locus encoding B hordein proteins was also mapped and designated as *Hor4* by Shewry et al. (1988b). Finally, the single D hordein band is encoded by the *Hor3* locus.

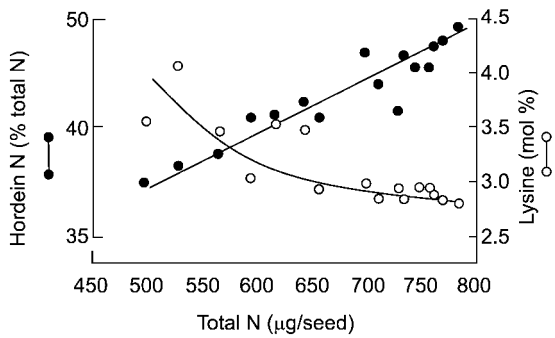

Fig. 13.6 The relationship between total seed N, the proportion of hordein proteins, and the grain lysine content. Taken with permission from Kirkman et al. (1982).

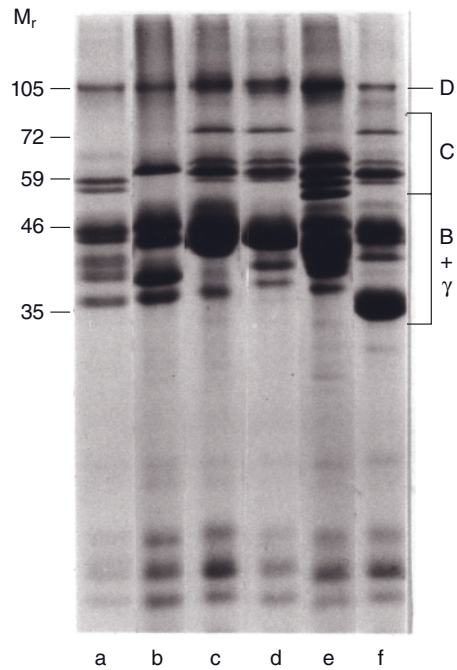

Fig. 13.7 SDS-PAGE of total hordein fractions from six barley varieties, showing the classification in B, C, D, and γ-hordeins. Taken with permission from Bunce et al. (1986).

Table 13.3 Characteristics of the four groups of hordein proteins

Group	Amount (% Total Fraction)	Form Polymers?	Loci	Partial Amino Acid Composition (mol%)								Repeat Motifs
				Gln	Pro	Phe	Gly	Cys	Lys	Met	Thr	
B	70–80	Yes	Hor 2 (Hor 4)	35	30	5	3	2.5	0.5	0.6	2.0	PQQPX(XXX)
C	10–20	No	Hor 1	40	30	8	0.3	0	0.2	0.2	1.5	PQQPFPQQ
D	2–4	Yes	Hor 3	30	12	6	16	1.5	1	0.5	8.0	PG or HQGQQ GYYPSXTSPQQ TTVS
γ	<5	Some	Hor 5 (Hrd F)	30	20	6	3	3	1.5	1	3.0	PQQPFPQQ

Based on data from various sources (see Shewry 1993; Shewry and Tatham 1997; Shewry and Darlington 2002).
Standard single- and three-letter abbreviations are used for amino acids: Cys/C, cysteine; Gln/Q, glutamine; Gly/G, glycine; His/H, histidine; Lys/K, lysine; Met/M, methionine; Phe/F, phenylalanine; Pro/P, proline; Ser/S, serine; Thr/T, threonine; Tyr/Y, tyrosine; Val/V, valine; X, other amino acids.

Many sequences of hordeins are available in sequence and expressed sequence tag (EST) databases where they can be readily accessed by the reader. Hence, only a brief summary is presented here.

All groups of hordeins are rich in glutamine, which comprises 30%–40% of the total amino acid residues (Table 13.3). B, C, and γ-hordeins also contain 20%–30% proline, but lower amounts of this amino acid are present in D hordein. However, D hordein also contains high proportions of glycine (16 mol%, i.e., 16 moles of glycine per 100 moles of amino acids) and threonine (8 mol%), while C hordein contains a higher proportion of phenylalanine (8 mol%). All hordeins are relatively low in lysine and cysteine, the latter being absent from C hordeins. These characteristic amino acid compositions result from the fact that all hordeins contain extensive domains comprising repeated sequences based on short peptide motifs (Table 13.3, Fig. 13.8).

The repetitive domains of the hordeins may also form regular spiral supersecondary structures based on β-reverse turns and poly-L-proline II structures. These structures have been studied in most detail for C hordeins of barley and the high molecular weight glutenin subunits of wheat (which are homologs of D hordeins) and are reviewed in detail by Tatham and Shewry (1995) and Shewry et al. (2003), respectively.

The differences in the number and distribution of cysteine residues in the hordeins have important effects on the ability of the proteins to form polymeric complexes stabilized by interchain disulfide bonds. The C hordeins lack cysteine residues and hence are present only as monomers. B hordeins contain six cysteine residues that are in conserved positions when B hordeins are compared with related proteins of wheat and rye and are thought to form three intrachain disulfide bonds (as discussed by Shewry and Tatham 1997; Shewry et al. 1999). In addition, B hordeins contain one or more additional "unpaired" cysteines, which probably form interchain disulfide bonds, leading to the presence of polymeric complexes. Similarly, γ-hordeins contain eight conserved cysteines which form four disulfide bonds as in the γ-gliadins of wheat, but some proteins also contain additional cysteines (as shown in the example in Fig. 13.8), resulting in both monomeric and polymeric forms. The former are sometimes called γ_3-hordeins and the latter γ_1-hordeins and γ_2-hordeins (Rechinger et al. 1993, Pistón et al. 2004). The B and γ_1/γ_2 hordeins probably form mixed polymers with D hordein, which contains a number of cysteine residues (Gu et al. 2003; Pistón et al. 2007) and is only present in polymers.

The polymers formed by D, B, and γ_1/γ_2 hordeins can be isolated as a "gel protein" fraction by stirring milled grain with SDS solution followed by centrifugation (Smith and Lister 1983; Brennan et al. 1998). This fraction may be similar

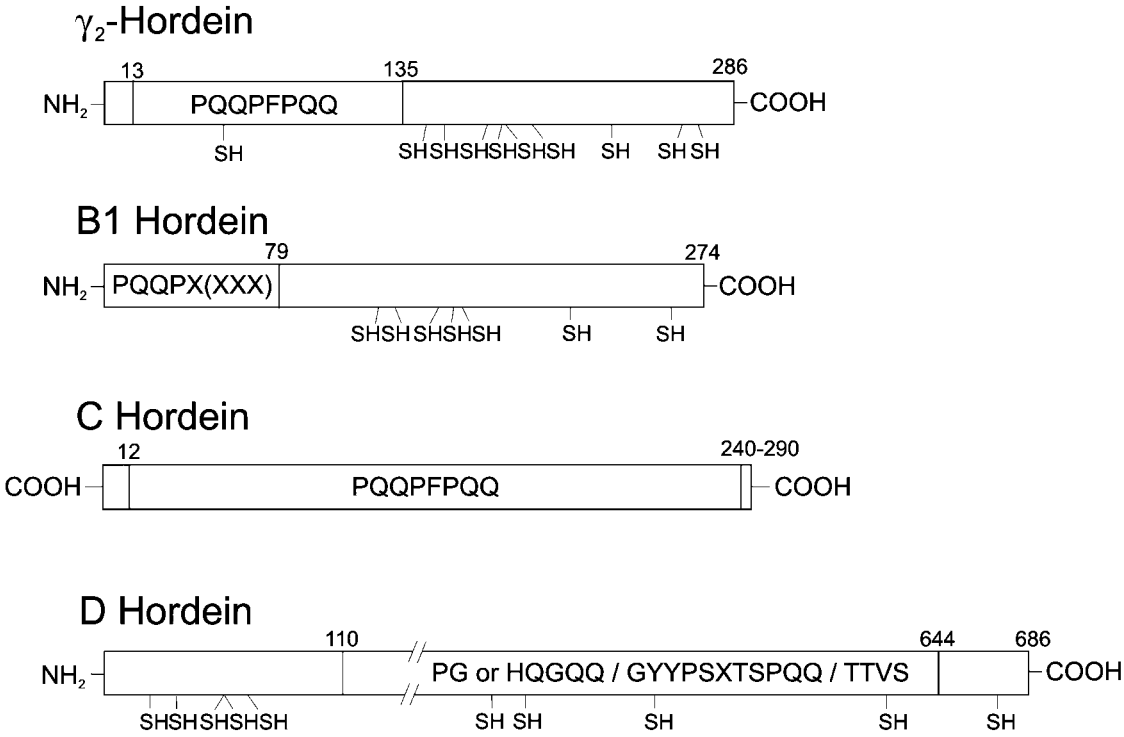

Fig. 13.8 Schematic comparison of the sequences of typical hordeins showing the repetitive motifs and positions of cysteine residues (SH).

in structure to the glutenin polymers formed by the related structurally gluten proteins of wheat (see Shewry et al. 2003 for a review). The gel protein fraction may also physically limit modification during malting, as suggested by Smith and Lister (1983).

The low contents of lysine in the hordeins result in the whole seed being deficient in lysine, which is the first limiting amino acid when barley grain is used to feed humans and monogastric livestock (reviewed by Shewry 2007). For example, whole barley grain contains about 3.5 g lysine/100 g protein compared to FAO recommended levels of about 4.5 g/100 g protein for adults (Protein and Amino Acid Requirements in Human Nutrition 2007). Furthermore, this deficiency is exacerbated if barley is grown with high nitrogen availability, with an increased proportion of hordein proteins resulting in a decrease in grain lysine from about 4 mol% to less than 3 mol% (Fig. 13.6) (Kirkman et al. 1982).

Low-molecular-weight (LMW) hordeins (avenin-like proteins)

This family of α-amylase and trypsin inhibitors was initially defined as chloroform methanol (CM) proteins on the basis of Aragoncillo et al. (1981) who showed that chloroform : methanol (2:1) extracts of barley grain contained a second group of proteins in addition to the CM proteins (trypsin/α-amylase inhibitors) discussed below. Two proteins were subsequently purified and were shown to have M_r of about 16,500 (Salcedo et al. 1982) and 22,000 (Festenstein et al. 1987), with high contents of glutamic acid/glutamine (over 25%), less than 10% proline, and low contents of charged amino acids. They were called LMW hordeins based on their similarity in amino acid composition, and this relationship has since been confirmed by more detailed studies of the homologs from wheat (Anderson et al. 2001; Clarke et al. 2003; Kan et al. 2006). These proteins are related to the major hordein storage proteins and are

classified in the prolamin superfamily (as indicated in Fig. 13.5). They have also been termed avenin-like proteins based on their nearest related sequences in database searches (Kan et al. 2006).

Storage globulins

Globulins are one of the Osborne fractions and are defined by their solubility in dilute saline. Storage globulins with sedimentation coefficients ($S_{20.w}$) values of 7–8 and 11–12 are present in many seeds and are often termed vicilin-like and legumin-like, respectively (see Shewry and Casey 1999).

Quensel (1942) demonstrated that barley contained a γ-globulin of about 8.15S and Danielsson (1949) showed that this was present in the barley embryo and in bran fractions of wheat.

Similar (probably identical) 7S/8S globulin fractions were prepared from embryos and aleurone layers of barley by Burgess and Shewry (1986) and Yupsanis et al. (1990), respectively. These comprised major subunits of M_r about 40,000 and 50,000, with minor subunits of 25,000 and 20,000, and partial amino acid sequencing showed homology with the vicilin-like globulins of cotton and legume species (Yupsanis et al. 1990). A more recent study of the biochemical and biophysical properties of the aleurone protein has been reported by Banciu et al. (2007).

Heck et al. (1993) identified a cDNA clone encoding a barley endosperm globulin 1 and designated the corresponding gene *Beg1*. The sequence encoded a protein corresponding to the aleurone globulin reported by Yupsanis et al. (1990), but the initial translation product was a protein of M_r about 72,000 with the smaller subunits being generated by posttranslational proteolytic cleavage (as in many other 7S-8S storage globulins). They further showed the presence of a second gene encoding a related protein. These genes, *Beg1* and *Beg2*, were both expressed in the embryo and aleurone, with the level of aleurone expression being about 25% of that in the embryo for *Beg1* but only 6% for *Beg2*.

The 8S storage globulins of barley embryo and aleurone form part of a family of embryo/aleurone-specific globulins, which have been characterized in most detail from maize (Kriz 1999).

Quensel (1942) also demonstrated the presence of a 12S δ-globulin in barley, which had a molecular weight of about 300,000. The identity of this protein is still not clear, but it could correspond to the legumin-like triticins of wheat endosperm (Singh and Shepherd 1985, 1987; Singh et al. 1988).

Enzymes

β-Amylase

Unlike α-amylase, β-amylase ((1 → 4)-α-D-glucan maltohydrolase) is synthesized during grain development and is stored in the mature endosperm in readiness for digestion of the starch reserves during germination. Most lines contain about 1 mg of β-amylase per gram dry weight (≈1% of the total seed protein), but higher amounts are present in the high-lysine line Hiproly, which also contains increased amounts of serpin (previously called protein Z as discussed below) and chymotrypsin inhibitors CI-1 and CI-2 (as discussed below) (Hejgaard and Boisen 1980). β-Amylase also acts as a storage protein in that the amount increases under conditions of high nitrogen availability (Giese and Hejgaard 1984).

β-Amylase occurs in the grain as free and bound forms (Hejgaard 1978). The free form is readily extracted in dilute salt solutions and comprises a mixture of forms, which include polymers with itself and with other proteins, notably serpins. In contrast, the bound form is only extracted using a reducing agent or by treatment with papain. However, the free and bound forms appear to comprise the same β-amylase isoforms (Shewry et al. 1988a).

Comparison of the sequence of the β-amylase protein with the sequences encoded by cDNAs indicates that β-amylase is synthesized without a signal peptide (Kreis et al. 1987), which is consistent with a cytosolic location (Nishimura et al. 1987). However, Hara-Nishimura et al. (1986) also showed that β-amylase becomes associated with the periphery of the starch granule during seed desiccation and suggested that this contributes to the increase in the proportion of the bound form, which also occurs during grain development.

Alternate forms of β-amylase occur in most, if not all, barley tissues (Shewry et al. 1988a), and genetic loci have been mapped to chromosomes 4HL and 2HL (Kreis et al. 1988). These loci appear to contain three genes, with *Bmy1* and *Bmy3* being located on 4HL and *Bmy2* on 2HL (Li et al. 2002). Although multiple forms of β-amylase occur in barley and malt, these all appear to arise from posttranslational processing of the *Bmy1* gene product, which includes the removal of a fragment of mass about 4000 from the C-terminal end of the protein during germination (Lundgard and Svensson 1986, 1987; Evans et al. 1997).

Electrophoretic analysis was used to identify two allelic patterns of β-amylase forms encoded by the *Bmy1* locus, designated *Bmy*-Sd1 and *Bmy1*-Sd2 (Evans et al. 1997). More detailed studies showed that the *Bmy1*-Sd2 allele comprised two forms, which differed in their thermostability: *Bmy1*-Sd2L (low stability) and *Bmy1*-Sd2H (high stability). The *Bmy1*-Sd1 allele exhibited an intermediate level of thermostability, while a fourth allele identified in *Hordeum spontaneum* (*Bmy1*-Sd3) also showed higher thermostability (Eglinton et al. 1998). Similar studies by Erkkilä et al. (1998) also identified two *Bmy1* alleles in cultivated barley and a third allele in *H. spontaneum*.

Ma et al. (2002) compared the amino acid sequences of the *Bmy1*-Sd1 and *Bmy1*-Sd2 alleles and identified five amino acid differences. However, site-directed mutagenesis showed that only one of these, the replacement of arginine 115 with cysteine, was capable of converting the Sd2 pattern to the Sd1 pattern (Ma et al. 2002). Ma et al. (2000) also showed that the proteolytically processed forms of the Sd1 and Sd2L alleles produced during germination had greater thermostability and substrate binding affinity, and that similar increases occurred when the four glycine-rich repeat regions (each comprising 11 residues) were deleted from the C-terminus of the recombinant protein.

Peroxidase

Peroxidase enzymes use hydrogen peroxide to oxidize a range of substrates and are of particular interest in cereal grains due to their role in the development of discoloration in pearled barley, flour, and processed food products and black point in barley.

Rasmussen et al. (1991, 1997) have characterized BP1, the major peroxidase of barley grain, and isolated the corresponding cDNA (Johannson et al. 1992). The protein has a mass of about 37,000, is synthesized only in the starchy endosperm during a short period starting at about 15 DAA, and appears to be directed to the vacuole by a C-terminal propeptide. The three-dimensional structure was determined by X-ray crystallography by Henriksen et al. (1998). March et al. (2007) used proteomic analysis to identify proteins associated with black point in barley and showed increased levels of BP1 in black-pointed grain.

Laugesen et al. (2007) reported detailed proteomic studies of barley peroxidases. They identified 13 spots as peroxidases, of which only two were forms of BP1 with the others corresponding to database sequences annotated as barley seed-specific peroxidase 1 (BSSP1) (nine forms) and barley peroxidase homolog (two forms). Although BSSP1 had not been identified before at the protein level, the amount clearly exceeded that of BP1 in some cultivars.

Further comparisons of 16 cultivars allowed them to be divided into groups based on whether they contained only BP1, only BSSP1, or both proteins. Studies of grain development also showed that the synthesis of BP1 was initiated before that of BSSP1. The putative peroxidase showed high sequence homology with a peroxidase from wheat. It was present in all 16 cultivars but expressed in other tissues in addition to the starchy endosperm. The two spots corresponding to this protein also differed from each other in their appearance during grain development, which was thought to reflect the effects of proteolytic processing.

Enzyme inhibitors

Barley grains, in common with other cereal seeds, contain a number of protein inhibitors of hydrolytic enzymes, most of which are active

against α-amylases and proteinases from exogenous sources (i.e., from insects or pathogens) rather than against endogenous enzymes. They are therefore considered to be largely protective, contributing to a fairly low level but broad spectrum defense against a range of pathogens and insects (as discussed by Shewry and Lucas 1997).

CM proteins

This family of α-amylase and trypsin inhibitors was initially defined on the basis of its solubility in chloroform : methanol mixtures (Salcedo et al. 1980) and are now known to be members of the "prolamin superfamily" of plant proteins (Jenkins et al. 2005; Finn et al. 2006) (Fig. 13.5). Subsequent studies, carried out primarily by Garcia-Olmedo and colleagues (reviewed by Carbonero and Garcia-Olmedo 1999), showed the presence of seven major subunits in barley with masses ranging between about 12,000 and 16,000. These subunits are present as either monomers, dimmers, or tetramers with the monomeric forms being active against trypsin (BT1-CMe) and α-amylase (BMAI-1), and the dimeric and trimeric forms being active only against α-amylases. None of the proteins inhibit endogenous barley enzymes, and the monomeric, dimeric, and tetrameric forms differ in their activities against amylases from different organisms (reviewed by Carbonero and Garcia-Olmedo 1999). The properties of the subunits and the designations and locations of their structural genes are listed in Table 13.4.

Chymotrypsin inhibitors CI-1 and CI-2

These two barley inhibitors belong to the widely distributed potato inhibitor 1 family of plant proteinase inhibitors (see Konarev et al. 2004) but differ from most other proteinase inhibitors, including related inhibitors from other species, in lacking disulfide bonds.

CI-1 and CI-2 were initially identified in barley as "lysine-rich" proteins, which were present in greater amounts in the high-lysine line Hiproly (Hejgaard and Boisen 1980). CI-1 and CI-2 are about 40% identical in their sequences, and both exist in at least two forms with masses of about 9000 (reviewed by Shewry 1993). The three-dimensional structure of CI-2 (Fig. 13.9) shows a

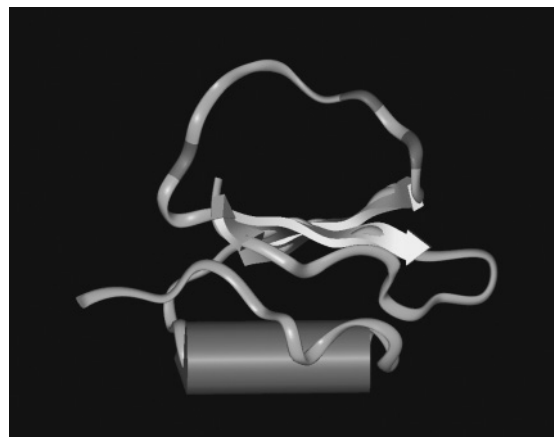

Fig. 13.9 Three-dimensional structure of the barley chymotrypsin inhibitor CI-2 showing the single α-helix (red cylinder), strands of β-sheet (yellow arms), and the reactive loop with three lysine residues (red segments). For color details, please see color plate section.

Table 13.4 The CM protein subunits present in barley grain

Inhibitory Activity	Aggregation State	Subunit	Gene	Chromosome
Trypsin	Monomeric	BTI-CMe	*Itr 1*	3HS
		BTI-CMc	Itr 2	7HS
α-Amylase	Monomeric	BMAI-1	*Iam 1*	2H
α-Amylase	Homodimeric	BDAI-1	*Iad 1*	6H
α-Amylase	Tetrameric	BTAI-CMa	Iat 1	7HS
	Subunit 1	BTAI-CMb	Iat 2	4HL
	Subunit 2	BTAI-CMd	Iat 3	4HL
	Subunit 2 (two copies)			

Taken with permission from Carbonero and Garcia-Olmedo (1999).

wedge-shaped disk, with a single α-helix, four strands of β-sheet with a left-hand twist, and a loop region containing the reactive site.

Since its discovery, CI-2 has become one of the most widely studied plant proteins. Its small size and lack of disulfide bonds have made it an attractive model for studying protein folding *in vitro*. The protein has also been used as a scaffold for the insertion of novel sequences within the reactive loop, and as a basis for designing and expressing nutritionally enhanced proteins in transgenic plants. These applications have been discussed by Campbell (1992) and Rao and Shewry (2009).

Serpins

The serpins are a family of serine proteinase inhibitors of M_r about 43,000, which include the mammalian blood plasma proteins antithrombin and α_1-antitrypsin. These and most other serpins have regulatory roles, inhibiting endogenous serine proteinases (Whisstock et al. 1998). In contrast, the cereal serpins do not inhibit endogenous enzymes, and their biological role is more likely to be defensive, inhibiting chymotrypsin-like enzymes of insects and pathogens (Roberts et al. 2003).

Two types of serpin, called BS24 and BS27, are expressed in developing barley grain and appear to be located in the starchy endosperm and, to a lesser extent, in the aleurone.

Barley serpins were initially identified as major allergens in beer (called protein Z) (Hejgaard 1984) and are also present in greater amounts in the high-lysine line Hiproly (Hejgaard and Boisen 1980).

Barley amylase/subtilisin inhibitor (BASI)

BASI is a protein of M_r about 20,000 and a member of the widely distributed family of Kunitz-type proteinase inhibitors (Richardson 1991). It is a double-headed protein, inhibiting both subtilisin and the endogenous group of α-amylase-2 (AMY2) enzymes, which are the major form synthesized in the germinating aleurone layer (Bønsager et al. 2005). Synthesis is largely restricted to the starchy endosperm during development and to the aleurone layer during germination (Leah and Mundy 1989). BASI may therefore play a role in the regulation of starch breakdown in the developing, mature, and germinating seed, including preventing breakdown during premature germination.

Other small S-rich proteins

Barley grain contains several groups of small sulfur-rich proteins, which may contribute to defense against pests and pathogens. Three of these, the CM proteins (discussed above), nonspecific lipid transfer proteins (LTPs), and hordoindolines belong to the same superfamily of proteins as the hordein storage proteins (Jenkins et al. 2005; Finn et al. 2006) (Fig. 13.5). The fourth group, the hordothionins, does not belong to this superfamily but has similar structural characteristics (being tightly folded, rich in α-helix, and stablized by multiple disulfide bonds).

LTPs

Nonspecific LTPs occur in many plant species and tissues. Although they have been widely studied, their biological role remains unknown. They were initially named based on their ability to transfer phosopholipids from liposomes to biological membranes *in vitro*. However, this activity lacks specificity (either for the phospholipids or the membrane) and is not now considered to be their biological role. They have since been suggested to contribute to defense, based on their activity against fungi *in vitro* (Gorjanovic et al. 2005) and in transgenic plants (Molina and Garcia-Olmedo 1997) and on the fact that the synthesis of some LTPs is induced by infection or damage. They have therefore also been defined as pathogenesis-related (PR) group 14 proteins (van Loon and van Strien 1999). Finally, the LTPs have also been suggested to transfer cutin and suberin monomers to the site of cuticle synthesis in epidermal cells (Douliez et al. 2000).

In common with many other plants, barley grain contains two classes of LTP, called LTP1 and LTP2, with *Mr* of about 9000 and 7000, respectively. Both are restricted to cells of the aleurone layer (Skriver et al. 1992; Kalla et al. 1994). However, these two types of protein show

little sequence identity except for the presence of a characteristic cysteine skeleton comprising eight residues: C—C–CC—CXC—C—C. Furthermore, the connectivity of these cysteines also differs between the two types of LTP (Douliez et al. 2001). Both proteins show the characteristic "four α-helical fold," which is characteristic of the prolamin superfamily (Douliez et al. 2000; Marion et al. 2004). They are able to bind lipids, and the three-dimensional structure and lipid-binding properties of barley LTP1 have been studied in detail (Heinemann et al. 1996; Lerche et al. 1997; Lerche and Poulsen 1998). Lindorff-Larsen et al. (2001) also showed that most of the LTP1 in the grain is modified posttranslationally by the esterification of an aspartic acid residue with a lipidlike component.

Many LTPs are potent food allergens (Marion et al. 2004), and this is the case for barley LTP1 whose high stability to heating and proteinases results in its presence as an allergen in beer (Gorjanovic et al. 2005; Perrocheau et al. 2006).

The addition of wheat LTP to beer has been shown to reduce lipid-induced foam destabilization (Clarke and Wilde 1994). The endogenous barley LTP1 is also present in beer foam fractions, but the isolated protein had only a small effect on foam potential and foam half-life (Sørensen et al. 1993). However, beer foam also contains a modified form of barley LTP1, called LTPb, which is reduced and glycated and is much more effective at promoting foam formation (Bech et al. 1995; Jégou et al. 2000).

Hordoindolines

Blochet et al. (1993) described the discovery of two related wheat proteins, which they called puroindolines, based on *puros* (Greek for wheat) and the presence of a characteristic tryptophan-rich sequence motif. It has since been shown that they play a major role in determining grain texture (reviewed by Morris 2002; Bhave and Morris 2008a,b; Shewry et al. 2009) and that related proteins, termed hordoindolines, are present in barley (Darlington et al. 2000, 2001; Beecher et al. 2001).

As in wheat, two types of hordoindoline are present in barley, called hordoindolines a and b. Also, as in wheat, they are encoded by genes at a locus on chromosome 5H, which is equivalent to the *Hardness* (*Ha*) locus on wheat chromosome 5D (Caldwell et al. 2004; Chantret et al. 2005). This locus is therefore also called *Ha* in barley. However, whereas single copies of the *Pina* and *Pinb* genes are present at the *Ha* locus in bread wheat (Chantret et al. 2005), the *Ha* locus of barley comprises two *Hinb* genes and a single *Hina* gene (Darlington et al. 2001; Caldwell et al. 2004). Furthermore, a comparison of the coding sequences of the *Hinb-1* and *Hinb-2* genes (also called *HoI-B1* or *HvIDa* and *Hol-B2* or *HvIDb* by other workers) with those of ESTs indicates that both *Hinb* genes are transcribed (Darlington et al. 2001).

The hordoindoline (Hin) a and b proteins are closely related to Pin a and Pin b, respectively, with amino acid sequence identities of about 85%. Although the precise masses of the mature proteins have not been determined, they are probably similar to those of the Pins, about 13,000 (Douliez et al. 2000). Hordoindolines a and b also both have the characteristic "tryptophan motif," comprising three tryptophan residues in Hin a and five in Hin b, which has been proposed to be a lipid-binding site in wheat (Kooijman et al. 1997). Extensive allelic variation in sequence also occurs, with Beecher et al. (2001) reporting three Hina and two Hinb sequences in only eight cultivars.

The mechanism and genetic control of grain texture is not as clearly defined in barley as in wheat, but adhesion between proteins and starch at the interface between the granule surface and the protein matrix appears to contribute (as in wheat) (Brennan et al. 1996). There is also evidence that hordoindolines are located at this interface and therefore may contribute "nonstick" properties as proposed for wheat (Darlington et al. 2000), and Beecher et al. (2002) and Fox et al. (2007) have shown that quantitative trait loci (QTLs) accounting for about 20% of the hardness in barley crosses map at or close to the *Ha* locus. However, unlike in wheat, no relationship has been established between grain texture and allelic differences in Hin sequences (Beecher et al. 2001; Darlington et al. 2001).

Lee et al. (2006) reported that Hins were predominantly expressed in the aleurone cells in late kernel development in barley. This conflicts with detailed studies of the Pins in wheat (Wiley et al. 2007) and the tryptophanins of oats (Mohammadi et al. 2007), which showed location solely in starchy endosperm cells.

Hordothionins

Thionins were first described from wheat in the 1940s with related components being reported from barley by Redman and Fisher (1969). Thionins are small proteins of M_r about 5000 and are rich in cysteine and basic amino acid residues (Florack and Stiekema 1994). The early literature on wheat and barley thionins has been reviewed in detail (Garcia-Olmedo et al. 1992; Shewry 1993; Garcia-Olmedo 1999) and will only be briefly discussed here.

Barley grain contains two thionins called α- and β-hordothionins, which comprise 45 amino acid residues with four disulfide bonds. They are both synthesized with signal peptides of 19 residues and with C-terminal extensions of 64 residues, which are presumably removed by proteolytic processing.

α- and β-hordothionins are toxic to a range of cultured cells and organisms and also inhibit cell-free protein synthesis (reviewed by Shewry 1993). They also inhibit the growth of pathogenic fungi and bacteria, both alone and in synergy with other plant proteins, when tested *in vitro* (Terras et al. 1993) and when expressed in transgenic plants (Carmona et al. 1993). They are therefore considered to play a role in defense (reviewed by Garcia-Olmedo 1999).

In addition to the α- and β-hordothionins, barley grain also contains two low-molecular-mass S-rich proteins, which were initially defined as γ- and ω-hordothionins (Mendez et al. 1990, 1996). However, although these proteins are similar to the hordothionins in size (comprising 47 and 48 residues, respectively) and disulfide content (both containing four disulfide bonds), they are related in sequence to the defensins, a widely distributed group of antifungal proteins, which also includes the sorghum S2α1 α-amylase inhibitor (reviewed by Osborn and Broekaert 1999).

Minerals

Cereals are major sources of minerals for human and livestock diets. Table 13.5 gives values for major minerals (P, K, Mg, and Ca) and nutritionally important minor elements (Fe, Zn, Mn, and Cu) in whole grain, grain fractions prepared by abrasion to remove 15% and 31% of the dry weight, and commercial pearl (i.e., abraded) barley. These analyses and similar abrasion studies reported by Liu et al. (2007) show that all of the minerals studied are concentrated in the outer layers of the grain and, in particular, in the aleurone layer and embryo (Stewart et al. 1988; Ockenden et al. 2004). Duffus and Rosie (1976) also showed that about 15%–20% of the Fe is located in the pericarp of the mature barley grain, with the endosperm (including the aleurone) containing about 70% and the embryo 7%–8% of the total.

Much of the phosphorus and other minerals in the whole grain is present as phytates. In an exhaustive review of phytic acid in plants, Lott et al. (2000) quoted values of 0.38% total phosphorus in barley grain (i.e., 3800 mg/kg) and 1.02% phytic acid. The data in Table 13.5 show that almost 64% of the phosphorus present in the whole grain is in the form of phytates but only 6% or less of the phosphorus that remained in the grain after abrasion to remove the outer layers.

Phytate is a mixed salt of phytic acid (*myo*-inositol-1,2,3,4,5,6-hexa*kis* phosphate). The six phosphate groups of the acid form negatively charged sites, which can form salts with mineral cations, mainly K^+ and Mg^{++} but also Ca^{++}, Mn^{++}, Zn^{++}, Ba^{++}, or Fe^{+++} (Lott et al. 2000). The number of mineral atoms bound depends on the charge, with binding of up to 12 monovalent cations such as K^+ or six divalent cations such as Mg^{++} per molecule. It is clear that phytate acts as a mineral reserve in seeds, forming globoid inclusions within the protein bodies (aleurone grains) of aleurone cells (see section above on aleurone cell composition).

The major interest in phytates is their impact on mineral bioavailability, with metals bound to phytic acid being unavailable or less available for absorption by monogastric animals (including

Table 13.5 Mineral compositions of barley grain and of fractions prepared by abrasion (pearling)

	mg/kg								
Sample	P	K	Mg	Ca	Fe	Zn	Mn	Cu	Reference
Grain	3160	4570	1360	259	26.1	20.1	14.9	4.15	Liu et al. (1975)[b]
Grain	3600 (2300)[a]	nd	nd	510	66	21	nd	3.8	Pedersen and Eggum (1983)[c]
Flour, 85% extraction	3000 (1700)[a]	nd	nd	310	24	17	nd	3.0	Pedersen and Eggum (1983)[c]
Flour, 69% extraction	1600 (<100)[a]	nd	nd	230	11	10	nd	2.4	Pedersen and Eggum (1983)[c]
Grain		5600	910	500	60	33	17	4.0	Holland et al. (1988)[d]
Pearled barley	nd	2700	650	200	30	21	13	3.0	Holland et al. (1988)[e]

[a]Amount present in phytates.
[b]Malting barley cv. Klages grown in Aberdeen, Idaho.
[c]Spring barley cv. Arkil grown in Denmark and milled by abrasive milling (pearling).
[d]Based on literature sources.
[e]Based on two commercial samples and literature sources.
nd, not determined.

humans). When the grain is used as a major component of the diet for human nutrition, this can result in deficiencies of iron, zinc, magnesium, and calcium. Similar deficiencies also occur in livestock with the added problem of environmental pollution, including eutrophication of waters, due to release of undigested phytic acid in animal manure.

One strategy to increase mineral availability and to avoid pollution is to reduce the level of phytate, either by the selection of low-phytate mutants or by genetic engineering to express phytase in the grain. Rayboy pioneered the former approach starting in 1990 via induced mutagenesis, first with maize and then with barley, rice, soybean, and wheat (reviewed by Rayboy et al. 2001; Rayboy 2006). Rayboy and coworkers and Rasmussen and coworkers were the first to screen mutagenized populations of barley (Larson et al. 1998; Rasmussen and Hatzack 1998; Hatzack et al. 2000). At least four types of mutants have been identified in barley, three involving *lpa* genes in the phytic acid synthesis pathway. Seed phytate reductions range from moderate (25%–50%, *lpa-1* and *lpa-2*) to large (66%–80%, *lpa-3*) and extreme (>90%, M955, gene unknown). The total phosphorus and other mineral contents of the mutant grain were similar or slightly higher than those in the control lines, with the exception of the lpa-1 type, which had 77% of the phosphorus of the control. Feeding trials with mutant grain in rats (Poulsen et al. 2001), growing and finishing pigs (Veum et al. 2002; Thacker et al. 2003), poultry (turkey, Li et al. 2001; chicks, Jang et al. 2003), and trout (Overturf et al. 2003) showed increased bioavailability of phosphate and zinc, iron, magnesium, and/or calcium.

The second approach has been adopted by Brinch-Pedersen and colleagues, although working with wheat rather than with barley. Most recently, Brinch-Pedersen et al. (2006b) have expressed heat-stable forms of fungal phytase in developing wheat grain, allowing the enzyme to remain active during grain processing.

The reader is referred to several review articles for more details of phytates and their role in mineral bioavailability (Maga 1982; Lott et al. 2000; Brinch-Pedersen et al. 2002, 2006a,b, 2007).

Phytochemicals

Cereal grains, including barley, are significant dietary sources of a range of vitamins and other phytochemicals, which are considered to have health benefits. However, most of these are concentrated in the outer layers and embryo with little present in the starchy endosperm. For example, Adom et al. (2005) showed that 83% of

the total phenolic content and 79% of the total flavanoid content of wheat grain were present in the bran and germ fractions (see also Ward et al. 2008).

Phenolic compounds

Phenolic compounds are probably the largest and most complex class of secondary products present in barley grain, with three groups occurring in significant quantities. These are phenolic acids, flavanoids, and alkylresorcinols, in order of decreasing amount.

Alkylresorcinols

Alkylresorcinols are phenolic lipids that are located in the outer cuticle of the testa and the inner cuticle of the pericarp of the grain (Landberg et al. 2008). They are particularly abundant in wheat and rye and are often used as biomarkers to monitor intake of whole grain products. Alkylresorcinols may also have beneficial physiological effects, but these have not been established conclusively (reviewed by Ross et al. 2004). The contents of alkylresorcinols in barley grain are low, between about 30 and 200 μg/g dry weight (Zarnowski et al. 2002; Ross et al. 2003; Zarnowski and Suzuki 2004; Andersson et al. 2008), compared to values up to about 1000 μg/g dry weight in wheat and 3000 μg/g dry weight in rye (Ross et al. 2003). Alkylresorcinols vary in the length of the lipid side chain, from C17:0 to C25:0, with the proportions of these forms also varying among genotypes (Andersson et al. 2008).

Flavanoids

The flavanoids are a large and diverse group of phenolic compounds based on a common 15-carbon ring structure (Jende-Strid 1993). Over 5000 types have been characterized from plants and classified into a number of groups. Components present in barley include the flavanols (flavan-3-ols), which comprise monomeric forms (catechin and gallocatechin) and polymeric proanthocyanidins and anthocyanins.

It is difficult to compare published data for flavanol content due to differences in the methods and fractions studied. Griffiths and Welch (1982) reported total flavanol contents ranging from 800 to 1700 μg/g in two collections totaling 116 barley genotypes for diversity. Perhaps surprisingly, the ranges in the two collections were not greatly different, from 1200 to 1700 μg/g for the U.K. lines and from 800 to 1500 μg/g for the more diverse lines. Lower levels of 345–527 μg/g flavanols were reported for a diverse selection of 16 barley lines, including hulled, hulless, waxy, and high-amylose types by Holtekjølen et al. (2006), and of about 700–1000 μg/g for a similarly diverse collection of eight lines by Quinde-Axtell and Baik (2006).

Monomeric catechin is a relatively minor component in barley, with amounts reported to vary from less than 20 to 80 μg/g. The major flavanol components are proanthocyanidins and, in particular, the dimeric procyanidin, prodelphinidin, and four trimeric forms (Jende-Strid and Møller 1981; Holtekjølen et al. 2006; Quinde-Axtell and Baik 2006). These components are all located in the testa (Aastrup et al. 1984).

The proanthocyanidins have been of particular interest because of their role in haze formation in beer (Jende-Strid 1993) and, more recently, because of their high antioxidant activity and potential health benefits (Beecher 2004). This interest has led to an extensive mutagenesis program in which almost 700 proanthocyanidin-free mutant lines were produced by chemical mutagenesis (Jende-Strid 1993). These lines contain essentially no proanthocyanidins (Jende-Strid 1993; Øverland et al. 1994) but may contain small amounts (less than 10 μg/g) of catechin (Quinde-Axtell and Baik 2006). It was recently shown in a survey of barley cultivars and lines that proanthocyanidin-free types produce and maintain brighter white food products than "normal" types (Quinde-Axtell and Baik 2006).

The same mutagenesis program also identified 75 mutants in anthocyanin synthesis (Jende-Strid 1993). Anthocyanins are minor components in barley compared to other phenolics. However, they do occur in the pericarp and aleurone of pigmented lines. Abdel-Aal et al. (2006) reported 35 μg/g total anthocyanins in a blue barley line, while Siebenhandl et al. (2007) reported 15.87 and 8.85 μg/g total anthocyanins in the bran of

two black barley lines, but only about 1 μg/g in the flour fractions.

Phenolic acids

The phenolic acids of barley and other cereals can be divided into two groups, which are derived from hydroxybenzoic or hydroxycinnamic acid. Both groups also exist in three forms, which can be extracted and analyzed as separate fractions. Free phenolic acids tend to be relatively minor components with most being present as soluble conjugates (esterified to sugars and other LMW components) or as insoluble bound forms, usually esterified to cell wall AX (as discussed above).

Typical values for these three fractions are shown in Table 13.6, which gives data for the whole grain of 10 lines selected to represent a range of types. Free phenolic acids account for about 3% or less of the total fraction and soluble conjugated phenolic acids for about 25%. However, there is a significant variation in amount among cultivars, particularly in the bound fraction, which varies by over threefold.

The values in Table 13.6 are also in broad agreement with those reported in some other studies in which similar methods were used: 8.1–17.5 μg/g free phenolic acids in nine lines (Goupy et al. 1999), 604–1346 μg/g bound phenolic acids in 16 lines (Holtekjølen et al. 2006), and 357–604 μg/g of total phenolic acids in 11 lines (Quinde-Axtell and Baik 2006).

The compositions of the individual phenolic acids in the free, conjugated, and bound fractions of the 10 lines were reported by Andersson et al. (2008). Although there was a significant variation among lines (Andersson et al. 2008), all free fractions were rich in ferulic, vanillic, syringic, and 2,4-dihydrobenzoic acids, the conjugated fractions in ferulic, 4-hydroxybenzoic, vanillic, sinapic, and 2,4-dihydrobenzoic acids, and the bound fractions in ferulic and *p*-coumaric acids. These analyses were also in line with published values (Jende-Strid 1985; Mattila et al. 2005; Holtekjølen et al. 2006; Quinde-Axtell and Baik 2006).

Phenolic acids are known to have strong antioxidant properties, and a number of recent studies have focused on relating the antioxidant properties of grain extracts to the amounts and compositions of phenolic acid fractions (Zieliński and Kozlowska 2000; Bonoli et al. 2004; Amarowicz et al. 2007; Kim et al. 2007; Liu and Yao 2007; Madhujith and Shadidi 2006, 2007; Zhao et al. 2006, 2008; Dvońáková et al. 2008).

Terpenoids

Sterols, tocols (including vitamin E), and carotenoids are terpenoids that are considered to have cholesterol-lowering properties (sterols) and antioxidant properties (tocols, carotenoids).

Table 13.6 Amount and composition of total, free, conjugated, and bound phenolic acids in a range of barley varieties

					Phenolic Acids (μg/g Dry Weight)			
	Hulled	Rows	Type	Starch	Free	Conjugated	Bound	Total
Diktoo	Yes	6	W	Normal	13.8	197.7	463.8	657.3
Plaisant	Yes	6	W	Normal	23.0	113.1	375.9	512.0
Igri	Yes	2	W	Normal	8.1	115.0	272.1	395.2
Rastik	No	6	W	Normal	4.6	87.6	212.0	304.2
CFL93-149	Yes	2	S	High amylose	5.4	101.1	442.5	549.0
CFL98-398	Yes	2	S	Waxy	7.0	94.6	276.2	377.9
CFL98-450	No	2	S	Waxy	7.6	113.0	132.9	253.5
Erhard–Frederichen	Yes	2	S	Normal	13.0	96.1	313.7	422.8
Borzymwicki	Yes	2	S	Normal	10.6	86.4	423.2	520.1
Morex	Yes	6	S	Normal	5.6	92.8	522.8	621.2

Based on data reported by Andersson et al. (2008).
S, spring; W, winter.

Total sterols in barley grain range from about 800 to 1150 μg/g with the major forms being sitosterol and campesterol and only 1%–2% of the total being stanols (saturated forms) (Piironen et al. 2002; Andersson et al. 2008). Tocols are present in lower amounts than sterols, with reported values of between about 30 and 70 μg/g. Cavallero et al. (2004) reported higher levels of tocols in hulled lines than in naked (hulless) lines, but this difference was not observed by Andersson et al. (2008). About 70%–80% of the total tocol fraction is tocotrienols (with unsaturated side chains) and the major form is α-tocotrienol (Panifili et al. 2003; Cavallero et al. 2004; Ehrenbergerová et al. 2006; Prýma et al. 2007; Andersson et al. 2008).

The levels of carotenoids in barley grain are low compared to those of sterols and tocols. Goupy et al. (1999) reported that whole grains of nine barley lines contained between 174 and 850 ng/g total carotenoids, with lutein accounting for about 90% of the total and zeaxanthin also being present. Siebenhandl et al. (2007) showed that lutein and zeaxanthin were present in milling fractions of barley with slightly higher levels in the shorts (fine bran-rich) and midding (large endosperm) fractions than in the bran and flour. However, the levels (less than 2 μg/g in all fractions) were too low to separate the two forms. Lutein and zeaxanthin both contain hydroxyl groups and are therefore classified as xanthophylls rather than carotenes.

Folate

Folate (vitamin B_9) is one of the most widely studied plant vitamins because of its role in neural tube development in developing fetuses (preventing spina bifida) and its possible role in reducing several diseases (Molloy 2002). Folate is a mixture of vitamers with total contents in barley reported to range from about 200 to 800 ng/g dry weight (Cerna and Kas 1983; Hegedüs et al. 1985; Gusjska and Kuncewicz 2005; Han et al. 2005; Andersson et al. 2008).

CONCLUSIONS

The mature barley grain is a highly complex system comprising organs, tissues and cell types that vary widely in their structures, compositions, and properties, including their processing and nutritional quality. The present review has focused on the mature grain, describing how developmental events have led to the final structure and discussing the major components that determine its composition and end use properties. Our knowledge is still far from complete, and limitations in time and space have precluded the inclusion of much information. Nevertheless, we hope that it will provide a good overview for the general reader while enabling the expert to access more detailed information where this is required.

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Chapter 14

Biochemistry, Physiology, and Genetics of Endosperm Mobilization in Germinated Barley Grain

Geoffrey B. Fincher

INTRODUCTION

The germinated barley grain has been used as a model system for studies on plant biochemistry and physiology for more than 40 years. In the 1970s, isolated barley aleurone layers were widely used to examine the effects of phytohormones on gene expression and on the secretion of hydrolytic enzymes from plant cells (Chrispeels and Varner 1967; Taiz and Jones 1970; Jacobsen and Higgins 1982). Where the isolated aleurone layers were treated with gibberellic acid (GA), activities of key enzymes involved in endosperm mobilization in germinated barley grain were secreted from the layers and detected in the surrounding media. These included α-amylases, amino- and carboxypeptidases, a range of endopeptidases, and various enzymes involved in the depolymerization of the major cell wall polysaccharides of the endosperm. Later, many of these enzymes were purified from extracts of germinated barley grain and were characterized in detail. During the first molecular biology period of the 1980s, amino acid sequence information obtained from enzymes purified from germinated barley grain was used to design nucleotide probes for the isolation of cDNAs and genes encoding the enzymes. Nucleotide sequence data from the cDNAs and genes allowed the full amino acid sequences of the enzymes to be deduced and opened the way for the definition of high-resolution three-dimensional (3D) structures for barley α- and β-amylases, and (1,3;1,4)-β-D-glucan endo- and exohydrolases. The characterization of enzymes from germinated barley grain, together with analyses of corresponding genes and cDNAs, soon revealed that many of the enzymes were encoded by multigene families.

Although the isolated barley aleurone layers provided a useful physiological system in its own right for the examination of enzymes involved in reserve mobilization in germinated grain, the interest in and adoption of the system were also driven by the central importance of germination in the malting and brewing industries, which represented the major end users of barley. For example, malt extract values are important for maltsters and brewers and indicate in general terms the degree of mobilization of the starchy endosperm of germinated barley grain. More specifically, delayed degradation of cell walls in the germinated grain could slow the solubilization of starch, protein, and other components of the endosperm and hence reduce extract values. Thus, low levels of the major cell wall polysaccharides in grain and/or the rapid production of enzymes that hydrolyze these polysaccharides were seen as highly desirable in malting and brewing. Diastatic power, which is a broad measure of the levels of starch-degrading enzymes in extracts of germinated barley, and fermentability of the final extract are dependent in large part on the levels of α- and β-amylases, starch debranching enzymes, α-D-glucosidases, and cell wall polysaccharide hydrolases in the germinated

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barley grain. The Kolbach index is another important malting quality parameter, and this will depend on the profile of proteolytic enzymes that develop in the germinated grain.

It is also worth noting that the importance of hydrolytic enzymes in the malting and brewing industries led to barley breeders selecting for the rapid and uniform modification of barley grain following germination, hence for the rapid synthesis of the key hydrolytic enzymes outlined above. Newly introduced breeding technologies, such as the quantitative trait locus (QTL) analyses that emerged in the 1990s, were quickly applied to the measurement of these barley quality traits and to the selection of desirable levels of the various enzymes in breeding lines. Barley also became a model system for studies of genetics in the Triticeae, not only because of the background information that was available on grain germination and the enzymes involved, but also because barley is a diploid species for which extensive genetic resources have been accumulated (Fincher and Langridge 2004). Indeed, an international consortium is currently making strong progress toward the provision of a complete physical map of the barley genome and toward the generation of DNA sequence information that will inevitably lead to the definition of the complete genome sequence for barley.

As a result of this broad interest in the physiology, biochemistry, enzymology, molecular biology, and genetics associated with barley grain germination, there have been numerous reviews of the literature over a long period (Briggs 1978; Fincher 1989; Bamforth 1999; Briggs 2002; Kuntz and Bamforth 2007). The central objective here is to review the more recent literature and to provide an update on the biochemistry, physiology, and genetics of germination in barley. Attention will be focused on major events in the process of grain modification after germination. In particular, cellular activities in the aleurone layer after the initiation of germination will be addressed, together with cell wall degradation, starch hydrolysis, storage protein mobilization, and other key events in endosperm modification. The potential applications of new technologies will be examined and new insights into barley grain germination that have been made possible by functional genomics and related high-throughput technologies will be presented.

BARLEY GRAIN STRUCTURE AND COMPOSITION

In discussing the physiology, biochemistry, and genetics of germination in barley, it is necessary to consider, in broad terms at least, the structure and composition of mature barley grain. Here, the discussion will be focused on the roles of the aleurone layer and the scutellar epithelium, which are key sources of hydrolytic enzymes, and the mobilization of the reserves of the starchy endosperm, from which sugars, amino acids, and other low-molecular-weight products are translocated to the embryo to support the growth of the young seedling. More detailed descriptions of barley grain structure and composition are available elsewhere (Fincher 1989; Duffus and Cochrane 1993).

Grain development

The fate of the various components of the endosperm of barley after germination depends to some extent on their levels in the mature, ungerminated grain. Following pollination, the barley grain follows a developmental pattern in which the nucleus of a single egg cell, or oospore, fuses with a single male nucleus to form a diploid zygote. When this diploid zygote divides, it forms an apical cell, which eventually develops into the embryo, and a basal cell (Duffus and Cochrane 1993). A second male generative nucleus and two polar nuclei fuse to form to a triploid endosperm nucleus (Bewley and Black 1983), which subsequently divides to generate a syncytium where multiple free nuclei are embedded in a cytoplasmic layer around the outside of a vacuolated central cell. After about 3 days, approximately 2000 endosperm nuclei have been formed (Duffus and Cochrane 1993), and cell walls begin to grow centripetally from the periphery of the central cell and eventually enclose individual nuclei to form individual endosperm cells. The endosperm is fully cellularized at about 6 days after pollination

(DAP) (Brown et al. 1994; Wilson et al. 2006). Deposition of starch and protein begin in the central endosperm cells at about that time. Cell division continues in the endosperm until grain filling is about half complete, and cell enlargement continues for some time after that (Cochrane and Duffus 1981; Berger 1999). Aleurone layer differentiation can be detected at about 9–10 DAP, when division of peripheral endosperm cells forms a pro-aleurone layer and an inner, sub-aleurone layer. After 15 days, the aleurone layer is clearly visible (Cochrane and Duffus 1981) and consists of small, isodiametric cells with relatively thick cell walls. In the mature grain, aleurone layer walls have a relatively thin inner layer and a thicker outer layer (Bacic and Stone 1981). In contrast, cell walls of the starchy endosperm are relatively thin compared with those of the aleurone.

In relatively recent work, the deposition of key cell wall polysaccharides in developing endosperm of barley has been monitored using light and electron microscopy coupled with immunocytological procedures (Wilson et al. 2006). Deposition of (1,3;1,4)-β-D-glucans, which can account for up to 70% of the final starchy endosperm walls (Fincher 1975), begins at about 5 DAP and slightly earlier than the deposition of arabinoxylans, which represent the other major wall polysaccharide in the endosperm of barley grain. Arabinoxylans are deposited after cellularization is complete and when starch begins to accumulate. Nine days after pollination, arabinoxylans are uniformly distributed throughout the starchy endosperm tissue (Wilson et al. 2006).

Thus, in the mature barley grain, the endosperm consists of two functionally and morphologically distinct tissues, the aleurone and the starchy endosperm, that are both of triploid origin but have dramatically different roles during grain development, dormancy, and following germination (Fincher and Stone 2004). Cells of the starchy endosperm of barley are nonliving and function as a storage site for starch and protein reserves that are mobilized following grain germination to provide energy and nutrients for growth of the young seedling. However, the aleurone cells remain alive in the mature grain and are packed with protein bodies, carbohydrates, lipid droplets, and various other storage molecules that support the energy requirements and the development of the protein-synthesizing machinery that are needed for the synthesis and secretion of the hydrolytic enzymes involved in starchy endosperm mobilization after germination (Fulcher et al. 1972; Fincher 1989). The aleurone layers of barley grains are one to several cells in thickness.

The embryo of the mature barley grain is diploid in nature and consists of the scutellum, which lies adjacent to the endosperm, together with other nascent tissues that form young vegetative organs after germination, including the embryonic leaf with its ensheathing coleoptile and the radical with its protective coleorhiza. The embryonic tissues might also be the source of key phytohormones that elicit aleurone and scutellar function following germination. The scutellum has a clearly discernible epithelial layer that is in direct contact with the endosperm, and which consists of cells that have the appearance, composition, and many of the functional characteristics of aleurone cells.

The tissues and organs that surround the embryo and endosperm in the mature barley grain mostly are nonliving and consist predominantly of residual cell wall material from maternal tissues, including the pericarp and the testa. They play an important role in the physical protection of the grain against pathogen attack, in the impermeability of the grain to water, and in the provision of overall structural rigidity and strength to the grain.

Finally, it is important to note that during the final stages of grain maturation, a broadly based system of defense strategies is initiated to protect the grain from infection by potentially pathogenic bacteria and fungi (Fincher 1989).

Composition of the mature barley grain

In the mature barley grain, starch accounts for about 65% by weight, while other polysaccharides can make up another 15% by weight of the grain. Proteins constitute 10%–12% of the grain (Table 14.1; Finnie et al. 2006). The non-starchy

Table 14.1 Composition of mature barley grain

Component	Dry Weight (%)
Carbohydrates	78–83
Starch	63–65
Sucrose	1–2
Other sugars	1
Non-starchy polysaccharides	9–11
Cellulose	4–5
Protein	10–12
Albumins and globulins	3–4
Hordeins	3–4
Glutelins	3–4
Lipids	2–3
Nucleic acids	0.2–0.3
Minerals	2
Others	5–6

Source: MacGregor and Fincher (1993).

cell wall polysaccharides usually constitute less than 10% by weight of the grain but, as will be noted later, can be key determinants of grain quality.

Mature aleurone layers of barley are packed with specialized protein bodies, which are referred to as aleurone grains, together with lipid droplets, mitochondria, and other organelles (Bacic and Stone 1981; Fincher 1989). Within the aleurone grains, phytin globules consisting of the potassium and magnesium salts of myoinositol hexaphosphate and niacytin particles are embedded in a proteinaceous matrix. The aleurone grains are surrounded by membrane-bound, lipid-rich spherosomes. Cell walls of the aleurone consist predominantly of arabinoxylans (65%–67% by weight) and (1,3;1,4)-β-D-glucans (26%–29% by weight); cellulose levels are low but some phenolic acids are present (Bacic and Stone 1981). The walls are characterized by a thick outer layer, which is rapidly removed following germination of the grain, and a thinner, inner layer which remains largely intact following germination (Taiz and Jones 1973; Bacic and Stone 1981).

The starchy endosperm cells of the mature barley grain are enclosed by thin cell walls that consist of approximately 70% arabinoxylan and 20% (1,3;1,4)-β-D-glucan (Fincher 1975). Again, cellulose levels are low and phenolic acids, in particular ferulic acid, are detectable (Fincher 1976).

Table 14.2 Composition of cell walls in mature barley grain

Component	Aleurone Layers (% by Weight)	Starchy Endosperm (% by Weight)
Heteroxylans	71	20
(1,3;1,4)-β-D-glucans	26	75
Cellulose	2	2
Glucomannans	2	2
Protein	6	5

Source: Fincher (1975) and Bacic and Stone (1981).

The low levels of cellulose in starchy endosperm and aleurone walls are attributable in part to the fact that walls of the starchy endosperm need to be rapidly removed following germination, given that cellulose is notoriously difficult to remove enzymatically, and because endosperm cell walls do not have a major load-bearing or structural role in the grain, which is supported largely by the surrounding pericarp and seed coat tissues. The compositions of aleurone and starchy endosperm walls are compared in Table 14.2 (Fincher 1975; Bacic and Stone 1981). The cells of the starchy endosperm are packed with a mixture of large lenticular starch granules 15–25 μm in diameter and smaller irregularly shaped starch granules that are less than 10 μm in diameter (MacGregor and Fincher 1993). The starch granules are embedded in a matrix of storage proteins.

The epithelial layer of the scutellum consists of cells that are morphologically similar to those of the aleurone layer (Fincher 1989). Similar protein bodies and lipid droplets can be observed in the cells, and the walls also consist of two distinct layers (Fincher 1989).

It is worth noting here that the cell walls of barley and other members of the Poaceae have a number of distinguishing features that become important following germination and make major contributions to the emerging interest in non-starchy polysaccharides from barley and other cereals as beneficial components for human health. First, walls of the Poaceae are relatively low in pectic polysaccharides and xyloglucans, compared with walls from eudicots (Farrokhi et al. 2006). While heteroxylans are widely

distributed in walls of higher plants, the other major components of walls in barley grains, the (1,3;1,4)-β-D-glucans, are restricted almost exclusively to members of the monocotyledon family Poaceae, to which the cereals and grasses belong, and to related families of the order Poales (Trethewey et al. 2005). Levels of (1,3;1,4)-β-D-glucans in mature barley grains are affected by genotype, the position of the grain on the spike, and environmental factors such as soil nitrogen availability and climatic conditions during grain development (Fincher and Stone 2004). Barley grains have high levels of (1,3;1,4)-β-D-glucans, which range from 2%–10% by weight (Fincher and Stone 1986) (Table 14.2).

HORMONAL REGULATION OF GERMINATION IN BARLEY GRAIN

The germination of non-dormant grain commences with the uptake of water, most of which penetrates the undamaged grain near the micropylar region. If physical damage has occurred to the cuticularized layers of the husk, pericarp, or testa-nucellus, water can also penetrate at the points of damage (Briggs 2002). The water diffuses through the grain at rates that are determined by the composition of the various regions. Once germination is initiated, a battery of hydrolytic enzymes is synthesized in the aleurone and scutellar epithelial layers and secreted into the starchy endosperm, where cell walls and reserves of starch and protein are depolymerized. Some hydrolytic enzymes, such as β-amylase, are deposited in cells of the starchy endosperm during grain maturation, and therefore preexist in those cells when germination is initiated (Fincher 1989). Degradation products released from starch and storage proteins diffuse along a concentration gradient that is created by the active transport of these products into the scutellar epithelial layer. Thus, the scutellar epithelium has a dual role in barley germination. In the first instance, it is involved in the secretion of hydrolytic enzymes (McFadden et al. 1988) into the starchy endosperm, and later it is involved in the active uptake into the scutellum of low-molecular-weight products of starch and protein degradation in the starchy endosperm. Once the products of endosperm mobilization are transported into the scutellar epithelium, they are translocated through a nascent vascular system to act as a source of nutrients for the developing seedling.

Following activation of the aleurone layer in germinated barley grains, the endomembrane system of the cells re-forms, mitochondria proliferate, the lipid and protein reserves of the cells are mobilized, and GA-induced gene expression commences. Key products of this gene expression are the hydrolytic enzymes that will mobilize the reserves of the starchy endosperm cells. However, the cell wall of the aleurone and scutellar epithelial cells represents a physical barrier to the secretion process, because many of the enzymes will be too large to freely penetrate through the walls. It has been shown that α-amylases, and presumably other enzymes, are released through channels that form in aleurone cell walls. During the secretion process, the thick outer layer of aleurone walls is degraded, but the thinner, inner layer remains intact, presumably to maintain aleurone cell integrity during enzyme secretion (Taiz and Jones 1970). This indicates that the outer walls are degraded by polysaccharide hydrolases secreted from the aleurone itself, but that the inner aleurone wall layer has a different composition that allows it to resist complete degradation (Fincher 1989). The composition of the resistant component of the aleurone wall is not known, nor is it clear how the hydrolytic enzymes penetrate the partially degraded walls. Finally, programmed cell death of the aleurone is observed as the aleurone reserves of nutrients become depleted (Fath et al. 2000).

The role of phytohormones

There is strong evidence that the antagonistic phytohormones GA and abscisic acid (ABA) are involved in a complex interplay of activities that regulate the onset and release of dormancy, the activation of transcription of genes encoding enzymes that are secreted from the aleurone, and the initiation of programmed cell death of

aleurone cells when their reserves are depleted. Overall, high levels of ABA and low levels of GA are associated with dormancy, while low levels of ABA and high GA concentrations result in the initiation of germination and the expression of genes encoding hydrolytic enzymes, but it is not yet clear how the processes of GA and ABA perception and signaling occur in plants. There are indications that GA binds to a soluble, nuclear GA-insensitive dwarf 1 (GID1) receptor that interacts with repressor proteins known as DELLA in a GA-dependent manner and thereby induces DELLA protein degradation (Schwechheimer 2008). While the GA and ABA hormone receptor proteins might be located within the nucleus in many plants (Razem et al. 2006), it has also been suggested that GA is perceived at the plasma membrane of aleurone cells (Lovegrove and Hooley 2000). Thus, there might be two types of GA receptors, namely soluble and membrane-bound forms (Ueguchi-Tanaka et al. 2007). Whatever mechanisms of hormonal signaling occur, barley aleurone layers appear to retain their sensitivity to ABA following germination, because subsequent addition of ABA suppresses expression of GA-inducible genes (Mundy et al. 1985; Koehler and Ho 1990b). This might allow the grain to temporarily suspend endosperm mobilization in unfavorable conditions, such as during partial dehydration (Fincher 1989).

Dormancy and aleurone activation

Although barley breeders have selected against grain dormancy so that the grain will germinate uniformly and rapidly in the field and in industrial processes such as malting (Simpson 1990), dormancy does persist to some extent in certain domesticated varieties and in wild relatives of barley that are used in breeding programs. A major disadvantage of the reduced level of dormancy observed in many elite barley cultivars is that the grain will be more susceptible to preharvest sprouting (Baskin and Baskin 1998; Gubler et al. 2005), where wet conditions during the late stages of grain maturation can cause grains to germinate and sprout in the head of plants in the field, prior to harvest. Thus, preharvest sprouting occurs as a result of low grain dormancy before harvest and depends not only on genotype, but also on environmental conditions (Rodríguez et al. 2001; Mares et al. 2005).

Dormancy is likely to be regulated by physical, hormonal, and environmental factors (Finkelstein et al. 2008). The rate of oxygen diffusion through the hull and the availability of oxygen to the embryo affect germination of barley grains (Bradford et al. 2008). The hormone ABA is known to be involved in both the induction and maintenance of dormancy in barley and other grains, and its concentrations relative to GA, which promotes aleurone activity in terms of the secretion of hydrolytic enzymes, appears to be important (Jacobsen et al. 2002; Feurtado and Kermode 2007). As noted above, the two hormones often play antagonistic roles in regulating plant growth (Gómez-Cadenas et al. 2001) and the ABA in barley grain may inhibit germination through the suppression of GA synthesis (Seo et al. 2006). Dormancy in barley is eventually broken in a process that again is related to ABA levels and signaling, and which can be induced by changes in such environmental conditions as temperature, light intensity and quality, oxygen levels, and nutrient availability (Simpson 1990; Jacobsen et al. 2002; Millar et al. 2006; Gubler et al. 2008). The complexity of dormancy and its release is reflected in QTL analyses in various barley mapping populations. Hori et al. (2007) reported 38 QTLs controlling dormancy clustered in 11 regions on all chromosomes except chromosome 2H, and there have been other reports of multiple QTLs for both the induction and release of dormancy in other populations (Gao et al. 2003; Prada et al. 2004; Prada et al. 2005). In a related work, Ullrich et al. (2008) showed in a six-row Steptoe/Morex cross that multiple QTLs for preharvest sprouting coincided with known dormancy QTLs, although some QTLs had a larger effect on preharvest sprouting than on dormancy. Similar results were reported by Li et al. (2003) in two-row Chebec/Harrington and Stirling/Harrington crosses. There is evidence for pleiotropy, linked genes, and epistasis among the QTLs identified for dormancy and preharvest sprouting.

Regulation of gene expression in the aleurone

Many of the genes transcribed in the aleurone during this period encode the hydrolytic enzymes that are secreted from the aleurone cells and mediate cell wall, starch, and storage protein depolymerization in the starchy endosperm. The GA that is synthesized in the embryo diffuses through the grain and progressively activates gene expression in the aleurone layer as it diffuses from the embryo toward the distal end of the grain. Based on mathematical modeling of diffusive transport, Bruggeman et al. (2001) concluded that GA diffuses through the apoplastic region of aleurone layers and moves more quickly than ABA.

There is now considerable information emerging on the regulation of gene expression in barley aleurone layers. The *cis*-regulatory elements in gene promoters and *trans*-acting factors are being identified, although the complete picture of these complex regulatory interactions is not yet clear. Gubler et al. (1995) showed that GA regulates expression of a barley aleurone *myb* transcription factor gene, which has been designated GAMYB and which transactivates α-amylase gene promoters, a (1,3;1,4)-β-D-glucanase promoter, and a cathepsin B-like protease promoter (Gubler et al. 1999). The GAMYB transcription factor is itself negatively regulated by protein kinases (Woodger et al. 2003; Moreno-Risueno et al. 2007). Transcription of the barley *Amy32b* α-amylase gene is effected by protein complexes that contain activators such as the HvGAMYB and SAD proteins or by repressors, which include HvWRKY38 and BPBF (Zou et al. 2008). Similarly, regulation of the ABA-dependent repression of aleurone gene expression involves transcription factors such as HvDOF19, and the activities of these transcription factors can be modulated by protein kinases and protein phosphatases (Shen et al. 2001; Moreno-Risueno et al. 2007). Several models have been proposed to explain GA and ABA regulation of genes and the antagonistic effects of the two hormones (Shen et al. 2001; Moreno-Risueno et al. 2007).

Programmed cell death of the aleurone

Although barley aleurone cells are living in the mature grain, the perception of GA is believed to initiate a program of events that leads eventually to cell death. This occurs after aleurone reserves have been mobilized for the synthesis of hydrolytic enzymes and the cells become highly vacuolated (Fath et al. 2000). Plasma membranes rapidly break down and organelles disappear, while levels of nucleases and proteases increase, but the more characteristic features of apoptotic cell death observed in animal cells are not manifest in dying barley aleurone cells (Fath et al. 2000). Although GA triggers programmed cell death in barley aleurone layers (Bethke et al. 1999), its antagonist ABA inhibits GA-mediated programmed cell death (Guo and David Ho 2008). Fath et al. (2000) report that ABA-treated barley aleurone protoplasts remain alive for several months, but that GA-treated protoplasts die in 5–8 days. It has been suggested that reactive oxygen species (ROS) are important components of hormonally regulated programmed cell death in barley aleurone cells, because GA-treated aleurone protoplasts are less able to tolerate H_2O_2 than ABA-treated protoplasts (Bethke and Jones 2001; Palma and Kermode 2003).

Caspers et al. (2001) have further proposed that programmed cell death of aleurone layers might represent a biological strategy that enables the late release of enzymes such as (1,4)-β-D-xylanases and limit dextrinases, perhaps to protect the aleurone wall against degradation in the case of the (1,4)-β-D-xylanases or for the late release of glucose from starch limit dextrins in the case of the limit dextrinase.

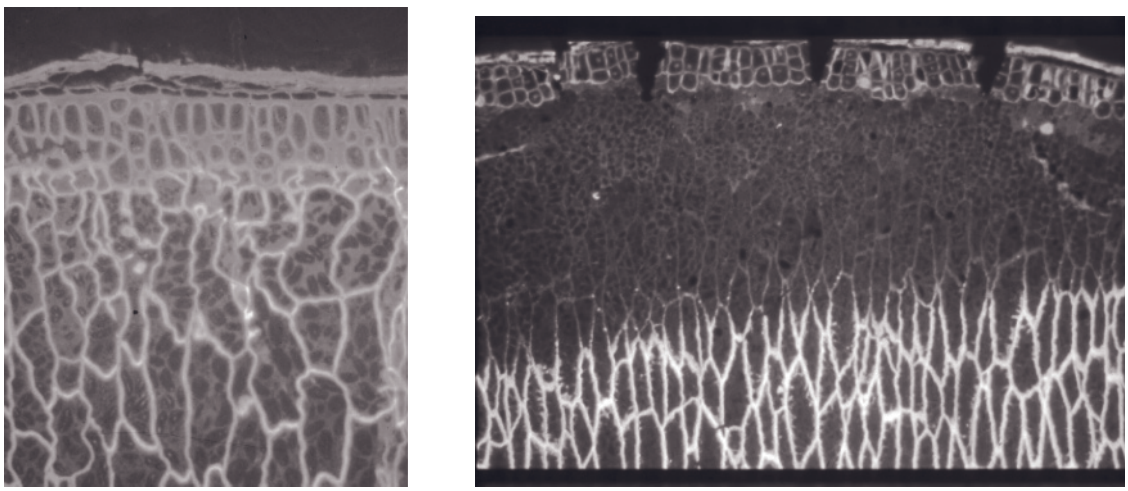
Role of the scutellum

In the scutellar epithelium of germinated barley grain, changes similar to those observed in aleurone layers also occur (Fincher 1989). Organelles such as endoplasmic reticulum (ER) and Golgi quickly develop, protein bodies and their phytin inclusions disappear, lipid bodies are mobilized, and the cells become more vacuolated. One might expect that the hormonal regulation of these

processes is similar to that of the aleurone layer. However, there are a number of important differences. The scutellar epithelial cells accumulate starch, at least transiently (Fincher 1989), they express peptide and other transporter genes (West et al. 1998; Potokina et al. 2002), consistent with their role in translocating enzymic degradation products of starchy endosperm reserves to the developing seedling, and they increase their absorptive surface area through the lateral disconnection of individual epithelial cells (Fincher 1989). Thus, their average period of activity is likely to be considerably longer than an aleurone cell, and it is not known whether or not the cells of the scutellar epithelium are subject to programmed cell death.

MOBILIZATION OF RESERVE POLYMERS OF THE STARCHY ENDOSPERM

Consistent with the site of GA synthesis in the embryo and its subsequent diffusion through the grain, endosperm dissolution in barley commences in the region immediately adjacent to the scutellum and progresses to the distal end of the grain. The "front" of endosperm mobilization moves away from the scutellum toward the distal end of the grain and reflects the progressive secretion of hydrolytic enzymes from the scutellum in the first instance (Gibbons 1981; McFadden et al. 1988), and later from the aleurone layer, along the length of the grain (Fincher 1989). During the dissolution of the starchy endosperm, cell walls, starch, storage proteins, and residual nucleic acids are hydrolyzed by a large number of endo- and exo-acting hydrolases. There is some evidence that the groups of enzymes are secreted sequentially from the aleurone layer, so that the physical barrier of the cell walls is removed first to enable access of α-amylases and peptidases, which may be secreted slightly later, to substrates that are initially packaged away inside the starchy endosperm cells (Fig. 14.1; Fincher 1989). In the sections below, the processes of enzymic depolymerization of walls, starch, and proteins are outlined.



(a) (b)

Fig. 14.1. Early stages of cell wall degradation in the starchy endosperm of germinated barley grain. The left panel (a) shows a low magnification micrograph of a section of ungerminated barley grain stained with Calcofluor White. The cell walls of the endosperm can be clearly seen, as can the starch granules in starchy endosperm cells. In the right panel (b), the degradation of walls progressively from the aleurone layer toward the center of the starchy endosperm can be seen. This degradation pattern reflects the diffusion of wall-degrading enzymes from the aleurone layer to the center of the grain. Photographs generously provided by Meredith Wallwork. For color details, please see color plate section.

Cell wall degradation in germinated barley grain

Non-cellulosic polysaccharides, in particular arabinoxylans and (1,3;1,4)-β-D-glucans, make up a large proportion of the walls of the aleurone and starchy endosperm of barley grain (Table 14.2). The walls generally have low cellulose contents and no lignin, and these will certainly facilitate enzymic penetration of the walls and hence satisfy a requirement to rapidly depolymerize wall components following germination of the grain. The following discussion will therefore be focused on the depolymerization of arabinoxylans and (1,3;1,4)-β-D-glucans of the starchy endosperm walls of the germinated barley grain.

Hydrolysis of arabinoxylans

Arabinoxylans of barley endosperm cell walls consist of a (1,4)-β-D-xylan backbone that is substituted with single α-L-arabinofuranosyl units, predominantly at C(O)3, but also at C(O)2 of the xylosyl units (Fig. 14.2). In some cases, α-L-arabinofuranosyl substitutions occur at both C(O)3 and C(O)2 (Fincher 1975). In barley, starchy endosperm walls the xylose:arabinose ratio of water-soluble arabinoxylans ranges from 1.1 to 1.3:1, while in aleurone layers, it is about 1.9:1 (Fincher and Stone 1986). A proportion of the α-L-arabinofuranosyl units in the arabinoxylans of barley are esterified with ferulic acid, and to a lesser extent *p*-coumaric acid (Fincher 1976). These hydroxycinnamates are found at C(O)5 of α-L-arabinofuranosyl units that are linked to C(O)3 of the Xylp units, and feruloyl residues constitute about 0.05% of barley starchy endosperm walls (Fincher 1976).

The backbone chains of arabinoxylans are extended and the arabinosyl substituents sterically hinder the aggregation of the linear (1,4)-β-D-xylan backbones and therefore enable the high-molecular-mass polysaccharide to remain soluble in aqueous media. As with the (1,3;1,4)-β-D-glucans, the asymmetrical nature of the arabinoxylans and their high degree of polymerization (DP) result in molecules that form solutions of high viscosity (Andrewartha et al. 1979). The arabinoxylans are believed to form a gel-like matrix between cellulosic microfibrils in the wall and many of the characteristics attributed to (1,3;1,4)-β-D-glucans in industrial processes and in human health and nutrition (Brennan and Cleary 2005) might partly result from the

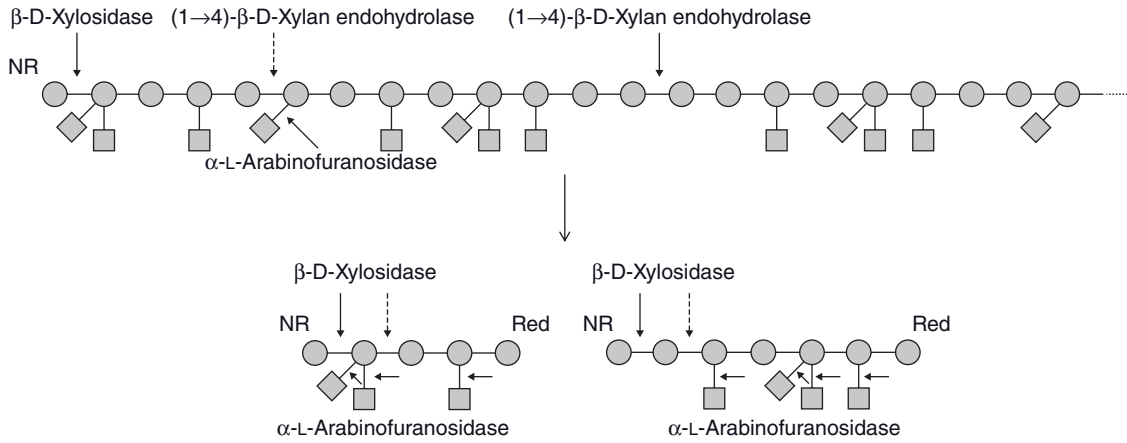


Fig. 14.2. Structure of arabinoxylans from the cell walls of barley grain. The circles represent the (1,4)-β-D-xylosyl residues of the (1,4)-β-D-xylan back-bone and the squares represent single α-L-arabinofuranosyl substituents, which occur predominantly at C(O)3, but also at C(O)2 of the xylosyl units. In some cases, α-L-arabinofuranosyl substitutions occur at both C(O)3 and C(O)2 (Fincher 1975). The enzymes involved in the hydrolysis of specific linkages in the polysaccharide are shown. Adapted from Viëtor et al. (1994).

contributions of arabinoxylans, which have similar physicochemical properties.

It is clear from these structures (Fig. 14.2) that the complete depolymerization of barley arabinoxylans in the germinated grain is likely to require the concerted action of a battery of enzymes, including (1,4)-β-D-xylan endohydrolases (Slade et al. 1989; Caspers et al. 2001), arabinoxylan arabinofuranohydrolases (Ferre et al. 2000; Lee et al. 2001), α-L-arabinofuranosidases and β-D-xylosidases (Taiz and Honigman 1976; Lee et al. 2003). The substrate specificities of each of these enzymes are shown in Fig. 14.2, and their properties are presented in more detail in the following section. Feruloyl esterases may be required for the hydrolysis of ester linkages between the α-L-arabinofuranosyl residues of the arabinoxylan and ferulic acid, and α-glucuronosidases might be required for the removal of α-glucuronosyl residues from the xylan backbone, but these enzymes have not been purified or characterized and will not be discussed further here.

(1,4)-β-D-Xylan endohydrolases

(1,4)-β-D-Xylan endohydrolases (EC 3.2.1.8) catalyze the hydrolysis of internal (1,4)-β-D-xylosidic linkages of the xylan backbone of arabinoxylans (Fig. 14.2). Xylanase activity is detected several days after (1,3;1,4)-β-D-glucanases in germinating barley (Slade et al. 1989), consistent with observations that endoxylanase genes are transcribed later than the (1,3;1,4)-β-D-glucanase genes (Banik et al. 1997). In early work, (1,4)-β-D-xylan endohydrolase activity was detected in the medium around isolated aleurone layers somewhat later than other hydrolytic enzymes (Taiz and Honigman 1976; Ashford and Gubler 1984; Caspers et al. 2001).

There is some uncertainty about the size of the active form of barley (1,4)-β-D-xylan endohydrolases (Simpson et al. 2003). When the enzymes were partially purified from the media surrounding isolated barley aleurone layers, (1,4)-β-D-xylan endohydrolases of about 29 kDa and 34 kDa were detected (Dashek and Chrispeels 1977; Benjavongkulchai and Spencer 1986). However, when extracts of germinated barley grain were used as a source of enzyme, a 41 kDa (1,4)-β-D-xylan endohydrolase was found (Slade et al. 1989). Two near full-length cDNAs and the corresponding genes allowed the complete amino acid sequences of the barley (1,4)-β-D-xylan endohydrolases to be deduced, but it remained difficult to confidently predict the NH_2-terminal residue of the mature, active enzyme (Banik et al. 1996, 1997).

In attempts to explain these apparent discrepancies in the molecular sizes reported for barley (1,4)-β-D-xylan endohydrolases, Simpson et al. (2003) used computer analyses to predict from the first 70 amino acid residues that the enzymes would occupy cytosolic locations. If the enzymes were targeted to the cytosol of aleurone layers they might not be detectable in soluble extracts of grain until relatively late after the initiation of germination, as observed by several groups (Fincher 1989; Slade et al. 1989; Simpson et al. 2003; Kuntz and Bamforth 2007). If the relatively thin inner layer of the aleurone wall were composed mainly of arabinoxylans (Fincher and Stone 2004), targeting the (1,4)-β-D-xylan endohydrolases to the cytosol would be expected to protect the inner aleurone wall layer from degradation. To ensure that starch- and protein-degrading enzymes could readily pass through the walls of the aleurone cells and gain access to their substrates in the starchy endosperm cells, it was suggested that (1,3;1,4)-β-D-glucan endohydrolases and other enzymes might degrade the thicker, outer layer of aleurone walls and a high proportion of walls of the starchy endosperm (Simpson et al. 2003). Following depletion of aleurone reserves, programmed cell death (Kuo et al. 1996) could lead to aleurone cell rupture and to the release of cellular contents, including the preformed (1,4)-β-D-xylan endohydrolases (Caspers et al. 2001; Simpson et al. 2003). If the enzyme were released in the 61 kDa form, endopeptidases in the starchy endosperm might process the enzyme to remove NH_2- and COOH-terminal regions; this would produce the 41 kDa and 30 kDa forms that are detected in the medium around isolated aleurone layers and in germinated grain. It is possible that a similar mechanism exists for delaying limit dextrinase release from barley aleurone cells (Burton et al. 1999).

To further investigate these possibilities, Van Campenhout et al. (2007) cloned cDNAs encoding the 34, 41, and 61.5 kDa forms and, following expression of the cDNAs in heterologous systems, assayed for (1,4)-β-D-xylan endohydrolase activity. The group recovered the 61.5 kDa form in the soluble fraction and showed that it had activity on arabinoxylans and on shorter xylo-oligosaccharides (Van Campenhout et al. 2007).

The barley (1,4)-β-D-xylan endohydrolases are members of the GH10 family of glycoside hydrolases (Banik et al. 1996; Coutinho and Henrissat 1999; Simpson et al. 2003). There appear to be three or more (1,4)-β-D-xylan endohydrolase genes in barley; these are clustered in a region on the long arm of chromosome 5H (Banik et al. 1997).

Xylanase inhibitors have been reported in wheat grain and have been studied in detail. Distinct classes of inhibitors are found in wheat, including the *Triticum aestivum* xylanase inhibitor (TAXI), the thaumatin-like xylanase inhibitor (TLXI), and the xylanase inhibitor protein (XIP) (Goesaert et al. 2004; Fierens et al. 2007, 2008). These inhibitors appear to target not only microbial (1,4)-β-D-xylan endohydrolases (Juge et al. 2004; Fierens et al. 2007, 2008), but also appear to inhibit barley α-amylases (Sancho et al. 2003). Endoxylanase inhibitors are also synthesized in the germinated barley grain (Goesaert et al. 2004; Beaugrand et al. 2007), and the unsuspected presence of inhibitors could certainly have complicated earlier interpretations of assays of (1,4)-β-D-xylan endohydrolase activity in grain extracts.

Arabinoxylan arabinofuranohydrolases

Enzymes that release L-arabinose from wheat endosperm arabinoxylan have been purified from extracts of young barley seedlings, which would include germinated grain that remained attached to the seedlings (Ferre et al. 2000; Lee et al. 2001). The enzymes are members of the GH51 family and have been designated arabinoxylan arabinofuranohydrolase, or AXAH. The enzyme appears to preferentially hydrolyze α-L-arabinofuranosyl residues linked to C(O)3 of the (1,4)-β-D-xylan backbone, but α-L-arabinofuranosyl residues can also be removed from doubly substituted xylosyl residues (Fig. 14.2). The complete amino acid sequence of barley AXAH-I was deduced from a near full-length cDNA: a cDNA encoding a second barley AXAH, designated AXAH-II, was also reported (Lee et al. 2001). It is important to distinguish the barley AXAHs from the bifunctional family GH3 glycoside hydrolases that have α-L-arabinofuranosidases and β-D-xylosidase activity (Lee et al. 2003). The family GH3 α-L-arabinofuranosidases hydrolyze arabinoxylans very slowly, if at all, but both the GH3 and GH51 groups of enzyme can hydrolyze 4-nitrophenyl α-L-arabinofuranoside (Lee et al. 2001).

The possible participation of barley AXAHs in cell wall arabinoxylan depolymerization germinated grain is not yet demonstrated. As noted above, the enzymes were purified from plant material that would consist of both germinated grain and young vegetative tissues (Lee et al. 2001). It has been proposed that the AXAHs participate in the modification of arabinoxylan fine structure during wall deposition, maturation, or expansion in coleoptiles (Gibeaut et al. 2005), or in wall turnover and the hydrolysis of arabinoxylans in germinated grain (Ferre et al. 2000; Lee et al. 2001). More evidence is required before we can conclude that the AXAH enzymes play any part in the hydrolysis of arabinoxylans in germinated barley grain.

α-L-Arabinofuranosidases and β-D-xylosidases

An α-L-arabinofuranosidase and a β-D-xylosidase, have been purified from extracts of 5-day old barley seedlings and characterized (Lee et al. 2003). The enzymes were designated ARA-I and XYL, respectively, and are members of the GH3 family of glycoside hydrolases (Coutinho and Henrissat 1999). The ARA-I was shown to be a bifunctional α-L-arabinofuranosidase/β-D-xylosidase, with an approximately similar catalytic efficiency on both substrates. However, the XYL enzyme preferentially hydrolyzes 4-nitrophenyl β-D-xyloside and has only low activity on 4-nitrophenyl α-L-arabinofuranoside (Lee et al. 2003).

The enzymes do not hydrolyze wheat flour arabinoxylan to any significant extent, but do

hydrolyze oligosaccharides released from arabinoxylans by (1,4)-β-D-xylan endohydrolases (Fig. 14.2). Thus, both enzymes hydrolyze (1,4)-β-D-xylopentaose and ARA-I can also degrade (1,5)-α-L-arabinohexaose (Lee et al. 2003), but neither will hydrolyze substituted polysaccharides.

The complete amino acid sequences of ARA-I and XYL deduced from cDNAs indicate that about 130 amino acid residues are removed from COOH-terminus of the primary translation product (Lee et al. 2003). The genes encoding the ARA-I and XYL have been mapped to chromosomes 2H and 6H, respectively (Lee et al. 2003).

In isolated aleurone layers, the α-L-arabinofuranosidases and β-D-xylosidases are secreted much earlier than the (1,4)-β-D-xylan endohydrolases (Banik et al. 1997) and, in this experimental system at least, secretion of the endohydrolases clearly does not occur at the same time as the secretion of the α-L-arabinofuranosidases and β-D-xylosidases. Lee et al. (2003) detected little or no mRNA encoding ARA-I in the aleurone layer of germinated grain but nevertheless concluded that the two family GH3 enzymes play important roles in cell wall degradation in germinated barley grain. It is not clear whether this observation might be explained in terms of a multigene family or by the possibility that the activity was not attributable to the family GH3 ARA-I or XYL enzymes, but rather to glycosidases from other families. Nevertheless, the ARA-I and XYL enzymes might participate in hydrolysis of the oligosaccharides released by the (1,4)-β-D-xylan endohydrolases in the germinated barley grain (Lee et al. 2003).

Hydrolysis of (1,3;1,4)-β-D-glucans

(1,3;1,4)-β-D-Glucans are polysaccharides found almost exclusively in walls of the Poaceae. Sugars released from (1,3;1,4)-β-D-glucans during wall degradation in the germinated grain make a major contribution to the total energy available for growth of the young seedling. In barley, it has been estimated that up to 18.5% of the total carbohydrate available to the young seedling, including that provided by the hydrolysis of the major storage polysaccharide, starch, is derived from the degradation products of cell wall polysaccharides (Morrall and Briggs 1978).

The (1,3;1,4)-β-D-glucans contain linear chains of β-D-glucopyranosyl monomers polymerized through (1,3)- and (1,4)-linkages. The ratio of (1,4)- to (1,3)-linkages is in the range 2.2–2.6:1 (Fincher and Stone 2004). The water-soluble (1,3;1,4)-β-D-glucans from barley endosperm cell walls consist predominantly of blocks of two or three adjacent (1,4)-linked β-D-glucosyl residues separated by single (1,3)-linked β-D-glucosyl residues. A small proportion of longer blocks of adjacent (1,4)-linked β-D-glucosyl residues, which might be over 10 residues in length and account for 10% by weight of the molecule (Woodward et al. 1983a), are also present in (1,3;1,4)-β-D-glucans from the starchy endosperm of barley grain. The (1,3;1,4)-β-D-glucans from barley grain can have DPs of over 1000 glucosyl residues (Woodward et al. 1983b).

Water-soluble barley (1,3;1,4)-β-D-glucans adopt an extended conformation with an axial ratio of about 100 (Woodward et al. 1983b). The asymmetrical conformations of barley (1,3;1,4)-β-D-glucans, coupled with their high DP, enable them to form a gel-like matrix in the wall and ensure that walls remain strong, flexible, pliable, and sufficiently porous to permit the transfer of water and other low-molecular-weight molecules across the wall. The same asymmetrical conformations of barley (1,3;1,4)-β-D-glucans lead to the formation of aqueous solutions of high viscosity and account not only for the undesirable characteristics attributed to (1,3;1,4)-β-D-glucans in malting and brewing processes, but also to the beneficial effects of barley (1,3;1,4)-β-D-glucans on human health and nutrition (Brennan and Cleary 2005). As a result, the high viscosity of soluble (1,3;1,4)-β-D-glucans represents a key quality factor in malting and brewing, where incompletely degraded (1,3;1,4)-β-D-glucans that remain after the controlled germination conditions during malting can increase wort and beer viscosity and cause problems in wort separation and beer filtration. The rapid synthesis of high levels of enzymes that depolymerize (1,3;1,4)-β-D-glucans during germination is a desirable quality char-

acteristic of malting grade barleys (Wang et al. 2004).

Several glycoside endo- and exohydrolases are required for the complete degradation of (1,3;1,4)-β-D-glucans to glucose in germinated barley grain, including family GH17 (1,3;1,4)-β-D-glucan endohydrolases (EC 3.2.1.73) and β-D-glucan glucohydrolases from glycoside hydrolase families GH1 and GH3 (Hrmova and Fincher 2002). Many of these enzymes have been purified from germinated grain or young barley seedlings, and their kinetics, substrate specificities, and 3D structures have been defined.

(1,3;1,4)-β-d-Glucan endohydrolases

The (1,3;1,4)-β-D-glucan endohydrolases (EC 3.1.2.73) are perhaps the most important enzymes for the rapid solubilization and complete degradation of (1,3;1,4)-β-D-glucans in endosperm walls of germinated barley grain. They only hydrolyze (1,4)-β-glucosidic linkages, and only where these linkages are located on the reducing end side of (1,3)-β-D-glucosyl residues, as follows:

$$\begin{array}{l} \quad\quad\ \downarrow \quad\ \downarrow \quad\quad\quad\ \downarrow \\ 4\,G\,4\,G\,3\,G\,4\,G\,4\,G\,3\,G\,4\,G\,4\,G\,4\,G\,4\,G\,3\,G \\ 4\,G\,4\,\ldots_{red} \end{array}$$

where G represents a β-D-glucosyl residue, 3 and 4 are (1,3)- and (1,4)-linkages, respectively, and red indicates the reducing terminus (Anderson and Stone 1975; Woodward and Fincher 1982). The enzymes therefore require adjacent (1,3)- and (1,4)-β-D-glucosyl residues. Reaction products released are mainly (1,3;1,4)-β-D-tri- and tetrasaccharides ($G4G3G_{red}$ and $G4G4G3G_{red}$), but they also release higher oligosaccharides of up to 10 or more contiguous (1,4)-β-D-glucosyl residues with a single reducing terminal (1→3)-β-D-glucosyl residue (e.g., $G4G4G4G4G4G4G3G_{red}$). These longer oligosaccharides originate from the longer regions of adjacent (1→4)-linkages that account for approximately 10% by weight of barley (1,3;1,4)-β-D-glucans (Woodward et al. 1983a).

Two (1,3;1,4)-β-D-glucan endohydrolase isoenzymes are present in germinated barley grain. The (1,3;1,4)-β-D-glucan endohydrolase isoenzyme EI is expressed in the scutellar epithelium early after germination and later in both the aleurone and scutellum (McFadden et al. 1988; Slakeski and Fincher 1992). In contrast, (1,3;1,4)-β-D-glucan endohydrolase isoenzyme EII is expressed almost exclusively in the aleurone of germinated grain, where mRNA levels of the gene are high (Slakeski and Fincher 1992). The two barley genes that encode (1,3;1,4)-β-D-glucan endohydrolase isoenzymes, designated HvGlb1 and HvGlb2, have been sequenced and mapped to the barley chromosomes 1H and 7H, respectively (Fig. 14.3). Nucleotide sequence elements associated with GA induction have been identified in their promoters (Litts et al. 1990; Slakeski et al. 1990) and, as noted earlier, the barley aleurone myb transcription factor, GAMYB, transactivates the (1,3;1,4)-β-D-glucanase isoenzyme EII promoter (Gubler et al. 1999).

The 3D structure of the barley (1,3;1,4)-β-D-glucan endohydrolase isoenzyme EII has been solved by X-ray crystallography (Varghese et al. 1994). The enzyme is a family GH17 glycoside hydrolase (Henrissat 1998) that adopts a $(\beta/\alpha)_8$ TIM barrel conformation. A deep substrate-binding cleft extends across the surface of the enzyme and can accommodate 6–8 glucosyl-binding subsites (Varghese et al. 1994). The open cleft enables the enzyme to bind at essentially any position along the (1,3;1,4)-β-D-glucan substrate and hence to hydrolyze internal glycoside linkages. This explains its endo-action pattern. The 3D structure of the barley (1,3;1,4)-β-D-glucan endohydrolase has been used to rationally redesign the enzyme, using site-directed mutagenesis, to enhance its thermostability under conditions similar to those encountered in the malting and brewing processes (Stewart et al. 2001).

(1→4)-β-D-Glucan glucohydrolases

The family GH1 (1,4)-β-D-glucan glucohydrolases in extracts of young barley seedlings can hydrolyze the β-D-oligoglucosides released from wall (1,3;1,4)-β-D-glucans by the (1,3;1,4)-β-D-glucan endohydrolases described in the section above (Simos et al. 1994). However, it has not been demonstrated unequivocally that the

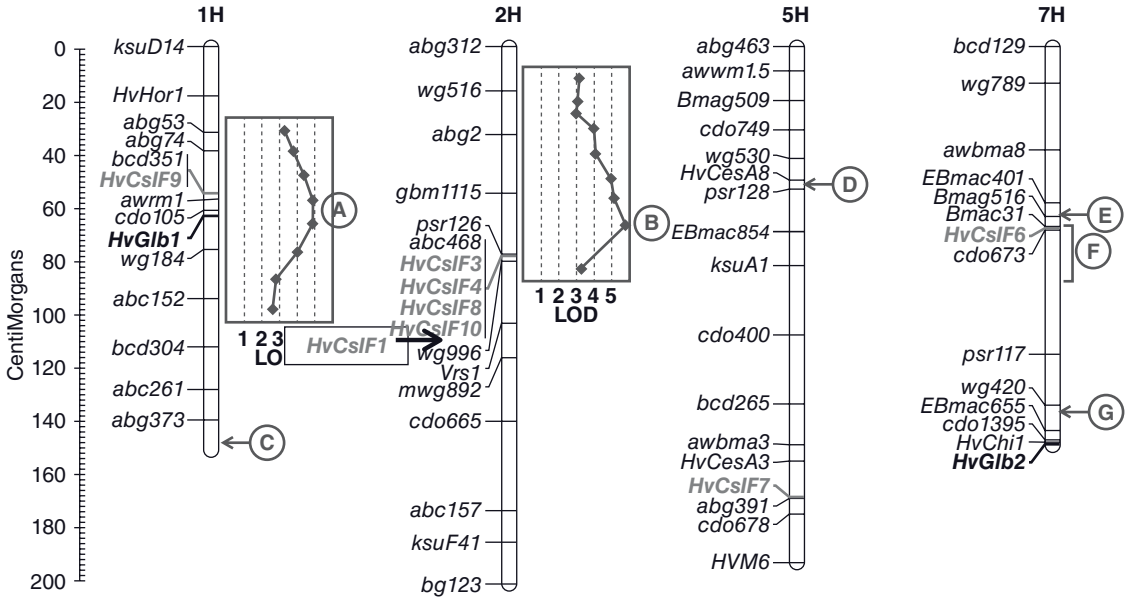


Fig. 14.3. Genetic maps of barley chromosomes 1H, 2H, 5H, and 7H showing the positions of barley *HvCslF* genes as mapped in a Clipper × Sahara population. Approximate positions of previously reported QTLs for grain (1,3;1,4)-β-D-glucan content are shown (A to G), based on alignment of the Clipper × Sahara map with genetic maps from other populations. Graphs A and B show statistically significant test statistics (LOD) values reported by Han et al. (1995) based on mapping in a Steptoe × Morex population. The letters C, D, and E show the approximate locations of markers at which Molina-Cano et al. (2007) detected QTLs in a Beka × Logan population, F shows the approximate location of the peak QTL test statistic value reported by Igartua et al. (2002) based on mapping in a Derkado × B83-12/21/5 population, and G shows the approximate location of a marker at which Kim et al. (2004) detected marker-trait association in a Yonezawa Mochi × Neulssalbori population. The approximate position of the *HvCslH1* gene is also shown (unpublished data). Approximate positions of the genes *HvGlb1* and *HvGlb2* are shown, based on mapping in other populations. (Figure reproduced from Burton et al. 2008.)

enzymes perform this function *in vivo*, because the precise location of the (1,4)-β-D-glucan glucohydrolases in young seedlings is not known and could be in vegetative tissues of the young seedlings from which they were purified, rather than in the germinated barley grain. The preferred substrates for the purified (1,4)-β-D-glucan glucohydrolases are (1,4)-β-D-oligoglucosides; (1,3)-β-D-oligoglucosides are hydrolyzed very slowly (Hrmova et al. 1996, 1998). The $G4G3G_{red}$ and $G4G4G3G_{red}$ oligosaccharides released by (1,3;1,4)-β-D-glucan endohydrolases have (1,4)-β-D-glucosyl residues at their nonreducing termini and a single (1,3)-β-D-glucosyl residue at their reducing termini. The barley (1,4)-β-D-glucan glucohydrolases can therefore hydrolyze an oligosaccharide such as $G4G4G4G3G_{red}$ (Hrmova et al. 1996), and their ability to slowly hydrolyze laminaribiose suggests that the (1,3;1,4)-β-D-oligoglucosides could be completely hydrolyzed to glucose by enzymes of this group (Hrmova and Fincher 2002).

β-D-Glucan exohydrolases

The broad specificity family GH3 (Henrissat 1998) β-D-glucan exohydrolases from barley rapidly hydrolyze glycosidic linkages in several polymeric β-D-glucans, such as laminarin and (1,3;1,4)-β-D-glucans, although their preferred substrates are (1,3)-β-D-glucans (Kotake et al. 1997; Hrmova and Fincher 1998). In addition, they can hydrolyze β-D-oligoglucosides containing (1,2)-, (1,3)-, (1,4)-, or (1,6)-linkages, aryl β-D-glucosides such as 4-nitrophenyl β-D-

glucoside (4NPGlc), and some β-D-oligoxyloglucosides (Hrmova and Fincher 1998; Kim et al. 2000). Glucose is released from the nonreducing termini of these substrates (Hrmova et al. 1996).

The 3D structure of barley β-D-glucan exohydrolase isoenzyme ExoI has also been defined (Varghese et al. 1999). Two distinct domains of the enzyme are connected by a 16-amino acid helix-like linker peptide. The first domain is a $(\beta/\alpha)_8$ barrel of 357 amino acid residues. The second domain folds to form a "β-sandwich" conformation that consists of a six-stranded β-sheet flanked on either side by three α-helices (Varghese et al. 1999). The active site of the enzyme is located in a shallow pocket at the interface of the two domains. The broad substrate specificity of the barley enzymes can be explained from the crystallographic data (Hrmova et al. 2002). The glucosyl residue of the substrate that is bound at subsite −1 is fixed through extensive hydrogen bonding with several amino acid residues at the bottom of the active site pocket (Hrmova et al. 2002). However, the glucosyl residue at subsite +1 is sandwiched between two tryptophan residues at the entrance of the pocket. The relative flexibility of this binding between the two tryptophan residues at subsite +1, coupled with the projection of the remainder of bound substrate away from the enzyme's surface, indicates that the short active site can accommodate a range of disaccharide constituents (Hrmova et al. 2002).

Two isoforms of the family GH3 β-D-glucan exohydrolases, designated isoenzymes ExoI and ExoII, have been purified from extracts of young barley seedlings (Hrmova and Fincher 1998). The total number of genes in the family has not been determined. As with the family GH1 (1,4)-β-D-glucan glucohydrolases, it is not known if the enzymes are located in the starchy endosperm of germinated grain or in vegetative tissues of young seedlings. However, the family GH3 group of enzymes has the potential to contribute to (1,3;1,4)-β-D-glucan hydrolysis at the polysaccharide level, and also to hydrolyze (1,3;1,4)-β-D-oligoglucosides released from (1,3;1,4)-β-D-glucans by the action of endohydrolases (Hrmova et al. 2002).

Starch degradation in germinated barley grain

Starch granules in the starchy endosperm cells of barley grain constitute about 64% of the grain on a dry weight basis and consist of about 75% amylopectin and 25% amylose (MacGregor and Fincher 1993). Following germination of the grain, these polysaccharides are depolymerized by the combined action of α-amylases, β-amylases, starch debranching enzymes, and α-glucosidases. The roles of each of these enzyme groups in starch degradation in germinated barley grain are summarized below.

α-Amylases

Barley α-amylases catalyze the hydrolysis of internal (1,4)-α-glucosidic linkages in both amylopectin and amylose, with an endo-action pattern. The enzymes have been classified into two groups on the basis of their isoelectric points. The low pI (AMY1) group is encoded by four genes in barley and the high pI (AMY2) group is encoded by six genes (Huang et al. 1992; Henrissat and Bairoch 1996). The AMY1 and AMY2 groups of isoenzymes exhibit approximately 70% sequence identity at the amino acid level, but much higher sequence identities of up to 95% are observed between members within each group (Bak-Jensen et al. 2007). Posttranslational modification, in particular COOH-terminal cleavage, increases the complexity of α-amylase profiles in barley tissues and leads to multiple forms of individual members of the two multigene families (Søgaard et al. 1991).

It has long been held that the levels of AMY2 in the endosperm of germinated barley grain are several fold higher than those of AMY1 (MacGregor et al. 1984). More recently, Bak-Jensen et al. (2007) monitored temporal and spatial aspects of barley α-amylase profiles following germination of barley, using 2D gel electrophoresis, Western blot analyses, and mass spectrometric identification of the various isoforms and their degradation products. The enzymes are secreted from the aleurone layer, and possibly from the scutellum (Gibbons 1981),

following GA-activation of the corresponding genes. It appears that the α-amylases secreted into the starchy endosperm are the products of one *AMY1* and two *AMY2* genes. Other members of the gene families do not appear to be expressed at high levels in the germinated barley grain (Bak-Jensen et al. 2007) but could be involved in starch turnover in chloroplasts in various photosynthetic organs. The enzymes expressed in the germinated barley grain are subjected to characteristic patterns of proteolytic degradation in the starchy endosperm, but these patterns do not appear to be related to malting quality of the barley variety (Bak-Jensen et al. 2007).

The activity of α-amylases of higher plants can be modulated by specific inhibitors. The barley AMY2 enzyme is specifically inhibited by the barley α-amylase/subtilisin inhibitor (BASI); the AMY1 isoenzyme is not inhibited by BASI (Mundy et al. 1983; Svendsen et al. 1986). The BASI protein is deposited in the starchy endosperm during grain development, while the AMY2 enzyme is synthesized *de novo* following germination. It has been suggested that BASI controls the activity of AMY2 under conditions that might promote premature germination or sprouting. In this way it could slow or delay precocious germination. Furthermore, BASI can inhibit proteases and might play a role in inhibiting serine proteases of potential pathogens and pests (Jones and Jacobsen 1991).

The interaction between the barley AMY2 enzyme and BASI has been defined in detail through X-ray crystallography (Fig. 14.4) (Vallée et al. 1994; Rodenburg et al. 2000). The molecular basis of binding of the inhibitor over the active site was established, and site-directed mutagenesis was used to increase the affinity of the interaction between BASI and AMY1, which is not normally inhibited (Rodenburg et al. 2000). The availability of the 3D structural details of barley AMY2/BASI binding presented a number of opportunities to engineer either partner to alter the kinetics of inhibition. Similarly, the 3D structure of the BASI protein in complex with a bacterial subtilisin protease has been solved, and the molecular basis for inhibition of microbial proteases has been defined (Micheelsen et al. 2008).

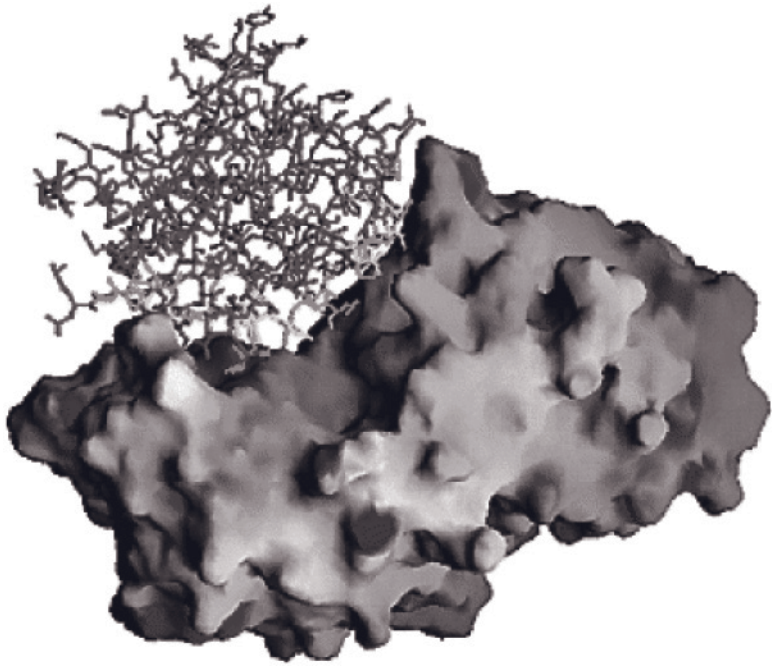


Fig. 14.4 The three-dimensional structure of AMY2–BASI. BASI is shown in stick representation with highlighted residues participating in hydrogen bond interactions (yellow) or in other contacts up to 3.9 Å (blue) with AMY2. The AMY2 surface electrostatic potential is color coded from negative (red) to positive (blue). From Vallée et al. (1994). For color details, please see color plate section.

β-*Amylases*

β-Amylase is a (1,4)-α-glucan maltohydrolase (EC 3.2.1.2) that hydrolyzes the penultimate linkage at the nonreducing terminus of both amylopectin and amylose, to release maltose. The enzyme can almost completely hydrolyze amylose to maltose, but is unable to bypass the (1,6)-branch points in amylopectin. The enzyme accumulates in the developing endosperm of barley grain and, at grain maturity, can account for up to 1% of total protein (Kreis et al. 1987). Some of the β-amylase becomes bound to proteins at the periphery of starch granules during grain desiccation. Thus, in mature barley grain, β-amylase is found in a free, active form and in a less-active form that is bound to other grain proteins, such as protein Z, via a cysteine residue near the COOH-terminus (Hejgaard 1978).

Proteolytic enzymes are believed to mediate the release of bound β-amylase during germination and this process is accompanied by the appearance of additional β-amylase isoforms (Guerin et al. 1992). These are probably generated by limited proteolysis of the COOH-terminal end of the polypeptide (Lundgard and Svensson 1987). The released, active β-amylase has little or no activity on insoluble starch granules, but in the presence of α-amylase released from the aleurone layer, starch degradation proceeds.

The barley β-amylase is critically important in malting and brewing, where the rapid production of high levels of the enzyme is considered an important quality characteristic. The activity of the β-amylase decreases quickly at temperatures above 55°C, but in the brewery, temperatures of up to 65°C are required for starch gelatinization, which in turn is essential for rapid starch degradation. As a result, there has been considerable interest in the thermostability of the barley β-amylase (Ma et al. 2000). Malting barleys with high β-amylase thermostability exhibit higher fermentability values (Eglinton et al. 1998). The crystal structure of a barley β-amylase has been solved and the structure has been used to evaluate thermostable forms of the enzyme (Mikami et al. 1999). Ma et al. (2000) showed that removal of the four COOH-terminal glycine-rich repeats of barley β-amylase greatly enhances its thermostability and substrate binding affinity.

Debranching enzymes

Starch-debranching enzymes can be divided into two groups, depending on their substrate specificities and action patterns. First, the pullulanases (pullulan 6-glucanohydrolase; EC 3.2.1.41) are endohydrolases that hydrolyze (1,6)-α-linkages in pullulan, a polysaccharide consisting of maltotriosyl residues linked by (1,6)-α-linkages. Second, isoamylases (glycogen 6-glucanohydrolase; EC 3.2.1.68) are endohydrolases that catalyze the hydrolysis of (1,6)-α-glucosyl linkages in glycogen and amylopectin but not in pullulan. Debranching enzymes might also play a role in starch synthesis, where an appropriate balance between branching and debranching enzymes might be required for amylopectin synthesis (James et al. 1995; Martin and Smith 1995; Ball et al. 1996; Nakamura et al. 1996; Rahman et al. 1998).

In germinated barley grain, debranching enzymes catalyze the hydrolysis of (1,6)-α-glucosidic linkages in amylopectin or in (1,4;1,6)-α-oligoglucosides released by α-amylases (MacGregor and Fincher 1993). The (1,6)-α-glucosyl linkages in these oligosaccharides, which are also known as limit dextrins, are not hydrolyzed by either β- or α-amylases. Debranching enzymes therefore play an important role in the complete depolymerization of starch to glucose. The debranched oligosaccharides are susceptible to further hydrolysis by amylases and α-glucosidases (Lee et al. 1971). The barley debranching enzyme, also known as the R-enzyme, has a marked preference for the hydrolysis of (1,6)-α-glucosyl linkages in oligosaccharides compared with polysaccharides and is therefore referred to here as limit dextrinase (Lee et al. 1971; Burton et al. 1999).

Burton et al. (1999) and Kristensen et al. (1998) have isolated a gene and cDNA encoding a single limit dextrinase from barley. The mature enzyme has close to 900 amino acid residues and a calculated molecular mass of about 97 kDa. The barley limit dextrinase mRNA was shown to be

abundant in GA-treated aleurone layers and in germinated grain (Burton et al. 1999). The mRNA was found at lower levels in the developing endosperm of immature grain, where the enzyme might participate in starch synthesis (Burton et al. 1999). As mentioned earlier in this review, the barley limit dextrinase cDNA encodes a pre-sequence typical of transit peptides that target nascent polypeptides to amyloplasts. This is difficult to reconcile with the secretion of the mature enzyme from aleurone cells into the starchy endosperm of germinated grain. It is possible that the enzyme is not released from the aleurone until programmed cell death occurs.

A specific inhibitor of the barley limit dextrinase is present in mature barley grain but disappears several days after the initiation of germination (MacGregor 2004). The limit dextrinase inhibitor can be modified posttranslationally through the addition of cysteine or glutathione to a sulfhydryl group of the enzyme. The inhibitor is believed to interact specifically with the active site of the barley limit dextrinase (MacGregor 2004). Stahl et al. (2004) used gene-silencing technologies to show that both limit dextrinase and its inhibitor might participate in starch synthesis as well as starch degradation.

Barley also has an isoamylase-like debranching enzyme. Sun et al. (1999) isolated a gene for a barley isoamylase and monitored its expression in germinated grain, developing endosperm, and vegetative tissues. Their results indicated that isoamylase is involved in starch synthesis in barley plants. Similarly, Burton et al. (2002) concluded that barley isoamylase is involved in starch synthesis and that the enzyme is an important determinant of the number, and hence the form, of starch granules.

α-*Glucosidases*

Enzymes capable of hydrolyzing maltose, and other small maltodextrins to glucose are necessary for the complete hydrolysis of starch. α-Glucosidase activity has been detected in barley malt and in the medium surrounding GA-treated barley aleurone layers (Tibbot et al. 1998). The enzyme was subsequently purified from extracts of germinated barley grain (Frandsen et al. 2000) and through heterologous of the corresponding cDNA (Naested et al. 2006), and characterized in detail. The enzymes have a molecular mass of 90–100 kDa and are detected as 101 and 95 kDa forms soon after the initiation of barley grain germination (Tibbot et al. 1998).

Again the thermostability of the barley α-glucosidase is important for the rapid conversion of starch to fermentable sugars, and site-directed mutagenesis has been used to significantly increase the thermostability of the barley enzyme (Muslin et al. 2002; Clark et al. 2004).

Storage protein degradation in germinated barley grain

The mobilization of reserve proteins in the starchy endosperm of germinated barley requires the action of endo- and exopeptidases. Amino acids and small peptides of two to five amino acid residues are released and diffuse along a concentration gradient to the scutellar epithelial layer, where they are transported into the developing embryo.

Carboxypeptidases

In barley, serine carboxypeptidases appear to be particularly important in storage protein hydrolysis. At least six isoforms are detectable in the starchy endosperm of germinated barley grain (Dal Degan et al. 1994). One carboxypeptidase isoenzyme appears to be deposited during grain development, and therefore preexists in the grain when germination is initiated, while all six isoenzymes are synthesized *de novo*, either in the scutellum or in the aleurone, once the grain has germinated and several of these are secreted into the starchy endosperm (Dal Degan et al. 1994; Potokina et al. 2002). Carboxypeptidases are also secreted from isolated barley aleurone layers treated with GA (Hammerton and Ho 1986). Most of the carboxypeptidases are synthesized as a single chain precursor and secreted in zymogen form. They are subsequently activated by the proteolytic removal of a central portion of the protein of approximately 50 amino acid residues

(Doan and Fincher 1988; Dal Degan et al. 1994). This leads to the formation of the active enzyme, which consists of two polypeptide chains linked by disulfide bonds. The battery of carboxypeptidases in the germinated grain quickly depolymerizes high molecular mass storage proteins, but activity on di-and tri-peptides is relatively slow.

The central role of carboxypeptidases in barley grain mobilization after germination has lead to analyses of transcription patterns of the corresponding genes. Potokina et al. (2006) examined in detail the transcription of the carboxypeptidase isoenzyme I gene, mapped the gene as an eQTL to chromosome 3H that coincides with the position of a minor QTL for "diastatic power," and defined its haplotype diversity. Their results showed that the gene is regulated in *cis* and that its expression level correlates with the presence of distinct single nucleotide polymorphism (SNP) haplotypes within the gene (Potokina et al. 2006).

Endopeptidases

In barley, several classes of endopeptidases are believed to participate in the mobilization of protein reserves of the starchy endosperm of germinated grain. Cysteine endopeptidases have been detected in isolated aleurone, where their synthesis and secretion is regulated by GA and ABA (Rogers et al. 1985; Hammerton and Ho 1986; Koehler and Ho 1990a; Martínez et al. 2003). Two of these cysteine endopeptidases, EPA and EPB, are expressed predominantly in the scutellar epithelial layer and in the aleurone layer of germinated barley grain (Koehler and Ho 1988, 1990a,b; Mikkonen et al. 1996). Transcription factors that are involved in the expression of cysteine endopeptidase genes have been described (Isabel-LaMoneda et al. 2003).

Serine endopeptidases have also been detected in germinated barley grain (Zhang and Jones, 1995; Terp et al. 2000; Fontanini and Jones 2002). One of these enzymes is found in ungerminated, mature grain and their activity is inhibited by the barley α-amylase/subtilisin inhibitor (BASI) (Nielsen et al. 2004). The activity of the serine endopeptidases increases after germination, but in the embryo rather than the starchy endosperm, and it is not clear that this group plays a role in storage-protein degradation.

Peptide transport into the scutellum

As noted above, the combined action of the exo- and endopeptidases in the starchy endosperm of germinated barley grains leads to the formation of amino acids and small peptides. The small peptides can be rapidly transported into the scutellum (Sopanen et al. 1977; Walker-Smith and Payne 1983), where they are hydrolyzed to amino acids prior to their transport to the young seedling (Enari and Mikola 1977). Uptake of the small peptides is mediated by transporter proteins that are located in the plasma membrane of the scutellar epithelium (Waterworth et al. 2000; Tsay et al. 2007). The transport appears to be proton-coupled, because it is strongly pH-dependent (Hardy and Payne 1992). It appears that while di- and tri-peptides are preferentially transported, tetra- and penta-peptides can also be transported (Hardy and Payne 1992). Peptide transport occurs early in the process and can be detected 6–12 h after imbibition, whereafter it increases rapidly to a maximum at about 24 h after imbibition is initiated (West et al. 1998; Potokina et al. 2002).

Possible roles of thioredoxins in germinated barley grain

There has been some evidence over many years that thioredoxins might play important roles in germinated barley grain (Baumann and Juttner 2002). Thioredoxins participate in thiol–disulfide reactions through two cysteine residues located in a conserved CXXC active-site motif (Jacquot et al. 1997). There are many thioredoxin genes in plants, and members of the thioredoxin h group are expressed in germinated barley grain, where redox-regulated processes might be essential for successful endosperm mobilization. It has been suggested that a thioredoxin system might regulate the activity of α-amylase inhibitors and trypsin inhibitors (Kobrehel et al. 1991), that they might enhance the activity of limit dextrinase (Cho et al. 1999), and that they might reduce

storage proteins and hence facilitate their depolymerization (Kobrehel et al. 1992). They are also known to activate thiocalsin, which is a grain-specific serine protease (Besse et al. 1996), and to enhance GA synthesis (Wong et al. 2002). Two of the barley thioredoxin h proteins have been expressed in heterologous systems and characterized (Shahpiri et al. 2008).

GENETICS AND FUNCTIONAL GENOMICS OF STARCHY ENDOSPERM MOBILIZATION IN BARLEY

Germinated barley grain is an important starting material for the malting, brewing, and distillery industries. As a result, barley breeders have selected for many characteristics that influence the rate and extent of starchy endosperm modification following germination. Molecular marker technologies have been used to locate regions of the barley genome that are important for malting quality and to accelerate selection of desirable traits related to germination processes. Thus, there are extensive QTL maps of quality characteristics (Fig. 14.5), and these are now being used for positional cloning of the major genes that contribute to particular quality traits. During the phenotyping of quality traits in germinated barley grain, it is important to remember that the traits are likely to be influenced not only by events that follow germination, but also by factors oper-

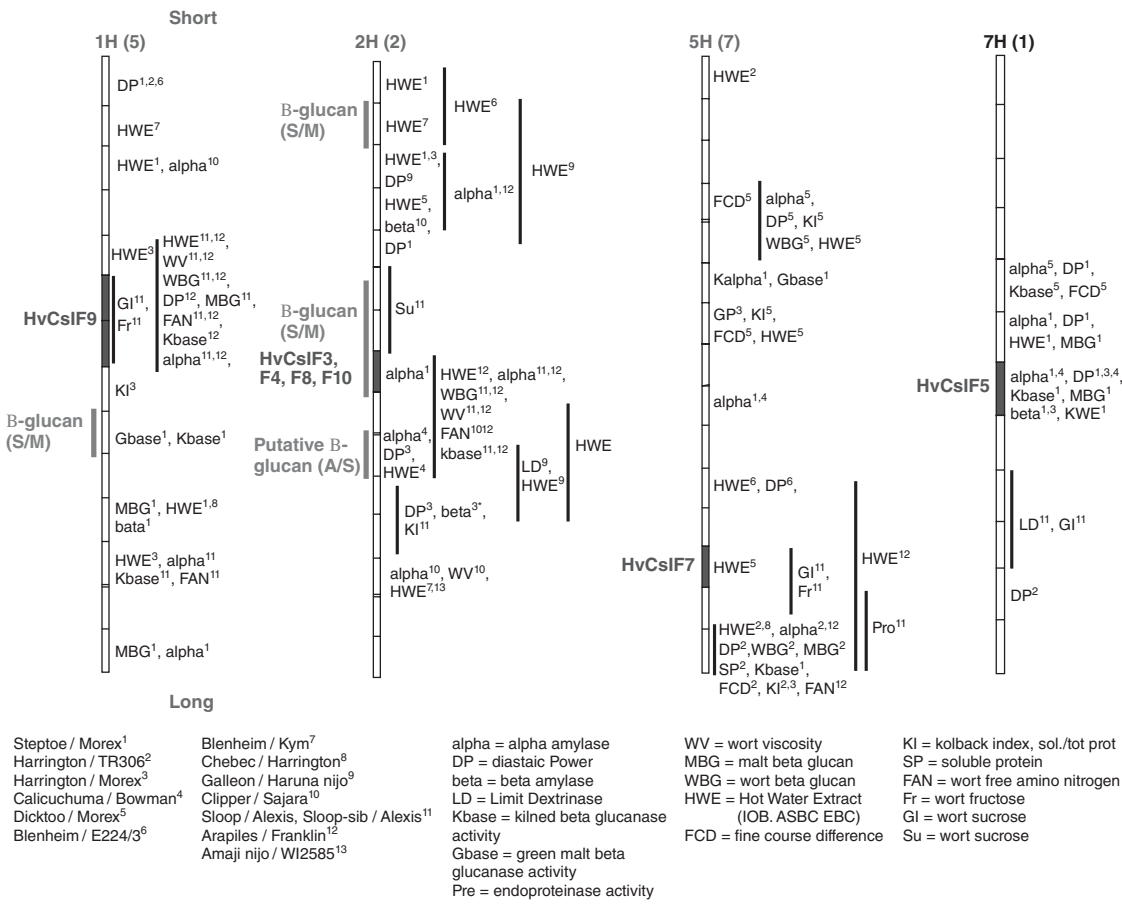


Fig. 14.5. Malting quality QTLs in barley. Diagram provided by Helen Collins.

ating during grain development. For example, breeders aiming for malting grade barleys generally select against high (1,3;1,4)-β-D-glucan concentrations in malt, because the polysaccharide can cause filtration problems in the brewery and can contribute to the formation of undesirable hazes in the final product (Bamforth 1999). However, the concentrations of (1,3;1,4)-β-D-glucan in malt will be the net result not only of the rate and levels of development of (1,3;1,4)-β-D-glucan endo- and exohydrolases following germination, but also of the initial concentration of the polysaccharide in the walls, the thickness of the walls, and the fine structure of the polysaccharide. Thus, genotypic effects on the synthesis of (1,3;1,4)-β-D-glucans in the developing grain will also influence residual levels of the polysaccharide in malt. As a result, attention in the area has been focused both on the selection of breeding lines with low (1,3;1,4)-β-D-glucan contents and/or on the selection of barley varieties that quickly produce large amounts of (1,3;1,4)-β-D-glucanases after the grain is germinated (Wang et al. 2004). The same principles apply for starch and storage protein mobilization. Furthermore, most of the malt quality characteristics under selection will be influenced by both genotype and environment. Using the same example, it is known that environmental conditions during grain development are key determinants of (1,3;1,4)-β-D-glucan content in the mature barley grain (Zhang et al. 2001), and high temperatures during the final stages of grain maturation appear to significantly increase grain (1,3;1,4)-β-D-glucan levels (Coles 1979).

The emergence of high-throughput functional genomics technologies is providing new opportunities for the discovery and description of genes that are important in barley germination and the subsequent mobilization of endosperm reserves (Finnie et al. 2004; Potokina et al. 2004, 2008). These procedures are still in the early stages of development, but in the sections below, well-defined examples are presented to illustrate how functional genomics can contribute to our knowledge of the biochemistry, physiology, and genetics of barley grain mobilization after germination.

Genetics of (1,3;1,4)-β-D-glucan degradation

QTL mapping has identified regions of the barley genome that control (1,3;1,4)-β-D-glucan concentrations in barley, and these have been placed on high-density genetic maps (Han et al. 1995; Burton et al. 2008). The multigenic control of (1,3;1,4)-β-D-glucan content in ungerminated barley grain is consistent with the mapping of QTLs to several chromosomes, including chromosomes 1H, 2H (Han et al. 1995), and 7H (Fig. 14.3) (Igartua et al. 2002; Molina-Cano et al. 2007). The barley *HvCslF* genes, which are believed to encode (1,3;1,4)-β-D-glucan synthases or other enzymes that are crucial for (1,3;1,4)-β-D-glucan synthesis (Burton et al. 2006), map to the same positions as known QTLs on chromosomes 1H, 2H, and 7H (Burton et al. 2008; Fig. 14.3).

As mentioned above, (1,3;1,4)-β-D-glucan concentrations in malt are likely to be under even more complex genetic control, because they will be affected not only by genetic factors that control their synthesis in the developing grain, but also by the speed and level of synthesis of degradative enzymes in the germinated grain. Thus, some of the QTLs for (1,3;1,4)-β-D-glucan content in malt extracts on barley chromosomes 1H, 3H, 4H, 5H, and 7H (Burton et al. 2008) may be related to concentrations of the two (1,3;1,4)-β-D-glucan endohydrolases, designated *HvGlb1* and *HvGlb2* in Fig. 14.3, which degrade the polysaccharides in germinated grain. Many of the genes that encode enzymes known to participate in (1,3;1,4)-β-D-glucan degradation, including genes for (1,3;1,4)-β-D-glucan exo-hydrolases and β-D-glucosidases, have not been cloned or mapped, and their possible involvement in the QTLs cannot yet be determined.

Genetics of malting quality

QTL mapping has been widely used to provide barley breeders with information on the extent of natural variation in a particular trait and on the genome location of genes that influence that trait. In this way, breeders have an objective basis on

which to follow and select for genes that contribute to important quality characteristics (Hayes et al. 1993; Mather et al. 1997; Marquez-Cedillo et al. 2000; Fig. 14.5). For example, a malting-quality QTL complex referred to as QTL2 has been mapped to barley chromosome 4H in a Steptoe/Morex doubled haploid population. The QTL2 complex affects malting quality traits such as malt extract, α-amylase activity, diastatic power, malt (1,3;1,4)-β-D-glucan content and seed dormancy (Gao et al. 2004). Fine mapping enabled the complex to be dissected into six QTLs, two of which influence malt extract and α-amylase activity, with one each for diastatic power and malt (1,3;1,4)-β-D-glucan (Gao et al. 2004).

FUTURE PROSPECTS

As indicated in preceding sections, the quality of barley grain to the malting and brewing industries is dependent not only upon the processes that occur during germination, but also those that have already occurred during grain development. Thus, both represent appropriate targets for the enhancement of barley for end-use quality. For example, biosynthesis of the major polysaccharide components of barley endosperm cell walls, including the commercially important (1,3;1,4)-β-D-glucans, is likely to involve many different types of enzymes and ancillary proteins, possibly organized into biosynthetic complexes. The complexes are likely to be sensitive to several developmental cues, including hormone levels, moisture availability and temperature, as well as the intrinsic controls exerted by the genetic makeup of each cultivar or line. Similar factors will control starch and storage protein synthesis during grain development. The very early stages of grain development might prove to be good targets for manipulation of malting quality characteristics of the mature grain, but we need to understand more about the enzymes and processes that are active at this early stage before rational attempts can be made to manipulate the processes for the improvement of grain quality. Transcription factors important in the initiation and control of grain development are likely to be critical in the next stages of defining grain development.

Equally valid targets may lie within the suite of enzymes involved in the hydrolysis of the various components of the barley grain during the germination or malting processes, whether those targets be wall degradation, starch depolymerization or storage mobilization. In all cases, recent identification of genes directly implicated in the mobilization of the various components of the starchy endosperm, coupled with emerging technologies that facilitate the identification of transcription factors and other key proteins, may provide more avenues to understanding the biology of grain germination and hence in the improvement of malting quality. The genome-wide analysis of expression quantitative trait loci (eQTL), which has been described for 16,000 barley genes using hybridization of Affymetrix arrays (Potokina et al. 2004, 2008), might prove to be a powerful tool for the identification of candidate genes for malting quality in the future. Association genetics, high-resolution QTL mapping, and comparative genomics, all supported by powerful bioinformatics programs, will create new opportunities for the rapid and easier positional cloning of genes important in barley starchy endosperm mobilization and hence in grain quality.

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Chapter 15
Malting and Brewing Uses of Barley

Paul Schwarz and Yin Li

INTRODUCTION

The largest value-added use for barley is the production of malt, which in turn, is mainly used in the production of beer. Malting is a process of controlled germination followed by drying. While any cereal grain can ostensibly be used to produce malt, barley accounts for the vast majority of malt production on a worldwide basis. In fact, for many, the term malt is used synonymously for malted barley. Lesser amounts of malt prepared from other cereals and pseudo-cereals are used in the production of several types of specialty beers or traditional beer styles (most commonly sorghum, maize, and millet in Africa, and wheat in Europe), and in the production of distilled alcoholic beverages. The baking, cereal, confectionary, and distilled alcohol industries also use small amounts of malt or malt flour as a source of flavor, aroma, and/or amylase activity.

The aim of this chapter is to provide background on the functions and utilization of malted barley in connection with a discussion of barley and malt quality. The intended audience is individuals working in barley varietal development and production, as well as those in supporting areas of research and technology. There is profusion of excellent texts on the science and technology of malting and brewing, and readers desiring more detailed information are encouraged to consult the following (Briggs 1998; Kunze 1999; EBC 2000; Briggs et al. 2004; Priest and Stewart 2006).

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MALTING AND BREWING

Functions of malt

Next to water, malt is the most abundant ingredient used in brewing beer, and its characteristics and quality have a preponderant influence on the brewing process and resultant beer quality. The malting process is essential in that it results in a large increase of hydrolytic enzymes, partial degradation of endosperm cell walls and protein, and structural changes within the grain tissues that render starch and protein substrates readily extractable. Malting barley typically contains approximately 60%–65% starch and 10.5%–13.5% % protein. These components are degraded and solubilized during the mashing stage of brewing, and contribute to malt extract. Typically 79%–82% of the malt is extractable under laboratory conditions. Starch is degraded to a mixture of fermentable sugars and non-fermentable dextrins, and these carbohydrates constitute the bulk of the extract. Proteins are solubilized and degraded during malting and mashing into a mixture of soluble protein, peptides, and amino acids. Soluble nitrogenous compounds account for 4%–6% of the malt when measured as soluble protein.

Thus, on a fundamental level, the major function of the malt is that it contributes the soluble extract required for brewing. Fermentable carbohydrates, which are the major component of malt

extract, are required by the yeast for alcoholic fermentation to proceed. Malt provides both the starch substrate and amylolytic enzymes necessary for conversion. In modern brewing practice, a portion of the malt is often replaced with other starch sources like maize or rice or sugar sources like sugarcane, but since the cereal adjunct materials do not contain enzymes, some malt is still necessary. Yeast require specific amino acids, as well as vitamins and minerals for growth, and these are in part supplied by the malt. Traditional adjunct materials contain little to no soluble nitrogenous components.

Malt makes important contributions to the organoleptic characteristics of beer. It is the major source of beer color, and can make a major contribution to beer flavor. Chemical components in the malt, including sugars, amino acids, lipids, and phenolics, serve as precursors to color, flavor, and off-flavor compounds. Specific process conditions determine which compounds are developed and/or are retained. The mouthfeel or body of the beer is dependent upon components derived from the malt including non-fermentable dextrins and cell wall polysaccharides. Beer foam is in large part dependent upon specific proteins derived from the malt. On the negative side, malt can sometimes contain factors that adversely impact beer quality, which can result in such things as gushing or premature yeast flocculation.

The husk (lemma and palea) of the barley grain is of importance in the malting and brewing processes. It serves to protect the growing coleoptile during malting. In addition, it serves as a filter aid in the separation of the extract from the insoluble spent grains, in the traditional lautering process. Retention of the husk at harvest is in fact one reason that barley is preferred over wheat for the production of malt.

Finally, one should consider that there are complex relationships between all ingredients, the malt, adjunct, hops, water, and yeast, and these can have significant influences on processing performance and beer quality. The balance between malt, yeast, and hop-derived flavor/bitterness is essential in defining beer character or style. Interaction between hop components and foam proteins from the malt are important in stability of beer foam. Minerals supplied by the water and malt, and vitamins and amino acids supplied by the malt, are required for yeast growth. The mineral composition and pH of the brewing water impact the rates of chemical and enzymatic reactions, solubilization and extraction, and perception of flavors.

THE MALTING AND BREWING PROCESSES

Malting and brewing are probably one of the oldest biotechnologies, and the use of sprouted grains in the brewing of beer-like beverages can be traced back at least 6000 years (Briggs 1998). While the production and sale of malt dates to the Middle Ages, it was practiced on a small scale and was technologically crude. The industrialization and scale of maltings began to increase in the mid- to late nineteenth century along with the development of the large-scale commercial breweries.

It is common practice in most texts to treat malting and brewing as strictly delimited batch processes. In modern practice, the processes are performed in separate facilities, the malthouse and the brewery, which are often at separate geographic locations. While some brewers still retain ownership of malting facilities, most malt is produced by independent maltsters. Nevertheless, the view of strictly separate processes is being questioned, and it may be more instructive to view, at least the initial phases of brewing, as a continuation of malting. Physical and biochemical processes that began with germination do continue during mashing when the malt is mixed with water. The processes were just temporarily suspended when the malt moisture content was reduced in kilning.

MALTING

For purposes of this discussion, the malting process will be divided into five stages. These are

grain cleaning and grading, steeping, germination, kilning, and malt cleaning and blending. Upon receipt, shipments of barley will be cleaned, graded, and stored. The preservation of varietal integrity is the normal practice in malting, and all operations are conducted with this in mind. Individual varieties of barley are stored and malted separately. Lots of specific varieties are also further segregated according to factors including protein content and farm production location.

Cleaning and grading

Cleaning is conducted to remove all materials that are not barley, including foreign seeds, stones, metallic objects, dirt, and dust. Broken barley kernels are removed as well. A variety of cleaning machines are employed in sequence, and these are covered by both Briggs (1998) and Kunze (1999).

Grading refers to separation based upon kernel size, and is generally conducted with the use of grading cylinders or a plansifter. Grading is necessary as kernel size impacts water uptake and modification rates in malting. Greater uniformity in the resultant malt can be achieved if different size fractions are malted separately. Kernels are separated into three to four fractions based upon sieve widths in the range of 2.6–2.0 mm. The plumpest fraction is those kernels that pass over sieve openings of 2.5 mm. Second or third grades are those kernels passing through the 2.5 or 2.6 mm sieves, but retained by progressively narrower sieves. Screenings are the materials passing through the narrowest sieve, such as a 2.2 mm. Plumper fractions are used for brewer's malt. Thinner kernels are malted and used for distiller's malt. The thinnest kernels, or screenings, are sold as animal feed. The necessity for grading is dependent upon the percentage of thin grain and uniformity of kernel plumpness within a lot. In general, it is more often necessary for six-rowed barley due to the greater distribution of kernel size. Two-rowed barley generally has plumper kernels and greater kernel size uniformity.

Steeping

The objective of the steeping process is to increase the moisture content of the grain from approximately 12% to 42% to 48% while maintaining seed viability. The grain will germinate as the moisture content reaches approximately 35%, but higher steep moisture levels are needed to promote a more uniform diffusion of enzymes throughout the endosperm, and in turn more uniform modification of the endosperm components.

Steeping is conducted in cylindro-conical vessels, flat-bottom vessels, or a combination of the two (Fig. 15.1). Conical vessels are the older design and are limited to a capacity of 50 t (metric tons) because of the high hydrostatic pressures encountered in the cones of large and deep vessels. The newer flat-bottom tanks can reach capacities of over 300 t as pressures can be controlled by maintaining a uniform bed depth (Briggs 1998; EBC 2000). Similar process steps are used in both vessels. The grain is initially added to the water, and temperatures of 14–16°C would be typical. The initial water uptake rate is quite rapid, but there is little metabolic activity (Kunze 1999). As such, the first steep is used as a cleaning process, which lasts from 4 to 6 h. During this time, the tank may be overflowed to remove floating kernels and dirt. Some undesirable components, including phenolics, are solubilized from the grain during this time. This is important as these can impact both beer flavor and cause hazes. Alkaline agents or lime can be added to the first steep water to promote extraction of phenolics (EBC 2000). Disinfectants such as hydrogen peroxide or sodium metabisulfite can be used with contaminated lots of barley, but actual use is likely not widespread.

As steeping time progresses, the rate of water uptake slows, but the metabolic activity of the grain and associated microbes increases dramatically. Oxygen which is dissolved in the steep water is rapidly depleted, and to maintain viability and efficiency of germination, it is necessary to supply oxygen and to remove accumulated carbon dioxide. This is accomplished in two ways. The first is to employ alternating cycles of

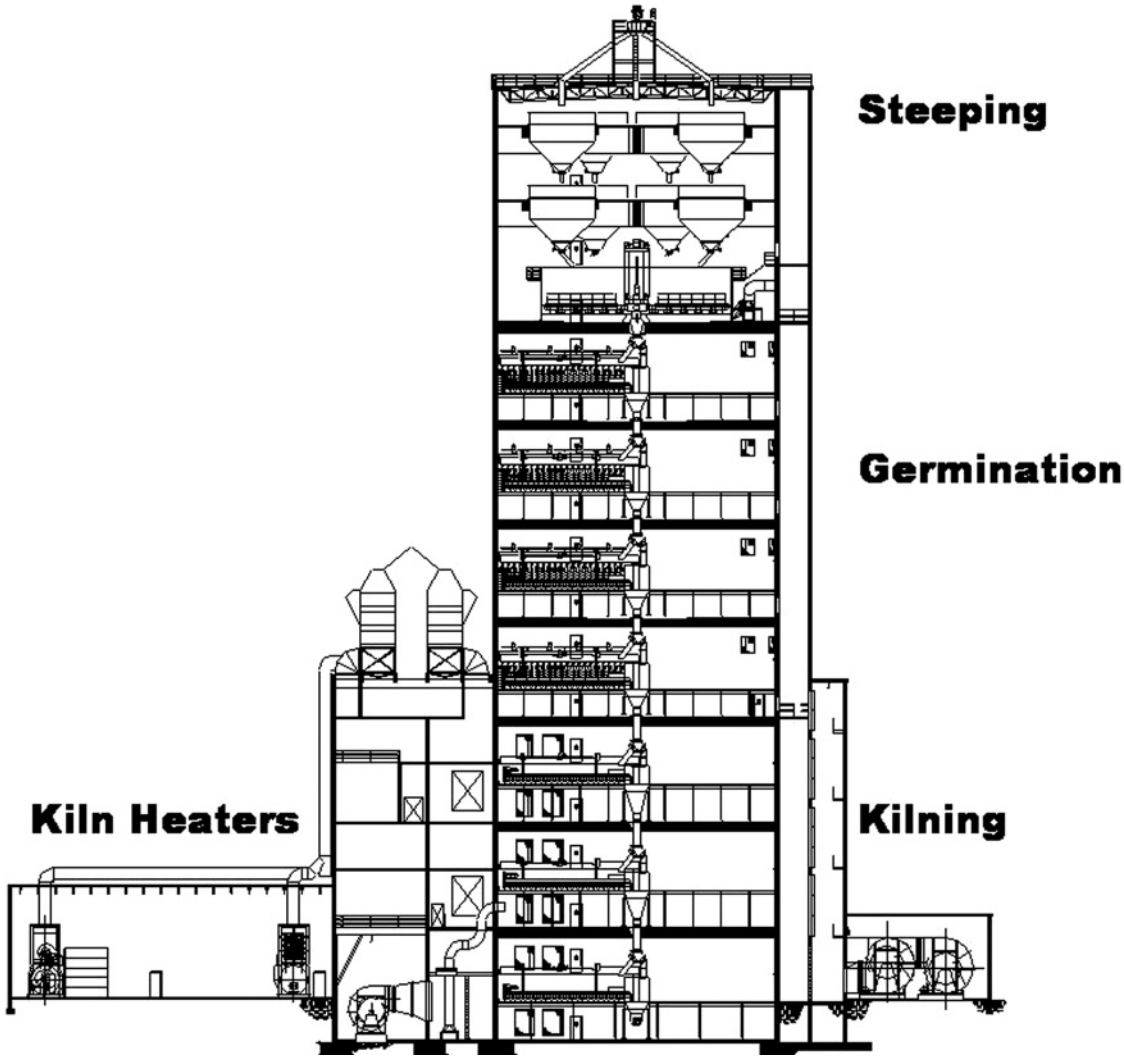

Fig. 15.1. Schematic diagram of a tower malthouse. Uppermost floors include several conical steep tanks and a single flat-bottom steep, or pre-germination compartment. The germination section includes four circular germination compartments. Helices on a central pivot are used to level and turn the germinating grain. The kiln section includes three kiln compartments. Diagram is used with permission of Rahr Malting Co., Shakopee, MN.

immersion and air rests. Water is drained during air rests, and carbon dioxide is removed using fans. Surface moisture continues to be absorbed during air rests, but the grain is exposed to atmospheric oxygen. During immersion periods, compressed air is injected into the steep water (aeration) to replenish dissolved oxygen.

Following the first steep, the water is drained and an air rest is conducted. This will be followed by two to three subsequent cycles of air rests and immersions. The specific lengths of the immersions and air rests can vary among maltsters, varieties, and crop years, but it is generally believed that shorter immersions (4–12 h) and

more frequent air rests (4–7 h) help to overcome germination problems such as water sensitivity. Highly vigorous barley may require only two immersion periods (EBC 2000).

The exact time of steeping is dependent upon the rate of water uptake to reach desired moisture levels. This is influenced by kernel plumpness, 1000 kernel weight, protein content, and other environmental factors. In general, steeping requires at least 24 h, and is generally complete within 48 h. The main change in the visual appearance of the grain following steeping is the emergence of the coleorhiza or root sheath. Maltsters refer to the coleorhiza as the chit, and following steeping the grain should be *chitted*.

In flat-bottomed steep vessels, the barley is distributed on a stainless steel plate, where 24% to 32% of surface consists of openings (Kunze 1999). A radial raking arm fitted with paddles is used to distribute and level the barley. Spray nozzles under the plate can be used to aerate the barley. Immersion and air-rest cycles are employed as with conical vessels, although immersions may be shorter (EBC 2000). In some operations, the first steep may be conducted in cylindro-conical vessels, after which it is transferred to a flat-bottom vessel (pre-germination compartment, Fig. 15.1).

Steeping consumes large volumes of water (5–7 m^3/t malt), and the cost of both water and wastewater treatment are of concern (Kunze 1999). Water from later immersion steps can be reused as first steep water. However, first steep water is not reused because of the large amount of dirt and dissolved solids. Spray steeping is a practice that can be used to reduce water use by as much as 70% (Briggs 1998). In practice, the moisture content of the grain is first raised by a traditional immersion, after which water is sprayed onto the surface of the grain bed. The water percolates through the bed and is absorbed. Water, which passes through the bed, can be recirculated. In addition to water conservation benefits, spray steeping may be of value in the processing of samples with poor viability or vigor, such as seen with preharvest sprouted grain. Uneven hydration and modification, however, are a criticism of spray steeping (EBC 2000).

Germination

The objectives of the germination process are formation and or activation of enzymes, modification of endosperm cellular structure, and the minimization of losses through respiration. On a simple level, the germination unit consists of the germination compartment, a conditioning plant to produce cool humidified air, and fans to move the air through the germinating grain. The main features of the germination compartment are a perforated stainless steel floor and a turning machine. In the past, germination compartments were rectangular, with the floor being surrounded by walls of reinforced concrete. This design is often referred to as a Saladin box, after J.A. Saladin, a nineteenth-century French engineer and manufacturer (The Western Brewer 1903). The turning machine travels along the long axis of the compartment by means of rails located on the top of the walls. In modern practice, circular compartments are a feature of tower malthouse design (Fig. 15.1). In this case, the turning machine moves on a central pivot.

Following the completion of steeping, the grain is transferred to the germination compartment and is allowed to germinate for 4–6 days. Transfer can be in a wet or dry state. In traditional, Saladin-type, malthouses conveyors are often used. In tower malting, the steep tanks are located directly above the germination compartments and transfer is by gravity. Once transferred, the screws or helices of the turning machine are used to level the grain bed. The depth of the grain bed does not typically exceed 1.5 m. Respiration in the germinating barley results in the generation of both carbon dioxide and heat. Excessive respiration can lead to a greater loss of endosperm components and in turn a reduction in malt yield and extract. As such, the grain needs to be both aerated and cooled.

Outside air is drawn into the conditioning plant where it is cooled (or heated) and humidified by water sprays. This is then passed through the grain bed. Depending on plant design, the direction of airflow can be either upward through the grain (updraft), or downward (downdraft). Airflow is in the range of 300–700 m^3/t germi-

nating barley/hour (Kunze 1999). Germination temperatures in the range of 14–18°C are common, with lower temperatures helping to limit respiration losses. Toward the end of the germination period, air within the germination compartment may be recirculated. This is because the carbon dioxide-rich air limits respiration losses, as endosperm modification is proceeding independently of germination at this point.

Humidified air limits loss of moisture from the germinating grain. Nevertheless, it is generally necessary to add additional water during germination, and this is accomplished with sprays located on the turning machine. Some maltsters will occasionally apply gibberellic acid as part of a water addition. The barley is turned twice/day early in germination and once/day on the last day. This is necessary to separate the growing rootlets and to maintain even airflow and germination conditions through the bed.

The main change in the visual appearance of the grain during germination is the growth of the rootlets and lengthening of the coleoptile (Fig. 15.2). Maltsters refer to the coleoptile as the acrospire, and the germinating barley as green malt.

Fig. 15.2. Barley at 4 days of germination. The acrospire, which grows under the husk, will typically reach 75% of the kernel length at this stage. In the case of several kernels, the husk has split or has been skinned, which allows the acrospires to grow outside of the husk. This is undesirable as damage to the acrospires can stop the progress of germination/modification.

The acrospire grows under the husk, and unless the barley is skinned, it is not readily visible. An old rule employed by maltsters was that at the end of germination, the acrospires should have, on average, reached approximately 75% of the kernel length.

Kilning

Objectives of kilning are to dry the grain to approximately 4%–5% moisture, preserve enzymatic activity, promote or limit the development of malt colors and flavors, and remove unwanted grain malt flavors. The appearance of the kiln compartment can be similar to the germination compartment, and they can be circular or rectangular in design. The kilning unit features the kiln compartment, a heating coil for indirect heating, heat exchanger for heat recovery, plenums or dampers for directing the flow of air (recirculation vs. exhaust), and fans for air movement (Fig. 15.1). Double-deck kilns (Kunze 1999), with drying of batches conducted in two stages on the two levels, were common in malthouses of the Saladin design. New tower malthouse construction tends to employ single-deck circular kilns (Fig. 15.1). Grain bed depths are lower than those used in germination, and as a consequence, the kiln floor area is larger.

Kilning is an energy-intensive process, and the conservation and recovery of energy is especially important in modern malthouse design (EBC 2000). Natural gas and fuel oil are common sources of combustion (Kunze 1999). Combustion gases are used to heat air in a heating coil (heat exchanger), which is then passed through the grain. This indirect method of heating is utilized to avoid contact of the nitrogen oxide-rich combustion gases with the grain, and thus limit the formation of carcinogenic nitrosamines. Sulfur dioxide gas is sometimes introduced during the initial stage of kilning by the burning of elemental sulfur (Kramer 2006). This has the effect of bleaching the malt and improving appearance, lowering the pH of the resultant wort, increasing soluble protein, and reducing nitrosamine formation.

Drying depends upon a number of factors, including the temperature of air entering and leaving the grain bed, relative humidity, the volume of airflow, surface area of the grain bed, depth of the bed, quantity of rootlets/grain shrinkage, water vapor pressure of the grain, and the moisture content of the grain. The precise manner in which kilning is conducted depends upon the type of malt being prepared and equipment design. However, maltsters utilize factors of temperature, airflow, and air recirculation to control drying. Kilning is conducted in gradual stepwise stages, in which temperature is progressively increased and the volume of airflow is reduced. The process is often categorized into two or three distinct stages (EBC 2000). In terms of drying stages, moisture is initially reduced from approximately 45% to 12% in a free drying stage, and then from 12% to 4% in a falling rate or slow drying stage. An intermediate stage is sometimes included in descriptions (Kramer 2006). In terms of chemical reactions, the process can be divided into germinative/enzymatic (withering) and chemical (curing) stages (Kunze 1999).

At the end of the germination period, the turning machine is used to unload the germination compartment, and the green malt is conveyed or dropped by gravity into the kiln compartment. After transfer, the kiln turning machine is used to level the grain bed, and there is no additional turning of the grain. Warm air is initially passed through the bed to warm the grain and kiln, and to establish air flow. As moisture removal builds, temperatures entering the grain bed are adjusted to 50–60°C (EBC 2000). Actual grain temperatures are below that of the incoming air, as evaporative cooling occurs as moisture is removed. This is important as enzymes are much more sensitive to heat denaturation at higher moisture levels. During this free-drying stage, evaporation occurs at a steady state rate, and the bulk of the moisture is removed without restriction. Airflow is very high (4300–5000 m^3 air/ton malt/hour) (Kunze 1999), and is adjusted so that that the air leaving the grain bed has a relative humidity of 90%–95%. Germination continues as long as grain temperatures are below 50°C and moisture is above 40%. Enzymatic activity continues longer. As the free drying stage progesses, the removal of moisture becomes more difficult. The point (breakpoint) where there is a sharp increase in air temperature leaving the grain bed, and concomitant decline in relative humidity, indicates that drying has entered a second stage. This typically occurs at approximately 20% moisture (Kramer 2006).

Moisture is now leaving the grain more slowly and it takes longer to establish equilibrium between the grain and the air. To accelerate moisture removal, the temperature is increased (70°C) and airflow is restricted. As grain moisture levels drop to approximately 10% to 12%, the remaining water is firmly bound and moisture removal further slows.

Temperature will again be increased (80–90°C for pale colored malts), and the air leaving the grain is often recirculated through the bed as the efficiency of drying is dramatically decreased. Moisture levels are reduced from approximately 12% to 4%–5% during this 2 to 3-h phase. Purely chemical reactions predominate during this curing phase. These include primarily the formation not only of melanoidins, but also caramelized sugars, and oxidized polyphenols. These compounds can be important components of malt flavor, aroma, and color.

Overall, the kilning process requires from 16 to 40 h, with the time being greatly dependent upon kiln design. At the completion of kilning, the grain is cooled and discharged for the removal of rootlets and then storage. Malt is generally stored for several weeks prior to shipment to the brewer, and malts may be blended prior to shipment.

BREWING

There are innumerable variations on the equipment and processes for brewing beer, but in general, the process can be divided into brewhouse operations, fermentation, cellar operations, and packaging. Light lager or pilsner style beers are most common on a worldwide basis, and this discussion will focus on this process, which often includes the use of adjuncts.

Brewhouse operations

The brewhouse is the portion of the brewery that is probably most familiar to the consumer, as it traditionally featured polished copper brewing vessels. In modern practice, however, brewhouse vessels are frequently fabricated from stainless steel. The primary objective of brewhouse operations is the conversion of malt and adjunct grains into a sweet and hopped extract that can be fermented. Principal components of the brewhouse are a mill and brewing vessels, typically including a mash tun, lauter tun, and a brew kettle. Breweries utilizing cereal adjuncts, such as maize grits or rice, will also feature a cereal cooker. Vessels are sized according to desired production capacity, and range from under 20 hL in some brew pubs to >600 hL. There can be considerable variation in equipment design from brewery to brewery.

The first operation in the brewhouse is milling of the malt and, if required, also of the cereal adjunct. Milling is conducted immediately prior to brewing in order to avoid oxidation. Fine milling favors maximum extraction of the malt and thus brewhouse yield. However, larger grist particles, with a greater percentage of intact husks, are more favorable for lautering, and may also reduce the solubilization and extraction of phenolics, non-starch polysaccharides, and off-flavor components. Roller, wet, and hammer milling are three methods utilized for grinding malt, and selection depends on the separation process that is to be employed (Kunze 1999). Roller milling, also referred to as dry milling, is the most common and traditional method. Here the malt is crushed between pairs of rollers, and the objective is to keep malt husks intact, while reducing the particle size of the endosperm. Roller mills are classified as two-, four-, five-, and six-roll mills, according to the number of rolls and their arrangement. Two-roll mills are only found in small-scale and pub breweries. Four- to six-roll mills are found in mid-size to large-scale breweries. Wet milling was developed to minimize damage to the husk and maximize reduction of the endosperm (Lewis and Young 1995). The technique involves pre-wetting or tempering the malt, which serves to toughen the husk. Hammer milling may be used when the lauter tun is replaced by a mash filter, as the integrity of the husk is no longer important for separation.

Following milling, the malt is transferred to the mash tun and combined with water, for the process of mashing. The primary objectives of mashing are extraction of the malt and conversion of starch to fermentable sugars. There are several traditional systems of mashing, but in modern practice, the double mash, upward infusion system is most common (Lewis and Young 1995). This system features both a cereal cooker and a mash tun. The mash tun is a circular vessel with a somewhat convex bottom and domed, vented top (Leiper and Miedl 2006). Height to diameter ratio is frequently about 1:1 (Dougherty 1988). This vessel is fitted with a low-shear agitator for mixing, and an outlet valve for removal of the mash. The smaller cereal cooker can be similar in design, and also is fitted with an agitator for mixing. Both vessels are often heated by means of a steam jacket, and can be of numerous designs.

In practice, the adjunct mash is started first. The adjunct and water are first heated to around 70°C and then boiled to gelatinize the starch. A small portion of malt or microbial amylase is included to help reduce the viscosity of the adjunct mash. Alternatively, pre-gelatinized adjuncts, such as flaked maize, may be added directly to the mash tun, and liquid adjuncts, such as cane or corn syrup, are directly added to the brew kettle.

Shortly after the start of cereal adjunct mash, water and malt are combined in the mash tun. The ratio of water to ground malt will typically fall in the range of 3–5 hL/100 kg depending upon equipment and beer type (Kunze 1999). Traditional mash-in temperatures were in the range of 40–50°C as this favors proteolysis, but with modern well-modified malts, mash-in temperatures may be higher. Transfer of the boiled cereal mash to the main mash raises the temperature of the total mash to approximately 60–65°C, where the bulk of the maltose is formed. There can be considerable variation in temperature ramps and rests utilized by brewers in mashing, as these are utilized to control composition and fermentability of the extract. At the completion

of mashing, the temperature is raised to a point where enzymatic activity is halted (~78°C) in a step referred to as mash-off. This increase in temperature also makes the mash more fluid by reducing the wort viscosity. The total mashing process will typically require several hours.

Following the completion of mashing, the mash is transferred to lauter tun for the process of separating the clear extract, known as wort, from the insoluble spent grains. The lauter tun is cylindrical vessel fitted with a domed, vented top. It is much wider in diameter and is shallower than the mash tun, and is fitted with a flat false or perforated bottom, where about 10%–20% of the area is open (Leiper and Miedl 2006). The false bottom allows the wort to be filtered or separated from the spent grain. A radial raking device, located on a central pivot inside the lauter tun, is utilized to distribute the mash, maintain grain bed porosity, and to remove the spent grain. Prior to the transfer of the mash, water which has been preheated to approximately 75°C is added to cover the false bottom. The mash is transferred and distributed across the bottom of the lauter tun to a depth of approximately 0.5 m. The wort that passes through the false bottom is recirculated back to the lauter tun until satisfactory clarity is achieved. Recirculation helps to establish a filter bed, and also to deposit small grain particles, which initially passed through the false bottom, to the top of the grain bed. When satisfactory clarity has been achieved, recirculation stops, and the clear wort is collected. Sparging, or the sprinkling of hot water, is conducted toward the end of lautering in order to increase the recovery of residual extract from the grain bed. Knives located on the radial raking arm can be passed through the upper surface of the grain bed to help maintain bed porosity and wort flow. When lautering has been completed (2–4 h), the spent grain is removed through a discharge port. These are commonly used as livestock feed.

An increasingly common alternative to lautering is the use of a mash filter (Kunze 1999). Mash filters are similar to plate and frame filters, in that they consist of an alternating series of filter screens separated by hollow filter frames. The mash is pumped into the individual filter sections under pressure, and also under pressure, the wort is filtered through the filter screens.

Wort is boiled in the brew kettle for a period of 1–2 h. The traditional high efficiency brew kettle is a cylindrical vessel with a vented domed top. The bottom of the kettle is concave, with the center being higher than the sides. This design increases the dynamic flow of wort during boil and speeds evaporation. The wort is heated by means of a steam jacket or an external boiler. Boiling accomplishes a number of goals including sterilization, concentration, precipitation of protein–polyphenol complexes, increased coloration, acidification, formation of reducing substances, volitization of unwanted flavor compounds, such as dimethyl sulfide, and finally the extraction and transformation of hop compounds (Kunze 1999). Concerns over energy utilization and beer quality have led to considerable advances in kettle design and a general shortening of boil times. In addition high gravity brewing, which is very common in modern practice, requires less evaporation of water, as a more concentrated mash is utilized. Dilution of the beer is conducted following fermentation.

Hops (female flowers, called cones, of *Humulus lupulus*) are added to impart bitterness, as well as flavor and aroma. The amount of hops added depends upon the desired beer bitterness, form of hops utilized, equipment design, and boil time. Bitterness is due to water-soluble iso-alpha acids that are formed through the isomerization of the corresponding hop alpha-acids during boiling (Peacock 1998). As the extent or efficiency of isomerization increases with boil time, bittering hops are generally added early in the boil. Components of hop essential oils contribute to hop aroma and flavor. As these components are volatile, aroma hops are added near the end of the boil. Hops may be added as whole hop cones, ground/pelleted cones, liquid extracts, or pre-isomerized extracts. Hopping rates for North American light pilsner beers are below 130 g hop cones/hL wort (Grant 1988). Hop pellet residue and coagulated protein is removed from the wort using a whirlpool tank following the completion of the boil.

Fermentation

After the wort is boiled, it is cooled to fermentation temperature using a plate heat exchanger. The wort is oxygenated, and yeast are added (yeast pitching) at approximately 20–30 million cells/hL (Kunze 1999). Modern fermenters are often of cylindro-conical design (Briggs et al. 2004). These closed vessels feature a cylindrical body, with a conical bottom that is used for collection and removal of yeast at the end of fermentation. Traditional lager fermentations (bottom fermentation) are conducted at temperatures from 7 to 14°C, and fermenters are jacketed so that cooling can be applied. They can range in capacity from 20 to 6000 hL. Multiple brews may be required to fill a fermenter.

During the initial (lag) phase of fermentation, the yeast assimilates oxygen, synthesizes metabolic enzymes, and also sterols needed for cell growth (Munroe 2006a). As the oxygen is consumed, the fermentation enters an exponential growth stage, and the cell population increases until the pool of sterols becomes limiting. Alcoholic fermentation begins, and glucose is first assimilated, followed by maltose and then maltotriose. As sugar concentration falls, the yeast enters stationary phase, and as the last of the fermentable sugars are assimilated, the yeast begins to flocculate or settle out of suspension. Wort specific gravity continues to drop throughout the fermentation process as sugars are consumed, and wort pH also drops as organic acids are excreted by the yeast. Lager fermentations require approximately 1 week. Traditional ale fermentations (top fermentation), which are conducted at higher temperatures, require less time.

While the primary reaction of interest during fermentation is conversion of sugars to ethanol and carbon dioxide, yeast also produce a wide range of by-products that can influence beer flavor and aroma. These include aldehydes, higher alcohols, esters, and sulfur compounds. Formation of these by-products is dependent upon the yeast genetics, initial cell numbers and health, and the pattern of yeast growth, which in turn is influenced by environmental factors such as wort composition and concentration, temperature, oxygen, and pressure. Diacetyl, which is formed by yeast as a by-product of amino acid synthesis, is an important flavor/aroma component in immature beer (Kunze 1999). At high concentration, it contributes a buttery flavor, which is undesirable in light pilsner/lager beers. Yeast will later reabsorb diacetyl and reduce it to butanediol, which has a much higher flavor threshold. This is one reason why yeast has traditionally been allowed to remain in contact with the beer during maturation. A modern alternative is to briefly raise the temperature of the fermenter following carbohydrate fermentation, which has the effect of hastening the conversion of diacetyl to butandediol.

Cellar operations

After the completion of primary fermentation, the fermented beer is far from suitable for consumption due to the presence of undesirable green beer flavors and aromas and also suspended yeast and colloidal materials. Cellar operations refer to the cold storage or lagering (Lager: German noun for storehouse or warehouse) of green beer for several weeks at −1 to 4°C for the purpose of flavor maturation. Reduction of diacetyl is primary among these reactions. In addition clarification, carbonation, stabilization against nonbiological hazes, and standardization/blending can be accomplished during this time (Munroe 2006b). Most of these operations were traditionally accomplished in storage tanks located in cellars or caves. Long maturation times (>4 weeks) were needed, not only for flavor development, but to allow yeast and suspended colloidal materials to settle by gravity. Secondary fermentations (krausening or green fassen) were traditionally conducted during maturation for the purpose of carbonation. In modern practice, maturation is often in cylindrical-conical tanks. Jacketed cylindrical-conical tanks, as for primary fermentation, can be used, and in some cases, primary fermentation and maturation can be accomplished in the same tanks (Unitank design, Briggs et al. 2004). Maturation can now be accomplished by a stepwise and gradual cooling that can be completed in a period of less than

2 weeks. Sedimented yeast may be removed once or several times during the process.

Stabilization against protein–polyphenol hazes is accomplished by the addition of silica gels and/or polyvinylpolypyrrolidone (PVPP). Silica gels act as protein adsorbents, and PVPP as a polyphenol adsorbent. Clarification is accomplished by centrifugation and filtration. Centrifugation is generally applied first for the removal of yeast. Smaller particles are removed by filtration through diatomaceous earth or filter sheets.

Packaging

Immediately prior to packaging, it is necessary to adjust the carbon dioxide content of the beer. Carbonation is typically accomplished by in-line injection or in-tank carbonation (Munroe 2006b) using carbon dioxide recovered from the fermenters. Beer is then packaged into kegs, cans, or bottles. Bottled and canned beer is often pasteurized to eliminate problems with spoilage microorganisms.

BIOCHEMISTRY OF MALTING AND MASHING

On a biochemical level, malting and mashing can be viewed largely as a controlled process of endosperm mobilization. These processes begin in steeping and are then suspended as moisture levels are reduced in kilning. Many of the processes are reinitiated as the malt is mixed with water in mashing. Endosperm composition and mobilization were extensively covered by Fincher in Chapter 14, and the primary intent of this section is to link this with the technology of malting and brewing.

Modification

The term modification is used to encompass the overall physical/biochemical changes that occur in the barley endosperm during the malting process (Lewis and Young 1995). As previously stated, a primary objective of malting is to modify the endosperm. The endosperm of well-modified malt is friable and easily crushed, while that of poorly modified malt is hard and steely. This physical change results principally from the degradation of cell walls and protein within the endosperm. On a simple level, an endosperm cell in mature barley can be viewed as large and small starch granules embedded in a matrix of protein. The cell is surrounded by a thin wall which is principally composed of β-glucans and lesser amounts of arabinoxylans. To use an analogy to construction, the protein matrix in barley can be viewed as the *cement* that binds the *aggregate* starch granules, resulting in a hard texture. The degradation of this matrix renders the endosperm easily crushed. Scanning electron micrographs of barley and malt endosperm are shown in Fig. 15.3.

The overall pattern of endosperm modification is important to maltsters and brewers (Bamforth 2002), as cell wall and protein degradation make the starch much more accessible to the amylases, and easier to extract and degrade in the brewing process. Incomplete modification limits extract availability. On the other hand, over-modification of the malt can result in both reduced malt and malt extract yields as more starch will actually be degraded, and the resultant glucose consumed in respiration.

Germination begins with water uptake in steeping. Water initially enters the embryo and later diffuses into the endosperm. As stated in Chapter 14, the synthesis of gibberellic acid (GA) in the embryo and subsequent diffusion through the grain initiates the synthesis and secretion of hydrolases including α-amylase, proteolytic, and cell wall degrading enzymes. Other enzymes (e.g., β-amylase) that are already present in the endosperm in bound forms are activated. Endosperm modification begins in the region in closest proximity to the scutellum, and then moves inward from the aleurone layer as enzyme synthesis and secretion progresses from the proximal to distal regions of the aleurone. The technological significance of this pattern is that a small region at the distal end of the endosperm often remains unmodified or only partially modified (Lewis and Young 1995). Extending germination time to promote complete modification of this region can

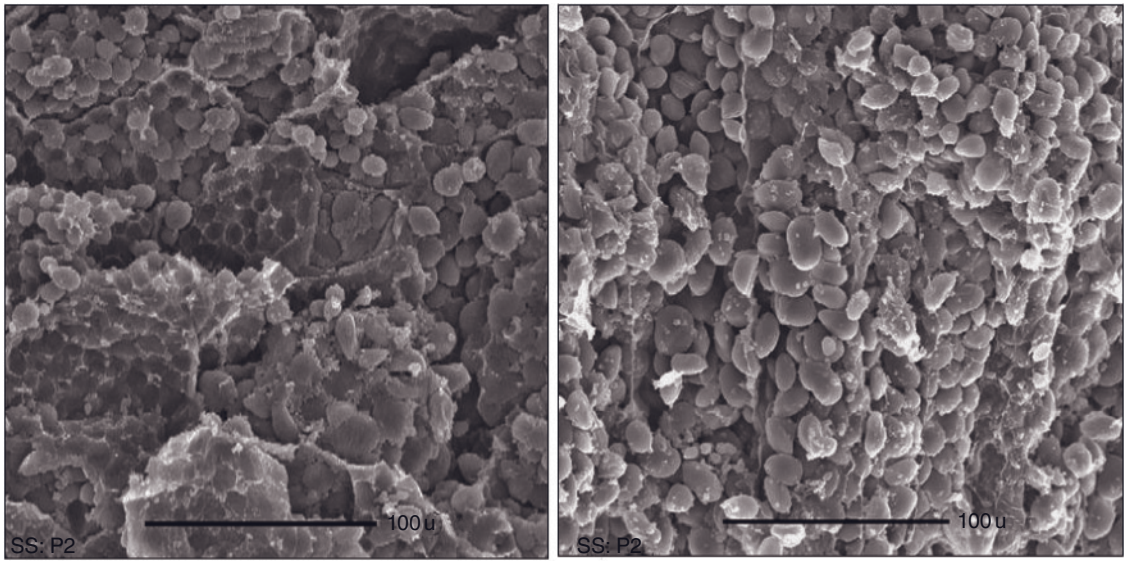

Fig. 15.3. Scanning electron micrographs of proximal endosperm tissue in sound barley (a) and 4-day malt (b). Large and small starch granules are clearly visible in both cases. In sound barley, the starch granules are embedded in a matrix of storage protein, whereas after 4 days of germination, much of this matrix has been degraded, and the starch granules are more exposed. Individual endosperm cells and cell walls are visible in the sound barley.

lead to excess degradation of starch in the region adjacent to the scutellum.

Factors of time, temperature, and moisture are all known to influence modification. The effect of time is straightforward, as the levels of many of the hydrolytic enzymes continue to increase throughout the 4–5 days of germination utilized in malting. Lengthening this period results in greater amounts of enzyme present and increased time for enzyme diffusion and interaction with substrate. Increasing steeping and germination moisture levels from 42% to 45%–48% generally favors overall modification (Smart et al. 1995), presumably by promoting greater and more uniform diffusion of enzymes into the endosperm. Increasing temperatures from approximately 12 to 15 to 20ºC not only speeds germination, but also has the effect of increasing respiration.

The extent and rate of malt modification must be balanced against respiration and rootlet losses. This is generally done by using lower germination temperatures and recirculation of CO_2-rich air toward the end of germination. Dry weight losses typically observed in the production of a pale brewer's malt would be around 10.5% (Kunze 1999) to slightly greater. This means 100 kg of cleaned dry barley yields 89.5 kg of malt on a dry weight basis. Losses are attributed to respiration, removal of rootlets, and steep solubilization in decreasing order of significance.

Endosperm modification can continue through the early stages of kilning (germination and enzymic stages), but enzyme activity is eventually suspended due to low moisture levels that reduce enzyme–substrate interactions, and by some thermal inactivation of enzymes. The amount of activity retained in the kilned malt is dependent upon the specific enzyme and the manner in which kilning is conducted. Again, the removal of the bulk of the moisture at lower temperatures helps to protect against denaturation.

Cell wall degradation

The importance of β-glucans ((1,3;1,4)-β-D-glucans) and arabinoxylans in malting and brewing has been reviewed by Jin et al. (2004) and

Egi et al. (2004). Composition, structure, and associated hydrolytic enzymes were covered in Chapter 14.

Arabinoxylans and β-glucans are present in barley in soluble and insoluble forms, and processes of both solubilization and degradation occur during malting and mashing. The β-glucans have received greater attention as they are the primary endosperm cell wall polysaccharides in barley. However, there is growing evidence that suggests that arabinoxylans should not be ignored, and this is particularly true for wheat malts. Both polysaccharides are polydisperse and their importance in brewing can mainly be attributed to the higher molecular weight fractions and their impact on viscosity. Failure to adequately degrade β-glucans can result in reduced malt extract and lautering and filtration difficulties.

The total β-glucan content of a number of historical and modern six-rowed varieties was found to range from approximately 3.5% to 5.0% (Schwarz and Horsley 1995). Corresponding malts contained 0.5%–1.5%, and when the malt figures are corrected for moisture and malting losses, this translates to approximately 65% to >80% of the total β-glucan being degraded during malting. The endo-β-glucanases ((1,3;1,4)-β-D-glucan endohydrolases) which develop during germination are largely associated with this degradation. These enzymes are relatively temperature sensitive and 40% to >70% of the activity developed during germination is lost in kilning (Kunze 1999; Georg-Kraemer et al. 2004).

Only a limited amount of β-glucan hydrolysis occurs during mashing. Endo-β-glucanase shows maximum activity levels at 40–45°C, and is very rapidly inactivated at temperatures above 50°C (Jin et al. 2004). Additional hydrolysis of β-glucans can be accomplished with low mash-in temperatures, but this can sometimes result in excessive proteolysis. As such, it is very important that breakdown of β-glucans be accomplished in the malthouse.

The total arabinoxylan content of barley has been reported to range from <4% to >10% (Egi et al. 2004). These figures, however, are a bit misleading as the bulk of the arabinoxylan is in the husk, and the brewer is only concerned with that in the endosperm and aleurone. When compared with β-glucans, only a very small portion of the arabinoxylans in barley is water soluble. Whereas more than 80% of the β-glucans may be degraded during malting, only a small portion (~20%) of the arabinoxylans are degraded. Several enzymes that are involved in arabinoxylan hydrolysis were discussed in Chapter 14, but we will refer only to endoxylanase ((1,4)-β-D-Xylan endohydrolases). Endoxylanase activity is synthesized during germination, but the increase in activity is not as pronounced as that of endo-β-glucanase. Endoxylanse activity, however, is more thermostable with only a reported loss of 5% during kilning. Additional solubilzation and degradation of arabinoxylans can occur during mashing. When wheat is included in the mash, the presence of xylanase inhibitors can reduce the activity of endoxylanase.

Protein degradation

The degradation of protein during malting and mashing is complex, and until recently was extremely poorly understood. The subject was recently reviewed by Jones (2005). Barley contains a large number of proteolytic enzymes and endogenous inhibitors. Endo-proteolytic activity is low in barley but increases dramatically during germination. Lower germination temperatures and higher moisture levels seem to favor protein modification (Smart 1995). Little endo-proteolytic activity appears to be lost in kilning (Jones 2005). However, while stable in kilning, this activity is rapidly denatured as mash temperatures increase from 50 to 72°C. Of the individual endo-proteases studied, the cysteine proteases seem to be most important in malting and mashing. Metalloproteases are important in mashing.

Worts for North American pilsner-type beers (25% adjunct) have been reported to contain 225 mg/L amino acids, 225 mg/L peptide, and 195 mg/L protein (Meilgaard 1999). A considerable amount of higher molecular weight protein precipitates during wort boiling. Worts contain around 17 amino acids derived from the malt, and these are classified into four groups based upon

assimilation rate by yeast (Jones and Pierce 1964). Proline, which is the predominate amino acid, is not utilized by yeast.

There has been considerable debate over the years as to the amounts of protein degradation that occurs during malting versus mashing. An elegant set of experiments by Jones (2005) has shown that in terms of soluble protein (N × 6.25), 32% is already present in barley, 46% is formed in malting, and 22% in mashing. Likewise, it was demonstrated that 15% of the free amino nitrogen was present in barley and 58 and 26% were formed during malting and mashing, respectively.

Traditional mashing was often started with a rest at 45 to 50ºC. This protein rest was conducted to complete protein modification, and the temperatures are in consideration of the poor thermal stability of the proteolytic enzymes. With the well-modified malts that are currently available, it may no longer be necessary. A lower temperature rest favors formation of additional amino acids, presumably through action of peptidases (Kunze 1999). It does, however, have a negative impact on foam. Additional discussion on the importance of protein follows in the section on quality testing.

Starch degradation

Germinating barley contains α-amylases, β-amylases, debranching enzymes (commonly referred to as limit dextrinases), and α-glucosidases, whose combined action can theoretically degrade amylose and amylopectin to glucose. Malting is important for synthesis and/or activation of these activities, but only a small amount (~12%) of the barley starch is actually degraded during the production of pale malts (Kunze 1999). Excessive starch degradation results in a loss of both malt yield and malt extract.

While there is little inactivation of α-amylase during kilning, approximately 40% of the β-amylase activity present in germinating barley is lost (Kunze 1999). Both limit dextrinase and α-glucosidase are believed to be relatively thermal labile in kilning and mashing. Sissons et al. (1995) reported that depending on the schedule used, 24% to 85% of limit dextrinase activity is lost during kilning.

A primary objective of mashing is the conversion of starch into fermentable sugars, and the amylolytic enzymes again become active as the malt is mixed with water. Alpha-amylase catalyzes the hydrolysis of (1,4)-α-linkages in amylose and amylopectin in an endo- manner to yield linear and branched (1,6:1,4-α-linked) dextrins. This activity is important as it greatly reduces the molecular weight of the starch and in turn, mash viscosity. It also provides additional substrate for β-amylase, which catalyzes the hydrolysis of (1,4)-α-linkages from the nonreducing end, to release maltose. Beta-amylase hydrolyzes amylose to maltose and a small amount of maltotriose, but cannot bypass the (1,6)-branch points in amylopectin. Limit dextrinase catalyzes the hydrolysis of (1-6)-α-linkages in branched dextrins, yielding linear dextrins. The primary activity of α-glucosidase is the hydrolysis of (1,4)-α-linkages in maltose and small maltodextrins from the nonreducing end, to release glucose.

Alpha- and β-amylases are of greatest concern to the brewer, and the temperature profile of mashing has a tremendous effect on the composition of the wort. Thermal stability of enzymes tends to be greater in mashing than in dilute buffer (Lewis and Young 1995), as colloidal components of the mash provide a protective effect. As such, thermal stability also tends to be greater in thicker mashes. As was the case for kilning, β-amylase is more sensitive to high temperatures. It works best at mash temperatures of 55–60ºC, while α-amylase can tolerate temperatures of nearly 75ºC. Solubilization of starch, and in turn extract, increases as mash temperatures are increased up to 70ºC. However, the fermentability of the wort begins to decline as temperatures exceed 65ºC and β-amylase is rapidly inactivated. Lewis and Young (1995) describe the critical range of temperatures that can be employed by the brewer to balance extract and fermentability. This range or "brewer's window" is dependent upon the modification level of the malt. Brewers desiring to maximize fermentabilty will extend

the time of the rest at 60–62°C. At the completion of mashing, enzymatic activity is terminated by raising temperatures to approximately 76°C.

The fermentable sugars, glucose, maltose, and maltotriose have been reported to account for 46%, 9%, and 14%, respectively, of the total carbohydrate in a North American pilsner wort (Meilgaard 1988). The use of rice or maize adjuncts does not appreciably change these figures, as the starch composition of these cereals is very similar to that of barley. The use of excessive amounts of adjuncts could, however, result in lower amounts of fermentable sugars, because malt enzyme levels can become limiting.

The remainder of the carbohydrate is largely branched (1,6:1,4-α-linked) dextrins. These compounds, which range in degree of polymerization from 4 to >11, result from the (1,6) branch points in amylopectin that were not degraded in mashing. The fact that dextrins can account for 20% to 30% of wort carbohydrate (Meilgaard 1988; Moll 1991) strongly suggests that limit dextrinase plays a very limited role in mashing. Brewers have traditionally believed that this is due to low thermal stability, but other factors may be involved (see Chapter 14).

Dextrins are the major contributor to the caloric content of beer, and also contribute to mouthfeel (Schwarz and Gordon 2002). The normal pilsner style beers that are popular in the United States typically contain between 0.14–0.15 kcal in a 355 mL serving. However, the major market in the United States is currently for low-calorie/low-carbohydrate beers. Most low-calorie beers are produced by reducing the dextrin content. Extending the (~60°C) saccharification rest of mashing and use of malts with high amylolytic activity may reduce this level to 0.11 kcal. However, further reduction in caloric content (to 0.09 kcal) is often achieved by the use of the microbial enzyme amyloglucosidase. This enzyme catalyzes the hydrolysis of both (1,4)- and (1,6)-α-glucosidic linkages, and is able to completely convert dextrins to glucose. Another option is replacement of portions of the malt with highly fermentable maize syrup.

Lipid degradation

Barley grain contains approximately 2%–3% lipid, which exists predominately as triacylglycerides in the embryo (Kunze 1999). Lipase catalyzes the hydrolysis of triacylglycerides to yield free fatty acids (FFA) (Schwarz et al. 2002). Increases in lipase activity of 40- to 80-fold have been reported following 7 days of germination. The FFAs liberated during the early phases of germination are metabolized through β-oxidation, and are an important source of energy. Alternatively, some linoleic acid is metabolized by the lipoxygenase (LOX) pathway. This pathway is of technological significance as the reaction products influence beer flavor stability. The LOX pathway is covered in more detail in a following section on nonstandard tests/flavor stability.

Flavor and color

The major reactions contributing to malt-derived flavor and color in beer are the Maillard reaction, caramelization of sugars, and degradation of phenolics and lipids (Hughes 2009). Kilning is the most important stage for flavor and color development and for removal of unwanted green malt flavors, as higher temperatures are generally required.

The Maillard reaction, which is often considered most important, involves interaction of amino acids and reducing sugars to form glycosylamines (Belitz and Grosch 1987). Glycosylamines are unstable and undergo rearrangement to form ketosamines. The ketosamines can further react to form reductones, short-chain hydrolytic fission products, or brown nitrogenous polymers (melanoidins). The reactions are favored by high temperature and low moisture levels (water activity levels of 0.6–0.7). Malt color can be attributed to polymeric melanoidins (Coghe et al. 2006). Lower-molecular-weight melanoidins predominate in paler malts, while higher-molecular-weight forms are predominate in darker malts. The higher molecular compounds are formed at higher roasting temperatures (157–166°C).

Maillard derived heterocyclic compounds are responsible for flavors and aroma in malt (Seaton 1993). Oxygen heterocyclic compounds such as furans contribute toffee-caramel flavors, while nitrogen heterocyclics, such as pyrazines are responsible for coffee-nutty flavors. Strecker aldehydes are derived from amino acids when heated with diketones or reductones. Isovaleraldehyde, derived from leucine, has a strong malty flavor.

Flavor substances derived from fatty acids include aldehydes, alcohols, and lactones. *Trans*-2-hexenol and *trans*-2-*cis*-6-nonodienal are responsible for the green or grassy aroma of green malts.

Dimethylsulfide (DMS) is an important flavor compound in pale malts. Its presence in beer is associated with cabbage or cooked vegetable flavors, and as such is generally considered undesirable (Kunze 1999). The flavor threshold of DMS is approximately 50–60 µg/L. S-methylmethionine (SMM) is the primary precursor of DMS in malt (Yang et al. 1998). The level of SMM in barley is negligible, but it is synthesized during germination in a reaction between S-adenosyl-L-methionine and methionine. This reaction is catalyzed by L-methionine S-methyltransferase (SMM synthetase). SMM is degraded into DMS under high temperatures during malt kilning and wort boiling. Dimethylsulfide is volatile, and variable amounts will be lost to the atmosphere, depending upon process conditions. The level of SMM in malt is influenced by barley genotype, germination conditions, and malting processing parameters. Conditions favoring protein modification appear to favor SMM. Higher kiln temperatures and longer wort boiling times favor reduction of SSM in the malt and DMS in the beer, respectively.

Pale malt flavors are due largely to DMS, Stecker aldehydes, and oxygen heterocyclics (Seaton 1993). The grassy lipid-derived aromas of green malt are largely volatized and lost in kilning. Although DMS is an undesirable flavor, its complete elimination through kilning is difficult. Pale malts are often well modified and kilned at lower temperatures. As such, some SMM persists into the finished malt. The task of completing conversion of SMM to DMS then falls to the brewer. Incomplete conversion and volatilization during boiling means that DMS may continue to form as the wort is transferred for cooling. This DMS can persist into the finished beer. Concerns over energy use are leading to shorter boiling times.

High temperatures used in production of darker malts completely eliminate lipid-derived compounds and greatly reduce SMM and DMS. Flavors in these malts are largely due to nitrogen and oxygen heterocyclics, as well as Strecker aldehydes.

MALT TYPES AND MALT USE

Malt types

The vast majority of malt produced around the world can be termed pale malt or brewer's malt, and brewer's malt will be used for purposes of this discussion. Brewer's malt generally constitutes the bulk of the malt used in formulation, and this is especially true for the pilsner or light lager styles of beer that are predominate in much of the world. This type of malt generally has the greatest levels of total extract, fermentable extract, and enzymatic activity, as the bulk of the green malt moisture is removed prior to significant increases in kiln temperature, and as relatively low curing temperatures are used (<95°C). Optimal modification and lower kiln temperatures, however, result in very light malt color (Fig. 15.4). Malt color refers to the color of Congress (laboratory) worts prepared from the malt. Congress wort colors for North American brewer's malts are generally below 3 SRM (Standard Reference Method) on the color scale used by the American Society of Brewing Chemists (ASBC 2004). Use of brewer's malt results in a pale yellow to golden beer color, and beers of the light lager or pilsner beer styles, are often below 5 SRM in color (Papazian 2006).

Addition of more color, malt flavor, and aromas requires the use of specialty malts. Specialty malting is often conducted by maltsters that focus predominately in this area, and it retains more of

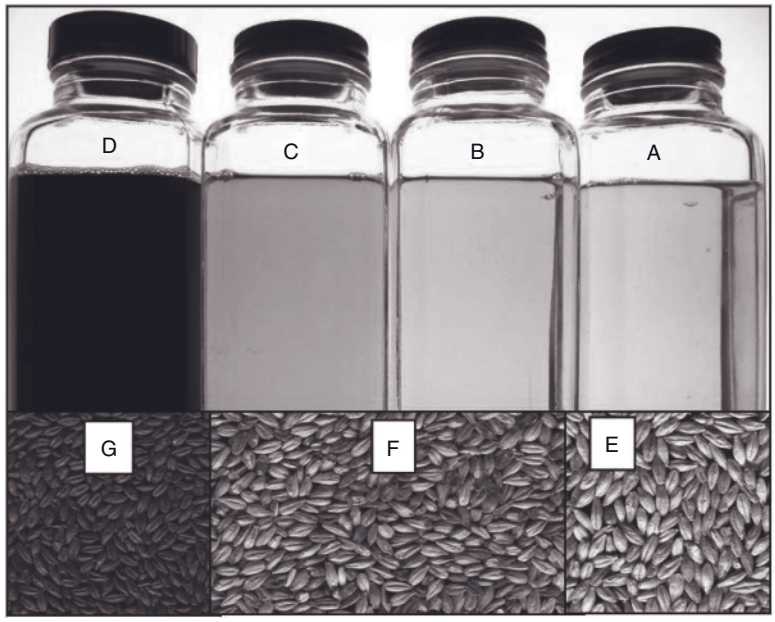

Fig. 15.4. Congress worts prepared from 100% brewer's malt (a), brewers malt and 5% caramel-60 (b), brewers malt and 10% caramel-60 (c), and brewers malt and 5% black malt (d). Brewer's malt, caramel-60 and black malt are shown in E–G, respectively. For color details, please see color plate section.

an artisinal image. A wide variety of products are available, and similar types of malts produced by different maltsters can have various names. However, the basic classification system used by Blenkinsop (1991) is useful in describing the general types of specialty malts. This system classifies malts as those produced through the traditional kilning processing, or those which require roasting. Within this system, malts are further classified by starting materials, which can include green malt at 40%–45% moisture, kilned malt at 4%–5% moisture, and unmalted barley or other grains.

Specialty barley malts that are produced through conventional kilning would include pale ale, Munich, and Vienna malts. These are characterized by yielding Congress worts of darker color, perhaps as high as 20 SRM. As higher temperatures are used in kilning, the resultant levels of α-amylase and DP are generally lower than those found in a brewer's malt prepared from the same barley. Nonetheless, adequate levels of amylolytic enzymes remain that these still can be used as base malts in formulation. These malts are characterized by distinct malty flavors, amber hues, and are utilized in the production of darker lagers, Oktoberfest beer, and in some ales (Briess Malt Product Guide). Additional color development in these malts is favored by a number of process modifications including the use of barley, with higher than normal protein levels (Kunze 1999). Higher steep-out moisture levels and higher germination temperatures can be used to favor more extensive endosperm modification, and the formation of reducing sugars and amino acids, which are color precursors. Kiln temperatures may be increased to higher than normal levels while the grain is still at ~20% moisture, which favors the production of additional sugars and amino acids. Finally, the higher curing temperatures (~105°C) used in kilning favors the formation of Maillard reaction products.

The development of even higher levels of malt color is generally achieved by roasting, and roasted malts can be prepared using either green malt or kilned malt as the starting material (Blenkinsop

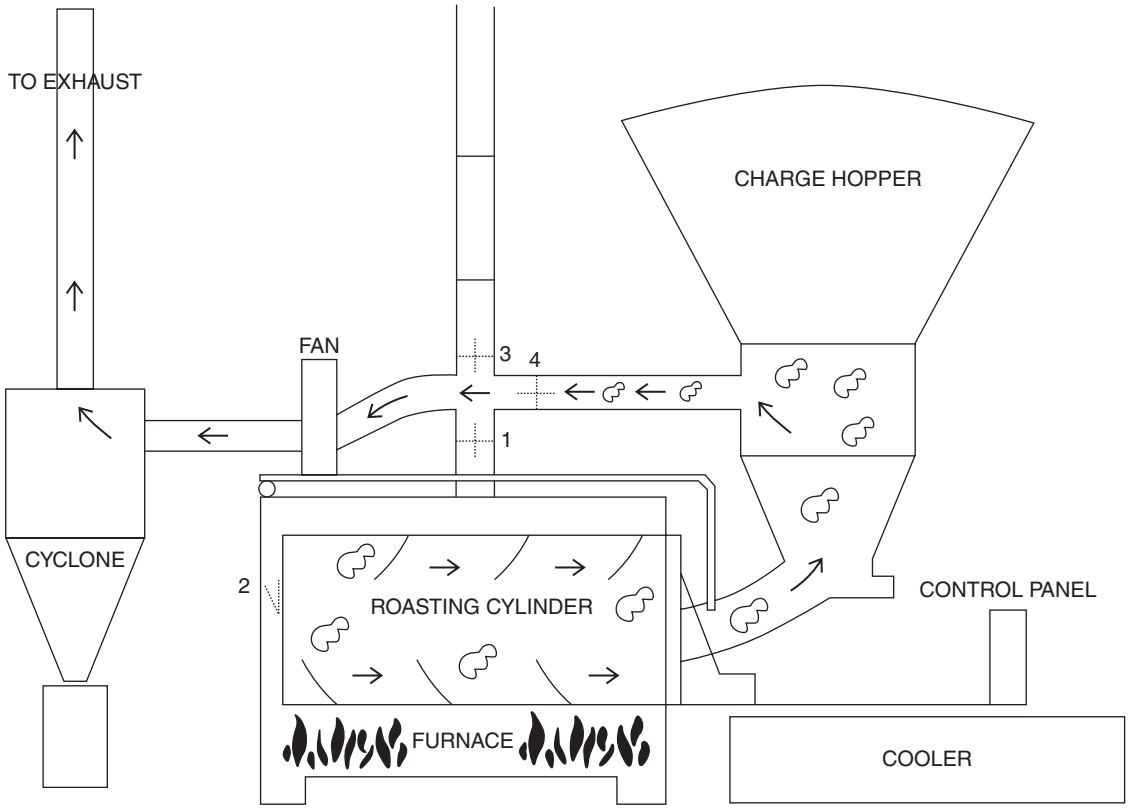

Fig. 15.5. Schematic diagram of a drum roaster used for the production of specialty malt. Arrows indicate the direction of air flow. Diagram is used with the permission of the Briess Malting Company, Chilton, WI.

1991). Roasted unmalted barley is also used in brewing, and while not truly malt, its production and use is similar. Roasted specialty malts are generally prepared in a drum roaster (Fig. 15.5).

The roaster consists of a rotating drum encased in concrete (or steel jacket). The grain can be heated by passing air, heated to various temperatures, directly through the grain in the drum, or indirectly by passing air around the drum. Roasting is conducted on a small scale, with drum capacities of two metric tons or less being common (Briggs 1998).

Caramel (also called crystal) malt is the most common type of specialty malt prepared through the roasting of green malt. To begin the process, the green malt is loaded into the drum, and the drum is sealed (Blenkinsop 1991, EBC 2000). In the first stage, which is called stewing, hot air (~350°C) is passed around the drum until the grain temperature reaches 65°C. The elevated temperature and high humidity bring about rapid breakdown of starch and protein. The endosperm may actually liquefy in some cases. In the next stage, which is called roasting, hot air (350°C) is passed directly through the grain bed until the grain temperature reaches 120–160°C. Maillard reactions occur rapidly during this stage, and when color/flavor development has occurred to the desired degree, the grain is cooled to 25°C. Malts from different batches can be blended to achieve desired traits. Caramel malts are available in a wide range of colors, from 10 to >100 SRM (Fig. 15.4). Color development is controlled by the degree of the stewing and roasting stages. Most roasted specialty malts have no enzymatic activity, and little if any contribution to ferment-

able extract. A caramel-10 malt might be used at low levels to impart additional color and sweetness to pilsner and amber style beers (Briess Malt 2007). It has a mild caramel flavor, whereas a caramel-80 would have pronounced caramel, to burnt sugar and raisiny flavors. Caramel-80 would be used in small amounts to contribute color and flavors to bock, porter, and stout beer styles. These styles can range from <10 to >40 SRM in color (Papazian 2006).

Kilned brewers malt can also serve as raw materials for specialty malt production. The most common types of malts in this class would be chocolate and black malt. In these cases, malt at 4%–5% moisture is loaded into the drum, and hot air is passed through the grain (~200°C) for period of time generally under 3 h. Rapid color development occurs, and factors of time and temperature control the final characteristics of the malt. As the grain may be near the point of combustion, water can be injected to halt the process. Chocolate malt will typically yield wort color in the range of 350 SRM, while black malt is darker (500 SRM) (Fig. 15.4). Chocolate malt has a roast coffee character, while black malt can have intense bitter flavors (Briess Malt 2007). Both are used at levels of ≤10% in porter and stout beer styles. Chocolate malt is sometimes used at very low levels for color adjustment. Roast barley (black barley) is prepared in a manner similar to black malt, although slightly higher temperatures may be used (~230°C). It is most commonly used in stout.

The final specialty malts to be discussed are those prepared from materials other than barley (Briggs 1998). For the most part, these malts are prepared similarly to brewer's malt. Wheat malt is widely produced and can constitute a significant portion of the formulation in wheat beers (EBC 2000). It has relatively high levels of extract and enzyme levels comparable to barley malt. Lack of husk can lead to wort separation difficulties in the traditional lautering process when used at high levels. Malted rye and oats are utilized in a few specialty beers and presumably in several types of distilled alcoholic beverages (Blenkinsop 1991; Jacques et al. 1999, Briess Malt 2007). There is growing interest in the malting of sorghum and pseudo-cereals such as buckwheat and quinoa for the use in gluten-free beers for individuals suffering from Celiac disease. Sorghum malts have long been used in traditional fermented beverages in Africa.

Calculation of malt use

On a fundamental level, the amount of malt required for the production of a given quantity of beer depends upon the style of beer being produced, the extract level of the malt(s) and adjunct(s), and the equipment/process being utilized. In terms of beer style and malt use, factors of original gravity (OG), adjunct use, and desired beer/wort color must be considered. Original gravity (OG) will be discussed first, while color and adjuncts will be discussed later in this section. The OG is a measure of the density of the wort prior to fermentation, and is expressed as %Plato (%P = g extract/100 g wort) (DeClerck 1958b). Values for %Plato are based upon tables constructed in the nineteenth century by Karl Balling and Fritz Plato, which relate specific gravity to the density of sucrose solutions (g/100 g solution) at specific temperatures. Increasing the OG of the wort increases sugar concentration, and thus in turn the potential alcohol content of the finished beer. Many of the pilsner or light lager types of beers produced throughout much of the world have OGs in the range of 10–11%P. However, it must be remembered that many brewers practice high gravity brewing where worts of >13.5%P are prepared (Kunze 1999), and the fermented beer is then diluted to proper concentration prior to packaging.

Once the desired OG of the wort is determined, the first step in determining malt usage is to calculate the amount of extract required. This can be done using the Lincoln equation shown below (Weissler 1995):

$$Extract(kg/hL) = 0.9974\left(\frac{1}{\frac{1}{P} - 0.00382}\right)$$

As an example, for the production of 1.0 hL of wort at 11%P, 11.45 kg of extract are required.

To convert this to actual malt use, the extract level of the malt and efficiency of the brewhouse operations must be taken into account. A typical value for laboratory or theoretical extract for a two-rowed malt is 81%, and division of 11.45 by 0.81 yields a value of 14.14 kg. However, the actual level of extract achieved in the brewhouse will be lower than that in the laboratory. Brewhouse efficiency is the percentage of theoretical extract that is actually achieved in production (Weissler 1995). This figure will vary somewhat depending upon the processes and equipment utilized by individual brewers. For purposes of this example, an efficiency of 78% will be utilized, and division of 14.14 by 0.78 indicates that 18.12 kg of the two-rowed malt would be needed for the production of 1.0 hL of wort at 11%P.

Extract is widely viewed as a factor of major economic importance to brewers, and the effect of incremental increases in malt extract levels on malt requirement is shown in Table 15.1. In this example, each 0.5 point increase in extract reduces malt requirement only by about 120 g/hL, which is less than 1% of the total. While this is undoubtedly of some economic significance to very large brewery operations, it is probably less important to smaller operations

The first example considered only a single malt and it must be remembered that beers are often produced from several malts and/or adjunct materials. Total extract requirement is still determined using the Lincoln equation as above, but the percentage of the total extract to be derived from each malt or adjunct material must then be assigned. Table 15.2 shows the grain requirements for 1.0 hL of 9 and 11%P worts prepared from 100% malt and a 20/80 blend of maize and malt. These examples clearly demonstrate that malt requirement can be significantly reduced by both reductions in OG and the use of adjuncts. The levels of cereal adjuncts like maize or rice are generally limited to no more than 45% of the total grain bill (Boyce 1986), as they contribute no enzymes and little to no free amino nitrogen (FAN). The use of cereal adjuncts above this level may result in poor starch conversion as levels of malt diastatic power (DP) becomes limiting, and poor fermentation results as the levels of FAN from the malt cannot support proper nutrition and growth of the yeast. A greater portion of malt can be replaced with cereal adjuncts when six-rowed malt is used in comparison to two-rowed malt (Schwarz and Horsley 1995). This is due to higher DP and soluble nitrogen levels. Still higher

Table 15.1 Amount of malt (kg) required to produce 1.0 hL of 11%P wort at various levels of malt extract[a]

Malt Extract (%)	Kg malt/hL Wort
82.0	17.90
81.5	18.01
81.0	18.12
80.5	18.24
80.0	18.35
79.5	18.46
79.0	18.58
78.5	18.70
78.0	18.82
77.5	18.94
77.0	19.06
76.5	19.19
76.0	19.32
75.5	19.44
75.0	19.57

[a]Total extract requirement of 11.45 kg/hL was based upon the Lincoln equation (Weissler 1995). The malt requirement (kg/hL) was calculated by dividing extract requirement by [(malt extract/100) × (brewhouse efficiency/100)]. A brewhouse efficiency of 78% was used for this example.

Table 15.2 Grain requirement for 1.0 hL of 11 and 9%P wort using two different formulations

Original Gravity (% Plato)	Formulation (% of Extract Derived from Malt or Flaked Maize)	Extract Required[a] (kg/hL)	Grain Required (kg/hL)
11	100% malt	11.45	18.12
9	100% malt	9.30	14.72
11	80% malt	9.16	14.50
	20% maize	2.29	3.91
9	80% malt	7.74	11.76
	20% maize	1.86	3.18

[a]Total extract requirement was based upon the Lincoln equation (Weissler 1995). The grain requirement (kg/hL) was calculated by dividing extract requirement by [(grain extract/100) × (brewhouse efficiency/100)]. A brewhouse efficiency of 78%, malt extract of 81%, and flaked maize extract of 75% were used for these examples.

levels of malt replacement are possible when corn syrups are used, as the starch has already been converted and as levels of malt enzymes are not as critical.

Specialty malt use

Specialty malts generally constitute a small portion of the grain bill, and are used as a source of additional color, flavor, and aroma. Malts that have been subject to high kiln or roasting temperatures make a limited contribution to fermentable extract, and probably contain little to no enzymatic activity. Their contribution to total extract can be taken into account, but color is perhaps more important in determining levels of use. Prediction of wort and beer color is complicated, and large commercial brewers are likely to utilize results from pilot-scale testing (Holle 2003). However, a formula in common use in the home- and micro-brewing literature can be used to demonstrate the use of specialty malts in making color adjustments.

$$WordColor\,(SRM) = \frac{(lb\ malt1 \times color\ malt1) + (lb\ malt2 \times color\ malt2) + (lb\ malt3 \times color\ malt3)}{(gallons\ of\ wort)}$$

The example for 11%P wort shown in Table 15.2 that used 80% malt and 20% maize would yield a color of 2.4 SRM using the above equation and color values of 1.8 and 0.8 for the malt and maize, respectively. A brewer wishing to increase beer color will use small amounts of specialty malts. Assuming the brewer wished to increase color to 10 SRM, the above equation indicates that this could be achieved by either the addition of 256 g of chocolate malt (350 SRM) or 1120 g of caramel-80. These additions represent only 1.4% and 5.7%, respectively, of the total grain bill.

World malt production and use

Values for world malt and beer production are shown in Table 15.3. The late twentieth and early twenty-first centuries have seen profound changes in the structure of the worldwide malting and brewing industries Mergers and purchases have resulted in a relatively small number of companies producing much of the world's malt and beer. Today, it is not uncommon for large companies to have ownership or significant interest in operations on several continents, and for some brewers to have very extensive product portfolios.

The consumption of beer has also been the subject of considerable change in recent years. Beer consumption has been stagnant to declining in many countries in Western Europe and North

Table 15.3 World beer and malt production by region[a]

	Production	
	Beer (million hL)[b]	Malt (million metric tons)[c]
European Union 27	408,884	8,900
Eastern Europe	159,835	2,350
North American (including Central America and Caribbean)	349,746	3,800
South America	172,334	1,600
Asia (Near, Far, and Middle East, but excluding China)	156,522	550
China	351,515	4,070
Australasia (Oceania)	21,959	86
Africa	78,807	400
Total	1,699,602	22,530

[a]Data is courtesy of Roger Martin, RM International, West Sussex, England.
[b]2006 data.
[c]2007 data.

America, but has shown considerable growth in other regions. Most notable of these are the so called BRIC countries, or Brazil, Russia, India, and China (Canadian Beverage Consultants. Beer Booming in BRIC Countries, Beer 2007. Available at: http://www.canadean.com/Default.aspx?TabId=191&section=PressReleases&newsId=72&cat=0, verified August 12, 2009). Total consumption across these four countries increased by almost 50% from 2002 to 2007 and has contributed to a growing demand for malting barley and malt. China surpassed the United States to become the world's largest producer of beer in 2002, and recently has become the largest producer of malt (Table 15.3).

The amount of malt used in the production of beer varies considerably from country to country, with estimates ranging from below 9 to over 19 kg/hL (Wesolowski 1995). This not only depends upon brewing traditions, products, and technology, but also to a degree on the world supply and demand for malting barley. Malt use tends to be highest in the countries of Western Europe with strong brewing traditions and perhaps lowest in newer markets. Use can also vary considerably among brewers within a country. In North America, craft brewers producing traditional beers using 100% malt will likely have a greater malt requirement/hL than the large mainstream brewers.

In the United States, the average use of malt has been declining since the early twentieth century. The average use of malt in 1934 was 14.7 kg/hL (38.1 lb/barrel) beer (USBF 1960), while in 1959, it had declined to 11.1 kg/hL (28.7 lb/barrel). In 2006, the figure was 8.6 kg/hL (22.2 lb/barrel) (Brewers Almanac 2007).

There are numerous factors involved in this decline, but probably the most pronounced is large market share for light or low-calorie/carbohydrate beers which are often of lower OG and use less malt. The increased use of both microbial enzymes and corn syrup in brewing has also decreased the amount of malt required. Improvements in malt quality and brewhouse equipment and efficiencies may be additional factors.

Average malt use in the world's largest beer producing country, China, was 7.0–7.5 kg/hL in 2008 (Xu 2009). This represents a significant decline from 1990 when malt use was 14.6 kg/hL (calculated from 1 t malt/10.5 t of beer) (Fung 2007). Chinese brewers have been increasingly using more adjuncts in brewing, and this has been particularly true in years when malting barley supplies were short and prices were high. In addition, brewers have lowered the OG of beer produced, in response to both supply issues and to changing consumer preferences. Today, beers of 7 or even 6%P OG are not uncommon.

OTHER USES OF MALT

Distilled beverages and fuel alcohol

Barley and malted barley can be utilized in the production of both distilled alcoholic beverages and fuel/industrial alcohol. These topics are easily as complex as the brewing of beer, and this section will present only a limited outline that is needed to explain the use of barley and malt in these processes. Readers desiring more detailed information on the processes and product characteristics are urged to consult Russell et al. (2003) and Jacques et al. (1999).

The production of distilled alcoholic beverages or fuel ethanol consists of a number of basic steps, regardless of the end product (Lyons 1999a). The first of these is cooking/mashing where the starch in the raw materials, such as cereal grains or potatoes, is gelatinized and then hydrolyzed to fermentable sugars and dextrins. Starch hydrolysis is accomplished with the use of malt and/or microbial enzymes. Mashing is bypassed if sugary raw materials, such as agave, molasses, sugarcane, or sugar beet are used. As in beer production, the sugars are fermented by yeast to yield ethanol, carbon dioxide, and various other by-products. Ethanol concentrations of 10% are traditional, but concentrations of greater than 20% are possible in modern practice. Distillation is used for additional concentration of ethanol. Some distilled alcoholic beverages require a maturation period, often in oak barrels.

Malted barley is a traditional ingredient in whiskies, and at minimum, it was used at lower levels (~10%–15% of the grain bill) as a source of amylolytic enzymes in the mash (Morrison 1999; Ralph 1999). The remainder of the grain bill, or primary source of fermentable sugars, typically consists of unmalted cereals such as maize, wheat, and/or rye. Distiller's malts tend to be higher in DP and α-amylase, as large amounts of unmalted grain is used (Briggs 1998). Malt extract levels are not of concern, and high enzyme levels can be achieved by the malting of thinner barley that is higher in protein. Gibberellic acid can also be used to increase enzyme levels, but its use is not permitted for Scotch whisky. Lower kilning temperatures also help to limit loss of enzymatic activity. In modern practice, many distillers also make use of microbial enzymes. These include thermal stable α-amylase that can be added during cooking, and amyloglucosidase that is often added during fermentation (Kelsall and Lyons 1999).

Scotch malt whisky can be produced only from malted barley, while Scotch grain whisky is produced with malted barley and up to 90% other cereal grains such as wheat or maize (Lyons 1999a). No exogenous enzymes are permitted. Since malt is the only material used in Scotch malt whisky, extract is important, and the total protein and enzyme levels are not as high as for the distiller's malt. Malt for some Scotch whiskies may be kilned with the introduction of peat smoke, which in turn imparts a distinctive peat flavor to the resultant whisky. Typically, a blend of peated and non-peated malts is used, as peating can reduce fermentability (Briggs 1998).

Irish whiskeys are also produced using cereal grains including malted barley. However, unlike Scotch whisky, the enzymes for starch hydrolysis can come from microbial sources, as well as malt. Peat smoked malts are not used.

A number of Asian distilled alcoholic beverages utilize barley as a raw material. As an example, shochu is a Japanese alcoholic beverage that is commonly produced from barley, rice, or sweet potato. A similar product, Shoju, is produced in Korea. Steamed cereals are first treated with a fungal culture (*Aspergillus kawachii*) to prepare molded cereal or koji (Fukuda et al. 2001). Enzymes in the mold accomplish starch breakdown. Yeast is used for alcoholic fermentation. Shochu is then distilled to typically around 25%–30% alcohol (Iwami et al. 2006).

While the goal of beverage distillers is generally a beverage, often very traditional in nature, with very specific organoleptic properties, the goals of industrial alcohol production are to maximize ethanol yield and process and cost efficiencies (Kelsall and Lyons 1999). Fuel ethanol may be produced in batch processes, but in terms of process efficiency, most production is moving toward continuous operations. Starchy raw materials are converted to fermentable sugars by the use of microbial α-amylase and amyloglucosidae. A key to achieving a high yield of ethanol is to manage stress on the yeast. Temperature control is very important, as is sugar delivery to the yeast. Amylogucosidase is used in the fermenter so as to more slowly deliver glucose to the yeast. Very high initial high sugar concentrations can cause stress and reduce yeast performance.

Selection of raw materials for fuel ethanol production is based upon numerous factors, which include availability, cost, the economics of processing, quality of the resultant distiller's grains, and ethanol yields. In terms of the largest producers of ethanol, sugarcane is primarily utilized in Brazil, maize in the United States, and cereal grains and sugar beets in Europe (Lyons 1999a). Barley is utilized in industrial ethanol production, but its use in North America has not been widespread. It has lower levels of starch than maize, and consequently yields lower levels of ethanol (Kelsall and Lyons 1999). In addition, high levels of soluble β-glucans can cause processing problems, the hulls are abrasive to processing equipment, and the resultant distillers dried grains cannot be fed to monogastric animals (Hicks et al. 2005). Hulless varieties have been proposed as offering advantages over hulled types for fuel ethanol production.

Interest in the use of lignocellulosic materials for ethanol production is growing; as these materials are often considered as wastes, they are renewable, and do not impact food and feed grain supplies. Considerable challenges exist to

the utilization of these materials, including removal of lignin and the hydrolysis and fermentation of sugars derived from non-starch polysaacharides (Katzen et al. 1999). Both barley husk (Palmarola-Adrados et al. 2005) and straw (Pahkala et al. 2007) are lignocellulosic materials that have been investigated for ethanol production.

Flavored malt beverages

Flavored malt beverages (FMBs) are brewery products that originated in the United States in the later portion of the twentieth century (DeBlauwe 1991). Although the base of the product is a beer derived from malt and other cereals or cereal products, they differ from beer in that they exhibit little to no beer character, and flavor is derived primarily from added materials. The alcohol content of FMBs is similar to that of beer (4%–6% alcohol by volume). The target consumer groups are generally non-beer drinkers, and introduction of FMBs can be seen as an attempt by brewers to capture additional sales in times of declining beer consumption. Flavored alcoholic beverages (FABs) that are similar in nature may be prepared using wine or distilled spirits as a base.

Although FMBs are produced by brewers, the methods of production likely differ significantly from beer. Information on actual production methods is difficult to obtain, but the base beers are undoubtedly brewed and/or treated to minimize color, flavor, and bitterness. Flavorings, sweeteners, coloring, preservatives, distilled alcohol, and other additives are then added to the base product. Citrus flavors and lemonade are common. While additional alcohol can be added, in the United States, FMBs that contain less than 6% alcohol (by volume may) can derive no more than 49% of their alcohol content from flavors and other non-beverage materials (ATTB 2005).

A U.S. patent for a colorless flavored malt beverage states that the malt beverage base is decolorized with active carbon following fermentation (Word et al. 1994). The decolorized base is then combined with a sweetener (corn syrup), an anti-foaming agent, tartaric acid, a buffer (sodium citrate), and a flavoring agent. An enzyme is used to reduce the amount of non-fermentable sugars in the base.

Food uses

The food uses of barley are covered in Chapter 17 of this book. Malted barley and products prepared from malted barley are used by the food industry for a variety of products, although the largest use is probably in the baking industry. Malt products for baking are available in a variety of forms including flour, malt extract, malt syrup, and dried malt syrup (Pyler 1988). Syrups and extracts are prepared by the extraction of malt as for brewing. The extract is then concentrated to a syrup or spray-dried to powder form. Bakery malt products are further classified as diastatic or non-diastatic based upon whether they contain active enzymes.

Malt flour is commonly used as a supplement in bread flour at 0.2%–0.4% of the flour, and used principally as a source of α-amylase. Alpha-amylase attacks damaged starch granules, thus providing additional substrate for β-amylase. The combined action of the two enzymes provide sugars in doughs that have little to no added sugar, and have a marked effect on increasing loaf volume. Malt syrups and extracts are available in both diastatic and non-diastatic forms. They can be used as an enzyme source, but as they contain high amounts of sugars, they can be used to impart a sweet or malty taste to various baked products. Malt syrup is sometimes added to the water in which bagels are boiled.

Malt vinegar is prepared by the acetification of beers prepared from lightly kilned malts (Briggs 1998; Grierson 2009). Cooled unboiled worts are inoculated with yeast and fermentation conditions favor maximum carbohydrate utilization and alcohol production. Following the completion of fermentation, the yeast is removed, and the beer transferred to acetifier vessels. Acetic acid bacteria (*Acetobacter* sp.) oxidize ethanol to form acetic acid. Malt vinegars contain 4% –8% acetic acid.

BARLEY AND MALT QUALITY TESTING

Standardization of testing

The origins of modern standardized testing by the malting and brewing industries can be traced to the late nineteenth or early twentieth centuries (Schwarz et al. 2007b), and several professional organizations evaluate and publish official methods. These include the Methods of Analysis of the American Society of Brewing Chemists (ASBC 2004), which are widely used in the Americas, the Analytica-EBC (EBC 1998) of the European Brewery Convention, which are used in much of Europe, as well as many countries around the world, and the Mitteleuropäische Brautechnische Analysenkommision (MEBAK) methods (MEBAK 2006) are used predominantly in the German speaking countries of Europe and in the Czech Republic. The Brewery Convention of Japan publishes Methods of Analysis of the Brewery Convention of Japan (BCOJ) (BCOJ 1998). The Institute of Brewing (IOB) Approved Methods of Analysis were once widely used in the United Kingdom, but were last published in 1993, and have since been harmonized with those of the EBC (1998).

In general, the above organizations accept methods only after several years of collaborative testing have assured adequate inter- and intra-laboratory accuracy and precision. A 2006 agreement between the EBC, ASBC, and BCOJ allows for the establishment of International Analytical Methods (IM) that are jointly accepted by all three organizations.

While the above methods are widely utilized in malting and brewing laboratories, they are not extensively used when barley is purchased from the producer. In the case of testing at a local grain elevator, barley standards and methods of Federal agricultural agencies such as the Canadian Grain Commission (http://www.grainscanada.gc.ca/main-e.htm, verified August 12, 2009) or the United States Department of Agriculture's Grain Inspection, Packers and Stockyards Administration (GIPSA) are more important (http://www.gipsa.usda.gov/GIPSA, verified August 12, 2009). These agencies also publish methods for sampling, which are critical in assuring that a representative sample is obtained for testing. Non-official or non-standard methods are also likely to be employed at the point of purchase. These may include buyer-specific stipulations on factors that are not seen every crop year (e.g., green kernels, visual blight, etc). In addition, protein testing by near infra-red reflectance is often by nonofficial methods, although handbooks are published by GIPSA http://www.gipsa.usda.gov/GIPSA/webapp?area=home&subject=lr&topic=hb-nirt, verified August 12, 2009)

Objectives of quality testing

Standardized testing methods for malting barley and malt are essential for both quality control/assurance and trade of these commodities. Discussion of testing should include not only details on the specific test and the significance of results, but also consideration of testing objectives at the various points along the producer to brewer supply chain. As objectives differ at the various points of the supply chain, so do the perspectives of those using the data.

It is also important to bear in mind that much of the testing that is performed is predictive in nature. Measurement of quality in an absolute sense would require pilot malting and brewing trials. However, it is assumed that when varieties that have been developed specifically for malting are used, the maltster and brewer are able to use tests for factors such as grain protein and kernel plumpness to predict that a sample will perform adequately within their processes. Aside from predictive measurements, the other major category of quality testing is screening for deleterious factors. These are contaminants or some form of damage that can render the barley or malt unsuitable for use.

Producers grow barley if it is an economically competitive crop within their environment and operations. From their perspective, barley testing is used to assure that contractual or market obligations have been met, and thus, ultimately to determine the price that is received. The grain buyer needs testing to ascertain whether the barley is of adequate quality for producing malt.

As this testing is often performed at delivery, methods must be simple and rapid, and as a consequence are largely predictive in nature (e.g., protein and kernel plumpness). Screening for easily determined deleterious factors (sprouting, broken kernels, insect damage) may also be conducted at this point, in order to eliminate undesirable samples.

The maltster will test barley shipments upon receipt, to assure that deliveries match purchase specifications, and to determine segregation of the shipments. Again these tests must be rapid, and are largely predictive. Actual testing of malt quality parameters is conducted as a means of internal quality control needed for process adjustments, to determine blending requirements, and finally to meet brewer specifications. Brewers evaluate malt quality upon receipt to confirm purchase specifications, determine segregation, and to establish the need for process adjustments.

STANDARD BARLEY TESTS

Kernel size and weight

Maltsters and brewers have long recognized a positive relationship between kernel size/weight and yield of malt extract, and these factors are important predictors of potential malt quality. As an example, Edney and coworkers (http://www.grainscanada.gc.ca/research-recherche/edney/bcsq-ctoq/bcsq-ct, verified April 21, 2009) investigated the relationship between both 1000 kernel weight and plumpness using commercial samples of the cultivar AC Metcalfe. When intermediate (>2.0 and <2.4 mm) and plump (>2.4 mm) kernel fractions were compared, the overall malt quality of the plump fraction was better. This included lower total and soluble protein and higher malt extract. Lower levels of protein and perhaps a lower ratio of husk to endosperm in the plump kernels contribute to greater extract. The 1000 kernel weights of the plump fraction samples ranged from approximately 40 to 50 g and a correlation coefficient (r^2) of 0.7 was observed with malt extract. The uniformity of kernel size within a samples is also important in terms of water uptake rate in steeping and uniform germination, as well as for milling in the brewery (Kramer 2006).

Thousand kernel weight is simply the weight of 1000 kernels following the removal of broken grains and foreign material. The test is generally performed with a seed counting device, and results are ideally expressed on a moisture-free basis, as values will increase with increasing moisture.

Determination of 1000 kernel weights can be somewhat time-consuming, and the determination of kernel assortment (plumpness) is performed more frequently. In the United States, barley (100 g) is mechanically sorted on three stacked sieves with 19.0 × 2.8, 2.4, and 2.0 mm rectangular openings. Sieving yields four fractions. The combined kernel fractions remaining on the 2.8 and 2.4 mm sieves are generally considered plump (%). The fraction passing through the 2.0 mm sieve is considered thin. Slightly different sieves sizes, 2.8, 2.5, and 2.2 mm, are utilized in Europe (Kunze 1999). Typical 1000 kernel weight and plump values are shown in Table 15.4.

Test weight, a density measure, is the amount of a specific barley sample required to fill a Standard Winchester bushel (Bu) measure of 2150.42 in^3 (ASBC 2004), and is expressed in lbs/bu or kg/hL. Test weight is a component of official barley grades in both Canada and the United States. As an example, the minimum test weights for U.S. No. 1 Six- and Two-Rowed Malting barley are 47 and 50 lb/bu, respectively. While test weight is utilized in grading, it is not widely utilized as a predictive test by maltsters and brewers (Kunze 1999). The actual test weight of sample is dependent upon many factors including cultivar, environmental conditions during production, sample cleanliness, presence of awns or awn parts, and grain drying. To facilitate trade, most grains are bought and sold based on a standard bushel weight (at a specific moisture content). In the United States, a standard bushel of malting barley is 48 lb (14.5% moisture), and a standard bushel of malt is 34 lb (Heyse 1981).

Table 15.4 Typical values for quality parameters in North American and European barley and malts[a]

	North American 2-Rowed[b]	North American 6-Rowed[b]	European 2-Rowed[c]
Barley			
1000 kernel weight (g, as is)	40–45	35–40	41–45
Plump kernels (%)	80–90+	75–80+	>85
Protein (%)	11.0–12.0	12.0–13.5	<11.5
Malt			
Extract (% fine grind)	80.0–81.0	78.5–79.5	79–82
Soluble protein (% of malt, dry basis)	5.0–5.4	5.5–6.0	
Kolbach Index (%)	42–47	42–47	35–41+
Free amino nitrogen (mg/L)	200–220	210–230	
Beta-glucan (mg/L)	100–130	110–160	
Alpha-amylase (DU)	50–65	45–60	
Diastatic power (°ASBC)	120–130	150–160	70–80

[a]Barley and malt quality data represent approximate ranges. It must be remembered that considerable variation can occur to changes in growing conditions, barley variety and quality, and malt processing. Typical germination times in Europe are often 1 day longer than those used in North America.
[b]Based on data from Schwarz and Horsley (1995).
[c]Based on data from Kunze (1999).

Germination and dormancy

Malting is a process of controlled germination, and as such, malting barley must display high levels of germination. In general, >95% of all kernels in a sample should be able to germinate under malting conditions (Kunze 1999). Kernels which do not germinate do not produce a full complement of enzymes and do not undergo endosperm modification. As such, samples with a higher percentage of non-germinative kernels are likely to have lower levels of malt extract. As the cell wall polysaccharides of non-germinative kernels are unlikely to be extensively degraded, these may contribute soluble higher-molecular-weight components that have been associated with higher wort visocity and filtration problems.

Dormancy, which was discussed by Fincher in Chapter 14, is a physical or physiological condition of viable seed that prevents germination even in the presence of favorable conditions. In barley, dormancy is usually overcome following a period of grain storage, and as a consequence, new crop barley has traditionally been stored for several months following harvest before being malted. However, dormancy is not typically a problem with modern malting barley varieties (Kramer 2006), as breeders have selected against it. A more common problem with freshly harvested barley is water sensitivity.

Water sensitivity, which is the failure of seed to germinate in excess moisture, may be a specialized case of dormancy (Briggs et al. 1981). It results not only from the direct influence of water, but also from the availability of oxygen for inhibition of the germinated seeds (anoxia) (Hosnedl and Honsova 2002). Unlike dormancy, it may or may not decrease with storage (Briggs et al. 1981). As the grain is normally submerged for long periods in traditional steeping, water sensitivity can be extremely detrimental. Steeping process parameters can be adjusted to overcome water sensitivity and achieve more homogeneous germination (Holmberg et al. 2002).

Germination tests can be classified as providing a measure of germinative energy, geminative capacity, or water sensitivity. Germinative energy is the ability of grain to germinate fully with vigor under optimal growth conditions. The test is typically performed by germinating on moistened blotter or filter paper. The number of kernels which have sprouted (chitted) after 24, 48, and 72 h is determined, and germinative energy is expressed as a percentage at a given time. This test is affected by dormancy.

Germinative capacity is the potential germinative energy at some future date, when dormancy has ceased to be a factor. The ASBC method (ASBC 2004) is performed by germinating seeds in a solution of 0.75% H_2O_2 to overcome dormancy. The percentage of the sample that has sprouted after 72 h is reported. Water sensitivity is measured by determining the difference in germinative energy (%) found between tests using 4 and 8 mL water. Presumably, the grain is covered by a surface film of water in the 8 mL test. Differences of <10% indicate that the barley is not water sensitive, 11%–25% are slightly water sensitive, and >45% (Kunze 1999) are profoundly water sensitive.

Kernel brightness (color)

Maltsters and brewers have traditionally used the visual appearance of barley as a means of screening out problematic samples, although these empirical measurements undoudtedly result in the elimination of some acceptable samples. Green kernels indicate immature grain that may have arisen from uneven ripening of primary and secondary tillers, or perhaps harvesting too early. Primary problems relate to poor or uneven germination. Discoloration can serve as an indicator of rain weathering and/or microbial damage. Numerous problems can be associated with the above, but poor germination and/or water sensitivity might be most common.

In general, barley that is light yellow-straw color and bright in appearance is desired. Barley color or actually lightness is sometimes determined with a colorimeter (ASBC 2004), and an example would be use the L-value of the tristimulus color scale (Shellhammer 2009).

Protein

The level of barley protein is a key predictor of barley and malt quality, and as such is very important in purchase agreements with the grower and in malt contracts. Typical malting barley protein levels are shown in Table 15.4. Protein levels are important in a number of respects, and most often problems result from excessive protein, rather than inadequate levels.

As previously stated, there is an inverse relationship between protein and starch content, and consequently, higher protein barley will yield lower levels of fermentable extract (DeClerck 1958a; Briggs 1998; Kunze 1999). High protein barley absorbs water more slowly in the steep (Kramer 2006), and this can lead to modification and uniformity problems, particularly if high and lower protein barleys are mixed to meet contractual specifications. High protein barleys tend to produce a greater amount of enzymes during germination, which can lead to over-modified malts, if not properly managed in the malthouse. This same factor can also lead to a more rapid conversion in mashing.

Higher total protein is generally assumed to be associated with higher levels of soluble nitrogen compounds in the wort, which can lead to a number of problems. Amino acids and lower-molecular-weight nitrogenous compounds, are heavily involved in the Maillard reaction (Bathgate 1973), and as such, high soluble protein levels can lead to excessive color development in malt and beer. Excessive levels of amino acids can also affect the growth and fermentation pattern of the yeast. High levels of barley protein and high levels of soluble protein in beer are associated with the formation of chill- or permanent hazes in beer (Leiper and Miedl 2009). These hazes often result from the cross-linking of proline-rich protein fractions with polyphenols (Siebert and Lynn 1998).

Low protein levels are not commonly a problem but do occur. Principal problems are an inadequate level of amino acids to support proper yeast growth and poor beer foam (DeClerck 1958a). Several barley proteins including protein Z and lipid transfer protein 1 (LTP1) have been identified as important contributors to beer foam (Evans and Bamforth 2009).

Total barley protein was traditionally determined by the Kjeldahl method as nitrogen $\times$ 6.25, based upon the assumption that barley proteins are 16% nitrogen (DeClerck 1958b). This method has fallen out of favor because of its time consuming nature and safety concerns over the use of

concentrated acid and caustic. Today, the determination of nitrogen by combustion analysis or the determination of protein by near-infrared (NIR) spectroscopy are more common. On a simplified level, combustion analysis involves heating the sample to high temperature under an oxygen environment, resulting in complete combustion of the sample (Foster 1989). Oxides of nitrogen are catalytically reduced to nitrogen gas, which is measured by thermal conductivity. The amount of nitrogen $\times$ 6.25 yields an estimate of barley protein. Modern instruments also allow for the automation of sample analysis.

The use of NIR spectroscopy for the determination of protein is very common in the grain trade. The NIR spectra of a sample are comprised of broad bands arising from overlapping absorptions involving CH, OH, and NH chemical bonds (Osborne 2006). In practice, the concentrations of chemical constituents like water, protein, fat, and carbohydrate are determined from the spectral data after calibration against a reference method for the constituent of interest using multivariate mathematics. Calibrations for barley protein, as well as other constituents are often supplied by the manufacturer of the instrumentation. NIR analysis is rapid and requires little sample preparation, with some instrumentation using non-destructive analysis of whole grain. However, the quality of the results is extremely dependent upon the robustness of the calibration used.

Deleterious factors

Deleterious factors encompass a wide range of damage or contaminants that have the potential to render the sample unfit for use. These typically result from adverse environment conditions, plant disease, or from poor practices in harvest, storage, or transport. Contamination with agricultural chemicals or microbial toxins can pose a food safety threat. As each of these factors occurs on a sporadic basis, and only under certain conditions, testing is generally performed on an as needed basis. Examples of some the more common problems are discussed below.

Damaged kernels

Damaged kernels can be the consequence of many different handling or environmental factors (American Malting Barley Association. Malting Barley Requirements. Available at http://www.ambainc.org/pub/index.htm, verified August 12, 2009). In general, damaged kernels (e.g., physical, heat, or frost damage) result in low germination or a reduction in vigor, leading to lower malt extract. Damaged kernels can also cause off flavors and reduce processing performance. Definition of various types of damage can be found in the U.S. Standards for Grain (USDA GIPSA, Subpart B—United States Standards for Barley, http://archive.gipsa.usda.gov/reference-library/standards/810barley97.pdf, verified August 12, 2009) and the Official Grain Grading Guide of the Canadian Grain Commission (Barley—Chapter 6—Official Grain Grading Guide, http://www.grainscanada.gc.ca/oggg-gocg/06/oggg-gocg-6-eng.htm, verified August 12, 2009)

A specification for skinned and broken kernels is included in most purchase agreements, and is part of the official grade requirements for barley in the United Statesand Canada. The loss of portions of the barley husk can have a dramatic effect on the malting process. The husk protects the coleoptile during germination, and if unprotected (Fig. 14.2), it may break off, thus ending modification. The husk helps to regulate water uptake into the kernel, and the loss of part of the husk can lead to uneven modification of individual kernels, which in turn can cause problems with the homogeneity of the sample. Broken kernels further contribute to uneven modification, as the non-embryo end does not germinate. Skinned and broken kernels are also more prone to fungal attack during storage or malting.

Sprouting

Pre-harvest sprouting is the premature germination of the grain while still in the field. It is most often associated with rainfall and prolonged wet weather after physiological maturity of grain, although prolonged ripening under cool and moist conditions can result in pre-germination,

without visible signs of sprouting (Hough 1990). The presence of α-amylase is frequently used as an indicator of pre-germination, and can arise several different ways (Flintham and Gale 1988).

Preharvest sprouting of barley has serious technological and economic impacts, as it can predispose barley to a relatively rapid loss of viability (Carn 1982). While barley may initially regerminate, the germinative energy can drop to unacceptable levels during storage. Sprouted barley often displays a high degree of water sensitivity, which requires modification of steeping. It can also absorb more water in steeping, may cause mold problems, and result in overall reduced malt quality (Sole 1994). Numerous methods are available for the assessment of pre-germination, but the use of pearling with visual assessment and the Rapid-Visco Analyzer (RVA) for determination of stirring number are most common in North America (Schwarz et al. 2004).

Molds and mycotoxins

While fungi are a ubiquitous component of grain microflora, most do not cause problems in processing or pose a safety threat. However, the presence of certain organisms can lead to off-flavors, reduce germination and cause processing abnormalities, produce mycotoxins, or cause beer gushing (Schwarz 2003). Because of these concerns, maltsters and brewers have long selected against samples that show visual signs of mold. However, several research studies have shown that visual factors, such as red grains, are not good predictors of fungal related problems like *Fusarium* mycotoxins or gushing. More specific testing is needed to eliminate problematic samples with a high degree of confidence.

Grain standards in most counties place very strict limits on the level of ergot permitted in samples (Schwarz et al. 2007a), and there is increasing regulation regarding allowable limits for mycotoxins on food and feed grain. Mycotoxins can be produced by a wide variety of fungal genera, and can develop in the field or in grain storage. The presence of mycotoxins presents one of the most significant food safety and consumer perception issues to the brewing industry. A number of mycotoxins have been detected in commercial beer (Scott 1996), with malt or adjuncts being the source. However, it is the trichothecene mycotoxins produced by species of *Fusarium* that are the most common problem on a worldwide basis. A relatively recent area of concern is that of bound mycotoxins. Bound mycotoxins may be defined as those that escape detection or are not extractable during routine analytical procedures (Zhou et al. 2007). This is of concern as they may be released during processing or in digestion. Binding may be through covalent or non-covalent associations with grain constituents. The presence of deoxynivalenol-3-glucoside has been reported in malt and beer (Kostelanska et al. 2009).

Gushing, or the spontaneous and often violent over-foaming of packaged beer upon opening, is another problem commonly associated with *Fusarium* species. However, gushing has also been reported to be caused by species of *Aspergillus*, *Penecillium*, *Rhizopus*, *Alternaria*, *Nigrospora*, and *Trichoderma* (Gabe et al. 2009). Peptides produced by the fungi, particularly the hydrophobins, have been implicated as gushing factors. The occurrence of gushing requires a critical concentration of hydrophobins, and tends to be more problematic in beers produced from 100% malt. The use of adjuncts can have the effect of diluting the gushing factors below critical levels. Gushing analysis is routinely performed on wheat and barley samples in years when *Fusarium* head blight is problematic in Germany.

STANDARD MALT TESTS

Friability

Friability, which is a standard test of both the ASBC and EBC, is a direct measure of malt modification. The method, which was developed in the late 1970s, requires a proprietary piece of equipment, the Friabilimeter. The principal of the method is based on the observation that the endosperm of well-modified malt should be easily crushed, while that of poorly modified malt is hard and steely. This principal has been long

recognized by maltsters and brewers, and mealiness (vs. glassiness) was once commonly measured by cutting kernels with a razor blade (ASBC 2004). In the Friabilimeter, a 50 g sample of malt is crushed by a rubber roller that is enclosed within a rotating screen. Kernels that are well modified are easily crushed, and most of the endosperm will pass through the screen. Friability is determined as 100% minus the percentage of the sample that did not pass through the screen. For pale two-rowed malts, friability values above 81% are considered very good, while values below 75% are considered unsatisfactory (Kunze 1999). A second test performed with the Friabilimeter is the determination of unmodified malt (partly unmodified grains). The malt that was retained within the screen is further fractionated by shaking on a sieve with 2.2×23 mm openings. The percentage of the sample that is retained on top of the screen is considered unmodified. Unmodified malt often originates from dead kernels, or the unmodified distal portion of the endosperm.

Growth count

As previously stated, maltsters frequently monitor the length of the acrospire during germination, as a measure of modification and the uniformity of germination. This continues to be a standard laboratory test and is also often part of contract specifications. In the laboratory, 100 kernels are selected and the lengths of the acrospires relative to total kernel lengths are determined. Acrospires can be visualized by cutting, peeling, or boiling the kernels (ASBC 2004). Lengths are classified into five incremental categories from 0 to 0.25, to above 1/1 (overgrown). It has traditionally been held that brewer's malts should have an average acrospire length of approximately 0.75 (Kunze 1999). However, this may be a low estimate for the extremely well-modified samples that are used by some brewers in the United States. The distribution of acrospire lengths is of interest, as it can indicate problems with uniformity of germination and modification.

Extract

Laboratory mashing for the determination of extract is one of oldest standardized malt methods (Schwarz et al. 2007a). The predominant method dates to the late nineteenth century and is frequently referred to as the Congress Mash, with the term Congress being derived from Congresses of the Malt Analysis Commission of the German Chemical Society. Extract methods of the ASBC and the EBC are very similar in that 50 g of finely (or coarsely) ground malt is extracted with 200 mL of water according to the following time/temperature profile; 30 min at 45°C, 1°C/min to 70°C, and 1 h at 70°C. An additional 100 mL of water is added when the temperature reaches 70°C, and the contents of the final cooled mash are adjusted to 450 g. The hot water extract (HWE) method, which is more widely used in the United Kingdom, utilizes an isothermal mash (65°C) for the determination of laboratory extract (EBC 1998).

Values for extract are expressed as a percentage of the malt on a dry basis, and for pale brewer's malt, values typically fall within the range of 79%–82% (Table 15.4). Six-rowed barley tends to be slightly lower in extract than two-rowed, and the inverse relationship between protein and extract, and positive relationships between kernel size and weight and extract were discussed previously. The determination of extract can be performed on both finely and coarsely ground malt, and the difference was once commonly used as a measure of malt modification. Extract will tend to be lower in the coarsely ground sample, as there is less surface area with the larger particles, and solubilization and enzymatic reactions will thus proceed at a slower rate. The differences are exacerbated with poor malt modification. Differences in fine-coarse extract levels of less than 1.8% are considered good for European malts (Kunze 1999), but acceptable levels for North American malts may be slightly below this value. In addition, as soluble protein is also expressed as a percentage of the malt on a dry basis, some individuals use the subtraction of soluble protein from total extract as a very approximate estimation of carbohydrate extract.

The methods for determination of laboratory extract have received criticism almost since their inception (Schwarz et al. 2007b). Principal among these are that the OG (water : grist ratio) of the wort and temperature profile used do not reflect commercial practice, and the method does not predict brewhouse performance. Laboratory worts are typically in the range of 8%P to 9%P. The higher conversion temperature (70ºC) was likely used in the Congress mash as extract is known to increase with temperature, even though the fermentability is lower (Lewis and Young 1995). In contrast, the isothermal HWE method yields lower extract values but higher levels of fermentable sugars.

It must be remembered that the original intention of the Congress mash method was to provide an estimate of theoretical maximum extract, which is needed for calculations in formulation and as a basis for the trade of malt. When this method was developed, few to no additional tests were performed on laboratory worts. However, the Congress mash is now used to provide wort for a battery of tests including determination of FAN, β-glucans, soluble protein, viscosity, and color. A recent study evaluated the influence of grind, OG (water : grist ratio) and temperature on the determination of laboratory extract and wort quality (Schwarz et al. 2007b). Malts of various modifications were used. The results indicated that while the alteration of the mash parameters did often change the magnitude of quality differences observed among samples, in most cases, these changes were unlikely to change the overall actual rank of samples. Notable exceptions were the effects of temperature on β-glucans and fermentable sugars, and these will be discussed in respective sections that follow.

The value for fine grind extract obtained by Congress mashing is entrenched within the industry, and changing this method seems unlikely, especially given the wide variations in equipment, formulation, and processes utilized by different brewers. The need to more closely predict actual brewhouse performance can be addressed by alterations in analysis, such as filtration methodology. This is briefly discussed under nonstandard tests. On the contrary, if the intent of analysis is only to accentuate the differences among samples, such as in a barley breeding program, a simple alteration would be to perform analyses on the coarse grind, rather than fine grind wort. Schmitt et al. (2006) recently reported a method for the preparation of Congress worts from <200 mg of ground malt. Again, such an alteration of methodology will likely be of use in breeding programs, where sample amounts are often limiting.

Soluble protein

Soluble protein is the most commonly used quality parameter to determine the acceptability of malt protein levels. The term is somewhat of a misnomer as it is actually the sum of all nitrogenous compounds in wort. These compounds are typically present in brewer's wort as 30% amino acids, 30% peptides, and the remainder as proteins in excess of 30 amino acid units (Meilgaard 1977). Soluble protein is determined as $N \times 6.25$, and is expressed as a percentage of the malt, on a dry basis. It represents components released during both malting and laboratory mashing. Soluble protein levels increase with the modification level of the malt, but it must be remembered that barley itself has some soluble protein (Schwarz et al. 2007b). Typical values are shown in Table 15.4.

As discussed in the section on barley protein, at minimum, the malt must provide a level of soluble nitrogenous constituents for proper yeast growth. However, excessive levels can cause problems. Acceptable levels of soluble protein vary from brewer to brewer, and also from beer to beer. Beers that utilize high ratios of cereal adjunct will require greater malt soluble protein as the adjunct provides virtually no soluble nitrogen.

Soluble protein was historically determined by the Kjeldahl method. Today, the determination is frequently by combustion analysis or UV spectroscopy. The spectrophotometric method is based on the difference in absorbance of a diluted wort sample at 225 and 215 nm (ASBC 2004). Values are determined from a standard curve, which is prepared using malt standards with known soluble protein levels.

Kolbach index

The Kolbach index or the ratio of soluble to total protein (S/T) is a direct measure of protein modification. It is expressed as the ratio of wort soluble protein to total malt protein × 100. Kolbach index generally increases with extended germination times and/or greater malt modification. Typical values are shown in Table 15.4. Values below 35% are considered undermodified, 35%–41% as well modified, and values in excess of 41% as very highly modified (Kunze 1999). North American malts tend to be very highly modified.

Barley protein levels are sometimes used in the Kolbach index calculation, as there is typically very little difference between the total protein in barley and malt when determined as N × 6.25. While the protein may be degraded to peptides and amino acids, only a small amount of nitrogen is actually lost with the removal of the rootlets from malt.

FAN

As the value for soluble protein does not provide any indication as to the composition of the wort, some maltsters and brewers will also determine FAN. The FAN method (ASBC 2004) can be viewed as an estimate of the total amount of assimilable free amino acids in wort. The method is based upon the reaction of ninhydrin with free α-amino nitrogen groups. The resultant blue color is measured at 440 nm and compared against a standard solution.

Constituents with a free α-amino nitrogen group include amino acids, ammonia, and the terminal amino groups of peptides. The fact that both peptide and amino acids contain only a single free amino group/molecule makes this test more sensitive to amino acids. In addition, the imino acid proline is not measured which makes the test a more accurate estimate of assimilable nitrogen, as yeast do not utilize proline during fermentation (Jones and Pierce 1964). A minimum of 150 mg/L FAN is necessary for proper yeast growth and health (Pierce 1966). Any amount over the minimum requirement can contribute to biological hazes and high diacetyl levels (Owades et al. 1959). Typical values are shown in Table 15.4.

Wort color

Color was historically measured by comparison to sets of chemical or colored glass standards (Shellhammer 2009). This has largely been replaced by measurement at a single wavelength (430 nm) using a spectrophotometer. The ASBC reports color in °SRM which closely matches °Lovibond. The EBC reports color in EBC units (EBC 1998). Color in °SRM can be converted to EBC by multiplying by 1.97 (Beer Color Laboratories, http://www.beercolor.com/index.htm, verified October 1, 2009). The method was developed to reflect the pale yellow or golden color beers that dominate the market. It is not really acceptable for darker and red hued products, and for these, measurement of tristimulus color would provide more information.

Color results to large degree from Maillard reaction products, and Maillard precursors increase with increasing modification. Other reactions contributing to color include pyrolysis, caramelization, and oxidation of polyphenols.

Beta-glucans

The role β-glucans in malting and brewing has been recently reviewed by Jin et al. (2004). The proposed impacts which include increased wort and beer viscosity, slow lautering, and membrane plugging, would appear to be a direct function of molecular weight (MW) or size and conformation. Although barley β-glucans are extensively degraded during malting, neither the β-glucan content of barley or malt have been found to be good predictors of brewing performance. However, increasing concentration and MW of β-glucans have been found to significantly increase wort viscosity and to decrease beer filterability. As such, levels in wort are routinely determined.

While several methods have been devised for determination of wort β-glucans, both the ASBC (2004) and EBC (1998) methods are based upon the reaction between β-glucans and calcofluor.

Both methods are automated using flow injection analysis (FIA). In these methods, the fluorescent brightener calcofluor is mixed with a sample of wort. The dye binds to higher MW β-glucans and fluoresces. The fluorescence is proportional to concentration, although the MW range measured by FIA-calcofluor depends on the ionic strength of the eluant (Manzanares et al. 1993). Wort β-glucans are extremely polydisperse, and it is likely that only fractions of higher molecular weight are truly problematic. The average absolute MW of β-glucans in worts from well-modified malts is generally <200 kDa (Sadosky 2007). Materials of >500 kDa generally account for <1% of the total β-glucan, while materials of 50 to 500 kDa, account for 10%–20%.

Differences in wort β-glucan content can also be attributed to the type of sample analyzed. Laboratory Congress worts will likely yield lower values than the corresponding brewery wort. This can be attributed largely to mash temperature schedule, as Schwarz et al. (2007b) observed that average β-glucans values were approximately 40% higher in a 65°C isothermal mash when compared to the Congress mash. While little β-glucanase is thought to survive kilning and mash temperatures in excess of 60°C, there was still apparently some degradation during the 45°C rest of the Congress mash.

DP

DP is a measure of the capacity of the malt to convert starch to fermentable sugars, and as such is of extreme importance. Both the methods of the ASBC and EBC are based upon the determination of reducing sugars following the incubation of a malt infusion with soluble starch. The ASBC (2004) method utilizes aqueous extraction at 20°C and alkaline ferricyanide for the determination of reducing sugars, while the EBC (1998) method uses extraction at 40°C and an iodometric determination of reducing sugars. The ASBC method reports DP in °ASBC, while EBC method reports Windisch-Kolbach (WK) units. WK units can be converted to °Lintner (approximately equivalent to °ASBC) by dividing (WK +16) by 3.5 (DeClerck 1958b). While the original official methods both involve wet chemical analysis, with considerable pipetting and titrations, most laboratories currently utilize FIA.

In theory, reducing sugars can be formed by any malt hydrolytic enzyme acting on starch or non-starch polysaccharides. However, as the starch substrate is present in excess in the assay, most reducing sugars are derived from starch. Further, some consider the measurement of DP to largely reflect β-amylase activity, as its turnover number (K_{cat}) is considerably higher than the other starch degrading enzymes. As such, the catalysis of the hydrolysis α-1, 4 linkages in starch to liberate maltose by β-amylase occurs at a much greater rate than the corresponding catalytic events of α-amylase, limit dextrinase, or α-glucosidase.

Typical values for DP are shown in Table 15.4. As β-amylase is sensitive to inactivation in kilning, pale malts tend to have much higher DP levels than colored malts. The higher levels of DP in six-rowed malts allow for the inclusion of a greater proportion of cereal adjuncts. A number of researchers have all shown a positive correlation between DP and grain nitrogen content (Hayter and Riggs 1978).

Alpha-amylase

Alpha-amylase is a measure of the dextrinizing capacity of the malt, and values are reported in dextrinizing units (DU). As α-amylase is an endo-enzyme, its primary importance is in the reduction of the molecular weight or size of starch and larger dextrins. This reduces viscosity and provides additional substrate for β-amylase.

Both the EBC and ASBC methods for the measurement of α-amylase in malt are based upon the 1939 paper of Sandstedt et al. (1939), which in turn is based upon an even earlier method. The substrate used is β-limit dextrin (BLD), which is prepared by exhaustively digesting a special lintnerized starch with β-amylase. The assumption is that only α-amylase is able to attack the BLD substrate following this treatment. The substrate yields a blue color when mixed with dilute iodine,

which is attributable to iodine forming a complex with helical side-chains of the BLD. As α-amylase degrades the BLD to smaller linear and branched dextrins, the ability to complex with iodine is progressively lost. When the degraded substrate is mixed with iodine, it will yield various shades of brownish red. The assay is conducted by mixing the malt infusion with substrate and incubating. Aliquots are removed at various times and mixed with iodine. The color of the reaction mix is compared to that of a standard glass color disk in a color comparator (fixed endpoint assay). When the color of the reaction mixture matches that of the disk, the reaction is considered complete. Activity is calculated by use of a formula. The color comparator is not widely used in modern practice, and the analysis is routinely conducted as fixed-time assay using flow injection equipment.

While the assay purportedly measures only α-amylase, it would seem that internal cleavage of A and B-chains within the BLD might provide additional points of attachment for β-amylase. However, the extent of this potential interference is not clear. Some laboratories analyze α-amylase activity using a ferricyanide reducing sugar assay following the inactivation of β-amylase by heat treatment, or with inclusion of a sulfhydral inhibitor.

Alpha-amylase values for brewer's malt are in the range of 30 to 60 DU (Kunze 1999). Brewers utilizing cereal adjuncts will have higher requirements, while α-amylase is of lesser importance to all malt brewers. Typical values are shown in Table 15.4. Levels of α-amylase in distillers malts can range from 70 to >90 DU (Cargill Food Ingredients: Distillers malt, http://www.cargill.com/food/na/en/products/malt/distillers-malt/index.jsp, verified October 1, 2009)

DELETERIOUS FACTORS

Several of the deleterious factors discussed for barley also apply to malt. These include contamination with agricultural chemicals or microbial toxins. Again, testing is generally performed on an as-needed basis. However, unique to malt is the presence of a number of process-derived contaminants that can pose a food safety risk.

Nitrosamines

The interaction of oxides of nitrogen and amino compounds of malt has been shown to give rise to carcinogenic nitrosamines in malt, *N*-Nitrosodimethylamine (NDMA) (Briggs et al. 1981). Oxides of nitrogen are present in the kiln combustion gases. This issue was of great concern during the 1970s, but has largely been addressed by adoption of indirect heating, where the combustion gases are no longer in contact with the malt. In addition, as stated in the section on malting, sulfur dioxide can be introduced to limit the formation of NDMA during kilning. Testing for NDMA often by GC-TEA (gas chromatograph interfaced with a thermal energy analyzer) (ASBC 2004) is routine, and limits are typically ≤2.5 μg/kg malt (Kunze 1999).

Acrylamide

In 2002, the Swedish National Food Administration reported the finding of high levels of acrylamide in prepared bakery and potato products (Hoenicke et al. 2004), which raised an alarm within the food industry, as acrylamide is known to be genotoxic and is a potential carcinogen. Acrylamide forms during the heating of starchy foods such as cereals and potatoes (INFOSAN 2005). A primary pathway is believed to be degradation of free amino acids and interaction with reducing sugars, as in the Maillard reaction. Mikulikova and Sabotova (2007) reported that acrylamide formation in malt increased from <400 μg/kg malt at kiln temperatures of 80°C to >1200 μg/kg at 160°C. Acrylamide levels were highest in roasted and caramel malts, and lowest in pale brewer's and wheat malts. While the levels reported were as high as 3080 μg/kg malt, it must be remembered that these specialty malts are used at relatively low levels and there is considerable dilution during the brewing process. The risk of acrylamide exposure would seem to be much greater with products like potato chips, bread crusts, and

coffee/coffee substitutes (Hoenicke et al. 2004). Concern over this contaminant within the brewing industry has diminished, and testing is not routinely performed.

LIMITATIONS OF STANDARDIZED TESTS

While malting barley and malt are subject to perhaps more quality tests than any other field crop, there is still concern that the battery of standard tests does not provide a full picture of processing performance. These concerns are understandable if one considers the real meaning of quality to a maltster or brewer. As the maltster derives income from processing barley to malt, any factor that impacts the economics of their operation will be important. These include the time required for malting (maltability) and malt yield, in addition to standard factors like dormancy and water sensitivity. Energy cost is key. For the brewer, the malt must be of acceptable quality for product and process considerations. Economic yield of fermentable extract, satisfactory beer flavor profile, and satisfactory shelf-life and colloidal stability are all very important. The malt must perform well within their processing schedule (e.g., so as to minimize changes). Processing considerations for malt include its use within a malt blend, use with adjuncts, timely conversion in mashing, timely wort separation, yeast performance and fermentability, and filterability. Many of these quality concerns are not directly addressed by the routine standard tests.

An additional concern is that that many of the standard tests do not directly measure sample homogeneity or consistency. In terms of homogeneity, the brewer desires little to no within-sample variation, or in other words, that each barley (malt) kernel be exactly the same. Consistency implies that there is little lot (batch) to lot variation. These factors are extremely important to large commercial brewers, as operations often include multiple lines with near continuous production. In this environment it can be difficult and costly to make process adjustments that might be required for changes in raw material specifications. Protein concentration is a good example on how homogeneity problems might arise. Standard protein tests provide an average value for the bulk sample analyzed. While natural variation does occur within a field, this variation (nonhomogeneity) can be greatly exacerbated by the blending of low and high protein samples. Beta-glucan concentration is another example of potential homogeneity problems. While the calcofluor method yields a concentration value for wort β-glucans, it might not be sensitive to very small increases in undegraded, high MW, β-glucan species that could arise with a few dead or poorly modified kernels.

As a consequence of these concerns, alterations of standard methods, or new tests for additional factors such as filterability, have been proposed. However, standardization, or widespread adoption, of these tests can be difficult in that formulation, equipment, and processing parameters can vary considerably among brewers.

NONSTANDARD MALT QUALITY TESTS

Numerous new tests or test modifications have been proposed over the years, and consideration of all of these is beyond the scope of this chapter. However, issues involving yeast performance, beer filterability/lautering performance, and flavor stability are all recurrent. Each of these and their relation to malt will be briefly discussed.

YEAST PERFORMANCE

Fermentability

Whereas extract was once the paragon of malt quality, fermentability is becoming an increasingly important issue in discussions of malt quality. Fermentability refers to the amount of extract that is available to, and is utilized by the yeast. Fermentability is measured by the apparent or real attenuation limits (AAL or RAL), also referred to as apparent or real degrees of fermentation (ADF and RDF). The AAL is the percentage of the original extract that was utilized during fermentation. Apparent refers to the fact that

alcohol in the fermented extract lowers the specific gravity. Determination of RAL involves the removal of alcohol or correction for its presence, as well as a correction for the mass lost by CO_2 evolution (ASBC 2004). Typical values for AAL fall between 75% and 85% (Carey and Grossman 2006). Brewing process parameters including mash temperature, yeast strain, wort oxygenation, fermentation temperature, and fermentation tank design and pressure have a significant impact on fermentabilty, but can be controlled. Since starch gelatinization occurs at ~60°C, and the fact that β-amylase is rapidly inactivated at temperatures above 65°C, the brewer will utilize mashing temperatures between 60 and 65°C to maximize fermentation.

Fermentability, as related to the malt is a complex process that is dependent on several factors, including malt enzymes, fermentable sugars, free amino acids, and minerals and vitamins. Malt modification has a substantial effect on fermentability because the extent of modification determines the rate and efficiency of starch solution. However, recent research has attributed differences in malt fermentability to viscosity level, rate of starch release during mashing, glucose supply level from more extensive β-glucan breakdown, and FAN levels (Edney et al. 2007). Good protein modification ensures adequate levels of FAN in the wort for the growth of yeast. On the other hand, Bathgate et al. (1978) has reported that the fermentability of over-modified malt can be reduced because of reduced levels of fermentable sugars.

Levels of DP have traditionally been used by brewers to estimate or ensure fermentability. Kaneko et al. (2000) proposed the improvement of β-amylase thermostability in barley as a means of enhancing wort fermentability. Variation in fermentability among barley genotypes has been attributed to differences in β-amylase thermostability (Gunkel et al. 2002). Evans et al. (2005) found that levels of α-amylase, limit dextrinase, and β-amylase activity, as well as β-amylase thermostability and Kolbach index, were able to explain much of the variation observed among malt samples for AAL. They have suggested that the conventional DP assessment could be replaced with the measurement of its component enzyme activities and malt modification level.

Estimation of fermentability (AAL or ADF) can be made by measuring the change in extract content during fermentation with an excess amount of yeast under specific conditions (rapid fermentation test, ASBC 2004). However, this type of measurement is time-consuming and labor-intensive, and is not readily amenable to screening large numbers of samples in a barley breeding program. Fermentable sugars in wort can be measured by high performance liquid chromatography (HPLC), but it must be remembered that they are not the only malt-based factor which influence ferementability. Fox et al. (2001) used correlation equations and the levels of fermentable and non-fermentable sugars in wort to predict fermentability. Sjöholm et al. (1996) reported the development of a NIR method that could be utilized for online determination of fermentability. As a final note, it is also important to consider that Congress worts are not ideal for the determination of fermentability or for fermentable sugar measurement, as the 70°C rest results in extensive inactivation of β-amylase and lower values for fermentable sugars (Evans et al. 2005; Schwarz et al. 2007b). Further, a primary factor influencing fermentability in commercial practice is the use of adjuncts. The use of all malt worts in small-scale fermentations may not be adequately discriminative to detect differences among genotypes that would be seen in commercial brewing, as factors such as amylase and FAN are present in excess when 100% malt is used.

Premature Yeast Flocculation (PYF)

PYF has been defined as a problem in brewing, whereby normally, yeast flocculates prematurely during fermentation in the presence of high sugar concentrations, leaving the wort under-attenuated (Fujii and Horie 1975). The significant negative aspect of the PYF phenomenon during fermentation is marked reduction in the number of yeast cells in suspension, resulting in a series of problems associated with beer quality, including undesirable flavor profiles (Stewart and Russell 1981), higher levels of diacetyl (Nakamura et al.

1997), lower alcohol content, and microbial infections (van Nierop et al. 2004).

Due to the sporadic occurrence of PYF in breweries, research on this subject has been intermittent over the past four decades. The first approach to solving the PYF problem was to explain the principle of yeast flocculation. Within a strain, flocculation depends upon four factors. First is the presence of flocculation [FLO] genes and their regulatory elements (Verstrepen et al. 2003). Second are the nutritional compounds in wort, including fermentable sugars, FAN, and divalent cations (Sampermans et al. 2005). Third are environmental factors which include fermentation temperature, ethanol levels, pH, osmotic pressure, and shearing forces (Jin and Speers 2000). Fourth are physiological aspects of the yeast cell including cell surface hydrophobicity, vitality, membrane integrity, and starvation (Smart 1995).

While the mechanism of PYF is not completely understood, one of the most promising hypotheses is associated with polysaccharides derived from malt (Fujii and Horie 1975; Fujino and Yoshida 1976). This assumption is based on the existence of lectin-like proteins on the surface of yeast cells which combine with polysaccharides, inducing premature flocculation under the conditions which develop during the later half of fermentation (Herrera and Axcell 1989). Most researchers have postulated that malt PYF factors consist of a high-molecular-weight polysaccharide containing arabinose, galactose, and xylose (Koizumi et al. 2008). The PYF factor is believed to be localized in the husk (van Nierop et al. 2004).

Several assays for assessing the PYF potential of malt have been proposed. A widely used flocculation assay is the Helm's test, in which the settling characteristics of yeast dispersed in a calcium-sulfate buffer are measured (Bendiak et al. 1996; D'Hautcourt and Smart 1999). Researchers have recently focused on the development of rapid, reliable, and small-scale methods for assessing the PYF potential of malt. Koizumi and Ogawa (2005) reported a rapid and sensitive method that does not require mashing or fermentation. A spectrophotometer (600 nm) is used to monitor yeast cells that remain in suspension when they are mixed with a buffered extract of the malt. The entire analysis only requires 3 h, and showed good correlation with fermentation tests. Small-scale fermentation methods have been recently reported by Jibiki et al. (2006) and Lake et al. (2008).

FILTERABILITY

The term filterability is somewhat confusing in that it is used to refer to issues associated with both wort separation and beer filtration. In both cases, barley cell wall polysaccharides are frequently cited as being problematic, and a common criticism is that neither is predicted by standard quality tests.

Wort filterability, which is somewhat associated with wort viscosity, is mainly determined by malt modification level and the composition of malt. Many factors have an influence including the degradation of starch and non-starch polysaccharide, proteolysis, viscosity, agitation, and filtration techniques. Much work relating to wort and beer filterability has been focused on β-glucans and arabinoxylans (Egi et al. 2004; Lu and Li 2006).

Wort separation performance is generally predicted using modified mashing and filtration techniques, as opposed to the gravity filtration of a dilute wort in the standard Congress mash method. Brown et al. (1990) proposed a thick mash filtration test based on isothermal mashing at 65°C. Researchers in Finland later modified Brown's method and developed a Büchner filtration test, which was able to reveal differences in wort filterability that could not be detected in conventional malt analysis (Stenholm et al. 1994, 1996). Tepral mashing uses thicker mashes and pressurized filtration at 75°C (Moll et al. 1989).

Beer filtration issues related to malt have been assessed by pressurized filtration through membrane filters and determination of filtration rate (Vmax). The value for Vmax is calculated by regressing the ratio of time (s) : filtrate collected (g) versus time(s) and taking the inverse of the slope. Stewart et al. (1998) found that the

viscosity and membrane filterability of beer was correlated with arabinoxylan content, whereas β-glucan was correlated only with viscosity. Sadosky et al. (2002) and Lu et al. 2005) later confirmed that the molecular weight of arabinoxylans and β-glucans were important to beer filterability.

BEER FLAVOR STABILITY

Beer flavor stability has been and continues to be one of the greatest challenges to brewers (Bamforth and Lentini 2009). Flavor stability determines the shelf-life of packaged beer, and as such is regarded as a very significant economic factor. The most widely investigated area involving malt and beer flavor stability is beer staling. Beer staling is often defined by the development of a cardboard-like off-flavor in aged beer, and this cardboard flavor is frequently associated with E-2-nonenal. However, beer staling also may be associated with other off-flavors that can be described as harsh after-bitter and astringent notes in taste. While the mechanism of beer staling is very complex and is still subject to debate, it has been widely accepted that the compounds responsible for the stale off-flavors most likely are oxidation products present in wort or beer.

In terms of malt-related flavor stability, lipid oxidation and the LOX pathway have been extensively studied. LOXs are a group of iron-containing enzymes catalyzing the dioxygenation of polyunsaturated fatty acids (linoleic and linolenic acids) to produce hydroperoxides or hydroperoxide radicals as primary products (Morrison 1993). The primary substrate in barley is linoleic acid, which is largely present in the form of triglycerides. FFAs are liberated through the action of lipase during germination (Schwarz et al. 2002). The hydroperoxides generated by LOX are further substrates for hydroperoxide dehydrase (formerly hydroperoxide isomerase) and hydroperoxide lyase, which lead to the formation of various carbonyl compounds including E-2-noneal (Bamforth and Lentini 2009). Oxidation of fatty acids may also occur through non-enzymatic means.

There are two isozymes of LOX in barley, with LOX-1 being found in both quiescent and germinating barley, and LOX-2 only being detected in germinating barley (Wu et al. 1997). Genotypic variation in the levels of the isozymes has long been known, and reduction of barley LOX was proposed as a means of reducing the staling potential of malt. The primary product of LOX-1 is the 9-hydroperoxide of linoleic acid, which in turn can be degraded to yield E-2-nonemal. Barley LOX-2 only catalyzes the formation of the 13-hydroperoxide. As such, levels of LOX-1 are thought to be much more important for flavor stability.

The elimination or reduction of LOX activity in barley and malt is one strategy that is being employed to improve flavor stability, and several breeding programs have carried out extensive screening for null- or reduced-LOX barley mutants. Sapporo breweries in collaboration with Okayama University identified barley germplasm that lacks LOX-1 (Hirota et al. 2006). Malt from the null-LOX-1 barley mutant yielded higher sensory scores with less cardboard flavor in brewing trails.

LOX activity in barley or malt extracts has traditionally been measured by monitoring the formation of hydroperoxides with a spectrophotometer at 234 nm, or by measuring the consumption of oxygen with an oxygen electrode. However, both methods are tedious and not suited to high-throughput screening of LOX for breeding programs. Recently, a high-throughput screening method for LOX that utilizes ferrous oxidation-xylenol orange for the determination of hydroperoxides was proposed (Li and Schwarz 2008).

The use of electron spin resonance (ESR) spectroscopy has become a common technique within the brewing industry for the estimation of oxidation and flavor stability. Specific techniques include the measurement of iron ions and free radicals (Kaneda et al. 1988, 1992), and 1,1-diphenyl-2-picrylhydrazyl (DPPH)-scavenging activity (Franz and Back 2001). Takoi et al. (2003) has applied ESR techniques for malt analysis.

FUTURE CONSIDERATIONS

While there have been tremendous advances in our understanding of the biochemistry of malting and brewing over the past 100 years, important gaps in knowledge remain. The relatively recent tools of biotechnology and genomics will likely lead to further advances. For example, the ability to manipulate the activities and stability of specific barley enzymes are needed for improvements in both product quality and consistency and processing efficiencies.

Economic, environmental, and political issues, however, also are likely to have a tremendous influence on barley supply, the use of malt, and views on quality. The acreage of malting barley has been declining in several countries that have traditionally been major producers. While multiple factors have been involved, political pressure or mandates regarding the production of biofuel crops have contributed to decreased acreage. Declining acreage coupled with increased demand for malt in developing beer markets has been a major factor in several recent global shortages of malting barley. The malting and brewing industries can respond to this situation by increasing prices for malting barley, lowering quality specifications for acceptable malting barley, or by lowering the use of malt in formulations. Malt use in the United States and China, both major beer producers, has been declining. Brewers, especially those in regions with less ingrained traditions, presently seem more amenable to the consideration of non-malt sources of starch and the use of exogenous enzymes in brewing. However, another approach to reverse declining malting barley production would be a renewed focus on agronomic research, and the investigation of production systems that lead to greater acceptance rates for malting barley producers, thereby making it a more attractive option.

Malting is an energy-intensive process, with the majority of gas and electrical use in the kilning stage (Davies 2009). Environmental and economic concerns are already impacting the malting and brewing industries, and compelling examination of carbon footprints. Energy efficiency and recovery as well as water usage are major considerations in the construction of new malt plants. Increasing the acceptable level of moisture in kilned malt from 4% to 5% can significantly reduce carbon emissions and energy costs. The development and acceptance of malting barley varieties that can be malted more rapidly is another option.

Finally, the recent consolidation within the global malting and brewing industries is also likely to have impacts. With relatively few entities controlling much of the production and consumption of malt, it is not unreasonable to speculate that there will be changes in views on quality, quality testing, and perhaps even within the organizations that currently support the adoption of standardized methodologies.

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Chapter 16
Barley Feed Uses and Quality Improvement

Tom Blake, Victoria C. Blake, Janice G.P. Bowman, and Hussein Abdel-Haleem

INTRODUCTION

Barley (*Hordeum vulgare* ssp. *vulgare*) is among our most versatile crops. Barley varieties have been developed that provide annual forage, grain for livestock, grain high in soluble fiber for human food, and grain suitable for malting. Barley competes with maize (*Zea mays*) and sorghum (*Sorghum bicolor*) as a feed grain, but does so at a disadvantage in the United States. The generally regarded as accurate National Research Council "Nutrient Requirements of Beef Cattle" (NRC 1996) claims that maize provides approximately 10% higher feed value for cattle than barley. We will critically examine this claim later in this chapter.

Over the 5-year period 2003–2007, estimated European Union production was about 41% of the world's barley or 58 of 142 million metric tons. Germany, France, Spain, and the United Kingdom ranked 3rd, 4th, 5th, and 9th in world production, respectively. The Russian Federation produced about 12%, Canada 8.5%, Turkey and Ukraine about 6% each, Australia about 5%, and the United States about 4% (Food and Agricuture Organization [FAO] of the United Nations 2009). Less than 20% of the world's barley crop is malted, and less than 5% is consumed as human food. Growing interest in renewable energy has led to modest use of barley grain for fuel ethanol production. Still, the largest use of barley is as animal feed. Over the past decade, several research groups have contributed to our growing appreciation of the diversity available in barley for traits that contribute to improved animal health and productivity.

Barley grain is utilized in the diets of ruminant and nonruminant livestock, poultry, and fish. Barley is commonly utilized as a feed grain for livestock species grown in temperate environments. Barley is a traditional grain for beef, lactating dairy cow, sheep, swine, goat, and poultry production. Barley is traditionally dry rolled or ground for beef cattle rations. Prize animals are often fed barley that has been tempered (soaked for 12 h) and then rolled. In Japan, barley is commonly steam rolled prior to feed composition. For poultry production systems, barley may be ground and β-glucanase and phytase added during feed composition (Kellems and Church 2002). Bregitzer et al. (2007) developed barley varieties uniquely suited for commercial fish feed. Barley is a versatile, high-quality feed grain. As food and feed resources become more valued in coming decades, we should do all we can to ensure that the best possible barley is available for each of its many uses.

Barley is a primarily inbreeding diploid ($2n = 2\times = 14$), and this characteristic enables barley to readily reveal the phenotypic impact of mutations. An examination of the landrace barley varieties collected in any of the global barley collections leads one to the conclusion that farmers long ago understood many of the uses barley

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could serve. Mutations in a few genes that impact morphology help define today's barley market classes. Forage barley is commonly defined by the hooded (*Kap*) gene on barley chromosome 4H that converts the lemma awn, a structure that can cause jaw infections in livestock, into a deformed floret with leaf-like projections (Muller et al. 1995). The *nud* gene on barley chromosome 7H results in non-adherent lemma and palea, a characteristic that results in grain that is more easily utilized in human food, and in poultry and swine feed (Taketa et al. 2008). These two traits are readily observed among landrace accessions in barley's germplasm banks, and were obviously prized by early farmers. The *vrsl* (Komatsuda et al. 2007) and *Int-c* (Komatsuda and Mano 2002) mutations result in 6-rowed barley with large, fertile lateral florets. The 6-rowed spike is used by barley growers in North Africa as a marker for feed barley. While 6-rowed barley is used in malt production in North America, the indigenous and improved 6-rowed varieties in North Africa are as valued for their water use efficiency and durability as they are for their feed value. Barley remains the temperate crop of choice for livestock producers in environments characterized by limited water, poor soil quality, or a short growing season. Much of the world's temperate zones, including the plains of Central Asia and North America's semiarid northern plains, provide the environmental constraints that make barley a competitive crop choice. In this chapter we will discuss barley's value and potential as a quality feed grain for beef and dairy cattle, poultry, sheep, fish, and swine production.

Most of the barley fed to cattle in North America is malting barley that failed to meet malting standards. Inadequate soil moisture results in barley with low grain starch content and increased grain protein percentage, resulting in low malt extract. Excessive moisture at harvest results in pre-sprouted grain and contributes to grain with excessive levels of aflatoxins. Breeding and selecting new malt barley lines that find favor with the malting and brewing industries is challenging, and the industrial testing required by industry often adds several years to the breeding cycle. Simply selecting for yield and adaptation leads to more rapid and predictable improvement in agronomic characteristics. Feed barley varieties currently tend to outyield malting barley varieties, but malting barley commands a premium over feed barley in most countries. In Montana, between 2000 and 2008, the average price of malting barley purchased through the open market was $185.74/mt, while the average price of feed barley was $125.20/mt (see http://wbc.agr.mt.gov/Producers/pricing_historical_mt.html for raw data).

Since barley's production advantages are its water use efficiency and its tolerance of short growing seasons, barley is a preferred crop in temperate environments with limited rainfall. In developed economies, the primary factors that determine farmers' crop and variety choices are economic and environmental. In the case of barley, these factors include the relative yield of the best-adapted feed and malt barley varieties, their price difference, the likelihood that the producer will be able to market his barley for malt, the cost to the farmer of storing and transporting barley to the buyer, and the relative production costs involved in producing feed barley or malt barley. Whether a crop of an industry-recognized malting barley variety will meet the standards required by the malting and brewing industry depends upon the crop's protein content and kernel plumpness, whether the crop has been damaged by threshing, disease, or weather, and the abundance of high-quality malting barley in the market. The selection rate of barley for malting in Canada (the percentage of the barley crop grown from recognized malting barley varieties that is purchased for malting) has traditionally been between 20% and 40% (http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/fcd7409). The yield differential between the highest-yielding feed barley varieties and the highest-yielding malt barley varieties tends to hover between 10% and 20%. The reason most barley grown in the United States, Canada, Australia, and the European Union is of an accepted malting variety is due to the price differential between feed and malt barley (Fig. 16.1). In 2007, this differential diminished due to a global shortage of feed grains, and farmers who

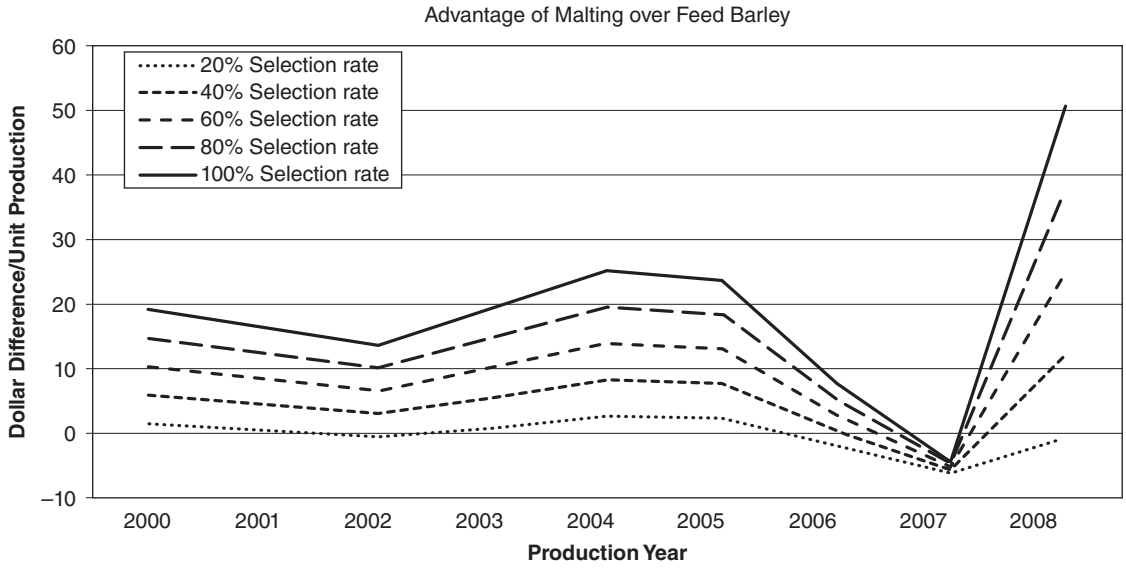

Fig. 16.1 Relative profitability to farmers growing malting versus feed barley varieties, assuming that feed barley outyields malting barley by 10%, and a range of selection rates (20%–100%). The Montana Wheat and Barley Committee provided the pricing data for Figs. 16.1 and 16.2 (http://wbc.agr.mt.gov/Producers/pricing_historical_mt.html).

planted the highest-yielding variety, regardless of potential malting quality, profited the most. Nonetheless, in most years, most farmers profit more by planting malting barley than by planting feed barley. Much of this price differential between malt barley and feed barley results from the discounted value of feed barley due to the nutritional values published by the National Research Council (NRC 1996). If we could improve barley to equal or surpass corn as a feed grain, the value of feed barley would increase, and feed barley acreage would likely also increase (Fig. 16.2).

Farmers producing grain that meets malting specifications 1 year in 5 would have produced grain of equal market value whether growing malt or feed grain, assuming the best available feed variety outyielded its malting counterpart by 10%. If the value of feed barley were to rise through revision of the NRC's recommendations, farms with a selection rate of 40% would have generated about as much per rainfed acre growing feed barley as they would growing malting barley, while farms with a selection rate of 20% would generate about $14 more per rainfed acre by growing feed barley. Improving the perceived value of feed barley should have a dramatic impact on barley's worldwide acreage and on the mix of market classes of barley in production.

FEED QUALITY

The characteristics of grain that contribute to improved animal performance vary among livestock species and their uses. While cattle effectively utilize the phosphorus and minerals contained in the salts of phytic acid, swine, poultry, and fish do not (Sugiura et al. 1999; Veum et al. 1999; Yi et al. 2001). Ruminant species depend upon their microflora for production of essential amino acids, while nonruminants require that sufficient essential amino acids be supplied in their feed. Barley, like other cereals, is primarily a source of starch-based calories when used in livestock feeds. Increasing the ratio of starch to protein could make barley grain more like corn, improving its market value. Large

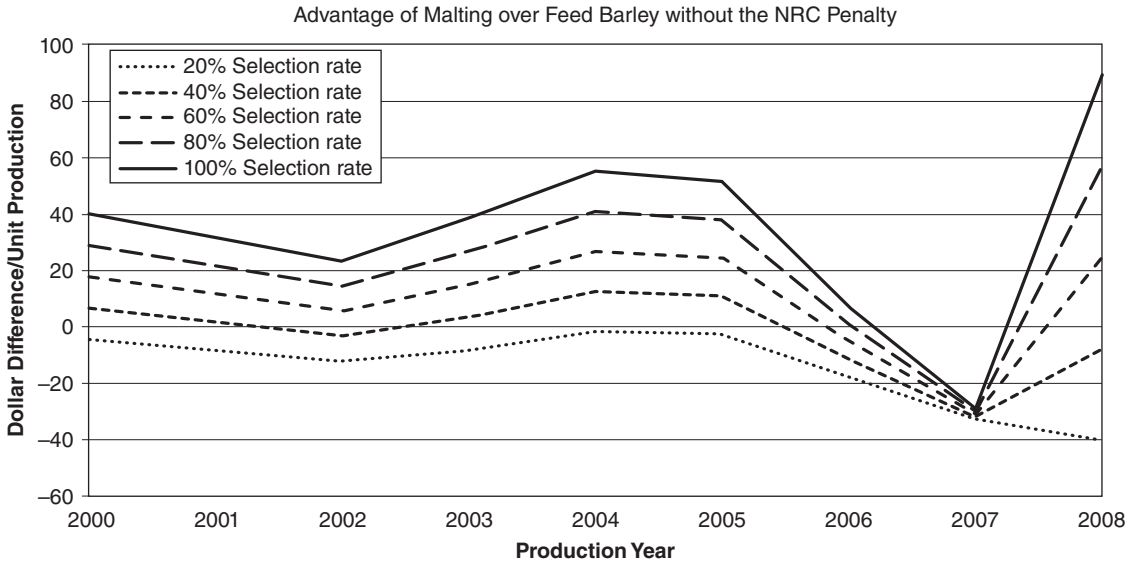

Fig. 16.2 Relative profitability to farmers growing malting versus feed barley varieties, assuming that feed barley outyields malting barley by 10%, and that feed barley is valued 10% higher through elimination of the NRC penalty relative to corn, and a range of selection rates (20%–100%).

ruminant species can suffer bloat, acidosis, and laminitis when fed diets composed mostly of barley. Rolled or steamed barley degrades more rapidly than desired in the bovine rumen, while small ruminants and nonruminants thrive on easily digested feed. Barley's extensive genetic resources and its ability to tolerate mutations that impact grain composition make it a good candidate for the development of feed barley varieties tailored to meet the specific dietary needs of different animals. Below are discussed five paths barley researchers have pursued in an attempt to improve barley grain quality for animal feed: reducing grain phytic acid content; improving grain protein quality; reducing β-glucan content in feed for monogastric animals; reducing *in rumen* grain fermentation rate; and increasing grain starch content.

Improving grain phosphorus availability

Plants store much of the phosphorus in their seeds as *myo*-inositol hexakisphosphate, commonly known as phytic acid. About 35 million metric tons of phytic acid are stored in plant seeds and fruits each year (Sharpley et al. 2003). While the phosphorus in phytic acid is readily available to ruminants, monogastric animals lack the phytases needed to liberate inorganic phosphate from phytic acid. Nonruminant diets containing large quantities of phytic acid are typically supplemented with inorganic phosphorus, and most of the phytic acid from grain is excreted intact. Phosphorus derived from environmental degradation of phytic acid in the waste of swine and poultry production systems pollutes ground and surface water and leads to eutrophication of streams and watersheds (Sharpley et al. 2003).

Phytic acid is a powerful chelator of divalent cations, reducing the bioavailability of iron, calcium, magnesium, and zinc. Over the past 20 years, the laboratory of Dr. Victor Raboy led the development and characterization of seed mutants with reduced levels of phytic acid (Bregitzer and Raboy 2006). These were selected utilizing a colorimetric assay that identifies individual seeds with increased levels of inorganic phosphate followed by reevaluation of the initial selections by either paper electrophoresis or thin layer chromatography (Larson et al. 1998;

Rasmussen and Hatzack 1998). These straightforward assays led to the isolation of low phytic acid (*lpa*) mutants in maize, rice (*Oryza sativa*), soybean (*Glycine max*), wheat (*Triticum aestivum*), and barley (reviewed in Cichy and Raboy 2009).

Mutations in at least six genes are now known to reduce barley seed phytic acid levels and consequently increase seed inorganic phosphate and divalent cation availability. These have been found to increase bone mineralization and phosphorus availability in turkeys, chickens, swine, and trout (Sugiura et al. 1999; Veum et al. 1999; Yi et al. 2001; Overturf et al. 2003; Linares et al. 2007). Fecal phosphorus output was reduced due to the reduced need to utilize inorganic phosphate as a feed supplement. This observation encouraged the development of low phytic acid barley varieties (Bregitzer et al. 2007, 2008), with the objective of providing a feedstock that would result in reduced surface and groundwater pollution from livestock. Since *lpa* mutations have also been isolated in maize and soybean, one might expect rapid adoption by the livestock feeding industry. Supplementation of grain-based feed with phytase can mitigate the need for phosphorus supplementation. Alternatively, transgenic laboratory animals and swine have been developed that express salivary phytase (Golovan et al. 2001a,b).

There are several routes available that would enable phosphate pollution reduction in watersheds that contain swine, poultry, and fish production facilities. The most straightforward of these is the use of low phytic acid grain. The *lpa* mutants in barley and other grains are well characterized and are readily available.

Quality protein barley

Barley, wheat, and maize store most of their grain nitrogen in the form of storage proteins. These storage proteins are characteristically deficient in essential amino acids, most notably lysine, threonine, tryptophan, and the sulfur amino acids cysteine and methionine. Since the discovery of the maize mutation *opaque-2* (Mertz et al. 1964) that increased grain lysine percentage, scientists have attempted to improve the nutritional quality of cereal grains through mutagenesis. The *opaque2* mutation resides in a transcription factor that regulates expression of zein genes, the genes encoding the main storage proteins in maize (reviewed in Presanna et al. 2001). Reducing the percentage of zeins as a proportion of total seed protein results in an overall increase in the percentage of essential amino acids, since zeins provide very little lysine and almost no methionine. The initial high lysine mutants selected in barley were in many ways similar to the *opaque* and *floury* maize mutants. The *lys* mutants developed at Risø achieved their high lysine character by impacting endosperm development, reducing seed storage protein content, and often negatively impacting starch deposition. The *lys 3a* mutation responsible for the dramatic alteration in Risø 1508 seed phenotype is caused by a failure to demethylate CpG islands in B- and C-hordein promoters during endosperm development, resulting in dramatically reduced transcription and translation (Sørensen 2002). These failed to achieve commercial success due to their poor agronomic performance and yield potential. To date, the only high lysine mutation in the cereals that has obtained commercial success is the *opaque2* mutation in maize, and that required the concerted effort of maize breeders and geneticists worldwide for 40 years.

More recently, targeted transgenic strategies have been developed that block essential amino acid catabolism and encourage synthesis of lysine, methionine, and tryptophan in the endosperm through the use of genes encoding key amino acid biosynthesis enzymes that are not feedback inhibited by their products (see Shewry 2007, for an excellent review). Dihydrodipicolinate synthase (DHDPS, EC 4.2.1.52) is the most commonly targeted biosynthetic enzyme to improve lysine biosynthesis in seeds. It is strongly feedback inhibited by free lysine, and bacterial forms of the enzyme that lack feedback inhibition have been isolated, and their genes have been appropriately modified and transformed into maize seeds. In a clever adaptation of single gene transformation, Frizzi et al. (2007) transformed maize with a construct containing an endosperm-specific promoter and a non-feedback inhibited DHDPS that

contained within an intron an siRNA-encoding sequence that downregulated lysine-ketoglutarate reductase/saccharophine dehydrogenase (LKR/SDH), a key enzyme in lysine degradation. The authors reported a 40-fold increase in seed-free lysine content. Several groups have targeted development of nutritionally improved storage proteins in small grains. Barley's hordothionin and chymotrypsin inhibitors 1 and 2 all appear to be strong candidates for use as high lysine storage proteins in transgenic cereals. It appears likely that simultaneous production of high levels of free essential amino acids, coupled with storage of these amino acids in novel seed storage proteins, will be required if barley is to become a true quality protein crop.

Protein quality is important for nonruminants, including humans. Barley is important as a food crop in high elevation environments with short growing seasons, primarily in the Andes and Himalayas, but the largest potential markets for quality protein barley remain poultry, swine, and fish feed. Plainly, human nutritionists, animal nutritionists, barley molecular geneticists, and barley breeders will need to collaborate if barley's potential as a nonruminant quality protein feed grain will be realized.

Finding the right level of β-glucans

Barley endosperm cell walls are largely composed of β-glucan. This highly branched glucose polymer has several interesting properties. Barley β-glucans are glucose polymers with β-1-3 and β-1-4 linkages. Since branch points are irregularly distributed, these polymers are hydroscopic and result in the production of gels in aqueous environments. The laboratory of Dr. Geoff Fincher (Waite Institute, Adelaide, South Australia) has led investigations into the synthesis, structure, and degradation of barley β-glucans for the past two decades. Utilizing comparative genomics, Burton et al. (2006) hypothesized that a cluster of rice genes encoding proteins similar to cellulose synthase, located in a region homoeologous to the β-glucan QTL on barley chromosome 2H, were responsible for β-glucan synthesis. They transferred one of these genes into *Arabidopsis thaliana*, and converted *A. thaliana* into a β-glucan synthesizer. Plainly, the branched β-glucans are produced by enzymes related to cellulose synthase.

Barley β-glucans are important to human health. They help lower blood cholesterol and glucose, they may help reduce the incidence of colon cancer, and they may help reduce obesity. Unfortunately, in feed barley, high levels of β-glucan result in poor rates of gain in poultry and swine. Fortunately, scientists at the Carlsberg Research Laboratory found a way to mitigate the negative impact of high β-glucan content on swine and poultry growth rates (for an excellent review, see Wettstein 2007). Swine and poultry raised on largely barley diets suffer from reduced feed intake and metabolic dysfunction largely due to the tendency of β-glucan to produce a gel in the digestive tract, reducing mobility. Wettstein et al. (2000) produced transgenic barley that expresses heat- and pH-tolerant β-glucanase that effectively reduces β-glucans in the poultry and swine digestive tracts to glucose oligomers, reducing their negative impact on ADG.

Reducing *in rumen* fermentation rate of barley

Barley grain is primarily a source of calories for livestock and often supplies more protein than is required for optimal growth. Barley grain as a whole does not differ widely from other grains based on chemical composition, although barley varieties vary dramatically. Barley grain tends to contain more total protein and higher crude fiber than corn and sorghum, but lower starch and lipid content (Kellems and Church 2002). Barley grain tends to ferment rapidly in the rumen and can result in acidosis. If unchecked, this can lead to bloat, liver damage, laminitis, and death.

Barley grain destined for beef cattle feedlots is often differentially priced based on grain bulk density. Heavy, high bulk density, high test weight grain is valued higher than is light, low bulk density, low test weight grain. Hinman (1978) reported a linear, positive response of bovine average daily gain (ADG) to increased bulk density using steam-rolled barley grain.

Grimson et al. (1987), McDonnell et al. (2003) and Boss et al. (2004) found no negative impact on bovine ADG related to barley bulk density when the barley was dry rolled, the most common feed preparation used in North America. The primary barley grain character (test weight) used to define barley feed value by the beef cattle industry appears to have little value in predicting bovine performance.

Owens et al. (1997) summarized and reanalyzed feedlot studies published between 1974 and 1997. These studies utilized a wide array of grains and grain processing technologies, but all provided complete metabolic datasets from yearling cattle study subjects. Metabolizable energy (ME) values for maize, sorghum, and wheat grain agreed closely with those provided by the National Research Council (NRC 1996). The NRC values underestimated ME for barley by 24%. More than 22,000 cattle participated in the studies summarized by Owens.

The Montana State University Ruminant Nutrition Laboratory, headed by Dr. Jan Bowman, summarized 18 bovine feedlot studies containing barley performed at Montana State University and the University of Idaho between 1993 and 1999. Fifty-six different barley-based diets were utilized in these experiments, and the analysis focused on identifying the characteristics of the grain that contributed to variation in animal performance (Surber et al. 2000). ADG, gain/feed ratio, net energy for maintenance (NE_m), and net energy for gain (NE_g) were negatively correlated with *in situ* dry matter digestibility (ISDMD). NE_m and NE_g were positively correlated with grain starch content. Utilizing multiple regression, barley NE_m could be well predicted by three grain characteristics, starch percentage (a positive factor), particle size after dry rolling (a positive factor), and ISDMD (a negative factor) ($r^2 = 0.87$, $p < 0.001$). ADG was well predicted from acid detergent fiber (ADF) percentage (a negative factor), grain starch content and particle size (both positive factors) ($r^2 = 0.75$, $p < 0.001$). Selecting new, improved feed barley varieties for use in bovine diets should be feasible using measurements of ADF, grain starch percentage, ISDMD, and particle size after dry rolling, if we can reduce *in rumen* grain degradation rates to acceptable levels.

These findings stimulated a search for barley lines that were degraded more slowly in the bovine rumen, that had high grain starch percentage, low ADF, and that produced large particles upon dry rolling. A search of available barley germplasm, based on particle size, ISDMD, and grain starch percentage resulted in the release of "Valier" barley (Blake et al. 2002).

The tendency toward overly rapid ruminal fermentation is an obvious target for improvement. Bowman et al. (2001) evaluated 1480 entries from USDA's barley core collection with the objective of determining whether variation could be found among barley cultivars and landrace accessions for *in rumen* digestibility. Substantial variation was observed among the landraces, and far less among widely grown modern varieties. Lines were identified that fermented far slower than standard cultivars (Table 16.1).

Table 16.1 The amount of variation in grain characteristics associated with feed quality for cattle available in the USDA's barley core collection (Table reconfigured from Bowman et al. 2001)

	Starch (%)	ADF (%)	Particle Size (mm)	ISDMD (%), 3 h *in rumen*
Low DMD entries	Mean: 50.1	Mean:5.7	Mean: 1.27	Mean: 33.3
	Range: 38.7–57.7	Range: 1.6–9.6	Range: 1.12–1.57	Range: 18.7–46.7
High DMD entries	Mean: 53.9	Mean:5.0	Mean: 1.19	Mean: 42.2
	Range: 43.4–59.3	Range: 1.5–8.6	Range:1.12–1.29	Range: 31.4–51.0
Cultivars	Mean: 55.1	Mean:4.6	Mean:1.17	Mean: 44.8
	Range: 51.8–57.0	Range: 2.2–6.5	Range: 1.14–1.20	Range: 38.5–51.7

ADF, acid detergent fiber; DMD, dry matter digestibility; ISDMD, *in situ* dry matter digestibility.

Most of the accessions that digested slowly in the *in rumen* digestibility assay did so because they were remarkably high in ADF percentage. However, six lines were identified that digested far less completely in the 3 h *in rumen* test, and which had >53% starch. One of these lines, PI370970, a six-rowed hulless landrace line from Switzerland, was low in ADF (2.2% vs. 5.0% for Valier), high in grain starch content (53% vs. 50.1% for Valier), slowly digesting (9.5% digested in 2 h *in rumen* vs. 33.4% for Valier), and produced reasonably large particles upon dry rolling (Valier and PI370970 produced particles with an average size of 1.4 mm upon rolling). Crosses were made between PI370970 and several locally adapted varieties, including Valier, "Baronesse," and "Tradition." As was expected, most of the variation for ADF percentage apportioned to segregation at *Nud/nud*. Since hulless grain has lost about 10% of the weight of the grain in nearly pure fiber, this trait also impacts protein and starch percentage. ISDMD variation largely apportioned to *Vrsl*, the gene controlling head type, or genes very closely linked to *Vrsl* (Abdel-Haleem 2004).

Increasing grain hardness tends to result in increased particle size after rolling. The puroindoline genes and their alleles are well characterized in wheat but until recently were less well characterized in barley. The laboratory of Dr. Mike Giroux at Montana State University addressed this deficiency by surveying a group of barley global germplasm lines, analyzing kernel hardness, ruminal digestibility, starch content, and particle size, and then sequencing hordoindoline alleles for the three barley analogues of the wheat puroindoline genes (Turuspekov et al. 2008). Significant associations were identified between *Hina* and *Hinb* allele states and particle size.

Improving grain starch percentage

Karl Klages, in an unusual choice of parents in the early 1960s, crossed Everest, a barley landrace from Nepal, with Good Delta, a landrace from Egypt. He topcrossed the F_1 to Traill, a then widely grown 6-rowed malting barley cultivar, and followed with two backcrosses to Traill. The variety "Karl" resulted. Karl's weak straw and marginal yield potential resulted in its failure as a commercial variety, but in the 1980s, Karl produced grain with the lowest protein percentage among the varieties grown in western North America.

Chee et al. (2001), following the work of Joppa et al. (1997), mapped a gene that impacted durum wheat (*Triticum durum*) grain protein percentage to chromosome 6B. See et al. (2002) mapped a gene impacting grain protein percentage in barley to a similar position on barley chromosome 6H. This allelic series in both barley and wheat appeared to impact on grain protein phenotype similarly. In barley, the high grain protein percentage allele is most common, while in cultivated wheat, the low grain protein percentage allele appears to be fixed. Both allelic series appear to be due to mutations in a transcription factor of the *NAC* family (Uauy et al. 2006). The low grain protein allele in barley was brought into modern barley cultivars through the variety Karl, from the Nepali parent Everest. The high grain protein allele was brought into cultivated durum and hexaploid wheat from an accession of *Triticum dicoccoides*.

In barley, the low grain protein allele from Karl results in grain with an average decrease in grain protein percentage and a similar increase in grain starch percentage. This transcription factor appears to have a major impact on plant senescence, with the low grain protein allele conferring senescence delayed by about 4 days.

These barley and wheat genes, and the variation their alleles confer, appear to be one of the clearest examples of Vavilov's law of homologous series in variation (Vavilov 1922). In this case, mutations from functional to nonfunctional forms of this transcription factor confer broadly similar effects in both barley and wheat.

SUMMARY

We have learned how to improve barley as a feed grain for bovine feedlots. We need to utilize the *vrsl* allele from PI370970 as a source of reduced

ISDMD. We need to utilize the *Hina2/HinB2* haplotype to increase grain hardness and particle size. We need to utilize *nud* to reduce ADF. In addition, we need to increase kernel starch content. Hulless, low fiber, high starch content grain should also result in improved ADG in sheep, swine, and poultry production systems. For poultry and swine production systems, employment of the heat and pH stable β-glucanase technologies developed by Wettstein at Washington State University should be helpful.

Those of us who are interested in improving barley as a crop are blessed with a wider range of genetic resources than are those working with most crops. As grain resources become limiting in the coming decades, we should, as a community, identify breeding targets that will help mitigate the problems that we will encounter as the human population grows and the climate becomes more challenging. Feed quality is one of these targets, and barley is uniquely suited for quality improvement.

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Chapter 17
Food Uses of Barley

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INTRODUCTION

As a food grain, barley is probably the least understood of the small grains and has been largely ignored by major cereal companies and consumers alike even though barley has many desirable attributes. This cereal grain can be successfully blended into many food products at various levels, and in many instances it adds texture, flavor, aroma, and nutritional value to the product. Regardless of the desirability of a food, acceptance is usually related to culture and social status of the consuming population. Breads and pastries of various types along with porridge have long been the primary use of cereal grains. As with oats, rice, and rye, barley is not comparable to bread wheat in providing gluten-containing flour required to make light white fluffy bread. Over the years, as nations became more prosperous, people's preference for grain in bread progressed from barley to rye and then to wheat (Taylor 1918). It is also evident that a similar change occurred in the greater use of rye and oats, especially the latter in porridges or hot breakfast cereals compared to barley. With the advent of ready-to-eat (RTE) breakfast foods, wheat and maize share this market along with rice, rye, and oats, with almost total exclusion of barley. Beyond the awareness of hulless barley and kernel weight differences, very little improvements were made to improve food quality parameters of barley in North America until the latter part of the twentieth century. The recently acquired awareness of the benefits of healthy diets that deliver fewer available calories, high in dietary fiber, low in fat, especially saturated fatty acids and trans-fatty acids, rich in antioxidants and other protective compounds, has triggered the reemergence of barley as a natural, inexpensive, available food source that meets these criteria.

Almost 20 years ago, a strong case was made for barley foods based on research showing health benefits and suitability for barley inclusion in food products (Newman and Newman 1991). Since that time, there have been minor changes in increased usage of barley as a food grain largely through the efforts and increased awareness of barley by cereal scientists, nutritionists, and grower-supported organizations. In 2006, barley joined oats in an approved health claim for reducing the risk of cardiovascular disease (FDA 2006). Success in obtaining the health claim was due largely to the team efforts of the National Barley Foods Council, the National Barley Growers Association, and the National Barley Improvement Committee. The health claim for both barley and oats is based on relatively high levels of soluble fiber in the cell walls of these two grains. Soluble fiber as found in barley and oats has proven effective in reducing cardiovascular risk by reducing serum cholesterol when consumed regularly and in small but adequate amounts.

Considerable variability exists in the concentration of soluble fiber components in both oats and barley due to cultivar and environmental growing conditions. Barley cultivars have been identified and selectively bred that contain two to four times as much of soluble fiber as regular

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market oats. A connection with the consumption of barley foods and control of diabetes has resurfaced after being all but ignored for hundreds of years. This attribute of barley is possibly more important than the cholesterol link given the epidemic levels of type 2 diabetes, which is currently being experienced in the United States and most other developed countries. In writing about the nutritional components of a food such as the barley kernel, it is most often forgotten that these compounds were first intended for the continuation of the plant's life cycle, not to feed some other organism. Thus, other biological systems simply take advantage of "a good thing." This is especially true for barley, with the exception of vitamins A, D, K, C, and B^{12}, and the mineral calcium. The barley kernel is packed with all of the other nutrients utilizable for the sustaining of human life. Specific compounds in barley are promoted for their roles in controlling cardiovascular disease and diabetes. In addition, protective compounds against invasion of microorganisms in the barley kernel are now recognized also as possible protective agents against various maladies in humans, including cancer.

This chapter covers a brief history of barley foods, structure, and nutrients of barley grain important for food uses, processing, and recent findings on the inclusion of barley in various food products, including challenges involved with this application of barley. Also discussed are physical and compositional grain traits directly associated with food processing and product quality which suggest directions for the development and improvement of food barley varieties.

HISTORICAL OBSERVATIONS ON THE USE OF BARLEY AS FOOD

Archaeological research concerning the lifestyle and history of mankind in ancient civilizations has provided convincing evidence that both *Hordeum vulgare* subsp. *vulgare* L. (domesticated barley) and *H. vulgare* subsp. *spontaneum* C. Koch (wild barley) were important foodstuffs along with Spelt, Emmer, and Einkorn wheat (*Triticum spelta*, *Triticum dicoccum*, and *Triticum monococcum*, respectively). It is generally accepted that modern domesticated or cultivated barley is a direct descendant of wild barley, both being subspecies of *H. vulgare* (Zohary and Hopf 1988; Nevo 1992). *H. vulgare* subsp. *spontaneum* is presently found growing in the Fertile Crescent (Southwest Asia), North Africa, and other regions of the world where cultivated barley is believed to have been first domesticated. Two recent papers describe the identification of wild barley and wheat seeds in a prehistoric site located on the southwestern shore of the Sea of Galilee in present day Israel dating to 22,000–23,000 years ago (Nadel et al. 2004; Piperno et al. 2004). Previous to this finding, the use of barley in foods and beverages in ancient civilizations dating as far back as 10,000 years had been documented by archaeological discoveries in many parts of Asia, North Africa, and Europe (Harlan 1978; Molina-Cano and Conde 1980; Xu 1982; Zohary and Hopf 1988; Diamond 1997). Of special note is the continued use of barley for food in Tibet other high altitude and mountainous areas in Asia, in much the same manner as hundreds or perhaps thousands of years ago (Shelton 1921; Tashi 2005).

As early as about 3000 BC, barley was a staple food utilized in various types of breads and cakes throughout Europe, especially in the British Isles and Scandinavia (Mikelsen 1979). The well-known Scottish bannocks and scones were probably first made almost entirely of barley (Gauldie 1981). Theobald et al. (2006) investigated the nutritional properties of *Bere*, a Eurasian landrace barley, reported to have possibly been introduced to the Orkney archipelago in the eighth century AD by Norse invaders (Jarman 1996). *Bere* barley is possibly the oldest-known barley retaining a varietal name designation. The composition of *Bere* barley is not remarkably different from modern barley with the exception of the content of β-glucan which is on the lower end of reported values (Theobald et al. 2006). Perhaps the extended use of *Bere* as food barley even though it is a hulled type is due to the low β-glucan characteristic. Barley remained a staple ingredient in porridges along with peas and oats in the British Isles but often was the only cereal ingredient in Scandinavian porridge and stew recipes up until

early in the twentieth century (Munck 1977). The development of bread wheat was the beginning of the decline of barley breads and pastries although barley flatbread, bannocks, and scones still remain popular fare in parts of Scandinavia and northern Scotland, especially in the Orkney Islands.

Although Stix (2008) and other scientists have presented solid evidence of human migration across the Siberian-Alaskan land bridge to the American continents from Eurasia, there is no evidence that either wild or domesticated barley was brought to the new world with these migrant people. Historical records show that barley's introduction to the Americas was at two separate locations and much later in time than the first human incursions of these continents through the northern route.

The Spaniards first brought barley to the North American continent through what is now Mexico and over a century later, English, German, and Dutch settlers introduced it along the East and Northeast coasts of North America (Weaver 1950; Wiebe 1978; Capettini 2005; Philbrick 2006). Columbus is credited with the first introduction of barley to the New World on his second voyage in 1493, and although records indicate some barley was seeded in the spring of 1494, there is no record of successful crops from that effort (Thacher 1903). Later efforts by the Spanish explorers to grow barley in Mexico were more successful. Barley lines that were introduced were adapted to the Mediterranean climates of Southern Europe and North Africa and thus were successfully grown in the similar climates of Mexico, Arizona, and southern California (Hendry 1931, 1934; Harlan 1978). It is most probable that barley moved into Central and South America from Mexico as barley represented a major food source in many high mountain regions of the continent. In the mountainous and rural areas of Ecuador, barley is still an important food (Villacŕes and Rivadeneira 2005). Immigrant settlers coming to what is now the United States and Canada, with the exception of the Mexican north border areas, were from western and northern Europe bringing barley types typical of those adapted to and grown in western and northern Europe at that period of time (Weaver 1950; Wiebe 1978). As the population moved inland across the midsection of the North American continent, barley production was concentrated near population centers primarily to provide grain for the inevitable breweries and to feed livestock. During those times of discovery and colonization of the New World, barley was the essential ingredient in the production of beer, providing a source of potable liquid whereas available water was generally recognized as contaminated. Beer was especially important as a liquid source on the sailing ships transporting the European explorers and immigrants across vast oceans. Often times, the availability of beer determined the length of the voyages and thus was a factor in determining the location of settlements (Philbrick 2006).

From purely a philosophical viewpoint, it becomes obvious when reading about the long history of barley foods and drink, just how versatile this grain has been through the ages in furnishing nourishment for humans across the globe. From the beginning of the written word (and no doubt much earlier), barley has been praised for its great utility in providing sustenance for the masses. Yet when times are good and there is plenty of wheat, rice, oats, and so on for all, barley falls into disfavor for food but not so much for drink. When times are hard, barley is there in the background as sort of a "safety valve." A notable relatively recent example of this occurred in Korea in the late 1960s and 1970s, when the government promoted consumption of barley through a national campaign and also mandated the incorporation of barley as a rice extender in school lunch boxes and restaurant meals to supplement the shortage of rice. Research efforts of the government and private enterprise were aimed at improving the quality of barley for its use as a rice extender and to expand the uses of barley in various food products, including noodles, bread, pastry, and snacks. Production of steamed and rolled grain as well as pearled and split grain were introduced to improve the appearance and organoleptic properties of cooked barley, and to shorten the required cooking time so that barley and rice grains could be mixed and cooked at the same time. However, synchronized research to define

the quality traits of barley for food use and to develop barley varieties uniquely fit for food use was mostly absent. Then very quickly, when a sufficient supply of rice in the 1980s became available due to increased agricultural productivity and economic prosperity, the consumption of barley in Korea plummeted, and the attention on food uses of barley has dissipated.

ANATOMY AND COMPOSITION OF THE BARLEY KERNEL

The nutritional value and health-promoting potential of barley foods depends first on composition of the kernel and second on processes applied to the kernel in preparation of products. Mechanical separation of the kernel during processing alters the components of the final products due to the marked differences in the anatomy and composition of the various parts of the kernel.

Anatomy

The mature kernel is composed of the hulls, rachilla, and caryopsis. These component parts of the barley kernel do not contain the same or similar amounts of nutrients. Thus, it is in the best interest of barley grain processors to recognize these differences and use them to enhance the quality and nutritional value of a product. The following is a brief description of the anatomical characteristics of the barley kernel as described by Reid and Wiebe (1978) and Reid (1985). The reader is referred to these publications for more details on the morphology and anatomy of barley.

In the developing floret and kernel, the ovary and stamens are enclosed within two flowering glumes. These structures, the lemma and palea become the hulls, the outermost portion of the mature kernel. They are principally composed of lignified cellulose, and silica providing protection against inclement weather and invading bacteria and fungi. Hulls are cemented tightly to the surface of the pericarp in hulled types and are not attached or are only loosely attached in hulless types at kernel maturity.

The caryopsis consists of the pericarp, epidermis nucellus, testa (seed coat), endosperm, and embryo (germ). The pericarp and the epidermis nucellus at maturity are thin tissue layers surrounded by the hulls. The testa, a tough insoluble fibrous membrane, lies beneath the pericarp and epidermis nucellus and on top of the aleurone layer, providing further protection against invading microorganisms and moisture. The testa almost completely encompasses the kernel, effectively separating the exterior from the interior of the grain.

The endosperm is the largest portion of the kernel, consisting of the aleurone, subaleurone, and the starchy endosperm. The endosperm contains a network of roundish, oblong, or globular cells extending from the outermost part of the aleurone to the center of the starchy endosperm. The cell walls are made up of a complex matrix of non-starch polysaccharides (NSPs) which vary in concentrations among the three tissues. This part of the kernel is the storehouse for nutrients and enzymes that are used for germination and growth of the new plant until emergence.

The embryo or germ is possibly the most complex tissue of the kernel although making up only a small portion of the total by weight. It is located on the dorsal side of the caryopsis at the end attached to the rachis. The embryo is attached to the endosperm by the scutellum, a flat connective tissue. Genetic material necessary for initiating the growth of a new plant is located in the cells of the embryo in addition to numerous subcellular structures. Subcellular constituents include mitochondria, protein bodies, spherosomes, Golgi bodies and rough endoplasmic reticulum, nuclei, and thin cell walls traversed by cytoplasmic threads.

An average mature barley kernel consists of approximately 13% hulls, 2% pericarp plus testa, 5% aleurone, 76% starchy endosperm plus the subaleurone, and 3% germ plus the scutellum, dry weight basis.

Nutrient components

Nutrient levels are controlled by genotype, environmental growing conditions, and the inter-

actions that often occur between these two modifying factors. For the most part, control of nutrient composition in the barley kernel is quantitatively inherited and is determined by multiple genes and alleles, although there are single alleles which enact major influences on selective nutrient levels. Environmental growing conditions that influence barley nutrient composition include available moisture, air and soil temperature, soil fertility, and cultural practices during critical growing periods. The latter includes items such as soil preparation, weed and insect control, planting date, and plant density. The most noteworthy effects are due to available moisture and nitrogen (assuming other factors are within acceptable limits), allowing growth of the plant with maximum filling of the kernel with starch, protein, and lipids. In the case of drought stressed plants, starch filling will be decreased, resulting in increased protein and fiber proportions. Barley kernels that are "filled" with starch granules are plump and heavy while those of drought-stressed plants are thin and light. There is a considerable range in kernel plumpness and weight which are also affected by row type, variety, date of planting, and climate during grain filling (e.g., frost). Cell wall thickness of endosperm tissues as well as major fiber component levels are significantly increased under drought conditions.

The major chemical (nutritional) components of the mature kernel are carbohydrates (starch, sugars, and fiber), protein (amino acids), lipids (fatty acids), and ash (minerals) that are generally reported as averages, when in reality, the levels may differ greatly (Table 17.1). Genetic removal of the hull has a major effect on fiber type and level and has small but significant effects on other nutrients. Mutant barleys have been identified and have been developed commercially that greatly differ from the reported averages, especially in carbohydrates (starch and fiber) type and level, protein, and amino acid levels. In some instances, the mutant barleys have unusually low amounts (<20%–30%) of starch and high levels (25%–35%) of fiber (Eslick 1981; Hofer 1985; Åman and Newman 1986; Munck 1992; Morell et al. 2003; Topping et al. 2003). Nevertheless, average composition data as presented in Table 17.1 are widely and routinely used in describing the nutritional value of barley.

Table 17.1 Typical composition (%) of hulled and hulless isotype barleys, dry matter[a]

	Hulled		Hulless	
Item	Mean[b]	Range	Mean[b]	Range
Protein[c]	13.7	12.5–15.4	14.1	12.1–16.6
Starch	58.2	57.1–59.5	63.4	60.5–65.2
Sugars[d]	3.0	2.8–3.3	2.9	2.0–4.2
Lipids	2.2	1.9–2.4	3.1	2.7–3.9
Fiber	20.2	18.8–22.6	13.8	12.6–15.6
Ash	2.7	2.3–3.0	2.8	2.3–3.5

[a]Adapted from Åman and Newman (1986).
[b]$n = 3$.
[c]N × 6.25.
[d]Glucose, fructose, sucrose, and fructans.

Carbohydrates: starches and sugars

Barley starch, the largest nutritional component, is composed of two high-molecular-weight polysaccharides, amylose and amylopectin. Amylose is a non-branching helical structure of glucose, linked α-1→4 in a long chain. Amylopectin consists of branched chains composed of 24–30 glucose residues also united by α-1→4 linkages in the chains and by α-1→6 linkages at the branch points. Amylopectin is visualized as existing in a cluster or tree-like structure as opposed to the helical structure of amylose. Normal barley starch contains ≈77% amylopectin and ≈23% amylopectin. The normal ratio of amylopectin to amylose is greatly altered by the recessive waxy gene (*waxwax)* located on chromosome 1(7H). When expressed, the *waxwax* gene produces starch containing 95% to 100% amylopectin. (Barleys containing starch with high levels of amylopectin are called waxy. The term "waxy" has no reference to the presence true wax compounds only to the high concentration of amylopectin and is not limited to barley as there are varieties of waxy maize, wheat, sorghum, and rice.) Zero amylose waxy barley was produced in a hulless barley breeding program in Canada (Bhatty and Rossnagel 1997) and in Japan by artificial induction (Ishikawa et al. 1995; Ajithkumar et al. 2005).

Table 17.2 Carbohydrates (%) and extract viscosity (cp)[a] of nonwaxy and waxy barley genotypes, dry matter basis[b]

	Nonwaxy		Waxy	
Item[c]	Hulled	Hulless	Hulled	Hulless
Starch	55.9	61.3	51.5	58.5
Sugars[d]	2.3	2.9	5.0	5.5
Fiber				
Total dietary fiber	17.0	13.2	19.6	13.8
Soluble dietary fiber	4.4	4.9	5.9	6.3
Arabinoxylans	6.2	4.4	6.7	4.6
Cellulose	3.8	2.1	4.4	1.9
Lignin	2.0	0.9	1.8	0.9
Total β-glucans	4.4	4.7	5.3	6.3
Soluble β-glucans	2.6	2.6	3.2	3.4
Extract viscosity	2.8	3.1	3.3	4.9

[a]Centipoise units.
[b]Adapted from Xue et al. (1997).
[c]$n = 6$ for hulled types and $n = 12$ for hulless types.
[d]Glucose, fructose, sucrose, maltose, and fructans.

On the other end of the spectrum, the *amo1* gene identified in a mutant of the six-rowed variety Glacier is located on chromosome 5(1H), and a gene in a mutant of Himalaya located at the *sex6* locus on chromosome 1(7H) produce barley starch containing 40%–70% amylose, respectively (Merritt 1967; Walker and Merritt 1969; Morell et al. 2003). The latter authors suggested that the starch synthase *IIa* gene (*sslla*) is altered in the mutant line Himalaya-292 leading to a decrease in amylopectin synthesis to less than 20% of the parent and consequent increased amylose content of the mutant line. These starches and barleys are referred to as "high-amylose."

Waxy barley traditionally contains less total starch than comparable normal starch types, but the reduced level of starch is usually accompanied by small increases in the simple sugars, sucrose, glucose, and fructose, and significant increases in the total dietary fiber (TDF) in cell walls. The TDF component increased is principally a mixed linked polymer of glucose residues commonly referred to as β-glucan (Ullrich et al. 1986; Xue et al. 1997). High-amylose barley also contains less starch and higher levels of β-glucans (Newman and McGuire 1985; Bird et al. 2008). The linkage between increased β-glucan levels and the starch types is not clear at this time, but it is probably a quantitatively inherited effect. Increased β-glucan levels are in part, if not totally, the results of increased cell wall thickness in endosperm and aleurone tissue. This has been shown in normal starch barley (Aastrup 1983) as well as in altered starch barleys. The carbohydrate composition of normal, waxy, and high-amylose barley genotypes is summarized in Table 17.2.

Carbohydrates: fiber

The definition of fiber in foods has been a controversial subject for many years, but most experts agree that fiber is composed of carbohydrate constituents that are not digested by mammalian enzymes. Although not a carbohydrate, lignin (not to be confused with lignan) is considered a component of fiber because of its close association with many plant fibers such as cellulose. Fiber in the barley kernel is principally composed of lignin and three large-molecular-weight NSPs, cellulose, β-glucans, and arabinoxylans that are structural components in the cell walls of the various tissues.

Cellulose is a linear NSP made up of glucose residues joined through β-(1→4) linkages in a long ribbon-like structure and is found in greatest concentrations in seed coat (testa) and hull, although small amounts occur in the germ, aleurone, and starchy endosperm. Most of the

lignin in barley is associated with cellulose in the hull.

The β-glucan as referred to here and in the paragraph above, is a mixed linked β-(1→3) (1→4) glucopyranosyl polymer where blocks of two, three, or more contiguous β-(1→4) linked glucose residues are separated by single β-(1→3) linkages (Parrish et al. 1960; MacGregor and Fincher 1993). The irregularly spaced β-(1→3) linkages interrupt the straight ribbon-like structure of cellulose-like sections, creating 90° bends in the molecule. More than 90% of the 40°C water-soluble barley β-glucan can be viewed as a copolymer of cellotriosyl and cellotetraosyl units linked by single β-(1-3) linkage (MacGregor and Fincher 1993). Approximately 10% of the remaining β-glucan consists of longer blocks of up to 14 adjacent β-(1→4) glucan blocks providing a more cellulose-like inner structure (Woodward et al. 1983; Edney et al. 1991; Wood et al. 1994). It has been postulated that these more lengthy units of β-(1→4) linkages may increase the extension of the molecule in space (Buliga and Brant 1982), which cause an increased radius of molecular gyration, increasing volume occupancy of the molecule (Robinson et al. 1982). The aqueous solubility of β-glucans is attributed to the internal molecular structure created by the β-(1→3) linkages and the increased volume occupancy created by interspersed longer spans of β-(1→4) linkages. It is thought that glucose residues with adjacent β-(1-3) linkages are nonexistent in barley β-glucan, or they only constitute a small amount of the total β-glucan.

Arabinoxylans are the third group of structural non-cellulosic polysaccharides of barley kernel cell walls consisting predominantly of the pentose sugars arabinose and xylose. Arabinoxylans in barley consist of a β-(1→4) xylopyranosyl backbone that carries α-L-arabinofuranosyl residues. Phenolic acids, ferulic and ρ-coumaric are covalently associated with arabinoxylans constituting approximately 0.05% of cell walls in the starchy endosperm (Viëtor et al. 1993) and 1.2% of aleurone cell walls (Bacic and Stone 1981).

Fiber components are under genetic control and may be severely modified by environmental growing conditions. Regardless of this fact, there is general agreement that genetic background is the more important of the two controlling factors (MacGregor and Fincher 1993). However, unlike the genetic control of the cellulose content (removal of the hull with the *nudnud* gene), the genetics of the barley kernel β-glucan content has proven to be complex and not completely determined (Ullrich 2002). Thickness of aleurone endosperm cell walls can be genetically manipulated, creating changes in β-glucan levels as well as arabinoxlan levels. Aastrup (1983) and Molina-Cano and Conde (1980) identified a simple inheritance factor that produced low-β-glucan mutant barleys. An additive genetic system of three to five factors controlling β-glucan levels was described by Powell et al. (1989), but chromosome location was not known. In a mapping study involving Steptoe/Morex crosses, Han et al. (1995) reported three quantitative trait loci (QTLs) located on chromosome 2(2H) and one QTL located on chromosome 5(1H) accounting for 34% of the total β-glucan content in that particular population. Less is known about the genetics controlling arabinoxylan concentrations in barley, but as with β-glucan levels, there is agreement among researchers that cell wall thickness is somehow involved. Henry (1986) suggested that variation in arabinoxylan content and the xylose-arabinose ratios varied somewhat with genotype but were more influenced by environmental factors. Small kernels with less endosperm due to genetics or drought conditions will produce higher levels of arabinoxylans, since this fiber component is more concentrated in outer tissues such as the aleurone.

Protein

Protein is either classified as true protein, a compound composed of a mixture of L-amino acids, or crude protein, which is a calculated value based upon the total nitrogen content. Analytical procedures for true protein are difficult, time-consuming, and expensive, whereas nitrogen determination is relatively simple, quick, and inexpensive. For estimating protein, the nitrogen content is generally multiplied by 6.25, as most proteins or mixtures of proteins contain fairly

close to16.0% nitrogen, that is, 100/16 = 6.25. Not all proteins contain 16.0% nitrogen, and errors are obviously made in these estimations; however, the blend of proteins from barley's major components usually contains the postulated 16% nitrogen. In the last 20 years, the analytical equipment and procedures for measuring amino acids in barley and other biological materials have become more practical and provide more informative and accurate evaluation of true protein. Therefore, when an accurate evaluation of protein is required, an amino acid analysis is recommended.

From a single nutrient standpoint, starch (energy) is the major nutrient in cereal grains although cereal proteins (amino acids) furnish significant amounts of energy as well as the nitrogenous needs of humans and other animals. When the nitrogenous factor is removed, the metabolic energy of protein is equal to that of starch. Total protein and starch are negatively correlated in barley; thus plump barley that contains high starch is relatively low in protein. The quality of dietary protein is as important as quantity, and both are superior in barley compared with maize and sorghum and may equal or exceed total protein found in wheat, rye, triticale, and oat. Barley generally contains more total protein than either brown rice or white rice, but the protein quality of brown rice is equal to or is greater than that of barley protein, especially when barley contains ≥12% total protein.

Quality protein may be briefly defined as a protein that contains enough of the 9, 10, or 11 essential amino acids (depending on species and age) to meet the needs of an individual, human or animal. Essential amino acids are those that must be preformed in the diet as they cannot be synthesized at all or in sufficient amounts by animal or human metabolism. Cereal grain proteins, including those of barley, are generally limited (insufficient amounts) in the essential amino acids lysine, methionine, threonine, and tryptophan. Lysine is the most limited of these, with some variation in the amounts of the other three depending on the cereal grain and the individual's (human or animal) requirements. In developed countries where other sources of protein, such as meat, milk, and legumes, are readily available in more than adequate amounts, the quality of protein is of less importance than in countries where a major source of the protein is from cereals. In the latter case, high-quality protein can make major differences in the growth curves of children and the health and well-being of the entire population.

Using QTL analysis, it has been determined that there are regions on all seven chromosomes associated in some way with the control of barley kernel protein content (Zale et al. 2000; Ullrich 2002). Protein level in the barley kernel is thus quantitatively inherited and is considered to be very complex. Shewry (1993) reported that all of the hordein proteins (storage proteins) are encoded by structural genes on chromosome 5(1H). Even without complete knowledge of which genes are involved, it is easily demonstrated that barley kernel total protein is dramatically increased with the application of nitrogen fertilizer providing the availability of adequate moisture and proper ambient temperatures. Unfortunately, the protein increase under these conditions is due primarily to increased hordeins, the storage proteins that do not contain a biologically balanced mixture of amino acids, being low in lysine, methionine, threonine, and tryptophan. The effect of total protein level on amino acid content of barley protein is shown in Table 17.3 (Newman and McGuire 1985). Protein increases in the kernels produced an increase in total quantity of all amino acids except cystine (as % of the kernel), which did not increase beyond the protein level of 12.7%. When expressed as a percentage of protein level, the amino acids tended to decrease with increased total protein. Lysine exhibited the greatest decrease, dropping nearly 24% from the low to the high protein level. Thus, it is not valid to make definitive comparisons of protein quality with other grains or barley varieties having different protein levels. Hulless barley will contain slightly more total protein than equivalent hulled varieties as hulls are low in protein. The barley kernel does not contain equal levels of protein or amino acids throughout it. Typically, the aleurone layer and the germ contain greater amounts of the essential amino acids, whereas the

Table 17.3 Amino acid composition of barley, expressed in terms of kernel weight and in terms of total protein as influenced by total kernel protein, dry matter basis[a]

	% of Kernel				% of Protein			
Protein level (%)	9.7	12.7	14.3	16.5	9.7	12.7	14.3	16.5
Amino acids								
Alanine	0.39	0.45	0.53	0.59	3.98	3.62	3.74	3.58
Arginine[b]	0.50	0.65	0.70	0.79	5.12	5.27	4.89	4.80
Aspartate	0.62	0.72	0.81	0.90	6.40	5.79	5.66	5.46
Cystine	0.25	0.33	0.32	0.32	2.56	2.67	2.21	1.95
Glutamate	2.39	3.24	3.90	4.50	24.60	26.18	27.28	27.30
Glycine	0.36	0.44	0.47	0.55	3.70	3.52	3.26	3.34
Histidine[b]	0.23	0.30	0.32	0.38	2.42	2.38	2.30	2.28
Isoleucine[b]	0.36	0.46	0.53	0.59	3.70	3.72	3.74	3.58
Leucine[b]	0.68	0.87	1.00	1.13	7.11	7.03	7.01	6.84
Lysine[b]	0.41	0.44	0.49	0.54	4.27	3.52	3.45	3.26
Methionine[b]	0.18	0.21	0.23	0.28	1.85	1.76	1.63	1.71
Phenylalanine[b]	0.48	0.63	0.75	0.90	4.98	5.07	5.37	5.46
Proline	1.05	1.42	1.69	2.08	10.81	11.48	11.81	12.62
Serine	0.47	0.55	0.64	0.76	4.84	4.45	4.51	4.72
Threonine[b]	0.36	0.45	0.49	0.58	3.70	3.62	3.45	3.50
Tryptophan[b]	0.19	0.24	0.27	0.32	1.99	1.96	1.92	1.95
Tyrosine	0.29	0.37	0.41	0.48	2.99	3.00	2.88	2.93
Valine[b]	0.48	0.62	0.70	0.77	4.98	4.96	4.89	4.72

[a]Means represent 4 samples, 2 two-rowed (cv. Compana CI 5438) and 2 six-rowed (cv. Unitan CI 10421) barleys grown in the same field in plots with increasing nitrogen fertilizer levels in the same year. Adapted from Newman and McGuire (1985).

[b]Considered to be essential amino acids for adult humans, but in some cases may be "spared" or partially replaced by other amino acids, that is, cystine for methionine and tyrosine for phenylalanine.

endosperm contains more of the nonessential amino acids. Even though hordein protein is of limited nutritional value to humans and other monogastric animals, the fact that the barley plant has the innate ability to quantitatively increase such large amounts of storage reserves is remarkable. Protein quality improvement in barley was intensively researched following the identification of the *Opaque-2* gene responsible for high-lysine maize by Dr. Ed Mertz (Mertz et al. 1964), later to be known as Protein Quality Maize (Bressani 1994). In almost parallel findings with barley, Lars Munck and coworkers at The Plant Breeding Center in Svalöv, Sweden identified Hiproly (*lys1*), a high-protein (17%–18%), high-lysine barley (4.5 g Lysine (Lys)/16 g nitrogen (N)) from Ethiopia (Munck et al. 1970). At that time, a number of Danish scientists at The Risø National Laboratory at Roskilde, The Royal Veterinary and Agricultural University, and Carlsberg Research Laboratory in Copenhagen were involved in developing, testing, and comparing high-lysine mutant barleys. One of the more successful of the many mutants produced was Bomi Risø 1508 (*lys3a*) containing about 5.5 g Lys/16 g N (Ingversen et al. 1973). However, from the very beginning of the high-lysine grain research, reduced kernel size and weight resulting in shrunken endosperm became major drawbacks due to improper starch synthesis and subsequent kernel filling, severely reducing yield. Dedicated research led to solving much of this problem in both maize (Bressani 1994) and barley (Munck 1992). The genetic base of normal barley protein containing about ≤3.5 g Lys/16 g N from high-yielding Danish malting varieties, Triumph and Nordal, was introduced into Bomi Risø 1508 (*lys3a*), and through a series of selection produced

a relatively high-yielding high-lysine barley, CA 700202 containing 6.5 g Lys/16 g N (Bang-Olsen et al. 1987; Munck 1992). At the time this barley was developed, it was nearly equal in kernel measurements and yield of varieties used as checks or controls. Whereas high-lysine maize has met with some commercial success, the high-lysine trait has not been utilized in commercial barley in any appreciable amount.

Lipids

Lipids are a class of nutrients that furnish concentrated energy (2.5 × more than that of soluble carbohydrates) which may be stored or used in metabolic reactions. Lipids are located throughout the barley kernel and although concentrated in the germ (18%), the greatest amount of lipid is located in the aleurone and endosperm. Åman et al. (1985) reported that the total extractable lipids in 115 barley kernels ranged from 2.1% to 3.7% of dry matter, having a weighted average of 3.0%. Major fatty acids in barley triacylglycerol are 23% palmitic (16:0), 13% oleic acid (18:1), 56% linoleic acid (18:2), and 8% linolenic acid (18:3) (Anness 1984; Morrison 1993). Nonpolar lipids, principally triacylglycerol and free fatty acids, represent about 75% of the total lipids in the whole grain, while the remaining 25% is somewhat divided between the polar lipids, glycolipids, and phospholipids (Briggs 1978).

There has been only limited success reported in genetic selection and breeding for increased total lipids in barley kernels. The high-lysine mutant barley, Risø 1508, was reported to contain higher than normal levels (3.5%–5.3%) of lipids (Bhatty and Rossnagel 1979; Newman et al. 1990b; Munck 1992), but this was attributed to the severely shrunken endosperm of this barley (Morrison 1993). However, it is worthy of note that the high-lysine cultivar developed from Risø 1508, CA 700202 (Bang-Olsen et al. 1987), has plump kernels with a higher starch content than the mutant parent, yet maintains the higher level (≥5.0%) of total lipids (Newman et al. 1990b; Munck 1992).

Vitamins

Among the many individual chemical compounds that are necessary for normal metabolism in humans and other animals are the water-soluble (B-complex) and fat-soluble (A, D, E, and K) vitamins. The original vitamin concept was that these compounds were required accessory food factors and could not be synthesized by human or animal metabolism. Whole grain cereals in general are good sources of the B-complex vitamins except vitamin B12 which is not found in higher plants. Barley is no exception, with these vitamins located in the outer layers of the caryopsis and in the germ. Nicotinic acid (niacin) levels are four to five times higher in barley than levels found in maize, oats, and rye. However, 85%–90% of the niacin in cereals is biologically unavailable as it is strongly bonded to a protein in all raw grains. Processing with alkali solutions, as in the preparation of tortillas, breaks the bonding of niacin with polysaccharides and glycopeptides, rendering it biologically available. Some processing involving heat can damage or destroy some of these B-complex vitamins that are heat labile. Average levels of B-complex vitamins from five sources of published data expressed in milligram/kilogram are as follows: thiamin (B1) 5.2, riboflavin (B2) 1.8, niacin 63.2, pantothenic acid 5.1, biotin 0.14, folic acid 0.43, pyridoxine (B6) 3.5, and choline 1290 (Newman and Newman 2008). Choline 1290 is not considered a true vitamin as it is synthesized in human and animal metabolic process to some extent.

Barley does not contain vitamin C (ascorbic acid) nor the fat-soluble vitamins A, D, and K, but does contain high levels of the vitamin E complex composed of four isomers each (α, β, γ, and δ) of tocopherol and tocotrienol. α-Tocopherol (8.7 mg/kg) and α-tocotrienol (32.3 mg/kg) are the isomers most recognized for their potent antioxidant capacity in mammalian metabolic systems. A weighted average from seven reports of the total tocols (tocopherols + tocotrienols) in barley was calculated to be 63.0 mg/kg (Newman and Newman 2008).

Ash

The ash or mineral matter found in barley ranges from 2.0% to 3.0% with hulless types trending toward the lower level and hulled types toward the higher level. The higher level observed in hulled types is due primarily to silicon, which is not considered a nutrient. Minerals are found throughout the human body providing structural support, and are integral components of metabolic systems such as energy transfer in metabolic reactions, acid–base balance, fluid balance, and are components of complex systems with hormones and vitamins. The minerals are classified into two groups, macro- and micro-minerals, by the amounts found in most foods and the amounts required by a biological system. The average amounts of macro-minerals (g/100 g) found in barley are: calcium 0.05, phosphorus 0.35, potassium 0.47, magnesium 0.14, sodium 0.05, chloride 0.14, sulfur 0.20, and silicon 0.33. Micro-mineral levels (mg/kg) in barley are: copper 6.25, iron 45.7, manganese 27.2, zinc 34.4, selenium 0.4, and cobalt 0.07. These data are weighted averages taken from eight research reports (Newman and Newman 2008). The high level of silicon (0.33 g/100 g) is indicative of hulled barley.

Calcium is the most limiting mineral in barley as far as the human requirement for this element. The phosphorus level in barley is also limiting in meeting nutritional requirements. However, when baker's yeast is added to bread or cookie dough, an enzyme (phytase) produced by the yeast releases the phosphorus from the bound state in phytic acid. A selective breeding program at the USDA Laboratory at Aberdeen ID has produced barley lines with low levels of phytic acid but at the same time maintaining the same level of free and nutritionally available phosphorus (Raboy and Cook 1999; Raboy et al. 2001).

Phytonutrients and grain color

Discoloration of barley kernels is most often attributed to humid or wet conditions during grain development that promote the growth of fungi and bacteria producing grains that appear to be covered with smut or soot. Li et al. (2003) suggested that barleys tolerant to discoloration by weather can be a solution to this problem. Genetic variability to discoloration tolerance existed among barley varieties studied by Miles et al. (1987), Young (1997), and Edney et al. (2002). Li et al. (2003) mapped QTLs for grain discoloration tolerance, detecting one to three QTLs for grain brightness in various populations, finding that each QTL accounted for 5%–31% of the phenotypic variation.

Numerous compounds and elements have been identified in the barley kernel that at first appeared to have no known structural role or participation in metabolic reactions other than to create kernels of various colors. It is now thought that their primary roles in the kernel are for protection against invading insects, bacteria, and fungi. A number of these compounds have been identified as biological antioxidants and when consumed, may possibly play roles as protective agents against certain maladies afflicting humans, including types of carcinoma, cardiovascular disease, and degenerative diseases such as arthritis. As a result, these compounds have been cast with the nonspecific title of phytochemicals or phytonutrients. By and large, the majority of these organic compounds are more concentrated in the outer tissues of the barley kernel. This particularly applies to proanthocyanidins (flavonols), ρ-cumaric acid, and ferulic acid (Zupfer et al. 1998). Holtekjølen et al. (2006) reported that ferulic acid was the most abundant phenolic acid in 16 cultivars, accounting for 52%–69% of the total phenolic compounds. Although most phytonutrients are nonstructural, ferulic acid is esterified to cell wall arabinoxylans and lignin in aleurone tissue (Bunzel et al. 2004). Proanthocyanidin and flavonol concentrations have been intensively researched (Holtekjølen et al. 2006). The main flavonols found were catechins, procyanidin B_3, and prodelphinidin B_3. Mazza and Gao (2005) is an excellent reference source for the genetics and characteristics of anthocyanins and other phenolic compounds found in barley.

Aside from the normal light yellow color of barley kernels, many shades of blue, red, purple, and violet are possible in kernels due to anthocyanins and their interactions with other phenolic compounds. True black pigmentation of barley is due to melanin-like compounds in the kernel (Choo et al. 2005). Although only light yellow and blue kernels are grown extensively, black or dark purple barleys are often grown as novelties and for use in special food recipes for color (Abdel-Aal et al. 2006).

When cooked, pearled, or milled, most barley products often develop gray, grayish blue, or other unattractive dark colors (Knuckles et al. 1997; Başman and Köksel 1999; Marconi et al. 2000; Quinde et al. 2004; Izydorczyk et al. 2005; Ereifej et al. 2006; Erkan et al. 2006; Lagassé et al. 2006). The darkening of cooked barley products is a real concern of food manufacturers and it is considered one of the major obstacles preventing the widespread use of barley as a standard food. Nutritional value is unaffected, but the dark discoloration is less appealing to consumers.

Colors may result either from enzymatic or nonenzymatic reactions or both. The latter effect may result from polymerization of endogenous phenolic compounds and Maillard reactions. Enzymatic browning is caused mainly by polyphenols oxidase (PPO) activity, which oxidizes phenolic compounds to *o*-quinones, which in turn condense and react with other phenolic compounds or amino acids causing dark discoloration. Extensive studies on the discoloration potential of barley grain and grain food products have been reported (Quinde et al. 2004; Quinde-Axtell et al. 2005; Quinde-Axtell and Baik 2006; Baik et al. 2008). A wide variation among barley genotypes was observed in the darkening potential or resistance, apparently related to the total phenol content, especially proanthocyanidins, and to polyphenol oxidase activity. Pearled kernels, gel, and flour of proanthocyanidin-free types tend to be brighter white and remain so longer with cooking than proanthocyanidin containing types.

As one would expect, the presence and type of color altering compounds are under genetic control. Genetic control of proanthocyanidins has possibly been more extensively researched than other compounds because of their known negative effects on clarity of beer. Specific genetic control of these compounds in barley and chromosome location of some of the controlling genes may be found in the *Barley Genetics Newsletter* (1996). More emphases may be placed on these compounds in future research because of their possible health-promoting potential. This is a fascinating and growing area of research that strongly supports the movement toward consumption of whole grain barley (Marquart et al. 2007).

PHYSICAL CHARACTERISTICS OF BARLEY KERNELS

Hulled versus hulless

The *Nud* (hulled)/*nud* (hulless) gene is located on chromosome 1(7H), (Franckowiak and Konishi 1997). This is a simply inherited characteristic that can be expressed in all types of barleys regardless of genetic background. In hulled barley, a glue-like substance is secreted by the caryopsis on or about day 16 after anthesis cementing the lemma and palea (hull) to the pericarp (Harlan 1920). The hull becomes firmly attached over the period of grain filling, but attachment is not complete until about 10 days before a kernel reaches maximum size. The hull completely surrounds the kernel but is not attached to the embryo (Gaines et al. 1985). In hulless barleys, the glue-like substance is not secreted, allowing the dry hulls to be removed from the rest of the kernel during combining at harvest. This is an overly simplified description of harvesting hulless barley with modern combines, as in reality as much as 15% of the hulls remain with the kernels unless the mechanical parts that separate the kernels from the chaff are set to remove total chaff. Under such conditions, cracked kernels and germ become part of the chaff and loss is practically unavoidable. To get maximum amounts of hulless grain, the harvesting machine cannot be set the same as with hulled barley. Thus, a grain processor must have cleaning equipment designed to carefully remove the

hulls that invariably remain with combine-run hulless barley in order to have uncracked whole grain (minus the hull only) hulless barley. "Whole grain" has become a popular term among cereal scientists and nutritionists, but with barley, it is necessary to recognize that it is a generally inaccurate terminology for hulled varieties. Hulled barley must be pearled or processed in some manner to remove the hull in order to make edible products. Removal of the hull by pearling also removes the germ and various levels of the caryopsis depending upon the degree of pearling.

Kernel hardness

Kernel endosperm hardness or softness is regarded as one of the major quality criteria of wheat in cultivar selection programs (Pomeranz and Williams 1990).

Grain hardness in wheat and/or barley has been measured as particle size, particle size index, the Perten Single Kernel Characterization System® (SKCS) (Fox et al. 2007), pearling index, grinding time, energy required for grinding, starch damage, crushing or slicing kernels, and near-infrared analysis (Halverson and Zeleny 1988; Osborne 2006), and milling energy (Fox et al. 2003). Although considerable variation in kernel endosperm texture has been observed in both wheat and barley lines, much less is known about barley than wheat. However, it is well established that soft kernel texture is preferred for malting barley (Allison et al. 1976; Fox et al. 2007).

Puroindolines are endosperm-specific proteins that control the majority of endosperm texture variation in wheat. These compounds are sometimes referred to as friabilin, a starch granule protein in wheat having multiple components (Morris et al. 1994). Jagtap et al. (1993) reported the presence of wheat friabilin homologues, hordoindolines in barley though in smaller amounts than in the homologues in wheat. The occurrence of hordoindolines in starch granules isolated from both hard and soft endosperm barley and the presence of both hordoindolines *a* and *b* in the mature barley endosperm suggested a role for these compounds in barley endosperm texture as with wheat (Darlington et al. 2000). However, Darlington et al. (2000) could show no clear relationship between the presence of hordoindoline and grain texture.

Hordoindolines have been mapped by QTL analysis in the Steptoe × Morex population revealing several areas of the genome possibly associated with grain hardness. The largest QTL mapped to the *HinA/HinB/Gsp* region of the short arm of chromosome7(5H) explained 22% of the variation in this population (Beecher et al. 2002). Through sequencing of single nucleotide polymorphisms, Fox et al. (2007) found that three hordoindoline genes showed only two haploids for each gene, and these showed no clear relationship with grain hardness. Thus, it appears that despite large variation that has been observed in the grain hardness of barley genotypes, the difference in hordoindoline gene sequences show relatively small or no association with barley grain hardness. Hardness or softness of barley endosperm is quite likely influenced by other components beside hordoindolines and structural characteristics of aleurone and endosperm cell walls. Concentration of the major cell wall constituents, β-glucan and arabinoxylan, has been shown to be significantly correlated with kernel hardness and water uptake (steeping) in an Australian study (Gamlath et al. 2008). Protein level had no effect.

Kernel size, kernel weight, volume weight, and kernel shape

As with the characteristics already discussed, these characteristics are also controlled by genotype, the environment, and their interaction. Approved methods for obtaining these measurements were developed with hulled (covered) barley and thus are not applicable for use with hulless (naked) barley until such time that approved methods are developed. These characteristics are used to determine the suitability of barley for processing and, to some extent, the basic nutrient levels, protein, and starch. These two nutrients are highly and negatively correlated, so kernels having high starch content will almost invariably have a low protein level and vice

versa (Åman and Newman 1986). High starch content correlates with larger endosperm; therefore, nutrients concentrated in other parts of the kernel will be somewhat reduced as a percentage of the kernel. The alleles controlling row type and the hulless characteristic are major factors in controlling kernel physical characteristics and are the most easily visualized. In two-rowed barley, only the central floret is fertile, and two lateral florets are sterile, resulting in a single seed at each node, producing a flat appearance to the spike. In six-rowed barley, all three florets are fertile, producing three seeds and giving the spike a rounded or tubular shape. Two-rowed kernels are symmetrical being from the central rows, and the principal variation in size is between larger kernels in the middle of the spike and smaller ones at the base and the tip of the spike. In six-rowed barley, approximately one-third of the kernels are symmetrical (centrals) as in two-rowed types, and the remaining kernels are asymmetrical (laterals), as they are twisted somewhat at both ends but with a more pronounced twist at the site of attachment on the spike. Symmetrical central kernels of six-rowed barley are slightly pinched in laterally at the attachment end in contrast with two-rowed kernels, which are usually broad at this site. Row type is controlled by two genes, *Vrsl* and *Int-c*, which are located on chromosomes 2(2H) and 4(4H), respectively (Nilan 1964; Kleinhofs and Han 2002). In commercial barleys, two-rowed barleys have the genotype *VrslVrsl int-cint-c*, and six-rowed barleys have the genotype *vrslvrsl Int-cInt-c*.

Kernel size or plumpness measurements are accomplished using specialized sieves in the United States, according to Federal Grain Inspection Service (FGIS) instructions primarily for malting barley. Plump malting barley is barley that remains on top of a 6/64th × 3/4 in. slotted-hole sieve. Thin malting barley is defined as six-rowed barley that passes through a 5/64th × 3/4 in. slotted-hole sieve or two rowed barley that passes through a 5½/64th × 3/4 in. slotted hole sieve. Thin non-malting barley or hulless barley is defined as two rowed barley, or barley that passes through a 5/64th × 3/4 in. slotted hole. A good plumpness score would be 90%–95% plump kernels with 10% or less thin kernels. Plump kernels will contain significantly more starch, less protein, and less total fiber than will thin kernels.

Thousand kernel weight (TKW) provides a measure for average kernel weight to the nearest 1 mg per kernel or nearest 1.0 g for 1000 kernels. This measurement is easily obtained by accurately weighing 1000 kernels. TKW complements kernel size or plumpness as both are highly and positively correlated. Heavy kernels will be high in starch and low in protein compared with low starch and high protein in light kernels. A good example of TKW measurements is data recently obtained in a breeding program. The TKW of 42 two-rowed barley ranged from 43 to 50 g (mean = 46 g) while 25 six-rowed barleys TKW ranged from 36 to 42 g (mean = 39 g) (Dr. Dale Clark, Westbred LLC, Bozeman MT, personal communication). The TKW of hulless barley is reduced 15%–20% less than comparable hulled barley.

Volume weight, commonly referred to as *test weight*, is referred to as *hectoliter weight when using the metric system.* This measurement is currently expressed as kilograms per hectoliter (kg/hL) and determined with standardized laboratory equipment. Previously, volume weight was expressed in pounds per bushel in the United States. The standard test weight of hulled barley is 62 kg/hL (48 lb/bushel), but may range from 52 to 72 kg/hL. Hulless barley volume weights may be as high as 80 kg/hL (Ullrich 2002).

Kernel shape, in most barley currently used in the industry, is rather standard in the United States and Canada, being somewhat oval, elongated, and wider at the middle than at the ends with a crease or furrow on the ventral side. Thin kernels will appear more elongated and plump kernels will appear more rounded. Kernel length is more constant within genotype than kernel width and thickness, which are dependent on environment and cultural practices. In the United States and Canada, commercial barleys length ranges from 7 to 12 mm. Among world barleys, kernels may be as short as 4 mm and as long as 15 mm. Barley kernels almost completely round (globose) were described by Eslick (1979). Effects

of the two-rowed and six-rowed genes on kernel shape were previously discussed.

HEALTH BENEFITS OF BARLEY FOODS

In ancient times, barley foods had a reputation as health-promoting and strength enhancing; however, as noted earlier, the desirability of wheat for bread and other baked foods has taken over barley's status as a staple food. In recent years, barley has been recognized as having significant benefits in human health functions: cholesterol-lowering, blood sugar control, and colon health (Newman and Newman 2008).

Barley soluble fiber and serum cholesterol

Elevated blood cholesterol is a major risk factor for cardiovascular (heart) disease (CDC (U.S. Centers for Disease Control and Prevention) 2007), and numerous studies have established that soluble dietary fiber, specifically β-glucan found almost exclusively in oats and barley, is associated with heart disease prevention (Rimm et al. 1996). DeGroot et al. (1963) was the first to report the cholesterol-lowering effect of oats, and this has been confirmed by numerous studies with oats and oat products, creating a huge marketing opportunity for oat foods, particularly RTE breakfast cereals. Barley contains equal or greater levels of β-glucan as oats, and numerous clinical trials have affirmed barley's equal or superior value as a hypocholesterolemic food compared to oats (Newman et al. 1989; McIntosh et al. 1991; Ikegami et al. 1996; Behall et al. 2004). These reports allowed barley foods as well as oat foods to carry a health claim that they may reduce the risk of coronary heart disease (FDA 2006). Foods made from eligible barley sources must contain at least 0.75 g of β-glucan (soluble fiber) per serving. Eligible barley sources specified were whole grain barley, pearled barley, barley bran, barley flakes, barley flour, barley grits, and whole or sieved barley meal. Minimum total and soluble dietary fiber for these products were specified. The initial ruling did not include barley β-glucan extracts produced through wet milling processes, although such products may become standardized in the future (Brennan and Cleary 2005). As stated above, β-glucan is recognized as the component responsible for the cholesterol-lowering functions in both oats and barley. Creation of viscosity in the small intestine slows absorption of dietary lipids and additionally, β-glucan also binds bile acids and carries them to excretion, resulting in breakdown of body cholesterol to replace them. The net result of β-glucan ingestion whether from oats or barley, is a lowering of blood cholesterol, particularly low-density lipoprotein (LDL) cholesterol (Tietyen et al. 1990; Kahlon et al. 1993). In oats, the β-glucans are concentrated more in the outer portion of the kernel while in barley β-glucans are found in the aleurone, sub-aleurone and throughout the starchy endosperm. Wood (2007) provided an excellent review of the role of β-glucans in diet and health.

Barley tocols and serum cholesterol

The hypocholesterolemic effect of tocotrienols and tocopherols has been investigated in chickens (Qureshi et al. 1991), pigs (Qureshi et al. 1991), and in humans (Lupton et al. 1994). Moderate reductions in serum cholesterol were observed; however, this research has not been aggressively pursued. Barley and oats contain higher levels of the tocols than most other grains, although there are major differences among barley cultivars in tocol concentrations (Ehrenbergerová et al. 2006). Extracted barley oil and barley spent grain from brewing are more concentrated in tocols than whole grain and have been suggested as sources of tocols for food products. Additionally, roller milling and pearling produce fractions that are enriched in oil and tocols, making them potential sources of these compounds (Wang 1992).

Barley carbohydrates and diabetes

Ajgaonker (1972) described an effective method used by ancient Indian physicians to stabilize type 2 diabetes some 2400 years ago. The treatment was remarkably simple and not really very different from recommendations given to people with diabetes in modern times (i.e., lose weight, change diet, and increase exercise). In the case of diet, the

major changes recommended were reduced calorie intake and substitution of barley for white rice.

More recent studies of Sato et al. (1990) and Rendell et al. (2005) have demonstrated the positive effect of barley foods on human glucose metabolism. Recent aggressive research of the glycemic lowering effects of barley have focused on two different aspects; β-glucan as a viscous fiber, and the ratio of amylose and amylopectin. The gut viscosity effect of foods containing β-glucan results in delayed absorption of carbohydrate and subsequent low, flattened blood glucose peaks, whereas many other carbohydrate foods such as white rice or white wheat bread produce rapid onset, and sharp peaks in blood glucose. White wheat bread is used as a comparative standard for rapid and total peak levels in blood glucose after test food consumption. Foods with soluble fiber such as in barley have been said to contain "lente" or slow carbohydrate release. Whenever blood glucose rises, particularly in sharp peaks, the pancreas quickly releases insulin, to lower glucose to normalcy. Frequent consumption of high glycemic foods can create a condition of insulin resistance, which is a hallmark of the metabolic syndrome and may also lead to type 2 diabetes. The wide range of blood sugar responses to foods was the basis of the Glycemic Index (GI), a system of classifying foods according to their immediate effect on blood sugar, compared to a white bread standard. The GI has some application in making food choices, but is controversial because of conflicting results in some studies (Jones 2007).

Differing amylose/amylopectin ratios in grains provide distinct differences in glucose and insulin responses, and these effects can be magnified by heat treatment of the grain. Amylose molecules tend to associate and link together by hydrogen bonding to form gels and/or insoluble precipitates when subjected to moisture and heat, as occurs in baking. The resultant resistant starch (RS) is defined as the fraction of starch that is resistant to digestive enzymes and enters the large bowel of healthy humans as an intact large-molecular-weight polymer (Asp 1992). Barley flours made from the genotypic series of the cultivar Glacier, of which amylose content ranges from 25% to 40%, were found to decrease *in vitro* digestibility and increase the RS content with increasing amylose content (Björck et al. 1990). Åkerberg et al. (1998) prepared breads with the Glacier series of barleys and demonstrated reduced blood sugar response in human subjects corresponding to the quantity of RS, which ranged from 2% in waxy barley to 10% in high amylose barley bread. Barley has been shown to not only produce a flattened glucose peak in subjects, but also have a prolonged effect of this observation extending to the next meal. Nilsson et al. (2006) fed barley foods at an evening meal and demonstrated a lowered glucose response following that meal and also the following morning, after a normal breakfast. These authors attributed their results to fermentable properties of β-glucan and RS in the barley, which caused slow digestion and absorption of the barley meal.

Barley and bowel function

Fermentation of β-glucan and RS occurs in the large intestine, producing short-chain fatty acids (SCFA) especially butyrate and propionate. The benefits of these fatty acids to the intestine are provision of an energy source for epithelial cells, producing healthy colonic mucosa (Topping et al. 2003). Bird et al. (2008) compared effects of foods made from a high amylose barley (Himalaya 292) or wheat in healthy subjects. Biomarkers of bowel health (fecal weight, butyrate concentration, SCFA excretion, and fecal *p*-cresol concentration) were all significantly different between groups and indicative of improved colonic integrity in those subjects who consumed barley. The beneficial results were attributed to the fermentation of resistant starch.

Heart disease, diabetes, metabolic syndrome, and cancer are challenges in public health. Incidence of these diseases occurs not only in aging populations, but are increasing in younger people. Clinical trials have proven that lifestyle, including diet choices, have a profound effect on prevention and/or control of these health threats. There is ample evidence that barley can have positive effects on blood cholesterol, glucose

control, and colon integrity. Implementation of this evidence, leading to more common consumption of barley, depends on consumer education and greater incentives to the food industry to develop products, such as RTE breakfast cereals that can be readily incorporated into the daily diet.

BARLEY FOOD PRODUCTS

As previously stated, one of the primary health-promoting components of barley is its β-glucan content. This, however, creates a dilemma for food producers since β-glucan has a detrimental effect on certain baked products, causing low volume, increased water retention, and high density. Also, the health beneficial phenolic compounds impart undesirable dark colors in products. In addition, barley does not contain the gluten protein, as wheat does, and therefore does not support the characteristic aerated structure of wheat bread. However, several uses of barley for food products are described below and several commercial products made wholly or partially from barley are depicted in Fig. 17.1.

Fig. 17.1 Barley food products: barley bob (cooked barley and rice mixture, upper left), barley steam bun (upper right), waxy barley walnut-cookie (bottom left), and waxy barley pancake (bottom right). Steam bun reprinted with permission from Bobo's Jeju (Jeju.com) corporation (Seoul, Korea); barley walnut-cookie and waxy barley pancake with azuki paste filling reprinted with permission from Mac corporation (Anyang, Korea). For color details, please see color plate section.

Barley in yeast bread

Various yeast bread baking studies using barley flour as partial substitution for wheat flour have been reviewed (Newman and Newman 2008). The upper limit for inclusion of barley flour is generally considered to be 30%, in order to be considered acceptable by objective and sensory testing. The most common functional deficits are volume and density. As noted above, baked bread is always darker in color when barley is an ingredient. Various milling techniques have been employed, including production of fiber-rich fractions which can be used in smaller quantities relative to wheat flour. Different barley cultivars, ingredient proportions, various additives, and modified procedures have been compared, with varying results (Izydorczyk et al. 2000). Recent technological innovations have shown that it is possible to produce barley-containing bread with qualities considered desirable to modern consumer tastes.

One important breakthrough was made by an Australian study testing three barleys including Himalaya 292, a barley with high amylose as well as high β-glucan content (Mann et al. 2005). In this study, different wheat flours were also tested for suitability of a wheat-barley blend to produce good quality bread. Two quality parameters that showed promise as predictors of baking quality of the flour mixtures were gluten : protein proportion of the base wheat and extensibility of the dough measured by a texture analyzer. Andersson et al. (2004) investigated molecular weight and structural units of β-glucans in dough and bread containing hulless barley milling fractions. The average molecular weight of β-glucans decreased with increased mixing and fermentation times. Since retention of molecular weight of β-glucan is important for the cholesterol lowering property of bread, it was concluded that mixing and fermentation times be kept as short as possible. Jacobs et al. (2008) demonstrated that superior yeast breads containing high-fiber barley fractions were achieved with a sponge-and-dough process. Further studies on β-glucan molecular weights in bread were reported by Cleary et al. (2006). These authors found that the inclusion of commercially prepared β-glucan having high and low molecular weights in breads resulted in attenuated reducing sugar release during *in vitro* digestion. They also reported that low-molecular-weight β-glucan was less susceptible to destruction during baking.

Gill et al. (2002) had used precooked barley flour which provided advantages to bread quality. This study compared two barleys, CDC Candle (waxy starch) and CDC Phoenix (normal starch), and demonstrated that starch type has a profound effect on bread characteristics, and pretreatment of barley starch can have beneficial effects on bread quality. Izydorczyk et al. (2001) compared four Canadian wheat flours with four Canadian barley genotypes varying in amylose content. This study suggested that interactions between gluten strength and barley starch may overcome the negative effects of wheat gluten dilution by barley. Jacobs et al. (2008) compared suitability of different baking processes for incorporating high-fiber barley fractions into yeast bread made with strong wheat flour. The best process was a sponge-and-dough technique in which the fiber fractions were presoaked and added after the dough was developed. Symons and Brennan (2004) showed lowered *in vitro* sugar release from breads made with barley fractions, suggesting lower glycemic response for human consumption.

A multicenter research program was established in Europe, the SOLFIBREAD project, entitled "Barley β-glucan and wheat arabinoxylan soluble fibre technologies for health promoting bread products" (Trogh et al. 2005). The project involved extraction of arabinoxylans from wheat and separation into water-extractable and water-unextractable fractions. Water-extractable fractions form viscous solutions that enhance bread quality, whereas the water-unextractable fractions are detrimental (Courtin et al. 2001). Endoxylanases cleave water-unextractable arabinoxylans, reduce their water-binding capacity and increase viscosity, making them desirable for use in bread dough. In this project, wheat-barley breads were made with a composite flour of strong wheat and milled hulless barley in a 60:40 ratio with xylanase in the formula. Highly acceptable loaves were produced with no impact on functionality of the barley β-glucan. Achievements of

this project are promising for future development of barley products having desirable characteristics and positive health benefits.

Barley flatbread

Flatbreads are the world's oldest breads and remain traditional in many cultures of the world. The nature of flatbreads, having little or no dependence on dough fermentation or volume development, are ideal for incorporation of barley flour. Indeed, flatbread is indigenous in many barley growing areas, indicating that barley was possibly the original grain used to prepare this ancient bread. Contemporary research reports reflect the regional variety of flatbreads. Two types of Turkish flatbread, bazlama and yufka, made with blends of barley and wheat flours, were reported by Başman and Köksel (1999, 2001). Chapatis, unleavened flatbread originating in India, were produced with partial substitution of barley flour (Anjum et al. 1991; Sood et al. 1992; Gujral and Pathak 2002). Balady bread, often called pita or pocket bread, is commonly eaten in Egypt and the eastern Mediterranean region. Barley-containing versions were successfully made by Ereifej et al. (2006). Tortillas, native to Mexico and Central America, were originally only made with maize meal, but wheat flour is now commonly used as well. Efforts have been made to prepare tortillas with 100% hulless barley flour (Ames et al. 2006). Several barley cultivars were evaluated to determine an appropriate genotype. A major factor involved in barley selection for good quality tortillas were amont of water required for optimum dough consistency and avoidance of stickiness in dough rolling. From this study, the Canadian waxy hulless cultivar CDC Candle, which has low amylose and high β-glucan contents, produced the highest rated tortillas.

Barley muffins, pasta, and noodles

Barley can be easily and successfully incorporated into chemically leavened baked products such as muffins, quick breads, and cookies, even as 100% of the flour content. However, genotype of the barley plays a large part of certain quality factors (McGuire 1984; Newman et al. 1990a; Berglund et al. 1992 Hudson et al. 1992: Newman et al. 1998; Klamczynski and Czuchajowska 1999; Ragaee and Abdel-Aal 2006). Color development can be masked in muffins with other ingredients such as caramel, chocolate, or berries. Disguising color in barley pasta and noodles can be accomplished with certain food colors (green for instance) that are currently popular in wheat pasta and noodles.

Pasta, in various forms, is an integral diet component in many societies and has gained popularity in recent years. Current interest in whole grains has stimulated development of new pasta products with whole wheat and barley flour. Pastas made with high β-glucan barley fractions have been produced with acceptable characteristics, although darker in color than all-wheat products (Knuckles et al. 1997; Marconi et al. 2000). Dexter et al. (2005) compared barleys with variable levels of amylose incorporated into pasta dough. The best results were achieved using 20% barley flour, except when using zero-amylose barley, which reduced firmness. Further refinements of pasta quality were achieved by Cleary and Brennan (2006), who added high β-glucan barley fractions, rather than barley flour, to wheat flour. The use of such high-fiber fractions may have an important role in development of healthful products with acceptable organoleptic properties.

Noodles are dietary staples among consumers of Asian ancestry and have become popular in other cultures as well. There are numerous research reports of barley inclusion in both white noodles (Japanese) and yellow noodles (Chinese) (Kim et al. 1973; Change and Lee 1974; Han 1996). Baik and Czuchajowska (1997) did a comprehensive study with pearled ground hulless waxy and nonwaxy barleys added to white salted noodle dough at 10%–30%. Noodles were intensively evaluated for physicochemical characteristics. Compared to 100% wheat flour noodles, noodles incorporated with waxy barley flour were much softer, while the addition of normal amylose content barley flour produced noodles of similar texture to wheat noodles. All barley noodles exhibited a microscopic open network on the

surface, attributed to starch gelatinization and water retention. Yellow alkaline noodles were enriched with eight hulless barleys of varying starch types by Hatcher et al. (2005). Barleys were pearled and roller-milled into flour and added to noodle dough at 20% and 40% levels. There were distinct differences between barley starch types as well as β-glucan content. Normal and high-amylose starch had increased firmness and chewiness which are desirable attributes in pasta. This same Canadian research group (Izydorczyk et al. 2005; Lagassé et al. 2006) further refined these Asian noodle investigations, using barley fiber-rich fractions as well as barley flour. *In vitro* digestibility studies indicated decreased glucose release from barley noodles, which suggests a low glycemic index value, beneficial for prevention and dietary control of diabetes.

Pearled barley and rice mixtures

Pearled barley is the most familiar form of barley for modern consumers, who invariably use it in soups. Pearled barley-brown rice and precooked pearled barley-polished rice blends can be used to prepare traditional meat or vegetable soups without cooking the grains separately. In many Asian countries, where rice is the staple starch food, barley has traditionally been used as a rice extender, especially when rice is in short supply. In Korea, the word *bob* means cooked rice, and a mixture of barley and rice is called *barley bob*. This practice produces a healthful advantage by increasing soluble fiber in the daily diet. Ikegami et al. (1991, 1996) promoted the use of a barley-rice blend for both cholesterol-lowering and glycemic control. Hinata et al. (2007) reported markedly improved diabetes metabolic control among Japanese male prisoners who were provided a barley-rice blend in place of white rice alone. The concept of combining rice and barley as noted above has application in any household, in that brown rice and pearled barley take about the same cooking time, and white rice can be combined with the product "Quick Barley" a precooked product of The Quaker Oats Company, Chicago, IL. In both of these cases, soluble fiber can easily be introduced in the diet with very palatable results. Pearling quality of barley for food use has been investigated by Edney et al. (2002), focusing on whiteness, and Klamczynski et al. (1998), who studied starch gelatinization and cooking quality. Bulgur, a cracked grain product usually made from wheat kernels, but also applicable to barley, was prepared by Köksel et al. (1999) from three Turkish barley genotypes with very acceptable results.

PROCESSING BARLEY FOR FOOD USES

Whole grain processing

Most cereal grains require processing in order to make them more easily consumable and applicable for use in various food products. As with other food grains, the first step of processing is cleaning to remove foreign grains, impurities, and broken kernels using various types of specialized equipment. Following cleaning, moisture conditioning, or tempering, is usually a pretreatment for most processes (Kent and Evers 1994). Processing barley normally includes pearling and/or one or more forms of dry milling to achieve products for food preparation. A detailed review of modern barley processing can be found in Newman and Newman (2008). Several forms of processed raw barley can be seen in Fig. 17.2.

Blocking and pearling

Blocking and pearling are abrasive and scouring processes used to remove the outer tissues of the kernel. Blocking, sometimes referred to as dehulling, is an effort to only remove the hull that represents 10%–13% of the dry weight of the kernel. It is difficult to selectively remove only the hull because of the strong attachment of the hull to the caryopsis and generally unequal kernel size. Blocked barley generally has a portion of outer tissues and germ removed as well as the hull. After cleaning, hulled barley kernels are dehulled generally by pearling which not only removes the hulls but also portions of the outer layers including the testa, aleurone, and subaleurone tissues, and may contain substantial amounts of the endo-

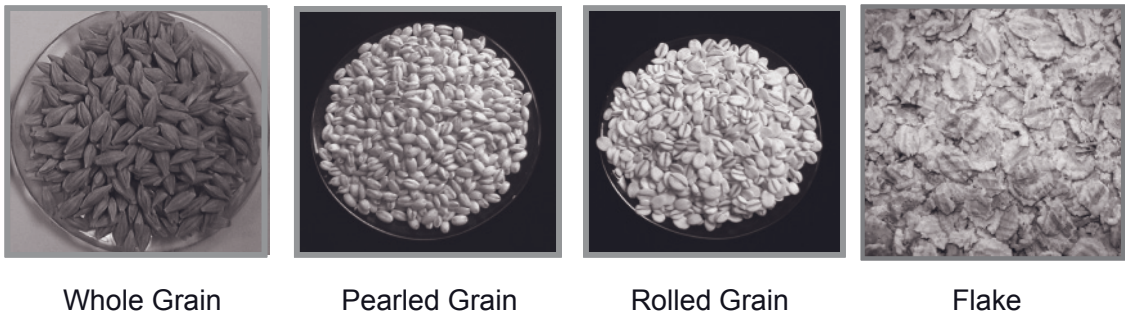

Fig. 17.2 Whole, pearled, and rolled barley grain, and flake. *Source:* Barley flake from http://www.sakthifoundation.org/kitchen_breakfast.htm. For color details, please see color plate section.

sperm. The product removed is generally called pearling flour or simply pearlings but is sometime referred to as "bran." This bran material is not the same as wheat bran or oat bran either physically or chemically. From this point, the kernels may be further rolled, flaked, or milled to flour. Hulless barley may be directly rolled, flaked, or milled to flour. However, hulless barley is often pearled for specific products or markets and to improve brightness of the final product. Pearling alters the composition and ratio of nutrients in the final products, given the varying concentration of nutrients that are present in separate tissues of the kernel. The pearling process is commonly practiced in three or more stages with less concern in retaining outer layers or germ. The degree of pearling is a percent of the kernel removed during the process. Differences in pearling characteristics within the same cultivar may be due to environmental factors affecting kernel size and hardness.

Successive pearling studies have shown that soluble dietary fiber and β-glucan levels increased in the pearlings by removal of up to about 30% of the kernel, while starch showed a continuous increase with pearling up to 80% of the kernel (Bach Knudsen and Eggum 1984). In another study by Pedersen et al. (1989), the hull and seed coat were in the first pearling fraction (11% of total), germ and aleurone made up the second pearling fraction (11%–25%), and the remaining material in the third fraction was composed primarily of endosperm. Extensive pearling studies comparing waxy and nonwaxy hulled and hulless barleys have been reported (Bhatty and Rossnagel 1998; Marconi et al. 2000; Zheng et al. 2000; Yeung and Vasanthan 2001). Pearled barley is an important rice extender in Asia, and barley quality requirements for that market were described by Edney et al. (2002). Many consumers, especially in Asian countries, demand symmetrical grains that resemble rice in size, color, and cooking time. To achieve specifications, the barley is often pearled, split, and pearled (or polished) again. These processes require barley grains with special texture, size, and color, and the authors of this study developed a ranking system for overall kernel desirability, which is based on pearling quality and visual appearance.

Grinding

Grinding is a form of dry milling for the production of whole grain flour or meal, which may be used as is or further separated into fractions. The earliest grinding in ancient times was carried out with stone mills, which are now used only for specialty products or historic display, such as the water-powered Barony Mill in the Orkney Islands of Scotland (http://www.birsay.org.uk/baronymill.htm). Hammer mills or pin mills are possibly the most common equipment for grinding (Kent and Evers 1994). Pearled barley is more appropriate for grinding with these types of mills as it is almost impossible to remove the small

parts of the hull from the flour without seriously reducing flour yield.

Roller milling

Roller milling, originally developed to mill wheat into white flour for baking purposes, can be used for barley by adjusting rollers, sifters, and screens. The cleanest flour, free from hull particles, will be produced from pearled barley. There are three objectives of roller milling: to separate the endosperm from the bran (outer portion of the kernel) and germ, to reduce the maximum amount of endosperm to flour fineness, and to obtain maximum extraction of flour from the grain. The number of parts of flour by weight produced per 100 parts of grain milled is called the flour yield or percentage extraction rate, which ranges from 72% to 80% for wheat. Barley has a much larger range in flour yield due to genotype differences due in large part to lack of breeding for milling quality (vs. wheat). There are two steps in roller milling: the break system which splits the kernels and crushes the outer layers, and the reduction system which reduces particle size of the kernel parts by a series of rollers and sifters. The by-products are shorts (intermediate) and bran, which includes the hulls from intact hulled barley.

The science of roller milling barley is young relative to processing wheat in this manner. Barley reacts quite differently from wheat, and techniques for milling barley are currently being developed. One major difference is that wheat bran is removed in large flakes whereas barley bran is brittle, breaking into small particles during the milling process. Small particles of the barley bran remain in the flour causing discoloration in the flour and bakery products (Jadhav et al. 1998). Furthermore, the widely variable characteristics of different barley genotypes complicate the development of standard methods for roller milling barley. Professor Ron Bhatty, a pioneer in processing barley with roller mills, conducted extensive studies in Canada during the 1990s (Bhatty 1993, 1997). Innovative newer research developments in barley milling have been reported recently (Kiryluk et al. 2000; Andersson et al. 2003; Izydorczyk et al. 2003; Ames et al. 2006). Roller-milling equipment designed for wheat milling was utilized to produce valuable barley fractions when tempering moisture levels were modified. Barley with varying starch and β-glucan levels required variation in milling flow steps as well as adjustment in degree of pearling prior to roller milling. Hulless barley produced high-fiber flour, shorts, and bran with large variation in composition among barleys, confirming the significance of variation between barley genotypes. Both β-glucan and starch type presented problems in production of standard straight-grade flour. The co-products, shorts and bran, offer options for quality fiber enhancement of an array of cereal-based products and other foods such as sausage.

Flaking

Flaking or flat rolling is a very popular type of processing for barley as well as oats. The grains are cleaned, sized, and tempered, then heated to deactivate undesirable enzymes, such as lipases that create off-flavors and odors during storage. The hot, moist grains are passed through flaking rollers to produce flakes of various thicknesses (Kent and Evers 1994). Barley flakes are similar to oat flakes, which are produced from dehulled oats or oat groats. It should be possible to make whole grain barley flakes using hulless types, but barley flakes are more routinely made with blocked or pearled barley. Flaking produces a desirable product with short cooking time and familiar porridge texture, and is appropriate for inclusion into cookies, muffins, bread/biscuit/roll topping, granola mixes, food bars, trail mixes, and other applications.

Secondary processing methods

Extrusion

Extrusion is a process used to convert dense grain formulations into lighter puffed or crisp products, such as RTE breakfast cereals or snack foods. The basic process uses high-temperature, short-term cooking, and forcing the product with rotating screws through a barrel. The combina-

tion of heat and pressure causes reorganization of the food molecular structure. As with other processing methods, barley presents unique challenges due to β-glucans and starch variability. However, extrusion offers the potential of desirable high demand products with enhanced health benefits (Berglund et al. 1994; Gaosong and Vasanthan 2000; Huth et al. 2000; Vasanthan et al. 2002).

Infrared

Infrared processing is an alternative heating technique described as thermal radiation. Infrared heating of barley grain causes starch granules to swell and gelatinize, permitting the grain to be pearled, ground, or flaked without the usual step of tempering or moisture conditioning (Skjöldebrand and Andersson 1987). In addition, infrared heat treatment has been shown to inactivate the peroxidase enzymes, as well as improve how the barley fractionates during milling, resulting in improved β-glucan levels in the fractions. Infrared treatment of hulless barley was reported by Ames et al. (2006), in reference to suitability of barley genotypes for roller-milling yields. The process is particularly suited to thin products such as chips and tortillas, because the dough dries quickly in preparation for further processing such as frying or toasting. Tortillas made from micronized flour had improved shelf stability and improved color.

Separation processing

Air classification

Air classification is a technique used to separate dry grain flour into different particle size groups. The procedure applies the technology of using moving air in a confined space to segregate a heterogeneous mixture into two groups, fine and coarse fractions. The barley must be hulless, blocked, or pearled, prepared as a whole meal, preferably by pin milling. Enrichment of selected components such as starch, protein, or β-glucan can be accomplished by air classification (Vasanthan and Bhatty 1995; Andersson et al. 2000).

Sieving

Sieving is an alternative method to air classification, for producing fractions with desired composition. Sieving refers to separation of ground grain into particle-sized classifications using size-designed sieves or screens, over which the material is moved. A combination of air classification and sieving may be more effective than either process alone. The application of different cereal processes for achieving specialty products has been reviewed by Jadhav et al. (1998).

Separation or isolation of important nutritional compounds in barley such as phenolic compounds, oil, tocotrienol, and particularly β-glucan, has been researched in recent years (Wang et al. 1993; Knuckles and Chiu 1995; Andersson et al. 2000; Lampi et al. 2004; Moreau et al. 2007; Zhang et al. 2007). Preliminary preparation of the grain may produce fractions with greater concentration of desired components, and further separation techniques or solvent extraction procedures are often employed. Recognition of the health benefits of parts of the barley kernel and ability to isolate valuable components provides a vast area for food product development. Increased levels of β-glucan, TDF, lipids, tocols, phenolics, and other compounds can be accomplished using standard pearling and roller milling, air classification, and sieving processes.

Products, the real need

Increased awareness of one's diet in improving health and longevity has created a tremendous amount of research searching for the perfect food. Such a food probability does not exist as a single ingredient, but barley has emerged as a cereal grain capable of contributing significantly to diets planned to be low in calories, high in fiber, and rich in antioxidants and other probiotic components. This knowledge has triggered a wealth of barley research as a natural, inexpensive, and healthy food source. Special attention has been focused on barley β-glucan, which has been scientifically proven to lower blood cholesterol levels and moderate blood glucose levels. Arabinoxylans in barley provide additional fiber creating a balanced fiber base. Body weight reduction and

strengthened immune system are other reported and/or claimed benefits of consuming barley.

One thing that is certain is that there is a wealth of knowledge available in the scientific literature concerning composition and health benefits of barley foods. However, there is a definite need of how to make barley products more available in food stores and more attractive to the consumer without compromising the nutritional and heath values. The authors are aware of current R&D efforts at some universities and private industry in barley product development. We do not wish to ignore these efforts nor seem ungrateful for current product happenings thus far. However, there have been some really exciting products developed by private industry that have not made it to the supermarket shelf.

SUMMARY

Barley, as with other cereal grains such as wheat and rice, has a long history of cultivation and consumption. Barley's popularity in cultivation probably comes from its genetic diversity and vigorous adaptability to various climes. Barley is readily available in the world and is relatively inexpensive compared with other cereal grains, but while its uses for feed and for brewing and distilling have been emphasized, its uses for food have been de-emphasized.

Cultural eating habits and lower preferences for barley compared to oats, rice, and wheat in many cultures have been formed over time. Possibly the major causes are tough fibrous hull that adheres tightly to the caryopsis, poor knowledge of hulless barley (even of its existence in many parts of the world), lack of wheat-gluten-like protein, high fiber in the aleurone and endosperm (β-glucans and arabinoxlans), and dark food product color. With an increase in awareness of human health benefits of consuming barley, however, there have been many recent attempts to boost the food use of barley by food and crop scientists, the barley industry, and food processors. The health benefits of consuming even low levels of barley soluble fiber are highlighted by the reductions of blood cholesterol and positive effects in diabetes control. Possibilities of reducing the incidence of obesity and improving bowel health through encouraging desirable microbial colonies in the digestive tract are other potential benefits of consuming barley. In addition, abundant tocols and phenolic compounds of barley are also considered as potential probiotic components.

For food use, hulled barley grain is dehulled and blocked or pearled to remove the outer fibrous layers; blocked or pearled grain may be roller milled, flaked, or ground to grits or flour. Barley flour can be air-classified into several fractions concentrated with specific components such as starch, protein, or fiber, and that can be fractionated to isolate β-glucan, starch, and protein. Dehulled and pearled barley is used as a rice extender, in soups and in pilaf. Barley grain that is flaked can be easily added to cookies, cereal bars, biscuits, scones, and bread, and may substitute oats and maize in cold and hot breakfast cereals. Barley flour can easily be incorporated into many wheat-based baked or non-baked products, such as cookies, bread, flatbread, tortillas, puffed snacks, noodles, and pastas.

The well-known health benefits of consuming barley have increased attention to the traits of barley required for processing and using barley in food formulations. Increasing the knowledge of genetically controlled traits in barley that are important for food processing and product quality will be essential for breeding new cultivars in the continuing efforts to increase the use of barley in foods. We have a relatively good understanding of the physical and compositional characteristics of barley grain, and of their variation among various types of barley. What we are not totally aware of is how to make the best use of these characteristics in food processing, new product development, and product quality improvement. Based on the identified processing needs, conventional food uses and other creative new potential uses of barley can be developed. Physical characteristics important in product development are volume weight, kernel size, shape, crease depth, hardness, and the hulless trait. Chemical components that have been identified as important in

product development include starch, protein, minerals, β-glucan, arabinoxylans, tocols, phenolic compounds, and polyphenolic oxidase. While there is much to learn about the relationships of these traits with processing and product quality of barley, the knowledge amassed thus far could be effectively utilized for the formation of guidelines for selecting, processing, and incorporating barley into foods.

Detailed information on the composition and physiochemical characteristics of barley as related to food uses is available in a review article "Barley for Food: Characteristics, Improvement, and Renewed Interest" (Baik and Ullrich 2008) and in the book *Barley for Food and Health: Science, Technology, and Products*" (Newman and Newman 2008).

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Chapter 18

Adapting Cereal Plants and Human Society to a Changing Climate and Economy Merged by the Concept of Self-Organization

Lars Munck and Birthe Møller Jespersen

INTRODUCTION AND SCOPE

It is most likely that the world population and the need for food and feed will increase by more than 50% up to the year 2050 to reach an annual grain production of 3.0–3.5 billion tons to support a population of 9–11 billion people. Such a change will force heavy strains on the environment of the entire planet, especially with regard to climate and the utilization of water resources. Cereal breeders, agronomists, and the industry need durable realistic predictions to match this change. The future utilization of cereals, which this chapter is focused on, with barley as an example, needs an improvement in efficiency by a diversified utilization of the whole plant for food, feed, and nonfood applications. In the years 2007–2008, grains have for the first time been in direct competition with fossil energy for fuel. The total result was rapidly increasing world market prices for grains. However, the world ecconomic recession in 2008–2009 reduced these prices back to normal in 2009. There is little doubt that food and energy prices in the long perspective will reach new heights, which are likely to be permanent, based on dwindling natural resources and increasing world population. Increasing prices will give a new interest in grading and payment for quality. The increase in payment to farmers creates entirely new incentives for the acceleration of agricultural development that is urgently needed to increase production. However, it also seriously affects the living conditions, especially for the poor people in the rapidly growing cities of the world that are increasingly depleting the resources from the countryside.

Industrialization based on cheap fossil fuels in industrialized agriculture has cut up the biological production chains and downgraded coproducts like straw as waste to be burnt in the field. This period in the history of mankind is now soon ending. In the last part of the chapter, we will discuss how a vitalized systems research effort focused on improving the symbiosis between the cereal plants and human society could implement sparing energy and stopping pollution by closing the industrial and biological production chains.

Today, science and the expanding electronic media in the rapidly changing globalized postindustrial society are focused on simple cause–effect relationships to find explanations and

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solutions for complex problems. Such a limited strategy is bound to produce serious environmental problems that rebound on human life by the impact of nature. Because the biosphere functions in many aspects as an integrated *emergent network*, surprising side effects of human causal interventions may appear as environmental problems that are more or less detectable. Likewise, on the molecular level, it has been proven that genes with designed functions transferred to plants, such as resistance to an herbicide, often have serious side effects, for example, on yield. However, the transferred gene may still be utilized in an adaptation of the genetic background by breeding for improved plant performance as a whole.

To understand the consequences of networking, the physical mathematical concept of "self-organization" as introduced by the Nobel laureate Ilya Prigogine (1997, 2003) comes to our assistance. The theory of self-organization implies that there are serious limitations in the present tendency to designed approaches in science built on limited causation. Instead, nature has to be asked by introducing pattern recognition technology or chemometrics (Martens and Martens 2000; Smilde et al. 2004; Munck et al. 2010), which enables overviews of the physical and chemical status of, for example, a cell, a plant canopy, and the biosphere. We fully acknowledge the impressive focused development of molecular biology that has led to a remarkable expansion in detailed knowledge of the cells and plants in nature, which is of great value especially to increase the genetic base of breeding. *However, the approach of molecular biology cannot stand alone for both theoretical and practical reasons. The effect of gene expression has to be considered in the context of the whole phenotype and its interaction with environment*. This chapter therefore focuses on new concepts, methods, and information that appear when spectroscopic and other instrumental overviews of cells, plants, and the biosphere are implemented. Science is here at the embryonic stage in the necessary development of new methods to tackle the possibilities and limitations of cause and effect in the self-organized networks of nature that are essential to build the future.

THE NEED FOR A CONNECTION BETWEEN THE MICRO AND MACRO ASPECTS OF THE WORLD

Uncertainty and probability are well established in the scientific view of the subatomic micro world as producers of the potential observables, the outcomes of which in quantum physics have a statistical nature. However, in the macro world of human sensing, the number of observables in nature is overwhelming and often confusing. Here, determinism (a specific DNA sequence, the sunrise) and uncertainty (length of life, the weather) seem to coexist in a complex, puzzling way.

The mathematical nonintegrability limit in modeling complex emergent systems

Mathematics shapes thinking in science. Theoretical physicists in the micro world are intrigued by the effectiveness of mathematics in natural sciences. Mathematics mediates both conservation and change. The classical physicists, in their search for mathematical formulations to understand the micro world in thought experiments, focused on ideal "integrable systems" where time was reversible and internal side effects such as collisions between particles were excluded so that equations could be solved. The great mathematician and physicist Henri Poincaré formulated more than 100 years ago (Prigogine 1997) a theorem, as an extension of the three-body problem, on "the nonintegrability of dynamic systems due to resonances between the degrees of freedom." Today, we can look upon Poincaré's theorem as a mathematical formulation of the environmental dilemma in connecting cause and effect. Consider a shot in a game of billiards that is bouncing off many balls in a cascade. According to Poincaré, such a cause–effect development cannot be predicted in mathematics because the reciprocal colliding events between several balls are producing *an environment of secondary collisions*, of which the impact on each ball is impossible to predict by mathematical modeling. However, by the assistance of a film from a video camera mounted above the billiard table, the

outcome of the play can be mathematically reconstructed and described backward as a unique phenomenon.

There is consequently a need for a dynamic overview of the complex parts in a system from nature. Such dynamic nonlinear developing systems are *emergent* (Kauffmann 1995; Penrose 1997; Lucas 2008), which signifies that the whole system cannot be described alone by following its components. Nonintegrability also implies irreducibility and is noncomputational. This implies that data gained, for example, from molecular analyses in the cell, cannot be used to make simulations of the dynamics of *the whole* performance of the cell in the computer (Penrose 1997). Kauffmann (1995, p. 23) concludes, "We must instead simply stand back and watch the pageant." That should be accomplished by a "global" analytical approach to describe the level of organization, structure, and entropy as is required in thermodynamics (Prigogine 1997). But is such a demanding approach at all realistic? And to what extent? Here we will aim at answering that question.

Remarkably enough, there is a rich parallel world of noncomputability in an analogous separate chaotic mathematic universe, different from Prigogine's quantum mathematics of physics and its chemical and biological applications that are built on emergent algorithms such as random Boolean networks (Kauffmann 1995), cellular automata (Wolfram 2002), and fractals (Kirilyuk 2005). The dynamic output from these algorithms can be visualized as intricate, artful patterns on a graphic display that can neither in detail be predicted nor exactly analyzed by mathematics.

Analyzing the macro world by classical statistics, biochemistry, and molecular biology as "surgery in networks"

J. Pearl in his visionary book *Causality* (Pearl 2000) defines causality thinking and data compression by mathematical modeling as surgery in networks. Causality is a man-made intervention focusing on a limited part of the network by and large disregarding the secondary effect of "a cause" for the whole network. *For causality to make sense an a priori man-made pathway model or hypothesis needs to be cut out from the whole network.*

The classical statistics of Pearson and Fisher is an excellent tool on the gamete level to study the segregation of qualitative Mendelian characters that, for example, are expressed as a visual pattern, such as the barley seed rachilla hair trait. The recombination of these genes in nature is more or less free and in accordance with the statistical model. However, when classical statistics is used in a quantitative trait locus (QTL) model to analyze gene expression on the phenotype (zygote) level, the assumptions of free variance of the variables in the model violate the strongly covariate network of gene interactions in data (Munck 2007, 2009). Consequently, Nadeau and Frankel (2000) argued that mutagenesis is a more efficient way than QTL to discover complex developmental and physiological processes because conventional QTL loses power rapidly as the complexity of gene expression increases. Then, chemometric pattern recognition models equal to multivariate analysis (Bjørnstad et al. 2004) and above all data inspection are less destructive and would lead to more consistent and reliable results.

At the 10th International Barley Genetics Symposium in Alexandria in 2008, computer data visualization methods without data compression were presented. DNA data from barley populations from gene banks were directly related to collection location and environmental response, interpreted as "association genetics."

J.D. Watson's book *Molecular Biology of the Gene* in 1965 started "the molecular movement" that introduced confidence in the causal "bottom-up" surgery in the network of gene expression by molecular techniques starting from the ultimate cause—the DNA sequence of a gene. Since then, the exploratory "top-down" skills of pattern recognition of gene expression by observation of phenotypes demonstrated by classical scientists such as Linnaeus and Darwin and further by plant breeders, botanists, and cytogeneticists lost attention in favor for a bottom-up causal analysis.

This paradigm shift was inspired by gene sequencing, molecular techniques, and by differential equation modeling in the Newtonian physicists' tradition (Omholt 2006). However, here, the mathematical limits in modeling emergent biological systems by destructive analyses were largely overlooked as in the QTL case.

At present, the rise of molecular biology has resulted in an underestimation of the necessity in phenotyping by careful observation of phenomenological characters in plants *as a whole*. The phenotyping skills that are needed to describe the mutant gene banks of barley collected in the last 80 years (Lundqvist 2009) are now on their way to be ignored and forgotten.

A need for a method to recognize the unique result of self-organization in an overview

We can now formulate the prevailing problem in genetic modeling of the phenotype as "the difficulty in finding suitable measurements and statistical models that can do justice to the qualitative and qualitative description of the *whole* phenotype."

Already in 1926, the prophetic geneticist S.S. Chetverikov wrote, "The concept of pleiotropic action of genes consists of the idea that every gene may influence not only the specific character corresponding to it, but a whole series of others; generally speaking the entire soma …. The very same gene will manifest itself differently, depending on the complex of the other genes in which it finds itself. For it (the gene), this complex, (and) this genotype will be the genotypic milieu, within the surroundings of which it will be externally manifested …. The concept of pleiotropic action of genes releases genetics from the extremely heavy ballast accumulated recently [in 1926!] in the shape of all kinds of special genes 'enhancers,' 'weakeners' or 'modifiers' of other genes, the number of which has now grown to absolutely threatening proportions." Obviously, Chetverikov's holistic concept of pleiotropy introduces a problem of *indeterminacy* in explaining how cause and effect is mediated to the phenotype through the internal "genotypic milieu."

The classical developmental geneticist C.H. Waddington (1970), who launched the term epigenesis for cell development and differentiation, focused on the genetic *indeterminacy* that is caused by the external physical milieu. He claimed that

> There is an essential indeterminacy in the relation between the phenotype and the genotype; the relation only becomes determinate if you take account of the environment. This sort of indeterminacy leads to a really interesting logical situation, which is not at all amenable to the kind of simple mathematical treatment that Haldane and Fisher tried to give it. It calls for mathematics much more like the Theory of Games.

Today, mathematical chaos theorists (Kauffmann 1995; Wolfram 2002; Lucas 2008) and physicists (Penrose 1997) have realized the implications of noncomputability in the mathematical modeling of emergent systems. Prigogine (1997) emphasized the scientific necessity of an overview of the outcome of self-organization but did not have a complete experimental model. The analysis of such an overview implies analyzing first and hypothesizing afterward to search for unexpected information without a specific causal hypothesis. Such an explorative investigation of data sets by induction is, in current systems biology (Allen 2001; Abel and Trevors 2006; Mustacchi et al. 2006; Wolkenhauer and Ullah 2007), not in accordance with the still ruling causal hypothetic deductive model favored by the influential philosopher Karl Popper (1995), which regards induction as nonfeasible. A "paradigm shift" in causal modeling is here introduced by a dialogue between the fine-grained bottom-up data of molecular biology with a coarsely grained top-down overview by spectroscopy (Munck et al. 1998, 2010) to account for the information that cannot be predicted bottom-up by deduction from an emergent network.

INTRODUCING AN EXPERIMENTAL MODEL OF SELF-ORGANIZATION BASED ON NEAR INFRARED SPECTROSCOPY (NIRS) OF THE BARLEY ENDOSPERM MUTANT MODEL INTERPRETED BY CHEMOMETRICS

Science is conservative and the consequences of the mathematical (Wolfram 2002; Lucas 2008) and physical (Prigogine 1997, 2003) aspects on causality in relation to self-organization are only slowly realized in other disciplines such as systems biology. The necessary overview of the system *is lacking in most disciplines* except in the satellite monitoring of geoscience. The near infrared (NIR) spectral barley endosperm mutant model (Munck 2006, 2007; Munck et al. 2010) presented here is probably the first attempt to confirm the theory of self-organization experimentally in biology. We will here aim at demonstrating how physical, chemical and genetic knowledge can take over to characterize the phenomenological expression of mutant genes with highly reproducible instruments and visual aids when destructive mathematical models fail as expected from the mathematical theory. To prepare for an interpretative discussion to connect to Prigogine's (1997, 2003) theory on self-organization, we have to describe the chemical and genetic interpretation of the model in detail in Section 3 including the use of the necessary visual data presentation tools that might be new to the reader. We will then in a generalized approach to self-organization discuss the climate issue and the symbiosis between the cereal plant and human society.

The feasibility of an exploratory strategy

The barley NIR spectral endosperm mutant model presented by the spectra in Fig. 18.1a gives a unique overview of the *cause* of a mutation in DNA as a *frozen dynamics* of the emergent physiochemical pattern of seed synthesis. The mutant seed phenotypes (approximately 80% endosperm tissue) are provided in a controlled homozygous, near-isogenic background that makes comparison possible between different mutants in the same genetic background and with the parent variety (Munck et al. 2004). NIRS has a broad chemical relevance as a "multimeter" in industry and plant breeding (Helm et al. 2008; Møller Jespersen and Munck 2009) that fully satisfies the characterization of the method as a "coarse-grained physiochemical overview on the level of chemical bonds of the composition of the phenotype."

It is now possible directly to hypothesize from spectral data evaluated by visual inspection and by score plots from the chemometric model principal component analysis (PCA) that finds its principal components (PCs) by self-modeling just as Prigogine's (1997, 2003) mathematical model on self-organization. The critical exploratory logistic process proceeds as "a selection cycle" (Munck 1991, 2006; Munck et al. 2010) among three *independent* data sets:

1. the NIRS data set;
2. a chemical data set for validation that partly can be induced from spectral data; and
3. prior genetic, environmental, and molecular data.

The NIR spectra of the barley endosperm mutants are not used here for monitoring specific components hypothesized beforehand. The size and high complexity of the 1400 wavelengths and 700 data points from NIR barley seed spectra multivariate scatter correction (MSC) $\log 1/R$ 1100–2500 nm (Fig. 18.1a) does not allow for any specific a priori hypothesis on what to find in an unsupervised analysis when samples are coded. Consequently, there is no bias in the initial spectral evaluation by PCA other than the limits set by measurement noise from the instrument, the linear mathematical model itself, and from the selection of the known and unknown samples for measurement. There is only a need for a broad *primary hypothesis* implying that NIR spectra would reflect the physiochemical genetic and environmental variation in an overview of the barley seed samples. The "multivariate advantage" (Munck et al. 1998) of a reliable fingerprint by a spectral overview and chemometrics allows *both* validation and falsification of patterns relative to a reference with a high degree of probability. Fingerprints were

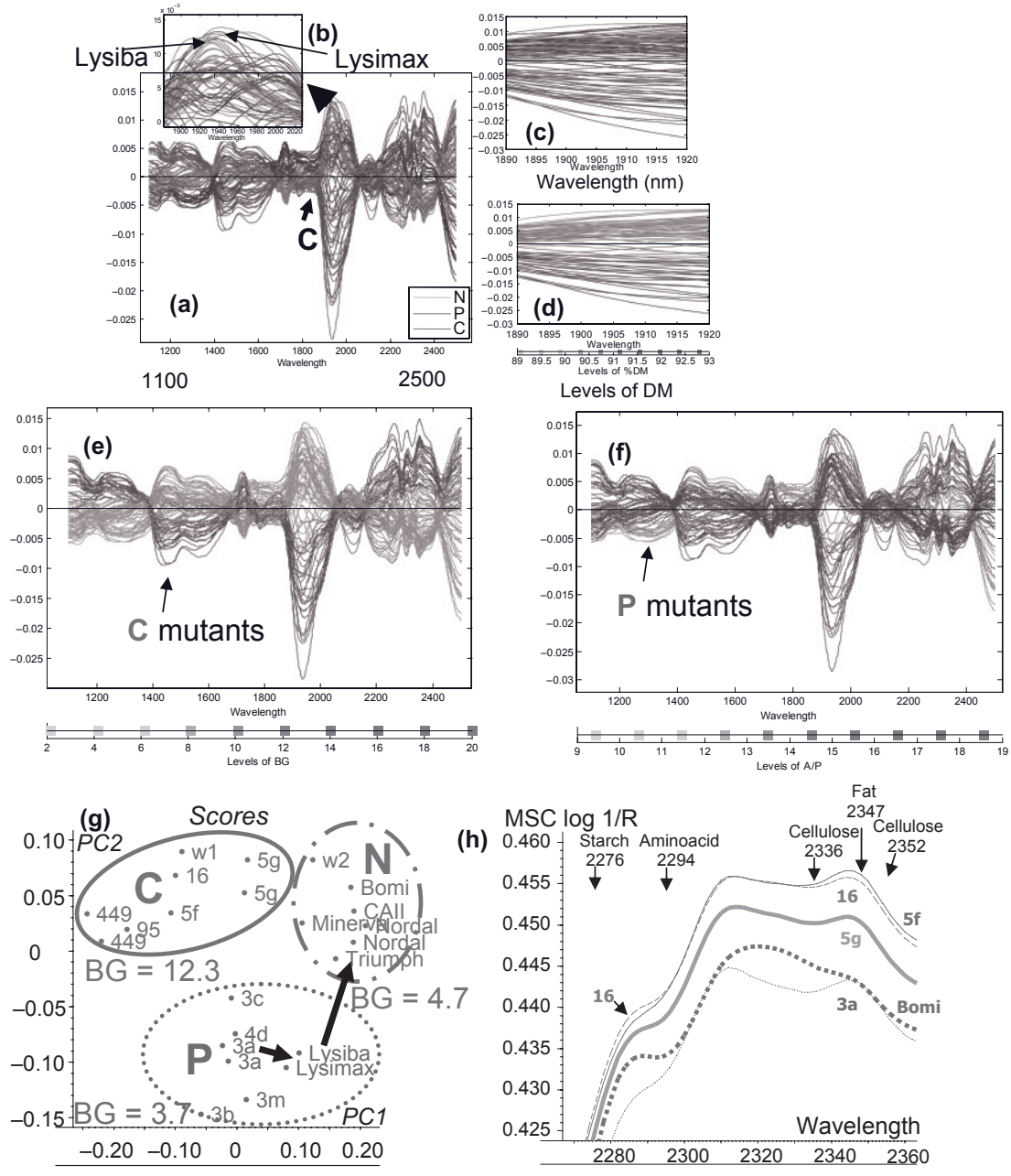

Fig. 18.1. (a) Mean centered log 1/R spectra 1100–2500 nm from 92 barley seed samples. Green, normal barley (N); blue, protein mutants (high lysine, P); and red, carbohydrate (C) mutants. (b) Enlargement of peak at 1935 nm; P outliers Lysimax and Lysiba. (c) Interval 1890–1920 nm from (a). (d) Marking of spectra from (c) for dry matter 89%–93% by color code. (e) Coloring for β-glucan (2%–20%) of the spectra in (a). (f) The same for amide/protein index (9–19). (g) PCA classification of 23 field barley NIR spectra (1100–2500 nm) for normal barley (N) and protein (P) and carbohydrate mutants (C). BG β-glucan % DM. (h) NIR spectra (2270–2360 nm) of Bomí (N) and its P lys3a and C mutants lys5f, lys5g, and mutant 16 grown in the greenhouse. (a–d) and (g–h) (Munck, 2007) by permission from Wiley & Sons Ltd. For color details, please see color plate section.

introduced by Scotland Yard inspired by Francis Galton already in the year 1900 (Pearl 2000) and are now in principle exploited in extended applications in the biometric computer industry.

Exploratory interpretation of the finely tuned spectral overview of self-organization by chemical validation and a priori genetic knowledge

The sample set (Munck et al. 2004) consists of 92 molecularly defined and undefined barley seed samples. There are 12 mutants of which 10 are identified as Risø high-lysine mutants (Doll 1983; Munck 1992; Munck and Møller Jespersen 2009a). Two unknown mutants were putatively assigned as waxy (high amylopectin). The original mutants and some crosses with normal lines and 14 normal barleys were grown both in the field ($n = 23$) and in the greenhouse ($n = 69$). Each seed sample was milled and measured by NIRS to produce 92 MSC log 1/R spectra from 1100 to 2500 nm (Fig. 18.1a). MSC is a gentle spectral pretreatment (Martens and Næs 2001) that effectively reduces stochastic scatter due to different particle sizes of the samples that is closely related to grain hardness. Every second wavelength was selected to produce a data set of 92×700 data points.

We start with an unsupervised PCA classification of the coded samples based on the full mean centered 92 NIR spectra (Fig. 18.1a) without *looking on spectra and in the field book*. The field book with the code is consulted after measurement. It was found that the first 1100- to 2500-nm spectral PCA score plot (not shown here) gives three clusters of samples from field and from greenhouse data that partially overlap. We therefore performed separate spectral PCA score plots for field (Fig. 18.1g) and greenhouse data (Fig. 18.3d) to obtain a full separation between the three clusters (Munck et al. 2004). A barley cluster with normal barley lines was first identified and labeled **N** (green) in Fig. 18.1g.

Surprisingly, the 10 high-lysine barley mutants come in two different clusters (Fig. 18.1g). The cluster **P** (blue) is assigned "protein" because it consists of the regulative very high-lysine allelic mutants *lys3.a*, *lys3.b*, *lys3.c*, and *lys3.m*. Blom-Sørensen et al. (1996) demonstrated that the *lys3.a* (Risø M-1508) mutant gene deregulates DNA demethylation of genes that drastically reduces the hordein storage proteins. The Risø mutant *lys4.d* is included in the **P** group and is therefore a putative regulative mutant.

The second mutant cluster (Fig. 18.1g) is called carbohydrate **C** (red) because it contains three structural gene mutants for reduced starch synthesis (see review by Rudi et al. 2006) in two loci, *lys5.f* and *lys5.g*, and Risø mutant 16 (not yet located on the chromosomes). They involve an adenosine-diphosphate (ADP)-glucose isoenzyme transporter through the plastid membrane and a defective ADP-glucose-pyrophosphorylase isoenzyme in the cytosol, respectively. The mutant 16 gene has been sequenced. Mutants 95 and 449 are previously selected for high lysine. They are now putatively classified as **C** mutants (Munck et al. 2004).

The NIRS patterns from ripe seeds of the *lys3.a* and *lys5.f* mutants (Fig. 18.1h) were compared to those during seed development (epigensis) by our research group (Fast Seefeldt 2008; Munck et al. 2010). The mutants are clearly differentiated from each other and from the normal control already at 16–20 days after pollination. Already at this stage, mutant-specific NIRS patterns appear, which are characteristic for the ripe stage of each genotype.

The two mutants of unknown origin, w1 (1201) and w2 (841878), were included with a note that they were selected for the waxy trait (high amylopectin starch). They are located in the **C** and the **N** cluster, respectively, in Fig. 18.1g.

Indication of a new regulatory pathway in glucan synthesis

It was a surprise when we identified by our in-house β-glucan (BG) analysis all the genotypes in cluster **C** (carbohydrate) as *BG compensating starch mutants* shown in Fig. 18.1g (including mean % BG for the three groups). This was luck; however, an experienced spectroscopist, from spectral inspection, can directly identify the involvement of BG using the assignments in the 2200- to 2500-nm region found in literature.

The extreme mutant *lys5.f* has a reduction in starch content of 61% and an increased BG of 296% compared to the Bomi parent. The partial break in the production of α-glucans (starch) by a mutant isoenzyme has apparently resulted in the overproduction of BGs. The starch biochemists that have exploited barley starch endosperm mutants since the 1980s (see review by Rudi et al. 2006) were not aware of the BG compensation of the starch mutants detected by Munck et al. (2004). The putative waxy w1 mutant in the **C** group is a starch–BG mutant. It was found that the waxy assignment was not correct because the level of amylopectin was normal. In agreement with the classification, the other putatively waxy mutant w2 placed as an extreme within the **N** group has a normal starch and BG content. w2 was confirmed to be waxy (Munck et al. 2004).

Genetic and chemical validation of spectral fine-tuning

We are now using the classification labels from the PCA score plots in Fig. 18.1g to color the mean centered MSC corrected log 1/R 1100–2500 nm spectra in Fig. 18.1a with green for normal (**N**) spectra, with blue for protein (**P**), and red for carbohydrate (**C**) spectra. The finely tuned spectra from the normal and mutant genotype categories presented in Fig. 18.1a ($n = 92$) are highly representative for each class. The environment (field or greenhouse) is influencing the offset from the baseline and to a minor degree the spectral form (Munck et al. 2001). Field spectra from the spectral band 2280–2360 nm are presented in Fig. 18.1h together with the chemical assignments from spectral literature. The peak at 2347 nm indicating an increase for oil (fat) for the mutant 16, *lys5.f*, *lys5.g*, and for *lys3.a* was confirmed by chemical analyses (Table 18.1), as well as the differences indicated in starch at 2276 nm, amino acids at 2294 nm, and cellulose at 2336 and 2352 nm (Munck et al. 2004). The spectra of the alleles *lys5.g* (BG = 8.9%) and *lys5.f* (BG = 16.5%) have a similar form; however, the large difference in the expression of BG introduces a different baseline response of their MSC-treated spectra. The spectra of mutant 16 (BG = 12.0%) and *lys5.f* (BG = 16.5%) in the **C** group are almost identical in expression in the 2280- to 2360-nm area in Fig. 18.1h in spite of the difference in BG and the fact that they affect two different mechanisms on the molecular level as previously described. It is possible by inspecting spectra from several replicates of the two mutants grown in different environments to find a consistent spectral region of genetic differentiation around 2150–2210 nm.

The spectral area 1890–1920 nm marked in Fig. 18.1a by a **c** was identified from literature as a band indicative for bound water. This area is magnified in Fig. 18.1c. The **C** (red) spectra below in the figure do not overlap with the **P** (blue) and **N** (green) spectra above. In Fig. 18.1d, the same spectra are colored by data inspection software (http://www.Latentix.com) with a gradient for dry matter (DM) in the narrow range of 89.5%–93.0%. The low DM content of the samples behind the **C** spectra below is verified. It reflects a mean value of 1.4% DM (Table 18.1) between the **C** and the **P** + **N** groups that confirm the sensitivity of NIRS on the 0.5–2.0 percentage level for water as explored *directly* by inspection of MSC-treated spectra. The chemical composition in Table 18.1 is further confirmed by color gradients 1100–2500 nm indicative of high BG for the **C** genotypes in Fig. 18.1e (range 2%–20%) and of low amide/protein (A/P) index indicative for **P** genotypes in Fig. 18.1f (range 9–19 units).

Confirmation of the chemical fine-tuning of spectral correlation curves

It was also surprising to find that simple correlations between single spectral wavelengths and the chemical analyses in Table 18.1 gave finely tuned chemical correlation curves specific for each chemical analysis shown in the local MSC-treated spectral area 1680–1810 nm in Fig. 18.2. A guide for the chemical assignments of the wavelengths from literature is given below the *x*-axis. Major correlation peaks at 1690 and 1740 nm for amide and for oil at 1724 and 1762 nm were in accordance with literature. The specific pattern of the chemical fine-tuning of BG 1100–2500 nm for the ripe seed material was confirmed in a separate experiment on seed

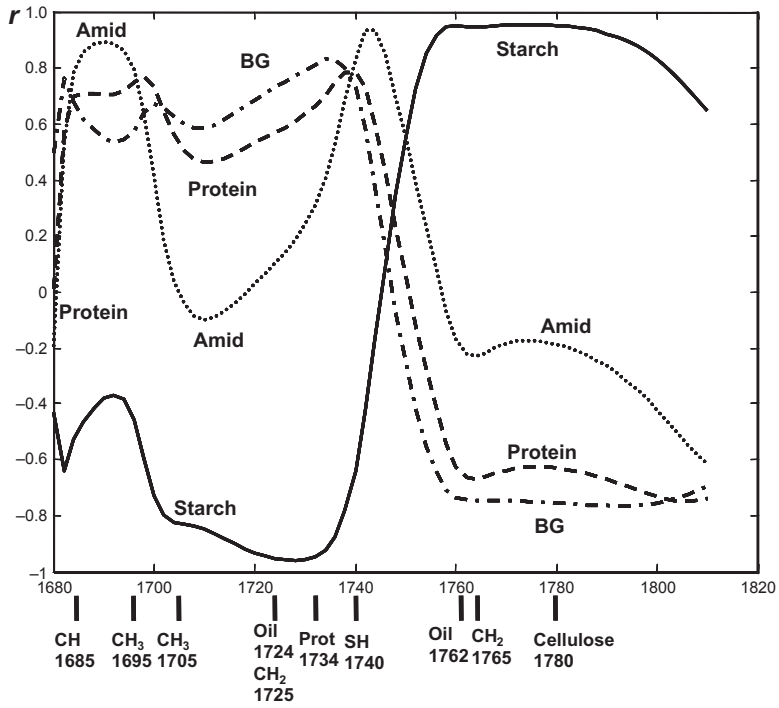

Fig. 18.2. Chemical fine-tuning. Simple correlation coefficients (*r*) between wavelength and chemical composition % DM for the material in Table 18.1. BG, β-glucan; SH, starch; Prot, protein. *Source:* AP/Elsevier Scientific Publishers (Møller Jespersen and Munck 2009).

development with **C**, **P**, and **N** genotypes by Fast Seefeldt (2008).

Transformation of quantitative chemical variables to qualitative genetic and food functional patterns by PCA

It is possible by chemometrics in a PCA to synthesize genotype specific *qualitative* patterns by combining *quantitative* chemical destructive analyses in PCs plotted in *x–y* combinations in score plots. In Fig. 18.3A, the chemical composition ($n = 6$) of 28 barley genotypes grown in the greenhouse from Table 18.1 are displayed as normalized "chemical spectra." They are evaluated by a PCA biplot in Fig. 18.3B. The genotype classification in **C**, **N**, and **P** groups by chemical spectra are confirmed by the corresponding classification of NIR spectra (Fig. 18.3C) in the PCA in Fig. 18.3D.

The chemical variables (loadings) are positioned in the PCA biplot in Fig. 18.3B. The symbols for starch, amide, and A/P index in the **N** cluster, for BG in the **C** cluster, and for protein near the **C** and **P** clusters indicate a high content of the samples near these symbols. Two double recessive 3a5g (*lys3.a lys5.g*) genotypes (not included in the material in Fig. 18.1, Table 18.1) in the PCA score plots in Fig. 18.3A,D are intermediates between the **P** and the **C** groups. NIRS in Fig. 18.3C,D constitutes a second *independent* view of the same 28 samples in the chemical data set in Fig. 18.3A,B to produce the same classification pattern for genetic validation.

Spectral definitions of phenome and pleiotropy (gene interaction)

The results given above confirm that *NIR spectra represent patterns of physiochemical information*, *a*

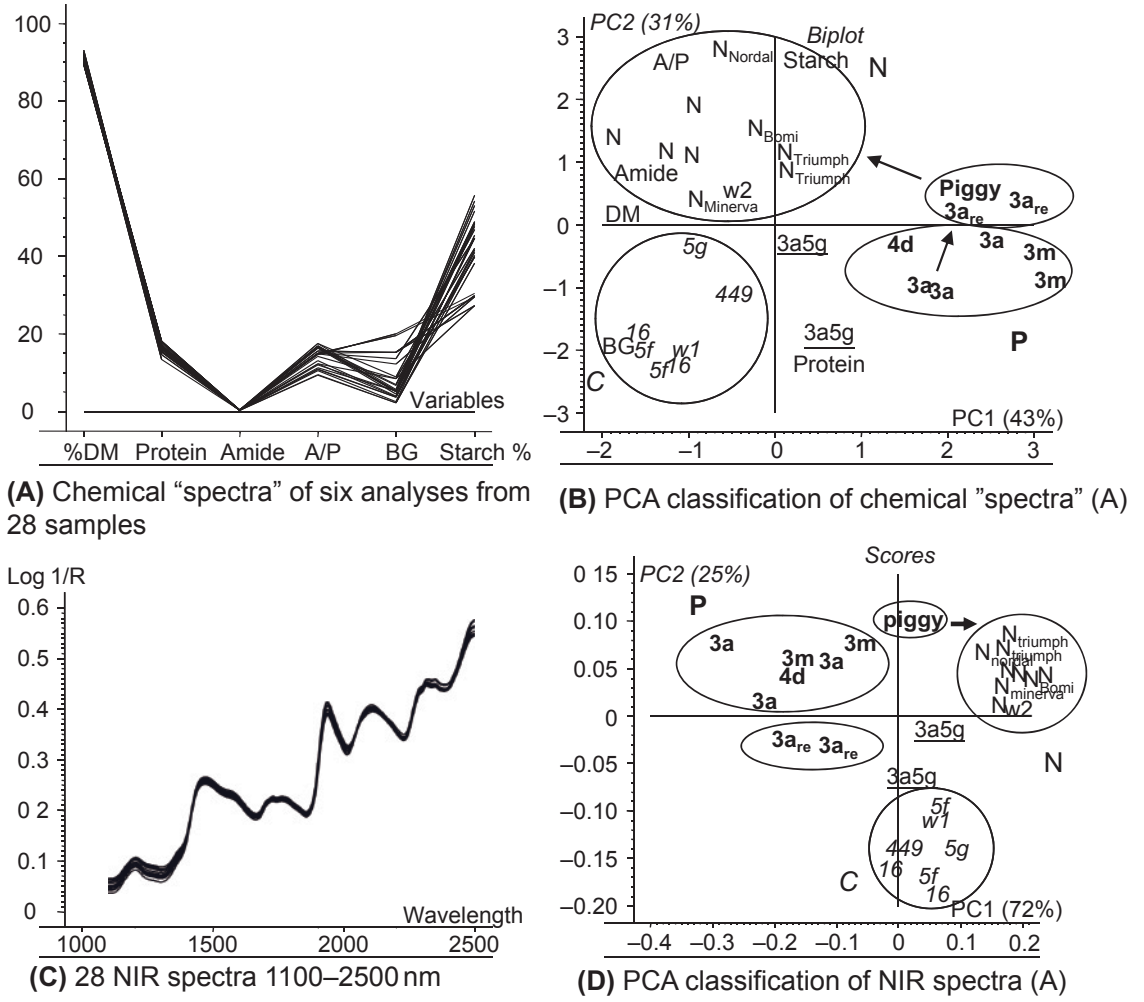

Fig. 18.3. Demonstration of transformation from quantitative chemical data displayed as spectra in (A) to qualitative genetic information of genotypes in the PCA biplot in (B). Loadings for dry matter (DM), protein, amide, A/P index, starch, and β-glucan (BG) are displayed. The chemical approach is compared to that of NIR spectra in (C) (1100–2500 nm) and (D). The spectra in (C) are classified in the PCA in (D). The material consists of 28 genotypes from Table 18.1 grown in a greenhouse. N, normal barley; C, carbohydrate mutants; P, protein mutants.

Table 18.1 Chemical composition (% DM) of the barley mutant seed material measured by NIRS in Fig. 18.2

		DM	Protein	Amid	A/P	BG	Fat	Starch
All	*n*	92	84	81	81	88	20	35
	Mean	90.66 ± 0.97	16.08 ± 2.22	0.4 ± 0.2	15.19 ± 2.22	8.06 ± 4.79	2.48 ± 0.76	45.18 ± 8.78
	Max	93.02	9.7	0.2	9.46	2.2	1.66	27.3
	Min	88.91	22.28	0.6	18.58	20.0	3.77	60.4
C	*n*	29	26	25	25	29	9	7
	Mean	91.62 ± 0.75	16.98 ± 2.13	0.42 ± 0.06	15.32 ± 0.81	14.21 ± 2.9	2.71 ± 0.76	32.1 ± 5.8
P	*n*	23	23	18	18	23	3	15
	Mean	90.18 ± 0.49	15.77 ± 2.33	0.29 ± 0.05	11.67 ± 1.54	3.79 ± 1.36	3.5 ± 0.6	44.75 ± 4.81
N	*n*	40	40	37	37	40	9	14
	Mean	90.22 ± 0.79	15.64 ± 2.09	0.42 ± 0.07	16.77 ± 0.78	5.72 ± 0.99	1.91 ± 0.16	52.16 ± 4.25

C, carbohydrate mutants; N, normal barley; P, protein mutants (Møller Jespersen and Munck 2009; Elsevier Science Publishing, with permission).

phenome (Munck et al. 2004) specific for each mutant and genotype. NIRS summarizes the causal pleiotropic physiochemical effect of an endosperm mutant in an isogenic background on all active genes during endosperm synthesis. By deducting the spectrum of the near-isogenic parent variety from that of the mutant, it is now possible for the first time to deliver Chetverikov's (1926) holistic view on pleiotropy (Munck 2007; Munck et al. 2010). In Fig. 18.4, the differential spectra 2260–2500 nm to the parent variety Bomi seeds of the mutant gene **P** alleles *lys3.a*, *lys3.b*, and *lys3.c* are presented. Each differential spectrum represents the total pleiotropic physiochemical expression of a mutant gene. Spectra from two *lys3.a* lines in Fig. 18.4 visualize the accuracy of the method at the positions marked I–V. There is an extremely high reproducibility of the differential spectra to Bomi of the two parallel *lys3.a* lines that have been separately propagated in more than 20 years. Even if spectra of the three *lys3* alleles resemble each other, there are significant differences. The higher level of BG of the *lys3.c* mutant (BG = 6.1%) compared with *lys3.a*

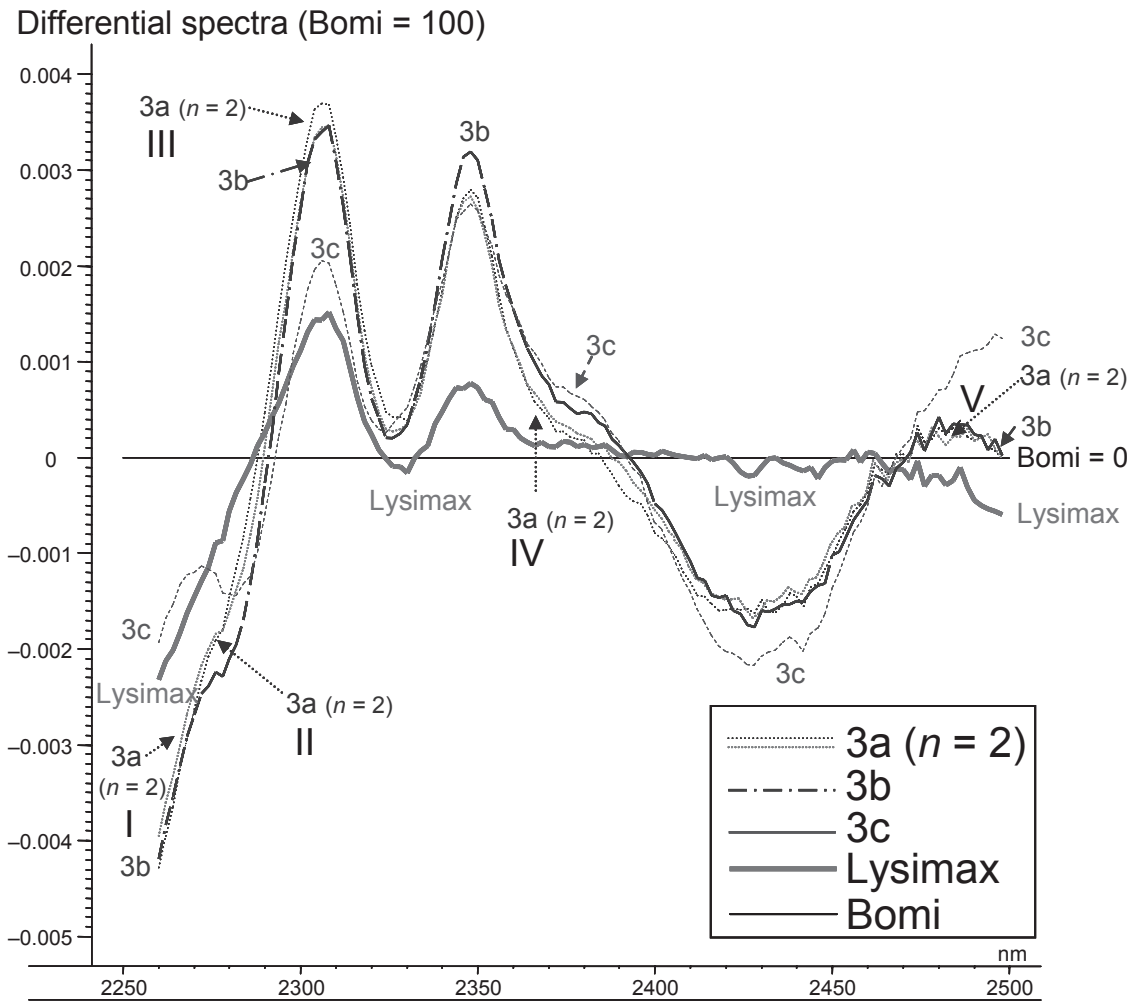

Fig. 18.4. Demonstration of pleiotropy for the *lys3.a* (*n* = 2), *lys3.b*, and *lys3.c* alleles and the effect of changed background in the *lys3.a* recombinant Lysimax. Differential spectra 2250–2500 nm to Bomi (zero baseline) are displayed. The spectral reproducibility of two parallel *lys3.a* lines are marked (I–V). The material was grown in a greenhouse.

(BG = 4.7%) and *lys3.b* (BG = 3.1%) is confirmed by the higher level of spectral absorption indicative for BG at 2500 nm for *lys3.c* compared with *lys3.a* and *lys3.b* (Fig. 18.4).

The physiochemical effect of an optimized gene background to a mutant overviewed by spectroscopy

Lysimax, Lysiba, and Piggy are *lys3.a* barley varieties that are improved in grain plumpness, starch content (approximately +10%), and yield by crossbreeding at Carlsberg in 1973–1989 (Munck 1992). The spectral PCAs in Figs. 18.1g and 18.3D demonstrate a movement of the improved lines from the position of the *lys3.a* mutant toward the normal **N** cluster as indicated by the arrows in the figures. This move is indicative of a change in chemical composition toward more starch as validated by the position of the "starch" label (loadings) in the PCA biplot for chemical spectra in Fig. 18.3B. The effect of a changed gene background is visualized by the finely tuned detail in the differential spectrum to Bomi of the improved barley line Lysimax in Fig. 18.4. The differential Lysimax spectrum is flattened out to approach that of Bomi, which is a straight line in the figure. It represents a physiochemical overview of the effect of the changed genetic background on Lysimax seed obtained from 16 years crossbreeding involving improved starch content and plump seeds while keeping the high lysine content intact but also many other variables such as a decrease in cellulose (Munck 1992).

Revealing genetic and functional trait patterns by NIRS for food and nutrition by "data breeding" and for large-scale single seed sorting for quality

The movement of the starch improved the recombinant of the *lys3.a* mutant Piggy from the **P** cluster toward the **N** cluster in the spectral PCAs in Figs. 18.1g and 18.3D demonstrates how NIRS and PCA classification can be directly used in an exploratory selection approach in plant breeding for quality called *data breeding* (Møller Jespersen and Munck 2009; Munck 2009). There is a need for positive (the starch-rich Triumph in the N group) and negative controls (the mutant parent *lys3.a*) grown in the same environment. Recombinants that approach the positive controls in the PCA score plot are selected and checked by chemical analysis. Now not only specific quality attributes that implies expensive calibrations can be predicted by NIRS (Helm et al. 2008), but also genotypes and complex baking and malting quality traits can be classified by NIRS in a PCA score plot with a minimum of costs (Møller Jespersen and Munck 2009). The use of spectroscopy can also be generalized to other tissues to trace genotypes in soybeans and in human cancer mutations (Munck 2007).

Selection by NIRS for baking quality in wheat was utilized by the Bomill TriQ pilot scale single seed sorter displayed in Fig. 18.5. Single seeds are positioned in an intended cylinder and are looked upon by NIRS fiber optics. The pattern of each single seed spectrum is analyzed in the computer, and a signal is given to a pneumatic system that pushes out the seed in one of the three fractions for low, medium, and high quality. The significant effect of single seed selection by NIRS for baking quality in a batch of a wheat variety grown in one location is confirmed by the analyses in Table 18.2, for example, differentiating gluten from 17.4% to 27.6%. Now the great variation in chemical composition due to environment can be exploited by value added pattern recognition sorting. Single seed sorting can also be used directly for selection for complex quality traits in crossbreeding populations. There are now industrial single seed sorters available that can sort up to 10 t an hour of wheat and barley.

CHEMICAL AND GENETIC INTERPRETATION OF THE NIR SPECTRAL OVERVIEWS EXPLAINS THE LIMITS IN MATHEMATICAL EVALUATION OF DATA FROM EMERGENT SYSTEMS

The implications of the theory of self-organization on the concept of probability

The crucial perspective in understanding physical-chemical self-organization in biolgy is to

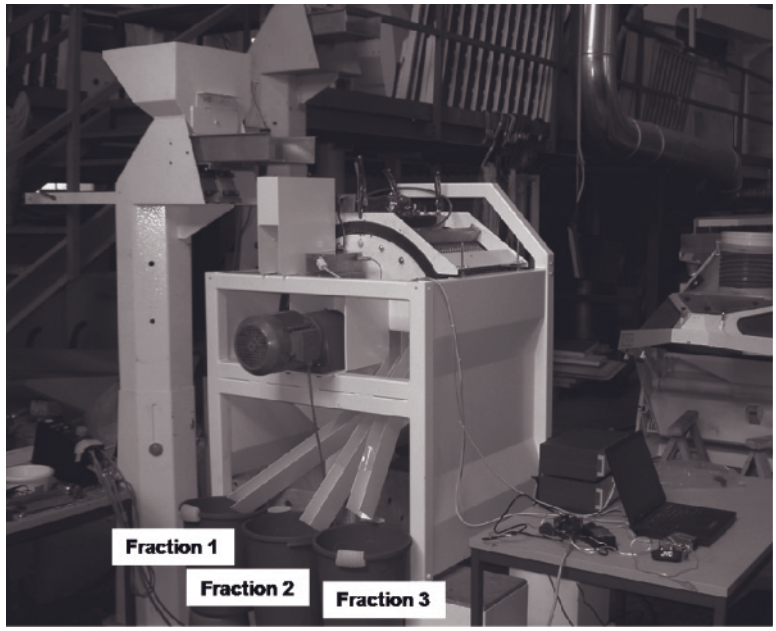

Fig. 18.5. BoMill TriQ single seed pilot NIR sorter for wheat and barley. See Table 18.2. *Source:* BoMill AB, Lund, Sweden.

Table 18.2 Sorting of wheat seeds from one sample (one variety, one field) with the TriQ pilot BoMill single seed NIR sorter in Fig. 18.5 (BoMill AB, Lund, Sweden, with permission)

		Fraction		
		1	2	3
Selection for		Feed	Bread I	Bread II
	Yield %	35	45	20
Farinograph	Dough stability time	1.7	5.5	8.4
	Water uptake %	53.1	56.7	59.7
Extensiograph	Dough elasticity height	100	129	146
	Gluten content %	17.4	22.7	27.6

recognize the existence of ordered irreversible, *persistent*, chemical reactions as a basis for a reformed probability concept based on nondistributivity (Prigogine 1997, 2003). Persistent ordered reactions are different compared to the probability of *transient* interactions in the relative simple chemical reactions that are considered in Newtonian dynamics and quantum chemistry. As explained by Prigogine (1997), "Persistent interactions mean that we cannot take a part of the system and consider that in isolation. It is at this (thermodynamic emergent) global level at the level of population (i.e., molecules, skies, and organisms) that the symmetry between the past and the future is broken and science can recognize the flow of time. This solves a long-standing puzzle. It is indeed in macroscopic physics that irreversibility and probability are the most conspicuous."

It is remarkable that Prigogine (1997, 2003), by his theoretical model on self-organization, succeeds without a direct experimental example to bridge the micro and macro aspects of emergent irreversible systems (Munck et al. 2010). In the tradition of the classical physicists, he used a combination of Newtonian dynamics and thermodynamics modified by quantum physics: The key problem for the validation of self-organization is

to find an experimental method that could provide a "global" overview that could account for the outcome of the probabilities that builds the persistent chemical reactions in the network and that cannot be modeled locally. Such a coarse-grained experimental overview on the level of chemical bonds is now available as demonstrated here by the NIRS barley endosperm mutant model.

The nondistributional probability of persistent reactions builds in self-organization "a narrow path between determinacy and arbitrary chance" (Prigogine 1997). Hierarchical, nonlinear, persistent ordered reactions are increasing the probability of complex structures to emerge (in mathematical terms in complex system biology as attractors) when the initial conditions are changed (e.g., by a mutation in DNA). Prigogine (2003) looked on the force of self-organization as a producer of correlations and patterns. Now, the statistical randomized, *normal distributional* version of probability, for example, used in climatology and population genetics, obtains a new definition of *nondistributivity* in self-organization. This has serious limitations in being predicted with regard to individual variables, although the outcome *as a whole system* may be observed and overviewed as is done by NIRS in the mutant endosperm model in a defined environment. This view is in full accordance with Chetverikov's (1926) holistic definition on pleiotropy by gene expression through the "genetic milieu" (internal environment) of a cell.

A global overview is always needed not only for mathematical but also for thermodynamic (Prigogine 1997) reasons to follow the development of entropy and structural organization in a dynamic system.

Interpreting the irreducibility of the finely tuned spectra observed from a self-organized system as output from a built-in "biological computer"

Because of the great genetic reproducibility of the complex NIR spectra from ripe seeds (see the spectra from parallel lines of *lys3.a* in Figs. 18.4 and 18.7) and from developing barley endosperm mutants (Fast Seefeldt 2008; Munck et al. 2010), we may consider the cell or a tissue as a biological computer (Kaneko 2006; Munck 2006). In the biological computer, software, hardware, and computational output are integrated by chemical affinity in the emerging biological structure of chemical bonds that can be observed by NIRS as a coarse-grained physiochemical representation of the phenome (Munck et al. 2010).

The precision of the finely tuned output from the "endosperm computer" measured by NIRS from correctly sampled parallel seed lines in a controlled environment is a staggering 10^{-4} to 10^{-5} MSC log 1/R absorption units (Munck 2007) as verified in the insert in the graph in Fig. 18.7 below. However, as seen in the PCAs on NIR spectra in Figs. 18.1g and 18.3D, the distances between genotypes in the score plots are not able to give the finely tuned spectra justice because linear data compression works destructively like surgery in networks (Pearl 2000).

The spectral scatter corrected output from the "cell computer" on the macro level in a constant environment looks *deterministic* in its stability in spite of being based on probabilistic reactions produced by chemical affinity on the micro scale. The self-organization observed by NIRS illustrates the limits in mathematical evaluation of data from emergent systems. *When data reducing mathematical models fail, data inspection and nonmathematical empirical evaluation of the unreduced phenomenological patterns are the only alternative.* The unreduced map of the "data landscape" (here a MSC corrected spectrum) is now the only guidance for maximal chemical and genetic information that remains (Munck 2007) as it is in the visual output from the analogous noncomputable mathematical example of cellular automata (Wolfram 2002). Phenomenological, highly reproducible spectral patterns directly read from cells and tissues in nature make *qualitative* sense and can be understood by proper controls that are chemically, molecularly, and genetically defined. The important data inspection, at a low level of data compression where stochastic variance is removed, can now be greatly facilitated by new data programs such as the Latentix software as shown in Fig. 18.1a–f.

Interpreting the indeterminacy in bottom-up path modeling in the NIR spectral endosperm mutant model

When now a holistic overview by NIRS of the outcome of self-organization is available, we can better understand the mathematical limitations in path modeling bottom-up extending from a gene sequence. In Fig. 18.6, a path modeling expedition in the tradition of molecular biochemistry is launched to follow the causal effect of the carbohydrate C mutant *lys5.f*. The mutation (**1**) reduces starch by 55% (**2**) due to a lesion in an isoenzyme for ADP-glucose transportation through the membrane of the plastid (**1**). From the metabolic scheme in Fig. 18.6, we can deduce that the lesion results in a potential piling up of ADP-glucose and glucose-1-phosphate that favors a shift in synthesis from sucrose–fructose to uridine diphosphate (UDP)–glucose and further to BG (**3**).

So far, classical biochemical and molecular methods should work to elucidate how this pathway is regulated. However, the *lys5.f* mutation results in a large increase in the production of BGs (**3**) from 5% DM in the control to 20% DM as a compensation for the reduced starch production. The change from crystalline starch to amorphous BG influences water activity because of a steady increase in the water content during seed synthesis (**4**) of the *lys5.f* mutant starting between 16 and 20 days post anthesis (d.p.a.) that amounts to 10 relative percentages (4%–5% absolute) higher than the normal control at 39 d.p.a. (Fast Seefeldt 2008; Munck et al. 2010).

The increased BG production derails a causal path modeling expedition (Munck 2007) to follow the metabolism from the *lys5.f* mutant DNA lesion because the increased water activity (**5**) in the endosperm tissue has the potential to change the activity of any enzyme that is active from about 20 d.p.a. during endosperm synthesis *in an a priori nonpredictable way*. The osmotic pressure of plant cells that varies between 7 and 10 atm (Cho 2004) should lead to a further amplification in water activity.

The *lys5.f* mutation is causing a change in *the whole internal milieu* of gene expression in Chetverikov's (1926) sense that is *indeterminable* in a path network model. It makes the scene for a pleiotropic cascade of secondary metabolic reactions in the *lys5.f* endosperm, which has been verified for the proteome (**6**: changed pattern of water-soluble proteins; Jacobsen et al. 2005), for fat/oil (**7**: +50%; Munck et al. 2004), and for the pattern of vitamin E components (**8**: α-tocopherol +60%, α-trienol −19%, γ-trienol +53%; Munck et al. 2010).

All the documented pleiotropic effects of the *lys5.f* mutation including the probably large number of undocumented ones are now summed up in its NIR spectrum (Fig. 18.1h) as a coarse-grained pattern of pleiotropic change on the level of chemical bonds as validated by the simple correlation between NIR absorption (MSC log 1/R) to chemical composition at specific wavelengths for the whole material (Table 18.1) in Fig. 18.2. The specific *lys5.f* NIR spectral pattern with the parent Bomi spectrum subtracted is a summary of the primary cause and the secondary causes of the mutant lesion. The spectral pattern can, *to some extent*, be chemically validated by experimental assignments from the spectral literature and by analyses (e.g., for BG), but *it is irreducible in much of its details and should be treated as a whole phenomenological event*. The results and discussion of the experiment fully verify Prigogine's (1997) characterization of self-organization: When associated with instability (e.g., a mutation in DNA), whether on the microscopic or macroscopic level, the new laws of nature deal with the possibility of events, but cannot to reduce these events to completely deductible, predictable consequences.

The NIRS barley endosperm mutant model gives an extremely simplified but *representative* coarse-grained overview of the outcome of self-organization (Munck 2007) that was confirmed in an experiment with developing seeds (Fast Seefeldt 2008; Munck et al. 2010). An arsenal of direct molecular methods can now measure the dynamics of the internal cell environment in Chetverikov's sense. Fluorescence imaging analysis is used to visualize single fluorescent molecules for internal signaling in cells that has a broad effect on many cell functions. Calcium and NADPH waves are propagated in living cells from 0.1 to 10.0 μm/s (Weijer 2003) and demonstrate

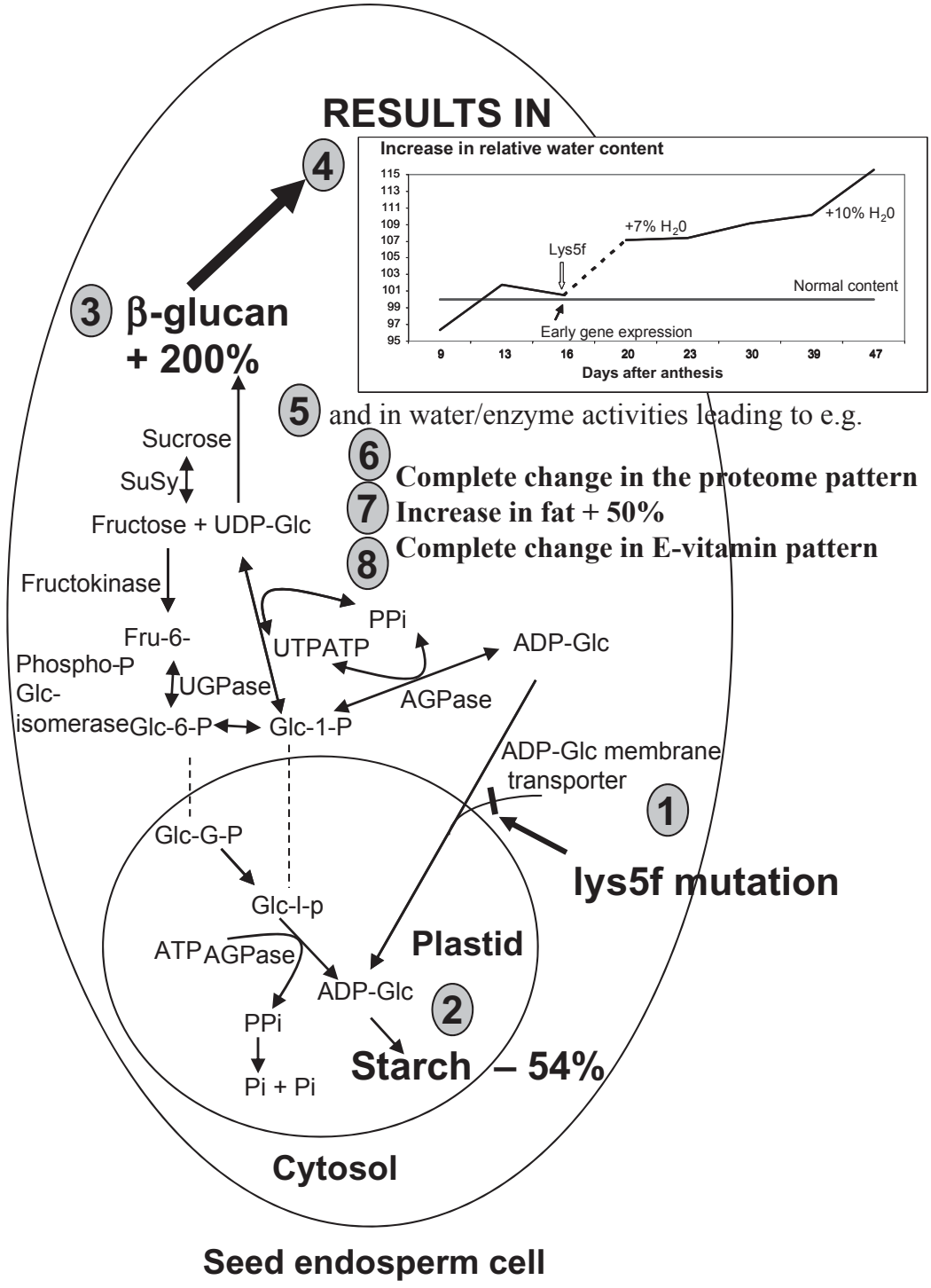

Fig. 18.6. Biological indeterminacy in path modeling exemplified by the action of the *lys5.f* mutant in relation to its parent variety Bomi. Water development from Fast Seefeldt 2008 by permission. See discussion in text.

persistent stochastic effects in "the genetic milieu" as with the indicated changes in water activity in the *lys5.f* mutant (Fig. 18.6, stage **4**).

The **C** and **P** barley endosperm mutants discussed here are major mutants with drastic effects on the seed metabolism. The effect of mutants with less pleiotropic effects on gene expression should also be considered. They may be localized in the periphery of the metabolic scheme. Thus, Frank et al. (2009) studied the pleiotropic effect on the metabolome level of two phytic acid mutants in rice and soybean grown in different environments involving the primary and secondary gene effects on phosphorous and phytic acid metabolism. Hundreds of low-molecular lipophilic and hydrophilic metabolites were studied by capillary gas chromatography. PCA score plots verified genotypic clustering for several of the components. However, it was only possible by *data inspection* to identify the four to six components that were consistently significantly different between wild type and mutant in different environments. They all belonged to the biosynthetic pathway of phytic acid. These findings confirm in principle the results from the barley model with respect to the need for data inspection to trace genetically reproducible patterns.

The *consistency of patterns* should preferably be judged with a coloring data inspection program as shown in the spectral data in Fig. 18.1a–f. The corresponding PCAs in Figs. 18.1g and 18.3D, because of data compression, give a faint reminiscence of the finely tuned reproducible MSC log 1/R spectra that are reflecting the physiochemical output from the biological computer in a controlled environment. The data-compressed PCA score plot is useful as a preliminary overview of a data set; however, a confirming data inspection is needed to evaluate the highly reproducible patterns in data.

Complementary top-down bottom-up data modeling in permutation experiments with cells and tissues as the best compromise to the unobtainable holistic model of gene expression

One should now consider that there is a two-way barrier of uncertainty in modeling complex biological systems as a whole—the partial irreducibility of a top-down overview at any omic level and the partial indeterminacy in bottom-up path modeling. The community of systems and molecular biologists such as Allen (2001), Abel and Trevors (2006), Mustacchi et al. (2006), Omholt (2006), Palsson (2006), and Wolkenhauer and Ullah (2007) seems not to have fully considered the consequence of these two limitations in prediction of gene expression. These scientists are not addressing the need for an overview of the phenome that could be provisionarily solved by spectroscopy. While it is possible to "design" a DNA sequence for a gene, design is, in principle, impossible on the organism level. Nature has to be consulted through the built-in self-organized biological computer to verify the total expression of the designed gene including the pleiotropic effects in a defined genetic milieu. The final aim could then be reached by adapting the gene to an optimized background by crossbreeding as discussed above.

The spectra determined by NIRS reflect not only the chemical composition but also the very high reproducibility of the underlying molecular development in a controlled gene background and environment. The extremely high specificity and resolution of the molecular description (however, *incomplete*) of functions in the networks of cells is just as stunning as the *complete* finely tuned, coarsely grained physiochemical endosperm overview by NIRS. As discussed by Mustacchi et al. (2006) in yeast systems biology, it stimulates to perform "in silicio design of advanced cell factories for production of fuels, chemicals, food ingredients and pharmaceuticals." However, if such a strategy is successful, it is not necessarily due to the skills of the scientist in "design." The result has probably been already calculated out there by "the biological computer" (Munck et al. 2010), the output of which can be registered by careful observation with high-throughput instruments.

The nearest we can come in complex systems modeling in biology is to build an experimental data bank on gene expression in the computer for prediction by artificial intelligence in a dialogue between, for example, spectral top-down and bottom-up molecular and genetic information (Munck 2007; Munck et al. 2010). This can be done by asking questions to the biological

computer through perturbations by mutations and environmental changes (e.g., addition of plant hormones). Specific questions to nature can be asked in a homozygotic background, for example, in barley or yeast, by targeted induced local lesions in genome (TILLING) or by endonucleolytic mutation analysis by internal labeling (EMAIL) to obtain a library of defined mutants and of complex gene combinations in a controlled genetic background. The bottom-up information is obtained by high-throughput techniques for the genome by snip markers, for transcriptomics by the Affymetrix chip, for proteomics by time-of-flight mass spectroscopy, and for metabolomics by high-resolution nuclear magnetic resonance (HNMR) spectroscopy or by gas chromatography.

One would then find a whole range of genes and gene combinations that behave differently with regard to the extent of pleiotropy. One extreme is the rigorous, deterministic-like, persistently expressed genes with a narrow pleiotropic effect (however still with a contribution of uncertainty) such as in the example with the phytic acid rice mutant (Frank et al. 2009). In contrast, there are genes with a broad pleiotropic effect that combine persistent "deterministic" changes in biochemical path models with large, stochastic persistent effects in "the internal milieu" of the cell, for example, through water activity that cannot be modeled bottom-up.

Even if many genes and their functions are comparable in many eukaryotic organisms, the task of using an expression data library from the biological computer from, for example, barley and yeast for prediction and "design" is of gigantean dimensions. There are always uncertainties because of pleiotropy and gene–environment interaction. One would never be sure how sensitive a selected gene background is to a surprising "attractor" reaction to a mutation similar to that of *lys5.f* where α-glucans were exchanged for BG, resulting in drastic pleiotropic effects.

We can conclude that there is *a philosophy of overkill in molecular biology* that emphasizes the need for fine-grained patchwise bottom-up causal path knowledge instead of the classical plant breeder's explorative pattern recognition view of manipulating the whole plant by selection. The breeder's strategy has now obtained a new extension on the level of chemical bonds by NIRS that should appeal to theoretical biologists as a complement to molecular data (Munck et al. 2009). As formulated by the complex systems biologist Kaneko (2006, p. 30), "Unfortunately … it is not always true that a field study benefits from an increased ability to observe microscopic phenomena …. However, it is not necessary to account precisely for each molecular process. On the contrary, it is often important to employ coarse-grained description in which insignificant details are removed. In fact, one of the approaches we propose here is that of seeking universal properties by using a coarse grained description of the systems under investigation."

Some advances have been made over the past 10 years in the exploitation of molecular markers in cereal breeding, but these have been most successfully used for simple quality attributes such as pathogen resistance, and little success has been achieved with more complex quality traits. Now the sense of pattern recognition by the sight of the plant breeder can be considerably extended to include chemical composition by data breeding of spectral patterns in a PCA and through spectral inspection.

In conclusion, the coarse-grained NIRS PCA pattern recognition model is *sufficient* to successfully quality grade cereal seeds in industry and plant breeding. Cereal seeds constitute the most economically important phenome for human consumption that is produced in over 2 billion tons per year.

From spectral overviews to evaluating qualitative–quantitative-physical structures of cells and plants

We will now widen the view of the coarse-grained spectral phenome to indicate how the NIR spectra 1680–1810 nm in Fig. 18.7 can be connected to a physical macro- and microstructural perspective of gene expression of the mutant *lys3.a*, a normal control, and a starch-improved *lys3.a* recombinant (Lysimax). The *lys3.a* (**P**) mutant causes a dramatic change by a shrunken seed (above to the right in Fig. 18.7) and an enlarged germ and scutellum plate. These changes contribute to high oil

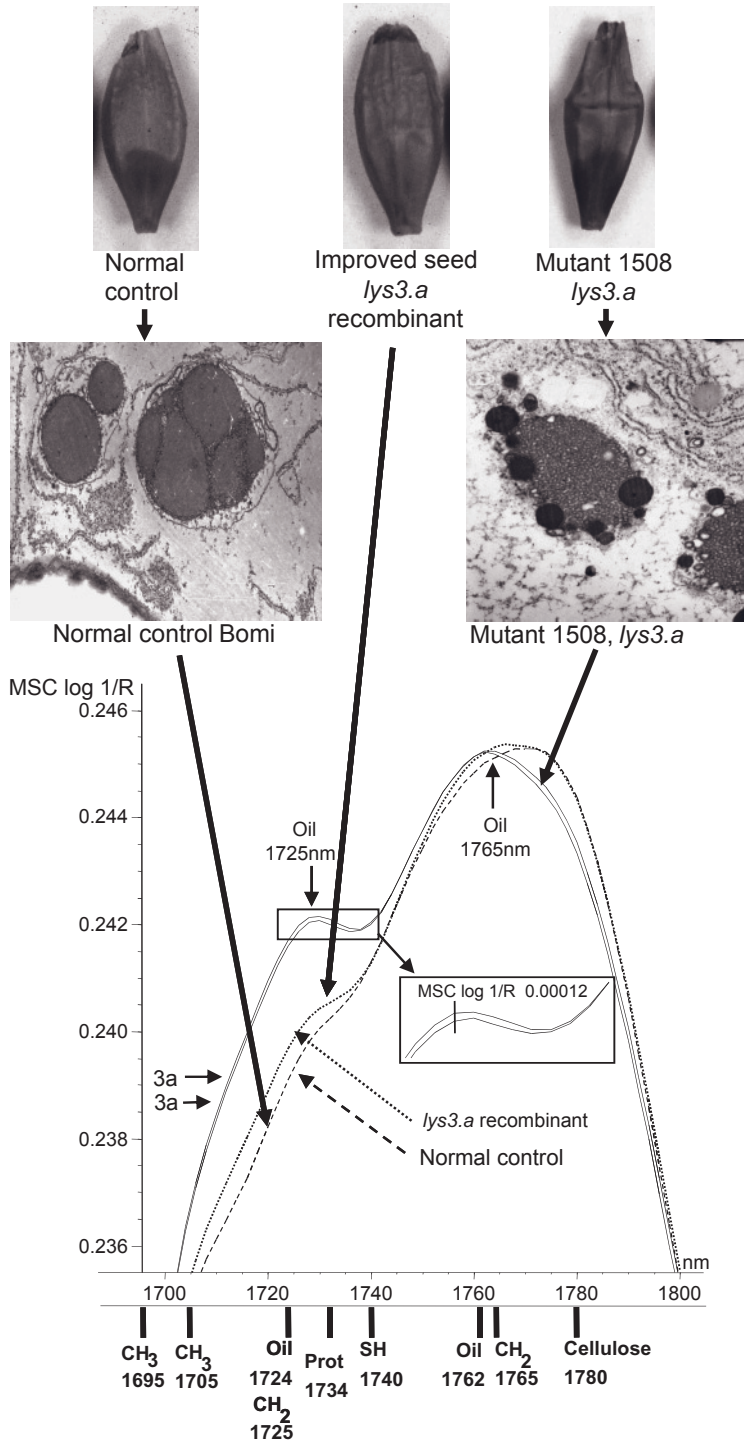

Fig. 18.7. From seed morphology and ultrastructure to NIR spectral representation of materials: *lys3.a* mutant (n = 2), normal control barley, Bomi, and *lys3.a* recombinant Lysimax.

content as indicated by the two spectral *lys3.a* replicates of parallel lines below in the graph and by the spectral assignments below. There is a drastic change in the endosperm micrographs in the middle of the figure comparing mutant *lys3.a* and its parent gene background Bomi (Munck and Wettstein 1974). The smooth, light gray protein structures in the protein bodies of Bomi is exchanged for a coarsely grained matrix and by the strongly stained globules in the mutant that reveals a dramatic change in the proteome from hydrophobic hordeins to hydrophilic water-soluble proteins (Jacobsen et al. 2005). The improvement in seed and starch content of the *lys3.a* recombinant Lysimax seed (above in Fig. 18.7 in the middle) is reflected in a normalized spectrum moving toward the N genotype in the graph. There is a reduction in the oil peak to the left that reflects a smaller embryo+scutellum, while the high-lysine trait is retained as checked by amino acid analysis.

Instead of one NIR spectrum per seed genotype in Fig. 18.7, it is now possible with advanced NIR-infrared (IR) and Raman scanning microspectrometers to scan two-dimensional images of endosperm cells with hundreds of spectral wavelengths "standing up" from each pixel (Thygesen et al. 2003; Mills et al. 2005). Due to the long wavelength, the resolution is approximately 10 μm with FT-IR and 1 μm with Raman microspectrometry. *Following the argumentation on the need for an overview to study self-organization, the limitation in resolution is of minor importance as long as a spectrum of a whole cell is obtained.* Combining imaging analysis with microspectrometry, the entropy and thermodynamics of differentiating tissues can now be studied in the microscope. In tissue cultures, the effect on the cell composition of hormones, signaling substances, and pharmacological molecules can be directly followed by interpreting overviews of whole cell spectra in analogy with the NIRS endosperm mutant model. The high water content in living cells that may affect the resolution in NIR and infrared spectroscopy can be overcome by Raman spectrometry.

In light microscopy including fluorescence, there is a wealth of targeted techniques (Stephens and Allan 2003; see Chapter 5 this book) to follow the dynamics of specific signaling mechanisms (Weijer 2003; Mills et al. 2005) in chromosomes and cells. General spectral overviews and specific signaling staining methods of cell and plant analysis can now be integrated in a new perspective on gene expression by self-organization on the nanostructural level.

Plant structures are built up as phytomeres in hierarchical macroscopic units (Forster et al. 2007). Organ differentiation can now be studied in depth by image analysis and microspectrometry to connect the microchemical to the macrostructural level by new specific "painting" techniques (Stephens and Allan 2003; Weijer 2003; refer to Chapter 5 in this book). In this work, the classical morphological barley mutants (Lundqvist 2009) that directly control phytomer development are of great significance. The study of symmetric morphology in plants is greatly inspired by fractal mathematical models focusing on the effect of differential mechanical stress during development that may lead to "cracks" mediated by the high intrinsic pressure in plant cells (Cho 2004).

Overviewing the physiochemical patterns of plant–environment interactions to quantify abiotic and biotic stress tolerance by spectroscopy

Spectrometric overviews by crop scanners combined with the concept of self-organization have a great future in evaluating the development of the whole barley plant canopy to forecast quality and yield (Hansen et al. 2002) and to understand abiotic stress tolerance. The spectroscopic view of the physiological status of whole plants on the canopy level should be a necessary complement to the molecular analyses on abiotic stress as presented by Langridge et al. (2008). In analogy, with the genetic impact on the *phenome* in Fig. 18.4, the environmental effect on the *environome* can now in the future be captured as a whole by differential spectra compared to a reference environment on the canopy and the seed levels. The variability of the environome of different varieties due to climate, soil, and plant

husbandry can be calibrated on the canopy level in *physiological terms* by the clustering of differential spectra in a PCA score plot for coarse-grained physiochemical difference and can be further interpreted by molecular and structural analyses (e.g., stomata function). The spectral information on the influence of the environment on segregating phenotypes can then be connected to markers on the genome by association genetics as checked by data inspection and labeling programs to save the reproducible information that otherwise is lost by mathematical data compression.

The varieties characterized by giving high yields and stable quality, also when grown in extreme environments, are likely to be identified by having a narrow and more consistent single seed quality distribution profile by seed sorting (Fig. 18.5 and Table 18.2). Seed sorting will have a large potential impact on the early identification and production of new varieties that can stand severe changes in the environment including temperature stress as well as resistance to fungal infection that also can be evaluated by the seed sorter. The limits of the precision of single seed sorting for complex quality traits with specific mutant genes and recombinant genotypes is not yet known, but a considerable enrichment of such genotypes should be expected by the use of a small-scale single seed sorter in an analytical version.

CROSSING THE FRONTIER BETWEEN THE MICRO AND MACRO ASPECTS OF EMERGENCE

There are vast problems in delimiting emergent open systems from the cell to the biosphere. It is an advantage that biologists have access to data in a controlled environment on the initial conditions (DNA) of a cell (tissue), on the fine-grained (molecular) gene expression and on a coarse-grained spectral overview of the resulting phenotype that can be interpreted not only by mathematics and statistics but also most importantly by spectroscopic, chemical, and genetic *prior* knowledge as phenomenological patterns.

The classical chemist J.J. Berzelius introduced already in 1837 the concepts of affinity and catalysis in chemistry, which are explained for simple systems by the theory of quantum dynamics (Clary 2008). Prigogine (1997, 2003), in his theoretical work on self-organization, modified the mathematics of quantum physics and the thermodynamics of the micro world to include the macro world in a unified model by introducing correlations. There has been a strong tradition of causality in science to overcome the interpretation problem of emergent systems by surgery in networks (Pearl 2000). Reductionism in high-energy particle physics, genetics, and molecular biology has traditionally been preferred rather than searching for the necessary complementary coarse-grained overview in the global dimension as attempted here. There is in these sciences a profound mistrust in the many parameters that are manifest in nature. Thus, in a book on string theory in physics, Green (2005, p. 5) points out in his chapter, "Roads to Reality," that "human experience is often a misleading guide to the true nature of reality …. the insights in modern physics has persuaded me that assessing life through the lens of everyday experience is like gazing at a van Gogh through an empty Coke bottle." From the evidence reported here, the physical method of NIRS can now be used instead of "the lens of an empty Coke bottle to assess life" because, as formulated by Prigogine (2003, p. 64), "The structures of life are formed in low energy. The influence of quarks on systems of low energy is negligible."

Models in quantum physics explain many facets of matter (Green 2005) including chemical transient reactions (Clary 2008). The conservatism and the problem of communication between scientific disciplines, however, should not be underestimated. The famous slit electron experiment of Young in classical quantum physics (Leggett 2005) demonstrates that the observation instrument is involved in determining which hole the electron passes. This physical model appears as *isolated* from a quantum network that should belong to the macro world of, for example, a cell. Then it seems likely, from the biological experiment demonstrated here, that if Young's model is

transferred to a quantum network, the choice of the electron between the superimposed, macroscopically distinct alternative states should be decided nonlocally by the whole self-organized network (biological computer) serving as an internal "observer" that could guide the electron as "a narrow path between determinism and pure chance" (Prigogine 1997; Munck et al. 2010). Therefore, the great probabilistic flexibility of the quantum world is the prerequisite for self-organization and life where "natural selection" already starts on the quantum and molecular level as affinity and catalysis (Munck et al. 2010). Self-organization is mediated on the level of chemical bonds by electrons *but directed nonlocally* by the impact of the whole system. Similarly, it seems natural that gravity should be regarded as an emergent global property of self-organization of the subsystems in the universe rather than a particle—the graviton (Green 2005). The physicist Jan Ambjørn at the Niels Bohr Institute in Copenhagen has recently discussed such a solution based on self-organization.

SURVEILLANCE OF SELF-ORGANIZATION OF THE BIOSPHERE BY SPECTROSCOPY AND IMAGE ANALYSIS AS A BASIS TO PREDICT CLIMATE AND GROWTH CONDITIONS FOR PLANTS

Remembering that the previously documented limits in mathematical modeling of emergence are often ignored by scientists in biology, we may expect that these limits also may be underestimated by climatologists and in presentations in media. Of the 3.3 billion hectare land above sea level available on planet Earth, 11.3% is under plow, 26.3% grassland, 29.3% forest, 31.5% deserts, ice, and mountain land, and 1.6% urban/settlement areas (http://geodata.grid.unep.ch/). With our background from studies on the emergent system of cereal seeds by spectroscopy and in chemometric pattern recognition modeling, we will refer to and discuss the climate issue based on literature and from a premeeting in June 2008 to the UN Climate Summit 2009 in Copenhagen (http://www.copenhagenclimatecouncil.com) in cooperation with the CITRIS Climate Navigator (2008) Center at Berkeley, California (http://www.citris-uc.org).

The giant wave example and the importance of overviews

It has long been known (see Google: giant waves) that every week, a large vessel is lost on the seven seas without a trace. Meteorologists had calculated that the probability for waves larger that 15 m is negligible, and ship builders have thus for economical reasons constructed the vessels accordingly. However, satellites have recorded local waves reaching 30 m, which could explain the frequent accidents. They are only rarely produced by tsunamis due to volcanic activity. Giant waves are most likely to be a probabilistic manifestation by self-organization of the chaotic wave system at sea. Now, by remote sensing through satellites, such local giant waves can be recorded and ships in the viscidity warned. In the longer perspective, "hot spots" on the map with a higher propensity of giant waves such as those in the Norwegian Sea can be identified.

Waves in the sea as well as weather and climate are examples of the chaotic extreme of self-organization, while those of organisms, tissues, and cells seem more deterministic as if guided by "an internal computer." Modern orbiting spectral imaging geosatellites for cartography have an imaging resolution of better than 1 m (Collins UK Staff 2006). They are able to estimate living plant biomass over vast areas and to predict yield and pest attacks on crops in farmers' fields. Weather satellites in stationary positions are equipped with sensors from UV to infrared wavelengths and are built to monitor a wide range of meteorological conditions such as cloud and ocean stream patterns, snow, dusts on ice, fires, as well as local surface temperature on land and seas. Top-down information from weather satellites has helped in validating bottom-up ground data and to better understand the nature of the self-organizing system of the atmosphere. Today, meteorological

predictions (May 2004) are based on satellite data and the Navier–Stokes (N-S) hydrological equations using the powerful computers of today. It is estimated that due to the consequences of chaotic dynamics, the predictions beyond 10–20 days can be effectively impossible regardless of the capacity of computers. The fact that the N-S equations have chaotic solutions as distinct from computer simulation that looks chaotic is still unsolved (May 2004).

The concern of the human contribution to climate change

When writing this chapter, we are met by information on dwindling ice masses, increasing local temperatures, and a seemingly increased instability of weather conditions involving storms, flooding, and drought, which all seriously affect the production of foods including cereals. On one side, climate records documented by ice strata and tree growth rings indicate that dramatic short-term climate changes occurred in the past when human influence was small or absent. These changes have been explained by changes in the activity of the sun and by radioactivity from supernova explosions from the universe that is reaching the atmosphere and that could affect the heat balance and cloud formation of planet Earth (Svensmark and Calder 2006).

On the other side, globalization and new satellite information technology (Collins UK Staff 2006) has created an increased awareness of the heavy load that human activities without any doubt are creating on the dwindling natural resources, including essential water for human consumption and for agricultural production. The yearly burning of organic materials that in total amounts to about 7 billion tons of carbon is released and included in the CO_2 balance between the atmosphere and the photosynthesis of plants on land and in sea. During the last 100 years, CO_2 in the atmosphere has increased from 290 to nearly 370 ppm. CO_2 contributes to global warming. Methane, which is an even more potent greenhouse gas, has doubled in the last 200 years. Increasing ruminant livestock, rice fields, and the thawing of permafrost areas are estimated to have a major effect on amount of methane and CO_2 released to the air.

Global warming, causing local fluctuation in temperature and precipitation between years, would put a pressure on plant breeders and agronomists to develop new varieties and improved growing conditions that will support high yield and quality even in extreme environments (see Chapter 9 in this book). At the 10th International Barley Genetics Symposium in 2008 in Alexandria, J. Spunar presented a paper on malting barley (Blumel et al. 2008) discussing the global warming impact suggesting winter barley as a reserve crop for spring barley because of higher yield stability and escape of high temperatures in the summer.

Predicting climate

When climatologist mathematical modelers in the beginning of the 1990s were predicting the causal elements of greenhouse gases on temperature change, they calculated a global average temperature change of 0.7°C for a period of 35 years. The International Panel on Climate Change (IPCC 2007) sponsored by the United Nations shared the Nobel Peace Prize in 2007. The panel claims in its fourth report the same year that "warming of the climate system is univocal" and "is very likely due to the observed increase in anthropogenic (human) greenhouse concentrations. World temperatures could rise between 1.1 and 6.4° Celsius and there is a confidence level >90% that there will be more frequent warm spells, heat waves and heavy rainfall. There is estimated an increase in droughts, tropical cyclones and extreme high tides on the confidence level of <66%."

At the 1997 UN Climate Summit in Kyoto, there were detailed discussions on how to reduce CO_2 emission by national programs and by dealing with CO_2 emission rights to control industrial emissions. In spite of IPCC's predictions, the global temperature since 1998 has not increased (http://www.cgfi.org) in spite of pronounced

local warming in Europe, in the Himalayas, and in the Arctic area. However, strong warming spells have been registered earlier in the Arctic that started in 1918 and in 1960 but then faded away. The predictive computer models should be looked upon as a mathematical approximation based on "surgery in a network of the parameters" (Pearl 2000) that has serious limitations (Lindzen 2008). Patterns, for example, defined by PCA score plots, are much better indications for a causal change than using single variables. As demonstrated by Lucio et al. (2007), temperature patterns by PCA are superior to simulations in modeling the local climate. It is questionable if it is at all meaningful, with the surprising ability of chaotic systems in mind, to focus on a yearly global average value for just one parameter at a time.

The long-range weather or climate is an emergent quality of the biosphere that in an open self-organized system is exposed by solar rays and radiation from the Universe. The climate can be affected by dramatic changes in the universe that have been recorded in geological and ice strata. In the latter, significant CO_2 peaks have been detected, but usually about 100 years after that, a period of warming has started because of microbial breakdown of humus (Svensmark and Calder 2006). At present, man-made CO_2 release is mainly caused by fossil fuel and wood burning including clearing of rain forests, for growing oil palms for food and energy, and for rearing cattle. These activities exceed the industrial contribution of CO_2 release that amounts to ~20%. In large parts of the Atlantic and in Western Europe, the warm golf stream contributes to a warm climate in spite of the northern latitude. The thermohaline circulation pump brings saltwater with high density back from Iceland to Saragasso. It has been shown from records in sediments that ice ages have been precluded by short warm periods. Melting ice from global warming will dilute salt concentration in the deep sea and weaken the thermohaline circulation that is needed for the Gulf Stream to function. A recent evaluation, however, claims that the Gulf Stream circulation is noisy but not stalling (Schiermeier 2007). Particles and soot on ice emerging from industrial activities (prevalent in the Northern Hemisphere), forest burning, and volcanic activity absorb solar radiation and are estimated to contribute to ice and glacier melting by ~30%.

The atmosphere absorbs longer wavelengths from the sun more effectively than shorter wavelengths. Greenhouse gases transfer energy from the sun to longwave radiation that produces "a greenhouse" warming effect. *Atmospheric water vapor has by far the greatest absorption bands in the infrared area as compared to carbon dioxide, oxygen and ozone, methane and nitrous oxide*. These spectral data are from reproducible measurements that can be checked in the laboratory. The modeling of influence of atmospheric water on the flux of temperature is complicated by the fact that molecular water vapor supports warming as a greenhouse gas, while water in the sky macrostructures has a cooling effect (Svensmark and Calder 2006). A comparison of the most used climate models (Trefil 1996) with current climate conditions reveals errors in prediction of 100% in cloud cover, 50% in precipitation, and 30% in temperature change.

There are claims that the IPCC report has underestimated the effect of the increasing atmospheric water vapor and cloud formation (Svensmark and Calder 2006) in their forecast on global warming. It seems likely that particle emission from burning is more important than the greenhouse gases including CO_2 in contributing to the significant increases in temperature in Europe, in the Himalayas, and in the Artic region. If the ongoing release in CO_2 is not the major factor behind global warming, then a high concentration of CO_2 in the atmosphere should be rather regarded as an asset from a plant breeder's point of view because it would increase the photosynthetic efficiency and yield of plants. One year before the 2009 UN Climate Summit in Copenhagen, there is a major focus on CO_2 and greenhouse gases as the main trigger of the climate change that now seems to be widely accepted also by industry and media. *It should be concluded that any attempt to explain the present changes in climate by one major factor alone must be rejected on scientific grounds.*

Freshwater: the limiting factor in maintaining the symbiosis between plants and human society

A recent book, *Fragile Earth* (Collins UK Staff 2006), delivers dramatic satellite views of our changing planet and the extraordinary effects of human populations on nature including expanding cities, pollution, deforestation, fire, floods, drought, advancing deserts, shrinking lakes, dying rivers, and increased salinity of agricultural land. Cities contained half of the world population in 2007, occupied less than 2% of the land surface, but used two-thirds of the resources. Deforestation is a major problem in the rainforests, for example, of Brazil and Indonesia, covering an area equal to Ireland per year. In China, an area of forest almost equal to that which is cut down in Brazil is planted. A vigorous cover of forests and plants that binds water is the prerequisite for local rains and conservation of water for agriculture and for human domestic use. Conservation of forests and plantations is especially essential in the mountain areas of the world in retaining precipitation for agriculture when glaciers and snow cover are vanishing. The importance of retaining water can be exemplified by the present changes in mainland China. There is a strong competition with regard to water resources between agriculture and cities. A significant part of the surface water that was previously used for agriculture in the arid areas in a wide zone around Beijing is now pumped to supply the capital. There are plans to pump water to Beijing from the Yangtze River at a distance of more than 800 km. It is not possible to use the nearby Yellow River 250 km from Beijing because it is overexploited by agriculture and industry and has 250 days/year of no flow(Collins UK Staff 2006). The recent gigantic Three Gorges Dam project in China created a lake of 632 km^2. It will moderate the severe problems of seasonal flooding in the Yangtze River and will highly improve the efficiency of water utilization for agriculture in analogy with the Aswan dam from 1970 in the Nile River in Egypt. The latter demonstrates that there are severe risks for increased levels of salt in the soil that could seriously limit agriculture if proper care is not taken in managing irrigation (Collins UK Staff 2006).

The 3rd United Nations World Water Development Report (2009) focuses on the low worldwide attention to conserve water, which is the limiting factor for food production and human well-being. About 2 million tons of waste per day is globally dumped within receiving waters. Projected climate change will increase water scarcity by 20%. There are 1.1 billion people, most of them poor, who do not have access to potable water. In 1998, in developing countries, irrigated land produced three-fifths of all cereals.

Freshwater is a fundamental source for retaining plant life and photosynthesis on land, which is essential for maintaining higher life on planet Earth. It is also a resource that is most easily wasted and where intelligent uses, such as drip irrigation, can save water by a factor of 50 or more. The fundamental role of water to maintain life, food production, and human health has been completely overshadowed by the CO_2 issue and global warming. It is likely that man-made CO_2 emissions have to be taken seriously, but not necessarily within a focus on global warming where other factors are more important.

The human impact by CO_2 pollution, deforestation, water redistribution, and urbanization is summed up in the self-organized "computer" of the biosphere and is merged with other inputs from the earth, sun, and the universe to form global and local climates. We can observe the total result of this "computation" as fluxes in climate that never can be causally assigned to one or a few causal elements alone because of the properties of emergent systems. Still, the human impact is well founded as observed on the ground and from satellites (Collins UK Staff 2006) and must be taken very seriously. Simplified causal arguments to "solve" the climate crisis (Lindzen 2008) must be succeeded by *a new strategy built on real data gathered in inventories evaluated by the fundamental principle of pattern recognition to interpret the self-organization that is expressed in nature*.

STRENGTHENING THE SYMBIOSIS BETWEEN THE CEREAL PLANT AND THE HUMAN SOCIETY

> Nothing will benefit human health and increase the chances for survival of life on Earth as much as the evolution to a vegetarian diet"
>
> Albert Einstein

The future perspective in improving food and feed production and utilization

Cereals constitute a "thread of life" as the main source of food for the world population and will continue to do so in the future if mankind succeeds in maintaining an environment that is friendly to plants and to the production of plant foods.

The last 200 years of industrialization, including agriculture driven by fossil fuels, have completely changed the basic living conditions for the growing human population that was about 0.8 billion in the year 1800, 1.5 billion in 1900, and 6 billion in 2000. The feeding of the population in the last century would not have been possible without the increased yields due to nitrogen fertilizers made by the Haber–Bosch synthesis. Industrial nitrogen fertilizers were introduced in the 1920s and facilitated the fixation of atmospheric nitrogen to hydrogen to form ammonia. Recent world production of cereals was just over 2 billion tons in 2006 (Table 18.3) and that of meat was 258 million tons in 2004. Meat consumption has doubled since 1970 and is rapidly increasing by more than 5% per year in the expanding economies in China and India. The increasing demands for meat and the high price of fossil energy, according to FAO statistics, have increased the price of cereals by 53% during the first four months of 2008. This turned out to be a short-term spike, but demonstrates the potential of high food prices to rapidly increase the number of people with an inadequate food supply. Six to sixteen kilograms of grains/soybeans are needed to produce 1 kg of red meat. A product life cycle analysis in the 3rd United Nations World Water Development Report (2009) demonstrates surprisingly that cereals use 1.5 m^3 water/kg, while fresh bovine meat needs 15 times more. The calculated total amount of greenhouse gases from meat production in Australia exceeds that from production of consumer electricity and from private motor vehicles (http://www.greenhouse.gov.au/).

Table 18.3 World production of cereals and lignocellulose (FAOSTAT 2006; http://faostat.fao.org/site/567/default.aspx)

	Million Tons
Cereal seeds	2,160
Barley	138
Cereal straw[a]	2,400
Internodes[a]	1,300
Leaf and marrow meal[a]	1,100
Barley straw[a]	150
Interndoes[a]	75
Leaf meal[a]	75
Paper production instead of pulp	
From wood	300
From straw and grasses	15

[a]Approximate values calculated from seed production.

Theoretically, the present cereal production would be able to feed a population of 14.7 billion individuals with 400 g/day. Retrospectively, we now understand how the vegetarianism built into the old cultures of India and China facilitated a near-sustainable high-population density. Changing eating habits is the single most effective way to support a growing population and to reduce pollution and save water resources if the right incentives could be produced. Therefore, the development of attractive and efficient plant foods by breeding and food product development is essential for future development. Here also, traditional uses should be documented and revived. Barley is a hardy classical food (see Chapter 17 in this book) that now is used to some extent for malt (see Chapter 15) but overwhelmingly for feed (see Chapter 16).

Plant breeders could contribute not only to increase plant yield but also to improve nutritional efficiency and to reduce pollution. By increasing available essential amino acids in high-lysine barley mutants (Munck 1992), nitrogen pollution in pig feeding can be reduced by

20% and protein utilization improved by the same figure. Likewise, phosphorous pollution/utilization in feeds can be decreased/improved by 50% with low phytic acid barley and maize mutants (Raboy 2009). It is significant that in both cases, the improved balance of nutrients introduced a more efficient energy utilization by animals (Munck 1992; Raboy 2009). Thus, overfeeding is as negative as underfeeding from a total metabolic efficiency point of view that also should apply to human physiology.

Of the world population of 6 billion in the year 2000, there were approximately 2 billion individuals with serious food health problems equally divided between under- and overfeeding. The *lys5.f* mutant is an example of how mutation breeding (Munck and Møller Jespersen 2009b) could contribute to improving dietary foods. Its record high BG content of up to 20% reduces caloric content and glycemic index by 40% and increases high-quality dietary fiber by 150%. In order to practically exploit the low-yielding *lys5.f* mutant, the gene background for improved seed quality and yield may be cost-efficiently changed by data breeding.

When low-quality damaged seeds are highly paid as an energy source, value added single seed sorting for bread and malt quality should be highly attractive. It should be acceptable to use single seed separated toxic seeds infected by mold for energy that cannot be used for food or feed. When in the future industrial seed sorting has been introduced in a large scale, distribution of quality of single seeds in seed batches is likely to be an important quality criterion in itself as related to total economic yield.

The complex yield trait is not quality neutral. Because protein and oil have 2.1 and 2.5 times the glucose (energy) costs of starch for plant DM production, selection for yield indirectly results in high starch and low protein and oil composition of seeds. Yield could consequently be improved through NIRS by selecting for a high content of starch combined with low protein and fat value. Hereby, cost-efficiency in yield evaluation in plant breeding could be improved by reducing the number of replications that are necessary in relative expensive yield trials (see Chapter 8 in this book).

Whole crop utilization for food and nonfood purpose

One hundred years ago, the manufacturing industry used relatively more cereal raw material than it does today. Starch, for example, was used extensively in the textile industry and straw was used as a main source for paper pulp. Since then, other materials from mineral oil, wood, and so on have reduced the use of cereals for nonfood purposes. In subsistence farming, all parts from crops like barley and sorghum (Munck 1993, 1995) are utilized. The combine harvesting technology driven by cheap fossil fuels, introduced in a large scale in Europe and in North America in the 1950s, downgraded straw to be burnt as a waste in the field. In the future, the biological chains that have been broken by fossil-driven technology will be partly reestablished by new technology taking care of the whole plant, including seed and straw (Bjørn Petersen and Munck 1993; Munck 1993, 1995, 2004). In Table 18.3, the total yield of 4.6 billion tons of cereal plants was preliminarily estimated from the world production in 2006 including 2.2 million tons of seeds and 2.4 billion tons of lignocelluloses. The cereal lignocellulose potential can be compared to the approximate world production of paper from wood of 300 million tons and from straw and grasses of about 15 million tons.

Up to one-third of U.S. maize grain production is estimated to be used for bioethanol from 2009 to 2010. An extensive use of sound cereal seeds for nonfood purposes will not be sustainable in the near future. The world production of crude oil was estimated in 2008 to be 5 billion tons yearly. Global crude oil burning is calorie-wise 2.5 times the calories contained in the whole cereal plant crop of 4.5 billion tons. Instead, cereal lignocelluloses that largely have been wasted in the industrial countries can be used in the second generation of bioethanol plants. Straw is now again collected in Denmark to be burnt in large heat–electricity–warm water generating

units near to cities. In 2006, 12% of Denmark's energy was produced by burning biomass of which one-third came from straw.

The first energy crisis in the 1970s stimulated research in the European Union (EU) and the United States on the use of biomass for nonfood uses (Pomeranz and Munck 1981; Munck 1990, 1993, 1995, 2004; Bjørn Petersen and Munck 1993). This research originally had its roots during World War II pioneered at the United States Department of Agriculture (USDA) laboratory in Peoria, Illinois. Starch from cereals has been traditionally used in the textile and paper industries, which in the EU amounted to about 5 billion tons per year. Starch (Fig. 18.8) can by minor chemical modifications be transformed to cationic and anionic starch for application as filler in paper and to degradable plastic that can be used for packaging. Starch and sugar fermentation has, depending on the price of petroleum, the potential to produce a wide range of chemicals that now are produced from fossil material.

The main use of straw today is feed for ruminants. The energy feed value can be greatly increased (+50%) by pelleting and heat treatment after the addition of sodium hydroxide or ammonia (Fig. 18.8). A surprising application of barley straw or straw extract (with polyphenols) is the control of algae in ponds including commercial fish ponds. Straw has traditionally been used for paper production before the wood paper process was introduced. Straw and grass today is utilized for paper in Spain, Egypt, Pakistan, and in other countries where forests are scarce. The efficiency of straw in paper pulp manufacture can be greatly improved by the principle of *value*

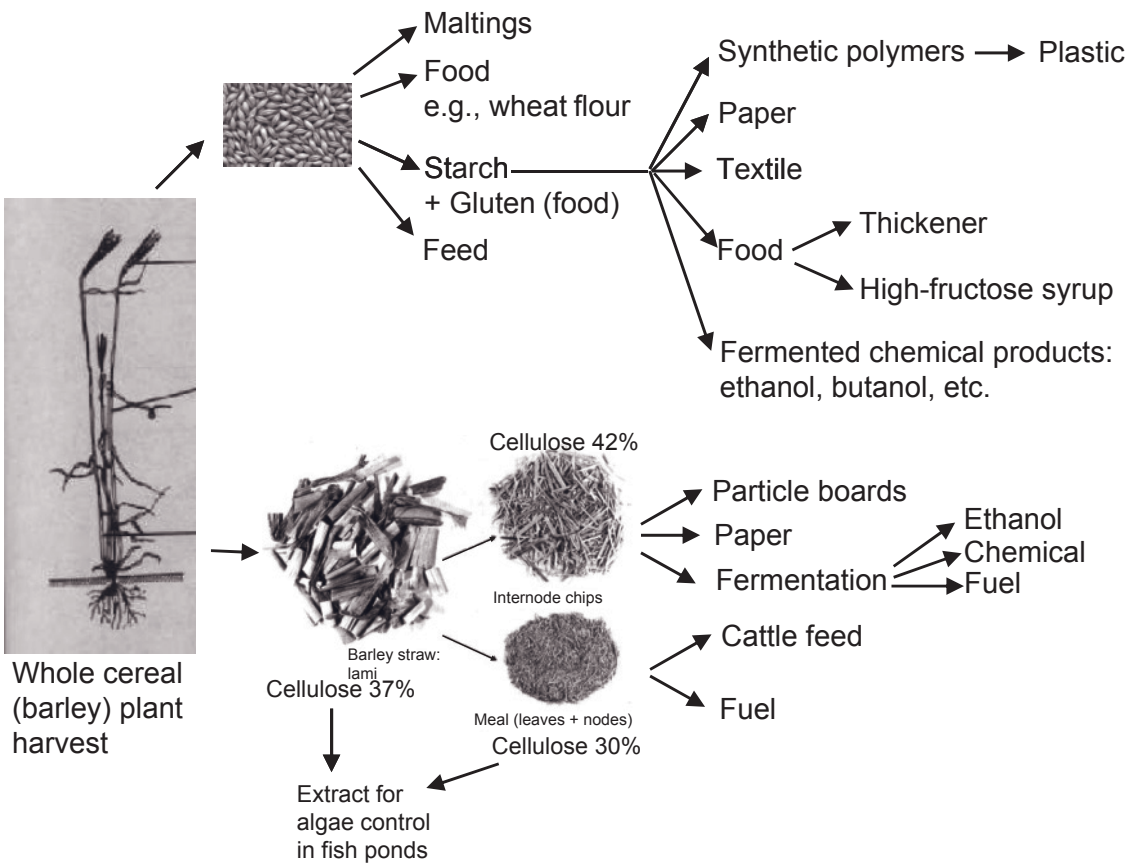

Fig. 18.8. Whole cereal plant utilization (see Fig. 18.10).

added sorting as shown in Fig. 18.8. By milling and sifting of straw (Table 18.4; Bjørn Petersen and Munck 1993), a coarse fraction consisting of internodes and nodes is obtained, which has 42% cellulose similar to wood (43%) compared to 29% for unseparated straw. The internode fraction is optimal for fiberboards and for paper pulp. On the other side, the leaf fraction has a higher value for feed than unseparated straw because of more than doubled protein content and higher digestibility (51%) compared with internodes (41%).

Silicon in straw is a significant problem in paper pulp manufacture because it is dissolved during the process and retards recycling of alkali in the black liquor after the removal of lignins. As seen in Table 18.4, silicon is reduced several times in the internode fraction by the milling and separation process. Plant breeding for increased fiber length and coarseness in cereal internodes should be considered in the future. The natural variation in fiber parameters from cereal crops grown in Denmark is displayed in Table 18.5 (Bjørn Petersen and Munck 1993). There is significant variation in fiber quality in barley with the classi-

Table 18.5 Yield of α-cellulose, average fiber length, and coarseness of chemical, bleached pulp from hand-dissected internodes of wheat, rye, oats, and barley from different varieties (adapted from Bjørn Petersen and Munck 1993; with permission from AACC, St Paul, MN)

Variety	α-Cellulose (%)	Average Fiber Length (mm)[a]	Coarseness (mg/m)
Wheat (Kraka)			
Internode	42.2	0.77	0.082
Leaf	29.3	0.63	0.112
Rye (Petcus)			
Internode	41.3	0.83	0.081
Leaf	29.8	0.74	0.085
Oat (Risø)			
Internode	39.2	0.91	0.103
Leaf	30.3	0.63	0.118
Barley	40.1	0.86	0.086
(Triumph)			
Internode	38.3	0.74	0.091
Leaf	28.2	0.53	0.114
Golf	40.1	0.83	0.160
Corgi	38.5	0.85	0.086
Golden Promise	42.7	1.02	0.092

[a]Average length-weighted fiber length.

Table 18.4 Analysis of hand-dissected botanical components of straw (Bjørn Petersen and Munck 1993; with permission from AACC, St. Paul, MN)

		Weight Distribution % of Total Harvested Straw (w/w)			Mean composition (% DM)			
		Mean	Max	Min	α-Cellulose	Protein	Ash	Silicon
Spring barley	Internode	50.4	55.1	44.7	38.3	2.0	4.5	0.5
	Leaf	41.6	48.4	33.9	28.2	3.9	6.3	1.4
	Node	5.4	4.0	1.0				
Winter wheat	Internode	55.0	63.0	49.8	42.2	3.0	4.6	1.0
	Leaf	38.7	44.2	31.6	29.3	5.2	8.4	2.3
	Node	4.8	7.0	3.4				
Winter rye	Internode	67.7	68.7	66.7	41.3	3.0	3.7	0.5
	Leaf	23.9	25.4	20.7	29.8	5.9	5.7	1.2
	Node	5.2	6.5	2.8				
Spring oats	Internode	50.4	53.4	47.3	39.2	2.6	4.6	0.2
	Leaf	42.1	45.5	38.7	30.3	3.5	7.4	1.9
	Node	4.4	5.1	3.7				
Maize	Internode	46.6	52.0	40.4	39.5	3.7	6.3	0.2
	Leaf	42.1	46.4	34.3		8.3	7.9	1.4
	Node	11.9	16.6	8.7				

cal variety Golden Promise leading with 9% higher cellulose and 19% longer fibers and thicker fibers compared to the Triumph variety. In conclusion, cereal lignocelluloses are a valuable resource especially after value added fractionation. Internodes/nodes and leaves from straw can be used for feed and for nonfood uses such as fiberboards, paper pulp, bioethanol, and energy without direct competition with food production.

Biorefineries: integration of whole plant utilization with human needs closing the production circles in balance with nature

During the first 5 years in the 1960s, agriculture in southern Scandinavia was in a serious climate crisis with an extreme cold and wet climate that affected storage and the quality of cereal produce. In Denmark, about 30% of the pigs had serious kidney problems due to toxins from fungal infection in barley grain that, in the case of humans, would have involved permanent dialysis. In this difficult situation, the concepts of "whole crop harvesting" and "biorefinery preprocessing stations" (Munck 1990; Rexen 1990) came up independently in Sweden and Denmark as a systems approach to increase crop reliability, production efficiency, and cooperation in the local farming community (see review by Munck 2004). In the 1960s, large oil drum dryers fueled by cheap oil originally designed for producing lucerne or alfalfa (*Medicago sativa*) feed pellets were then converted to dry cereal crops harvested whole by field choppers. It was found in full-scale experiments that whole crop harvesting and drying of a wide selection of crops could greatly increase flexibility in plant husbandry by safe harvest and conservation of the whole biomass. For the first time, crops with long growth periods such as maize and broad beans could then be reliably harvested in Scandinavia. The increased oil prices during the first energy crisis in 1973 made the traditional use of large drum dryers uneconomic, and the lucerne pelleting industry vanished.

The increase in energy prices in the 1970s made society and science focus on renewable sources as documented in the American Association of Cereal Chemists (AACC) book *Cereals: A Renewable Resource* (Pomeranz and Munck 1981). However, in the 1980s, energy prices were not high enough to reduce the increasing surplus of agricultural products by nonfood uses. In Fig. 18.9, the world market prices in 1965–2008 of maize, crude oil, and pine paper pulp are outlined showing the dramatic increase for crude oil since 1998. The very high-energy costs in extracting lignocellulose fiber from the forest are reflected in the steady high price of paper pulp in relation to both maize and crude oil. The straw internode fraction should be highly competitive with wood as a raw material for paper because of the more energy-efficient harvest in a biorefinery system. Since 2005, the price of maize has followed that of crude oil (Fig. 18.9).

The extraordinary low fuel prices up to the 1970s had drastically lowered the energy efficiency in agriculture and forestry by favoring energy expending equipment. Thus, in 1986, the EC-12-cereal harvest demanded half a million combine harvesters worth 3 billion euros that were used no more than 100–200 h/year (Rexen 1990). The European Commission in 1986 (Sargeant 1990) organized an agroindustrial development program (http://www.ienica.net/) to stimulate innovation involving whole crop harvesting and biorefineries (Munck and Rexen 1990) that was launched under the FAIR EU program in the 1990s. A "Bioraf" demonstration project financed by EU was developed at the island of Bornholm, Denmark, based on equipment from previously established industries—a feed processing unit, a lucern drum dryer, and a fiberboard factory (Rexen 1990). A pilot plant was built involving milling and separation equipment for cereal seeds and straw, also including a rapeseed oil extraction plant. It was realized that the whole crop drum dryer process could be self-sufficient in energy by using one-third of the straw as a fuel—preferably the leaf meal that could be sprayed directly into the oven.

The concept of the agricultural biorefinery is outlined in Fig. 18.10A. The biorefinery unit using up to 2000 ha from cooperating farmers is a local harvest machine station combined by drying and preprocessing equipment combined with a feed factory/seed elevator. A biogas unit based on animal and human manure/urine produces gas for driving harvest equipment, while the rest of the product is recycled as fertilizers to the field.

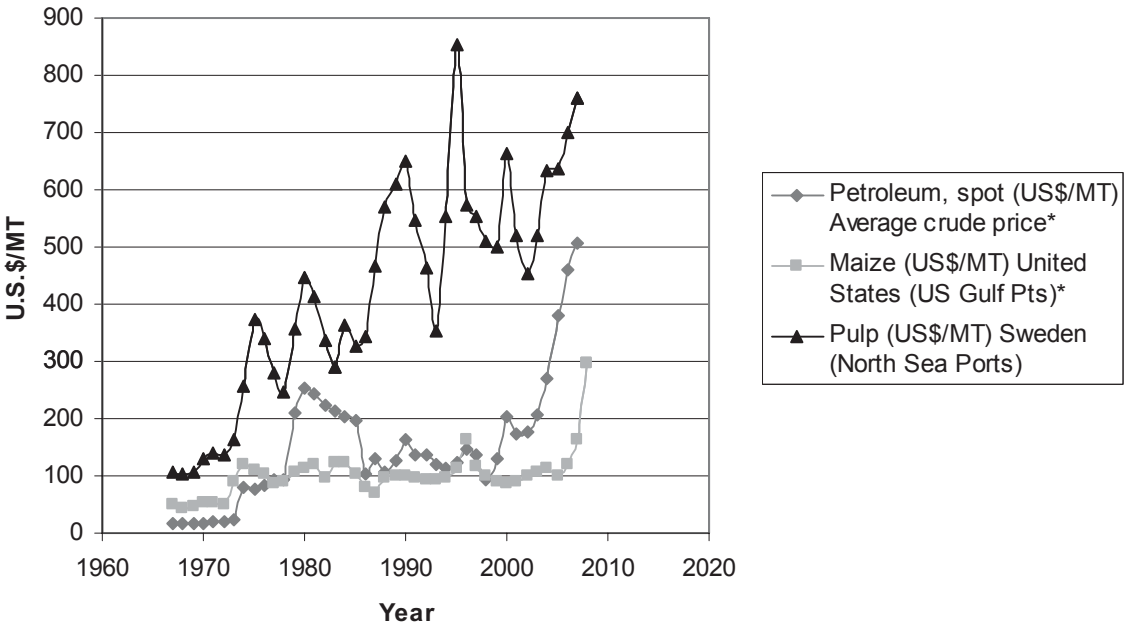

Fig. 18.9. Market prices of crude oil, maize, and pine pulp (IMF 1996, 2006).

The unit was planned to be self-contained with renewable energy and exports seeds, straw internodes, and feed products out from the area to large industries.

Paradigm shifts in infrastructural networks are seldom and expensive. The difficulty to implement the biorefinery concept is due to the fact that the society is married to its earlier investments in infrastructure. A large number of biorefineries have to be implemented together in large enough scale as a whole network of hundreds of units that together could mediate a cascade of changes in the agroindustrial infrastructure. Because harvesting and drying is secured independently of weather, a wide range of old and new crops covering the whole growing season can then be produced and adapted to the needs of the market. The wider variety of crops improves disease control. Machines can be used 8–10 months instead of a few weeks. The local biorefinery unit can provide work during the whole year and makes the area less dependent on energy prices.

In 2008, at the wake of plummeting crude oil prices, the Danish state gas and energy company Dong announced a demonstration plant (http://www.inbicon.com/index.htm) for bioethanol processing from lignocelluloses yearly of 30,000 t of each of straw and feed grain to produce 13,500 t of ethanol, 10,000 t of biofuel, and 9500 t of dry weight feed molasses with a CO_2 reduction of 40,000 t/year. The research behind the project has been partly funded by EU and can be seen as a first step to an urban version of the biorefinary (Fig. 18.10B) to utilize low-grade energy from power stations.

After hydrothermal cellulose treatment (Fig. 18.10B), the cellulose is hydrolyzed by enzymes and the glucose is fermented to ethanol. The lignin fraction is burnt for energy. The newest idea is to recover low-grade heat from power stations to be used for the energy-demanding distillation step in making bioethanol after yeast fermentation of the hydrolyzed celluloses from straw and household waste. The difficult step of fermentation of hemicellulose is avoided by producing high-quality molasses for ruminant feeds that are sold back to agriculture.

Pure CO_2 obtained from fermentation is a valuable raw material to combine with hydrogen to produce methanol (Fig. 18.10B) for fuel and as a raw material for the chemical industry. Hydrogen can be produced by electrolysis to regulate the output from the windmill power stations that are in a steady increase. By closing the urban and

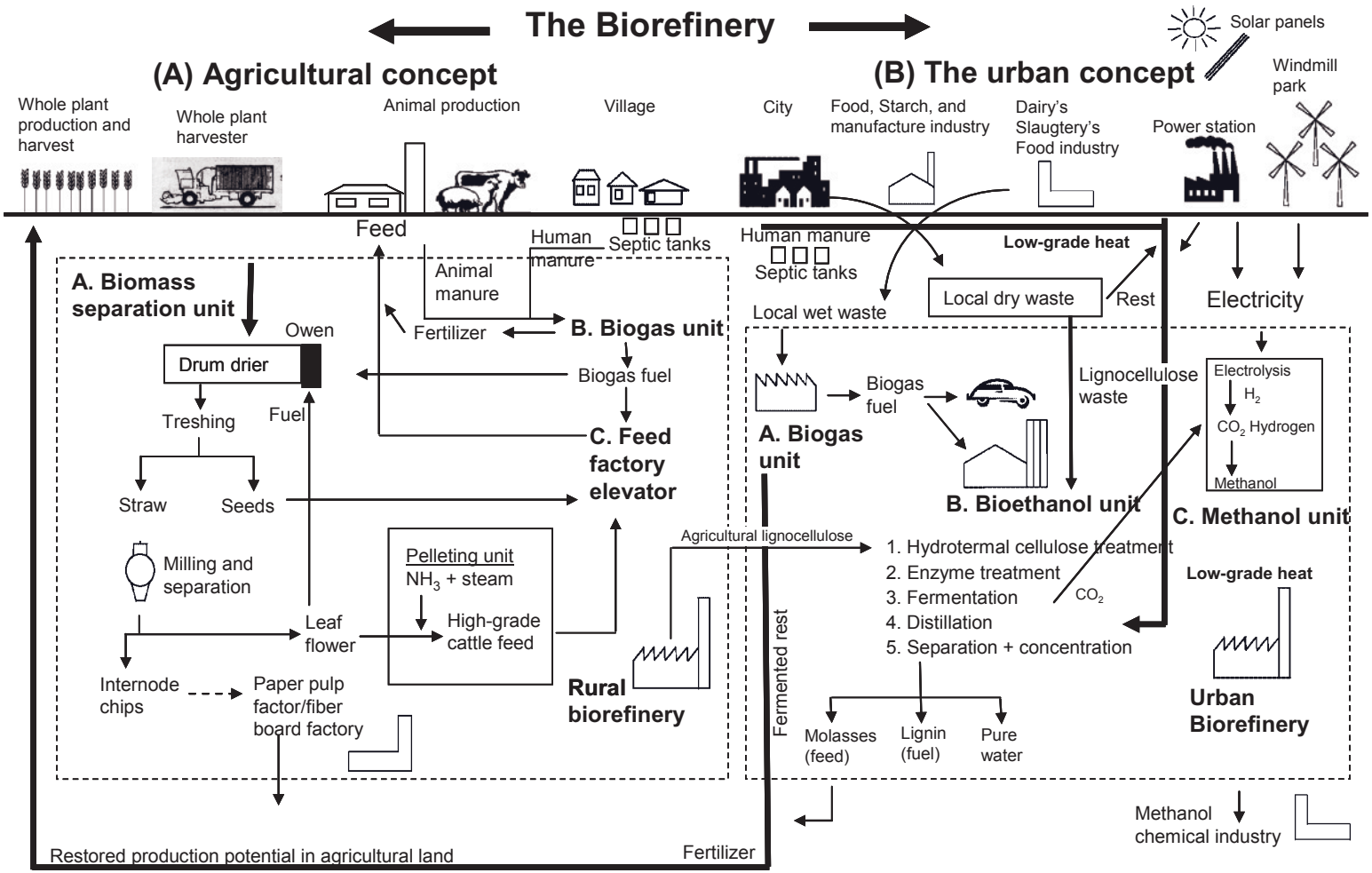

Fig. 18.10. The agricultural and urban biorefinery.

rural biological production chains (Fig. 18.10A,B), human manure/urine is collected in septic tanks instead of wasted in the sewage system to be processed in biogas units to fuel and to fertilizers that are recycled to agriculture.

It is now clear that there is advanced technology for each of the many biological processing steps that are needed to be combined in a paradigm shift to make a major change from a wasting to a recycling society. The question is now how the self-organizing society and market as a whole can select, combine, and optimize all these options in an efficient network focusing on improved sustainability.

Integration: stabilizing the infrastructure of the chaotic emergent global society to obtain an improved balance between self-organization in nature and in human activities and innovation

In Western society since the 1970s, there has been an increased focus on causal relations that could promote short-term profit. Before 1970, companies were investing in long-term diversification to obtain higher economic endurance during recessions and to fulfill a social responsibility to the network of society. Since about 1978, industry focused on their own field of business with the aim to produce short-term cash to the shareholders and the management. Competition in a deregulated market was thought to secure efficiency. The main services in the backbone of society such as electricity and water supply were privatized, leading to problems regarding supply and price because the profit strategy of the time did not allow long-term investments and enough resources for maintenance and renewal. *The persistent structure building that was needed to increase the adaptability of the emergent society network in real life was compromised for the transient catabolism of energy and resources focused on entertainment and virtual life* (Fig. 18.11).

There is need for a long-term interdisciplinary approach in a scientific analysis of (self-organizing)

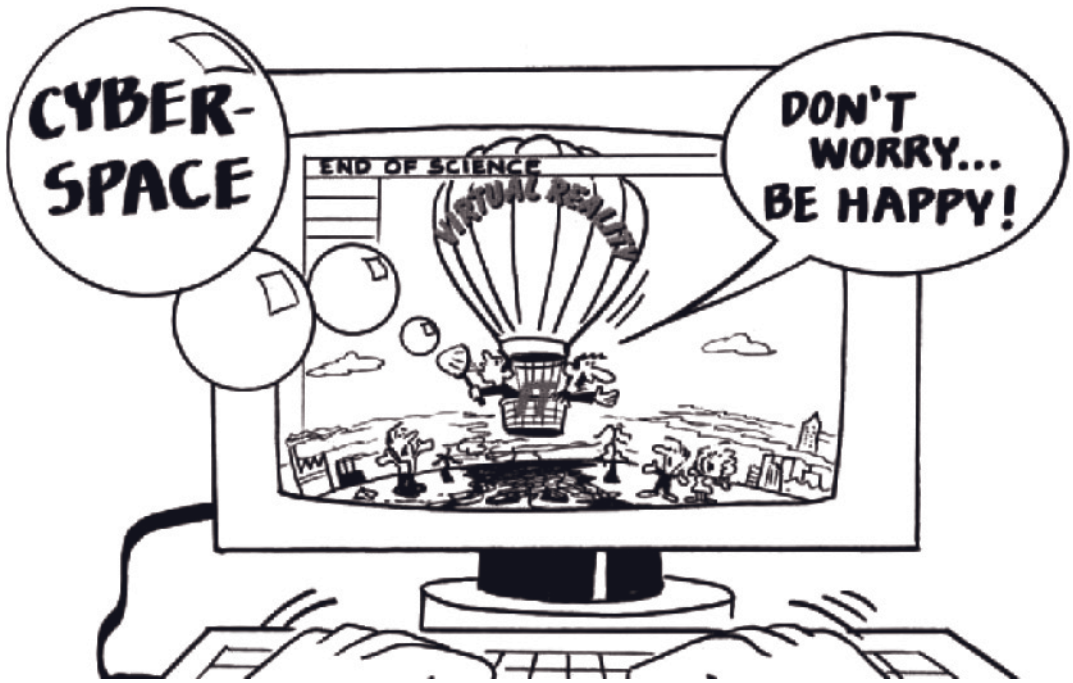

Fig. 18.11. In silico veritas: the selected participants in the computer modeling expedition into virtual reality wave farewell to the inhabitants on Earth. What is to going to happen with those who cannot join the expedition but are longing for the perfection in the freely designed universe? Who is going to survive? © T. Newlin/L. Munck.

systems comparing the pros and cons of government and market control of the basic infrastructure. The feasibility of large demonstration projects for a multifactorial improvement in infrastructure such as the "biorefinery" concept should have high priority. There is no lack of scientifically well-motivated point "solutions" to improve infrastructure. But neither single states nor the free market is capable to select among the available solutions *to improve infrastructure in the direction of an optimal combination* without pragmatic testing and implementation in an international effort. On the other side, the market is rapidly responding to earning short-term money on consumer and financial products. These consumer products have until recently been in focus instead of the needs to improve the efficiency of the infrastructure of basic supplies. Even if "global warming" is on the agenda and crude oil is reaching US$150 per barrel in the spring of 2008, the American car industry had not yet fully accepted that hybrid electric cars, combined with a battery charging and exchange system, is now already economically viable (Lemoine et al. 2008). But who will invest long-sighted in infrastructure when the price of oil went back to the US$40–US$50 per barrel range in the spring of 2009, when there are other less risky ways to earn quick money?

The shortsightedness of the stock market is at present reflected in the extremely low share prices of energy-producing companies. It seems that inputs from governments are the last hope to secure a long-sighted development of the crucial infrastructure.

At the climate premeeting in Copenhagen in June 2008 with scientists from the CITRIS Center at Berkeley, California, and leading Danish companies, the economists seemed to have left their earlier individualistic targeted strategy for industry and agreed on the need for long-term broad investments in *all* the main aspects of the biorefinery network discussed above (Fig. 18.10).

It seemed that both CITRIS (Kammen 2007) and the industrial representatives have accepted IPCC's (2007) global warming concept including CO_2 and greenhouse gases. In 2008, a major international oil company was reported to cut funding to climate change denial groups. The present focus on CO_2 should be seen as a symptom—a measure of excess human activity that is scary as such. However, it is likely that intelligent utilization of sparse and erratic water resources will constitute the limiting factor to survive a warming climate where CO_2 is just one contributor. The human symbiosis with cereals and with the entire ecosystem has to be supported as a first priority. It would be a catastrophe if the present suggested global CO_2 reduction program would divert the full attention that is urgently needed to be given to water conservation and development. *An eventual global CO_2 tax does not stop the wide misuse of water but could make sense if the money gained could be transferred without deduction to long-range water conservation to improve the local climate including agriculture.*

CONCLUSION: MAN AS SELECTOR—A DARWINIAN BOOMERANG STRIKING THROUGH NATURAL SELECTION

We are living in a stacking Russian doll-like situation in the biosphere with dynamic emergent self-organizing networks outside and inside us, as depicted in Figure 18.12 (Munck 1991). Here, over 6 billion fellow human agents with a causal focus are living their lives by selecting resources, breeding plants, the environment, and their future with a virtual entity "money" based on the communication of confidence as the most visible selection criterion. Biologists are impressed by the high reproducibility and stability in self-organization of organisms as compared to the climate of the biosphere and the prices on the stock market that indeed are unstable self-organizing systems.

The human brain is a self-organized computer that exceeds the capacity of most digital computers. The brain can receive and evaluate several megabytes of information per second from the senses, for example, by visualization, in comparing two or more fingerprints. The limit of the brain is centered on the narrow path of communication by language and text. The long-term memory of the human brain can store the

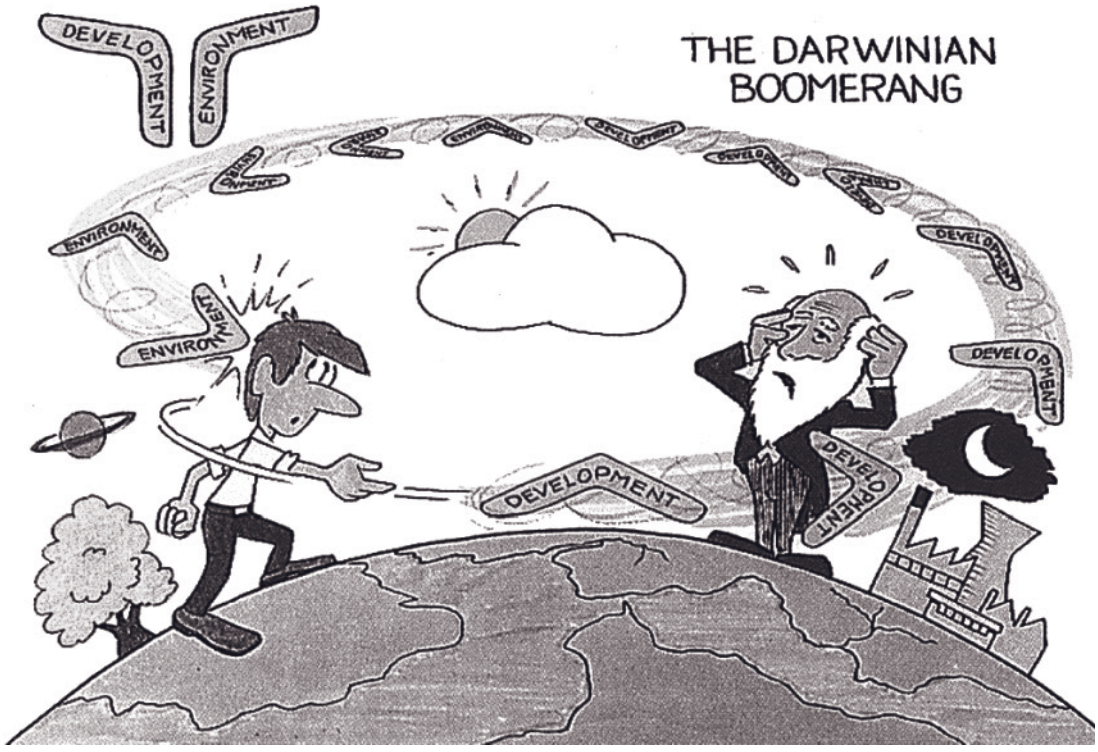

Fig. 18.12. Man as selector driven by the attraction of cause and effect aims the Darwinian boomerang targeted for specific development. The boomerang hits his back with the punch of environment. Charles Darwin looks terrified on observing this aspect of his "natural selection." © T. Newlin/L. Munck. For color details, please see color plate section.

meaning of 100,000 words passively and 10,000 actively. The short-term memory can only handle up to 20 letters a second. This is why human communication through language and numbers also in science is a gigantic communication problem where mathematical modeling plays a central role in order to improve precision and to automate complex sequences of logical operations. However, retrospectively, the seductive attraction of the relation between cause and effect has distorted our course, causing surgery in the networks (Pearl 2000) manifested as environmental problems.

Supported by the previously defined limits in modeling self-organization derived from mathematics and verified in our experiment with barley endosperm mutants, we can conclude that direct inspection by the human eye directly or indirectly by instrumental methods is the last resort, when mathematical modeling and evaluation becomes incomputable and destructive to data. Martens et al. (2007) has shown in mathematics that perhaps the best way to evaluate the output of the unique patterns from the Delta–Notch cellular automata model is by visual inspection executed by a trained sensory panel.

It is surprising to many that a sensory panel may discriminate between 50 and 100 different sight, taste, smell, and mouthfeel variables from, for example, beer, with an error of a few percent. The main prerequisite for success is to obtain a consensus among the participants in training a panel in defining the linguistic elements of quality that can be presented graphically as a "star" pattern or by PCA score plot (Martens and Martens 2000). Pattern recognition with spectroscopy and chemometrics uses the great

capacity of the visual sense of the human brain by evaluating spectra and score plots rather than numbers as in statistics.

In contrast, logic and causal thinking in classical philosophy is based on word concepts, such as *deduction* and *induction* (Popper 1995), that can be seen as "a linguistic trap" (Munck 2007, p. 422) due to the explanatory limits of words. The great American chemist and philosopher Charles Sanders Pierce, the father of semiotics, the interpretation of signs (Hoffmeyer and Emmeche 1991), founded a new exploratory multivariate logic for patterns more than 100 years ago. Pierce introduced the term *abduction* for interpreting signs from nature. He claimed that human beings could not think without signs and the signs we have are to be developed because they are vague. We have here focused on the benefits of interpretation of spectral signs and PCA score patterns by abduction (Figs. 18.1 and 18.4). *Spectroscopy and chemometrics can now be introduced, as a much-needed experimental method in semiotics and biology that provide an improved abductive scientific strategy for the interpretation of signs and patterns from nature*.

It is remarkable that causality, which earlier has been neglected in classical statistics, now is discussed (Aalen and Frigessi 2006), while the mathematical limits in the causal evaluation of emergent systems have not yet been fully considered in statistics. Chemometric fingerprinting precluded by removal of stochastic variation (Martens and Næs 2001; Smilde et al. 2004) is indispensable in the initial phase of such analyses for classification to visualize the hot spots for evaluation by data inspection. However, chemometric models (Section 3.5) are only giving a crude idea of the fine-tuning of NIR spectra. Whenever the mathematical model is not in harmony with the modeling of the "self-organizational computer," there is "a surgery" in the network based on Pearl (2000) sense. That is a large camel for science and mathematic modelers to swallow.

Now science has to give up to penetrate self-organization by reductionistic methods only because "the multitude of factors are fenced in a cage of covariance—a black hole which is swallowing up all the efforts of the scientists without rendering any information of the sequence of events hidden to them" (Munck 1992, p. 592). Nonlinear dynamics cannot be understood by causal bottom-up local modeling in biology even if simulation models can give valuable inspiration (Palsson 2006). The decision is not taken by the modeler but by the whole emergent system as reflected by its state in a time window. A phenomenological overview of self-organization is needed, which, in combination with advanced molecular data, can make biological sense in a combined top-down–bottom-up modeling approach. *Science and society have to realize that causality in nature's sense is what is happening in the whole network* (Munck 2007; Munck et al. 2010).

The scientific society today is, to a large extent, ignorant of the fundamental need for data inspection to save the finely tuned deterministic-looking information in emergent systems. Too many data sets are investigated by mathematical modeling only. Data compression destroys the finely tuned information from self-organization that is visualized when stochastic variation has been removed. There is a lack of pattern recognition thinking in interpreting reproducible emergent systems by data inspection. *It requires much more a topological sense of patterns and a physical/chemical/biological experience including genetically defined reference samples than skills in mathematical modeling*.

Remembering that language is the most narrow communication link in human communication, the question is now what are the precious words to be communicated to the human agents to make them moderate their selection activities in a world with limited resources (Munck 1993)? We will suggest here two concepts, "vegetarianism" and "the creative uncertainty of nature," the latter implying that we are all participants in a remarkable chain of surprising creative events that should also apply to every single human being. All these creativities are emerging from the self-organizational property of matter that started 14 billion years ago and are still proceeding.

We can envisage the major principles behind the various forms of self-organization. Natural selection on all levels from molecules to organisms introduces creativity *in nature as a whole* that produces a wealth of unique individuals and

surprising events regarding climate *that to some extent can be understood but never fully predicted.* The limits of causality in the human dimension and in interpreting the intrinsic *uncertainty* of the universe should be respected and rationally faced without fear. An awareness of self-organization in science and society mediated by a grand educational process starting with the universities would allow a healthy respect of humbleness for *the creative uncertainty in nature* and would be the best argument for a free science in basic research with a dedication to society.

It is comforting that uncertainty can be largely disarmed by making pattern recognition decisions based on careful overviews in continuous inventories of the dynamic natural and social systems with the new technology that is now available. The patterns observed from data in inventories (as in the barley endosperm mutant NIRS model) have a much higher diagnostic value in emergent systems than single-factor causality and simulations (Lindzen 2008) that are biased by surgery in networks (Pearl 2000).

Vegetarianism introduces automatically a new pattern of consumption. It is estimated that a reduction in meat consumption in the United States by 10% would improve health and potentially feed 1 billion extra people. There is solid evidence for the environmental benefit with a factor of 10–50 times to reduce red meat consumption and increase plant foods in a further move toward vegetarianism that should be promoted locally and internationally. While CO_2 is just a symptom, the label vegetarianism stands for a pattern of forward-directed effects that are pulling in the same positive direction. These are improved human health combined with a radical relief of land resources and water consumed per unit of food that implies a drastic decrease in greenhouse gases such as CO_2 and methane.

Today's pressure from politicians and media on technology and science to find simple causal solutions has never been higher (Lindzen 2008). Human normative design solutions communicated by the limited bandwidth of language are favored in a virtual volatile world boosted by the Internet (Fig. 18.11). The question is how could the global society be more earthbound in thinking and action? The collective experience of humanity proves that nature indeed is intelligible by human senses. The impressive empirical results of selection by humans in plant and animal breeding can be seen as a personalization of Darwin's emergent concept of "natural selection." However, now at the age of globalization, billions of innovative human individuals who are living far from nature are active as "selectors" (Munck 1991, 1993). They are focusing on cause–effect relations attractive to them that hit back as environmental problems in the emergent network of nature (Fig. 18.12). It is time for a paradigm shift in science, technology, and society where "nature's creative uncertainty" in self-organization, explored as signs, is fully acknowledged and respected as "a law of new possibilities" (Prigogine 1997, p. 155) that could give us a new direction to vitalize our future lives. The signs from nature should be explored and conserved as true patterns of variables and should not be washed out by destructive mathematical models. Because uncertainty in nature is essentially creative, science has to be practiced in a free critical dialogue with nature and with a currently improved nonfundamentalist theory that gives the basis for lasting investments in infrastructure and the survival of society.

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Note: Page numbers in *italics* refer to figures; those followed by "T" refer to tables.

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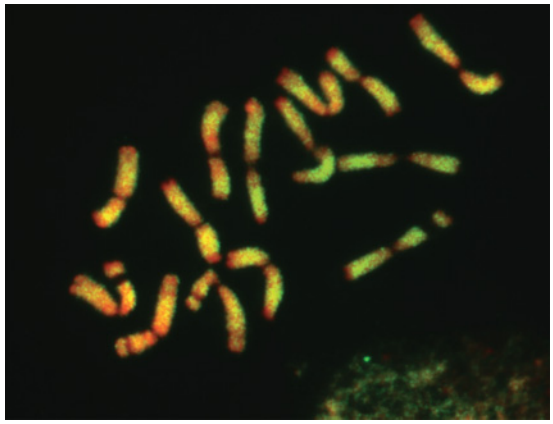


Fig. 3.1. Metaphase cell of barley cv. Morex after fluorescence *in situ* hybridizations with a probe specific for the *Gypsy*-type transposable element *BAGY2*. Hybridization signals (in yellow) are distributed throughout all chromosomes except the subtelomeric and pericentromeric regions (Wicker et al. 2009).

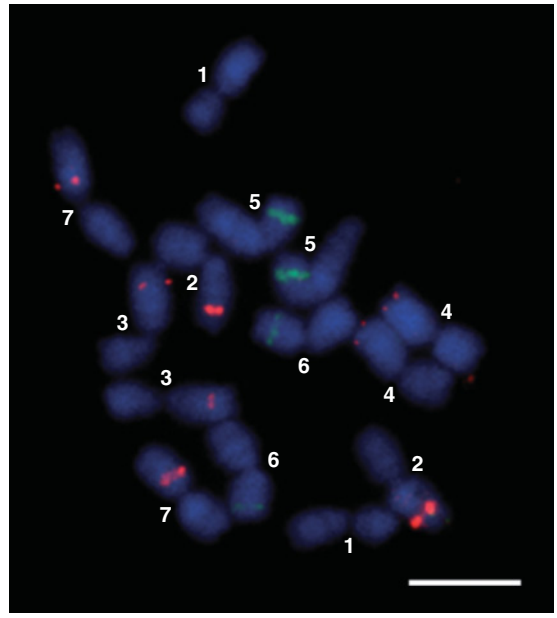


Fig. 5.2. DAPI-stained somatic metaphase chromosomes of barley cv. Bonus, showing signals after FISH of 25S (green) and 5S (red) rDNA probes, labeled with digoxigenin-11-dUTP and immunodetected with FITC-conjugated antidigoxigenin antibody and with tetramethyl-rhodamine-5-dUTP, respectively. Numerals designate chromosomes 1H–7H (Leitch and Heslop-Harrison 1993; Brown et al. 1999). Bar = 10 µm (courtesy of Łukasz Kubica and Robert Hasterok, Department of Plant Anatomy and Cytology, University of Silesia, Katowice, Poland).

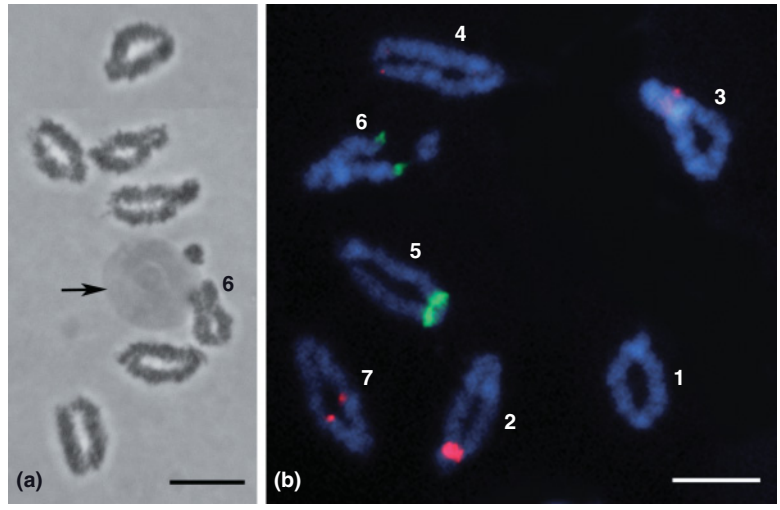


Fig. 5.3. Diakinesis bivalents of barley cv. Bonus. (a) Stained by Snow's carmine. A large nucleolus (arrow) associated with the secondary constriction of chromosome 6H. (b) FISH and chromosome numbering as in Fig. 5.2. Bar = 10 µm. Fig. 5.3b (courtesy of Łukasz Kubica and Robert Hasterok, Department of Plant Anatomy and Cytology, University of Silesia, Katowice, Poland).

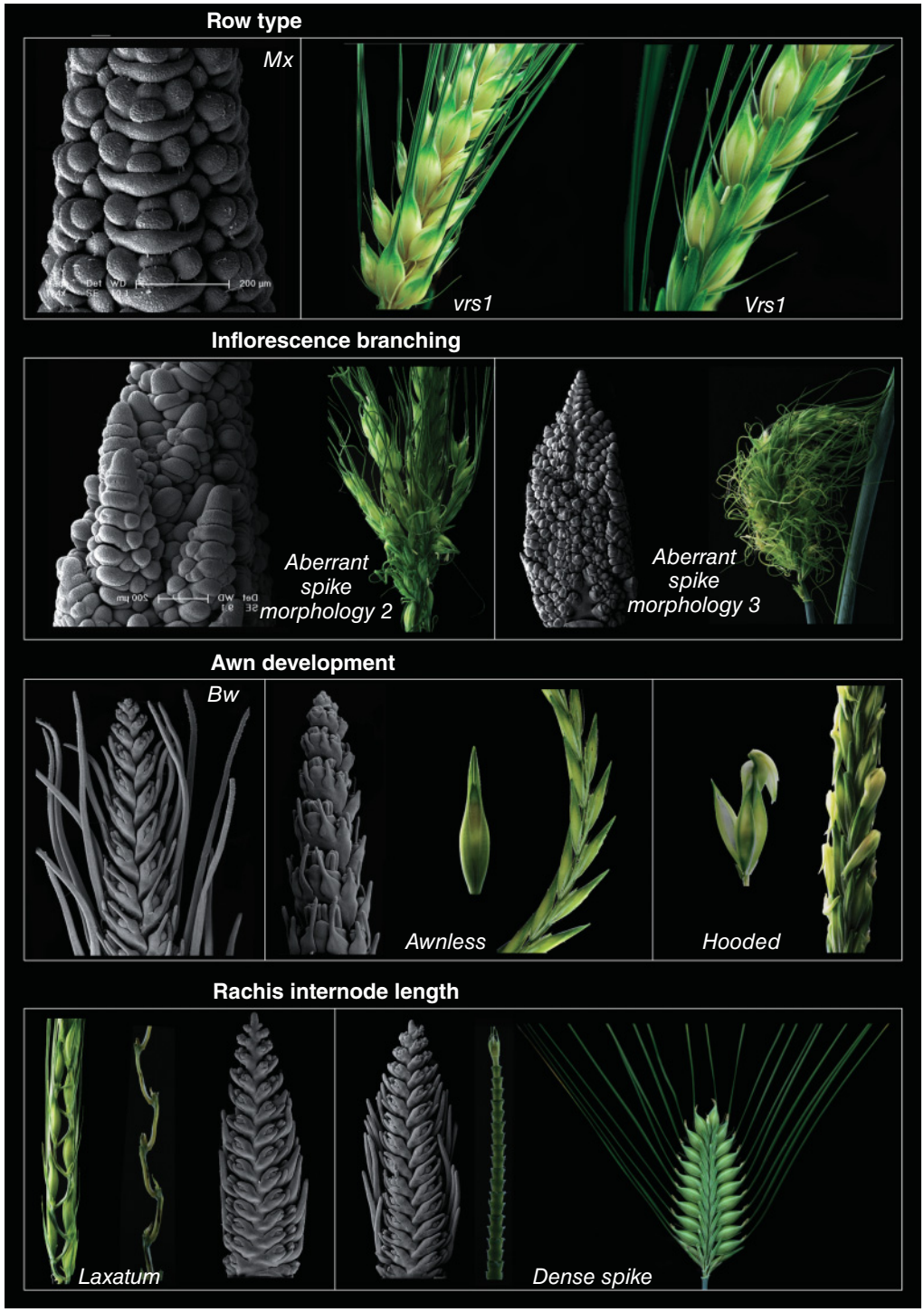


Fig. 4.2. Phenotypes of barley inflorescences representing row type, inflorescence branching, awn, and rachis development traits. The *vrs1* and *Vrs1* alleles represent six-row and two-row inflorescence types, respectively. The *aberrant spike morphology 2* and *aberrant spike morphology 3* mutants represent examples of excessive inflorescence branching mutants. The *awnless* and *Hooded* mutants represent mutants that exhibit altered awn development. The *laxatum* and *dense spike mutants* represent mutants exhibiting different rachis internode lengths. The rachis with spikelets removed is also shown for both mutants. Scanning electron microscopy images of developing barley inflorescences dissected from cv. Morex (Mx) and cv. Bowman (Bw) and the mutants are also shown.

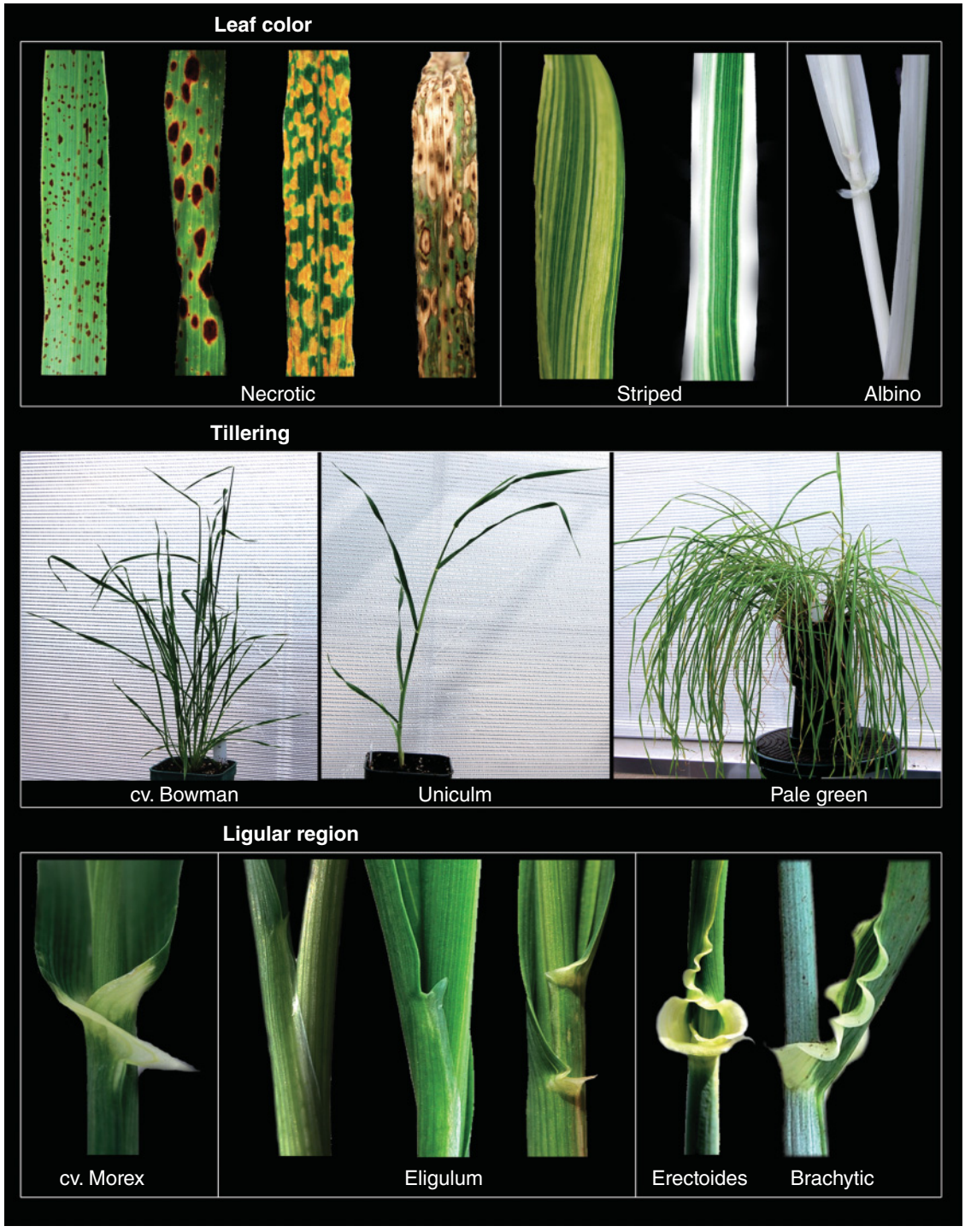


Fig. 4.3. Phenotypes associated with barley vegetative development representing leaf color, tillering, and ligular region development traits.

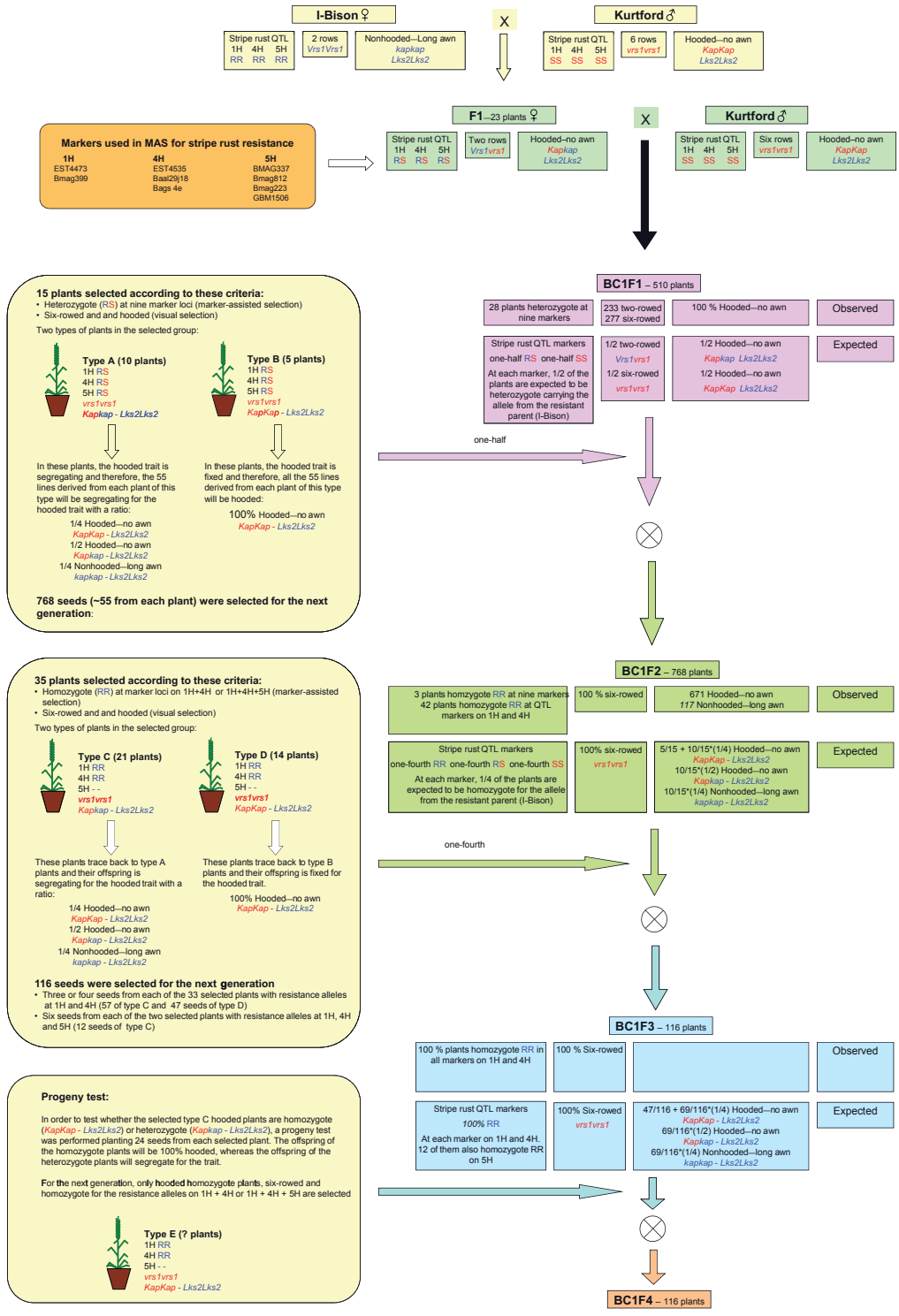


Fig. 6.6. Strategy for introgressing stripe rust resistance into Kurtford.

Fig. 7.1. Medium-term storage in the National Small Grains Collection, USDA-ARS, Aberdeen, Idaho. Rooms are maintained at 5–6°C and at 25% relative humidity (RH). *Source:* H. Bockelman.

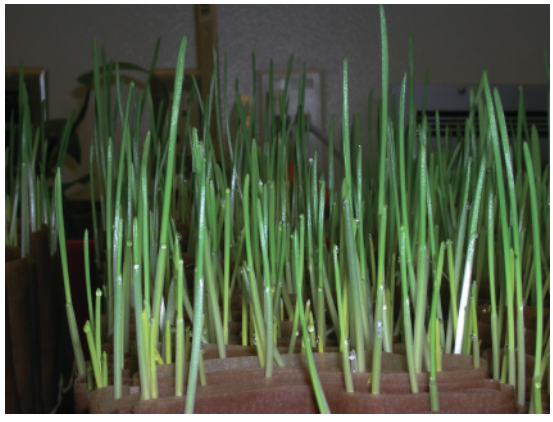


Fig. 7.2. Viability testing of barley accessions in the National Center for Genetic Resources Preservation, USDA-ARS, Fort Collins, Colorado. *Source:* D. Ellis, NCGRP.

Fig. 7.3. Cryogenic storage tank in the National Center for Genetic Resources Preservation, USDA-ARS, Fort Collins, Colorado, holding safety backup samples of NSGC barley accessions and other germplasms throughout the NPGS. *Source:* D. Ellis, NCGRP.

Fig. 7.4. Seeds of 20 different landrace barley accessions from Ethiopia—an illustration of diversity from the USDA-ARS National Small Grains Collection. *Source:* H. Bockelman.

Fig. 9.2. Modern drills can go from stubble to drilled crop in a single pass (photo courtesy of Vaderstad Verken AB).

Fig. 11.1. Severe Fusarium head blight infection on two-rowed barley in the field.

Fig. 11.3. Barley spike heavily infected with fusarium head blight collected from the field. The base of the third kernel on the left from the bottom shows salmon to pinkish masses of *Fusarium* mycelium and conidia, whereas the lower right kernels show the black perithecia of the *Gibberella zeae* sexual stage.

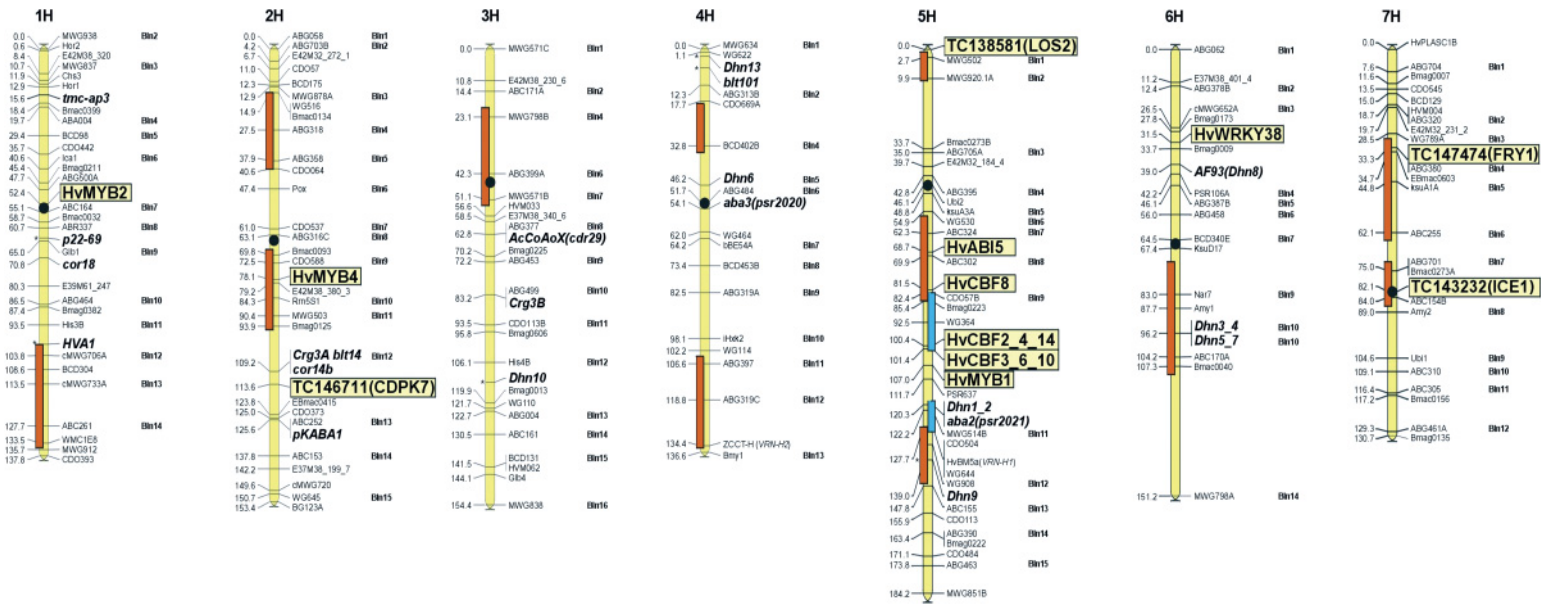


Fig. 10.1 Summary of the main QTLs mapped in barley related to frost tolerance in the vegetative phase (blue bars; data from Francia et al. 2004) and drought tolerance (brown bars; data from Teulat et al. 2001a, 2002, 2003; Diab et al. 2004). On the right side, known stress-related genes and transcription factors controlling stress-related gene expression are reported (data from Tondelli et al. 2006).

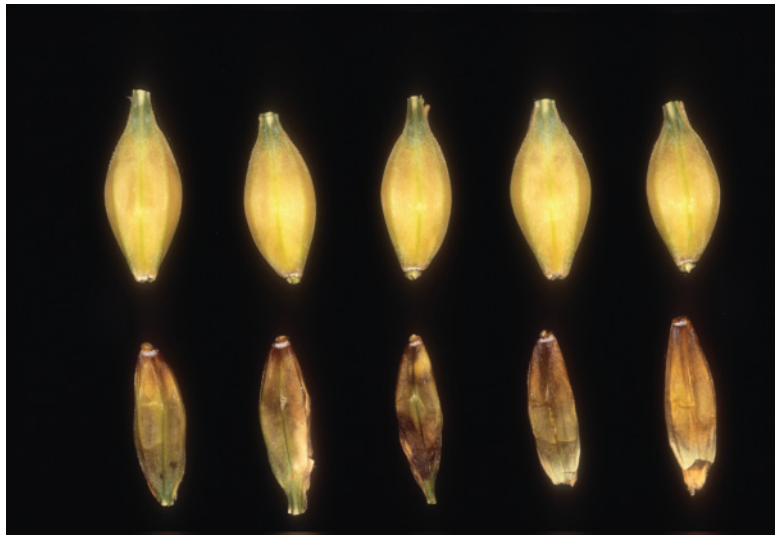


Fig. 11.2. Fusarium head blight-infected barley kernels (bottom row) in comparison to healthy, noninfected kernels (top row) from inoculation tests conducted in the greenhouse with *F. graminearum*. Note the dark lesions on infected kernels and their much smaller size.

Fig. 11.4. Barley spike heavily infected with *Fusarium* mycelium in the field.

Fig. 11.5. Stem rust infection on barley in the field.

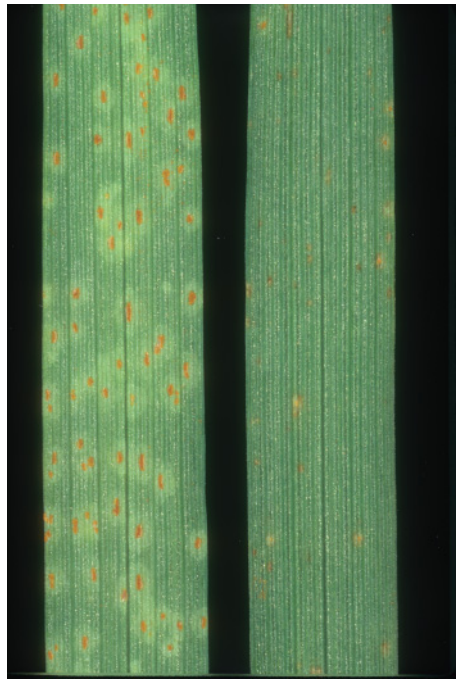


Fig. 11.6. Leaf rust infection on seedling leaves of a susceptible (left) and resistant (right) barley cultivar from the greenhouse.

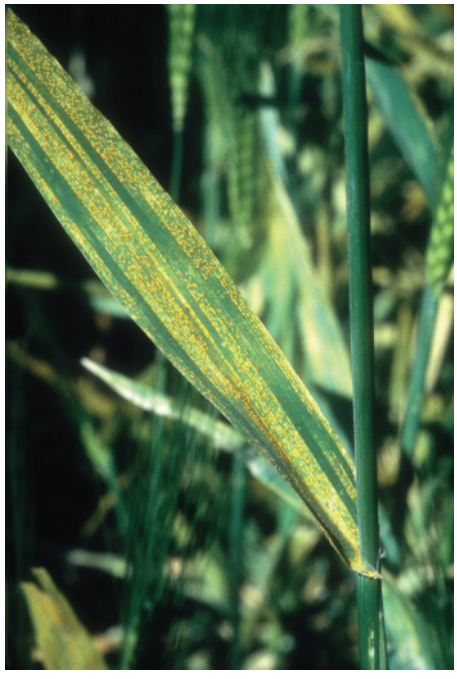


Fig. 11.7. Stripe rust infection on barley in the field.

Fig. 11.8. Powdery mildew infection on barley in the field.

Fig. 11.9. Powdery mildew infection on seedling leaves of a susceptible (left) and highly resistant (right) barley cultivar from the greenhouse.

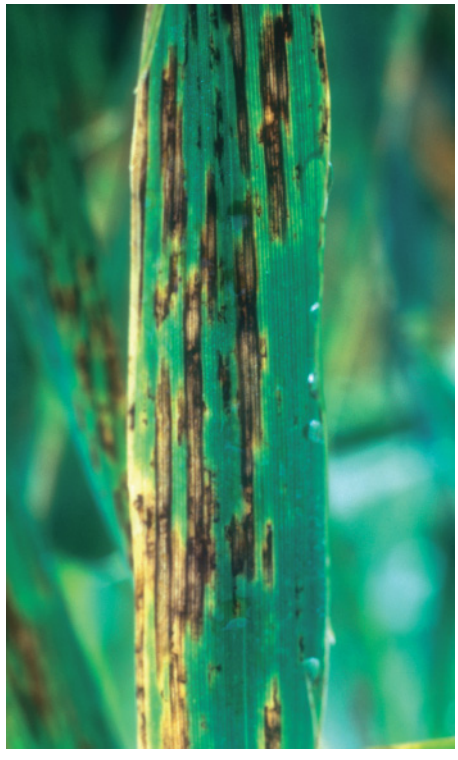


Fig. 11.10. Net-form net blotch infection on barley in the field.

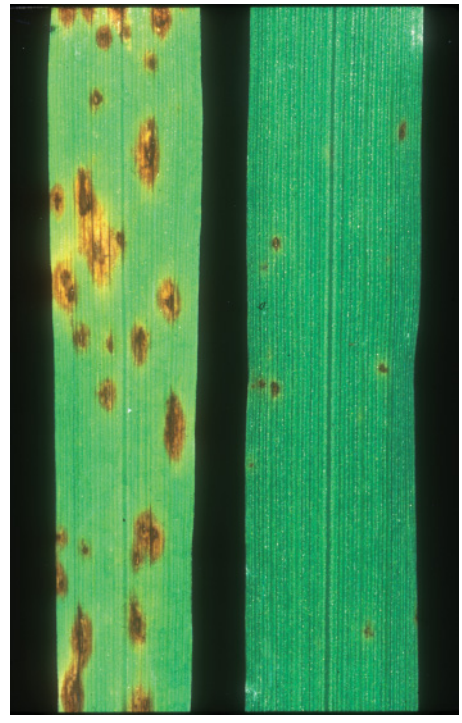


Fig. 11.11. Spot-form net blotch lesions on seedling leaves of a susceptible (left) and resistant (right) barley cultivar from the greenhouse.

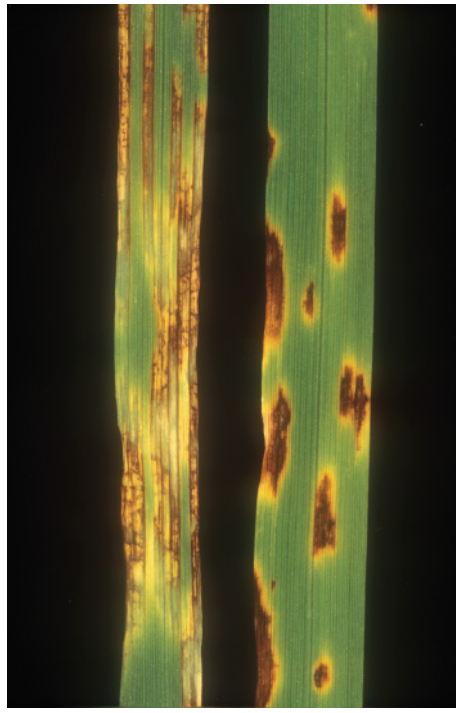


Fig. 11.12. Comparison of net-form net blotch (left) and spot blotch (right) lesions on barley seedling leaves from the greenhouse. Net-form net blotch has lesions with dark brown longitudinal and transverse striations, whereas spot blotch has uniform chocolate brown lesions.

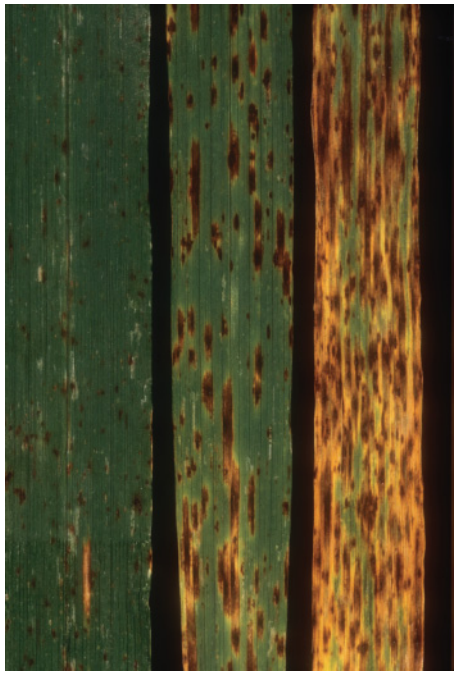


Fig. 11.13. Spot blotch lesions on a resistant, moderately resistant, and susceptible barley line collected from the field.

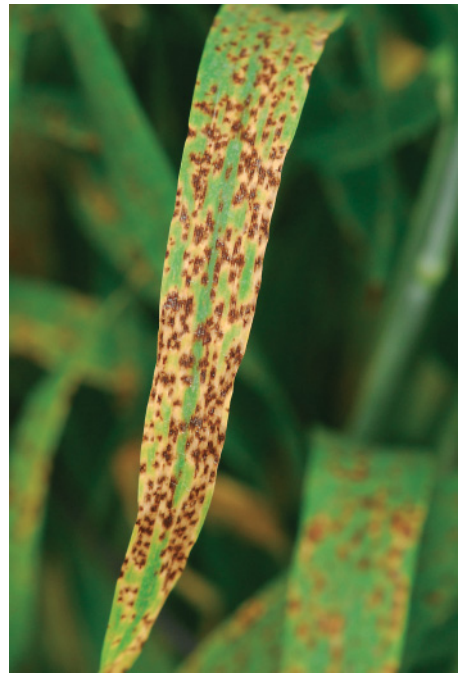


Fig. 11.14. Ramularia leaf spot on barley in the field (courtesy of Hans Pinnschmidt).

Fig. 11.15. Leaf scald infection on barley leaves collected from the field.

Fig. 11.16. Severe Septoria speckled leaf blotch infection caused by *Septoria passerinii* on barley in the field.

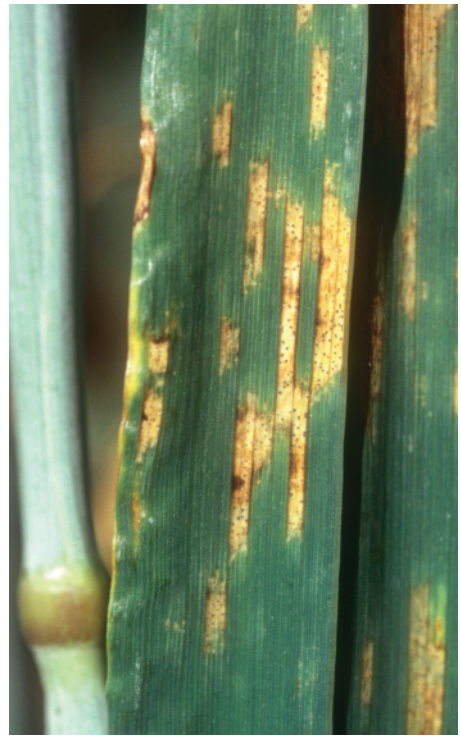


Fig. 11.17. Pycnidia of *Septoria passerinii* forming in lesions of an infected leaf in the field.

Fig. 11.18. Barley yellow dwarf infection on barley in the field.

Fig. 11.19. Bacterial blight infection on barley in the field.

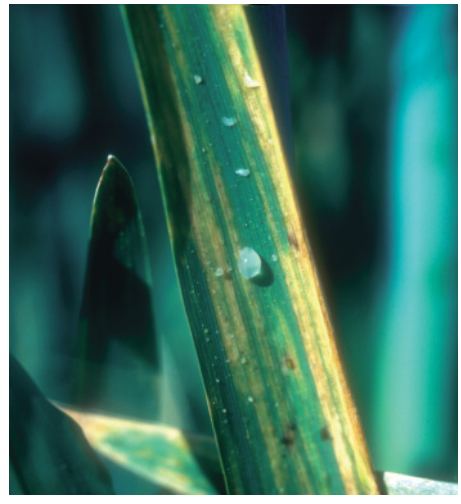


Fig. 11.20. Bacterial blight infection on barley in the field showing a droplet of bacterial exudate.

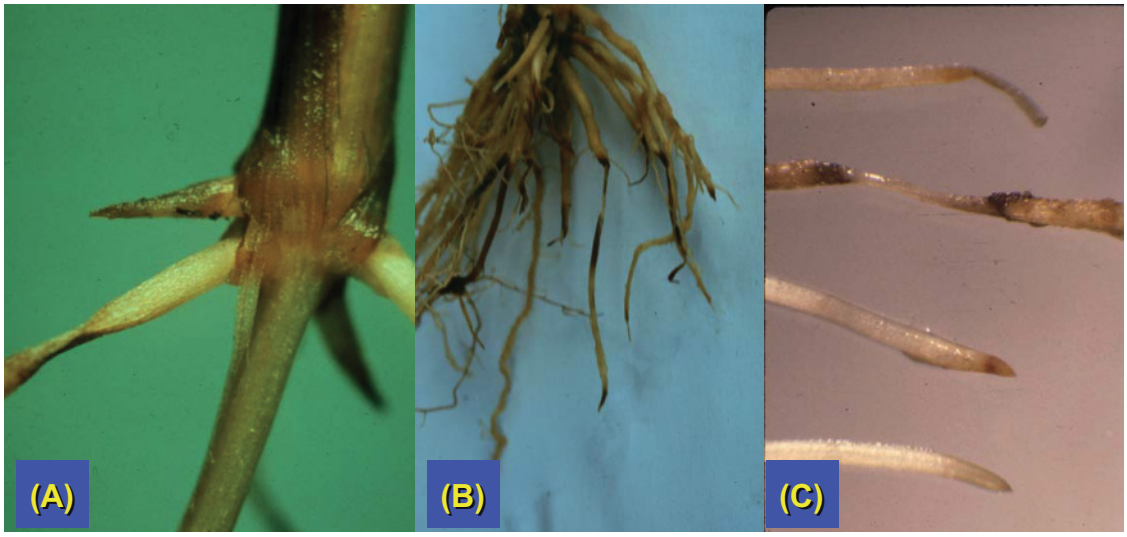


Fig. 11.21. Typical root symptoms of rhizoctonia root rot, including spear tipping, root lesions (A,B), and constriction of the root cortex (C) (A and B, courtesy of R. J. Cook and D. Weller; C, courtesy of R. Smiley). Reprinted from Bockus et al. 2010 with permission from the American Phytopathological Society.

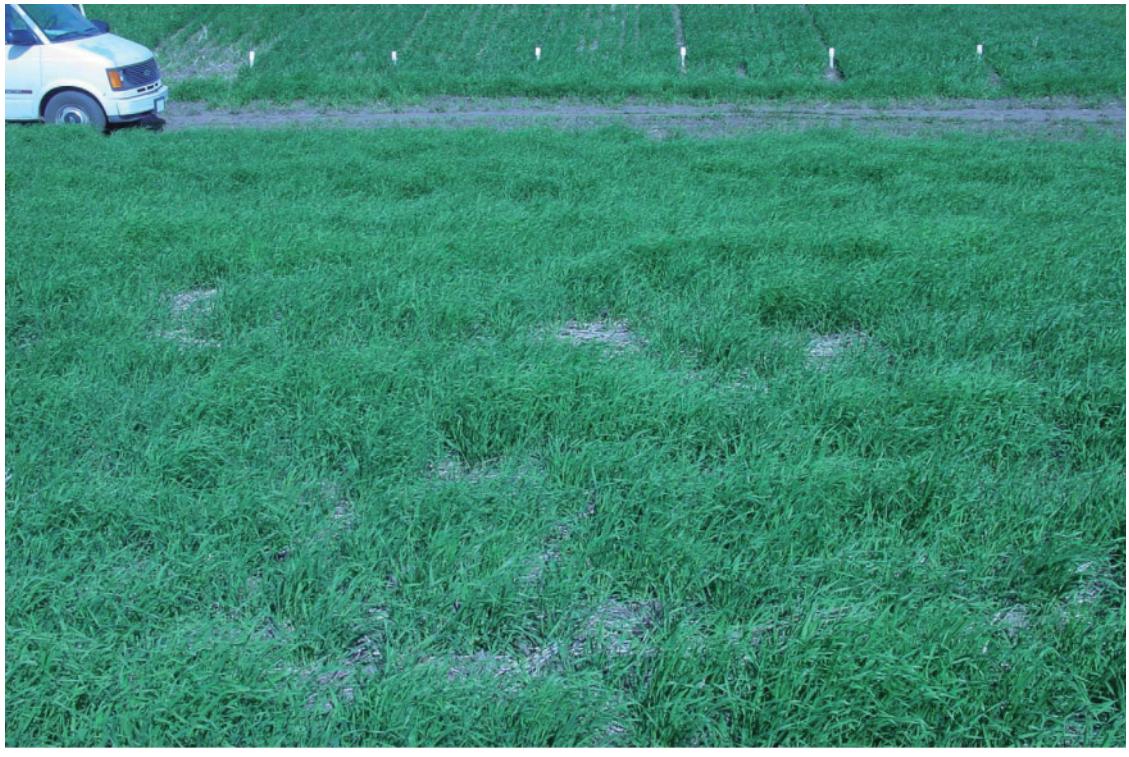


Fig. 11.22. Field symptoms of rhizoctonia root rot on spring barley. This field was direct seeded. Note uneven stand with varying heights of plants.

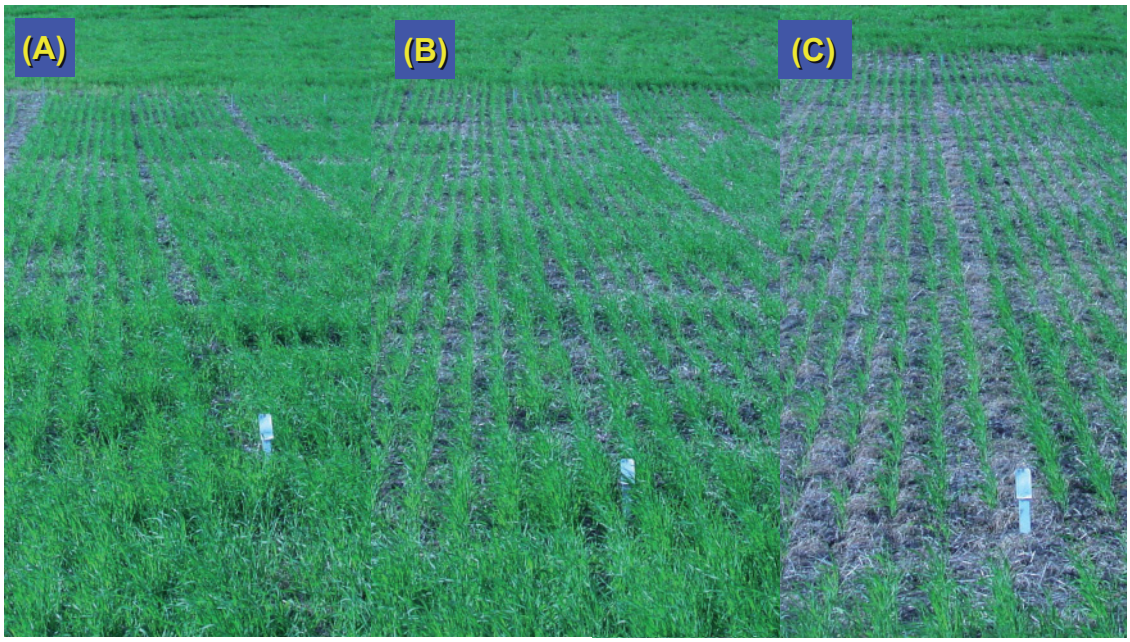


Fig. 11.23. Effect of greenbridge management on the rhizoctonia root rot of barley. All three plots of spring barley were in the same field, direct seeded at the same time. (A) Weeds and volunteers were killed with the herbicide glyphosate in the previous fall. (B) Weeds and volunteer sprayed out with glyphosate 3 weeks before planting. (C) Weeds and volunteer sprayed out with glyphosate 3 days before planting.

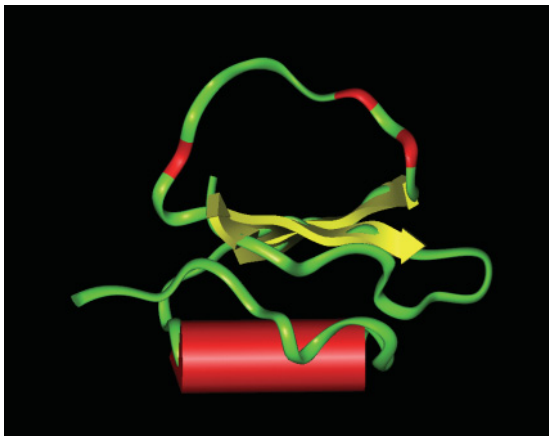


Fig. 13.9 Three-dimensional structure of the barley chymotrypsin inhibitor CI-2 showing the single α-helix (red cylinder), strands of β-sheet (yellow arms), and the reactive loop with three lysine residues (red segments).

Fig. 13.1 Changes during barley caryopsis development. Light micrographs of sections of developing barley caryopses (cv. Optic) at anthesis (panel a) and before (panel b) and after (panel c) cellularization of the endosperm and during starch accumulation (panel d) shown in cross sections (left panels) and longitudinal sections (right panels). Color bars at the margins indicate the positions of the pericarp (p), chlorenchyma (ch), inner epidermis of the pericarp (red), outer (oi) and inner (ii) integuments, nucellar epidermis (ne), nucellus (nu), central cell (cc), and endosperm (e). At anthesis (panel a), the nucellus (nu) is prominent. The nucellar epidermis (ne) consists of small cells. Inner (ii) and outer (oi) integuments are clearly visible. The narrow inner epidermis of the pericarp (indicated by red color) is best observed at this stage. Cells in the pericarp are small; a frontal vascular trace in the cross section is indicated by an arrow. The longitudinal section shows the inner integument coming from the left, right, and behind (arrow) at the micropyle close to the egg cell. Before cellularization of the endosperm (panel b), the nucellus is already reduced and a thin layer of endosperm with free nuclei is shown. The nucellar epidermis and chlorenchyma have increased in size, while the outer integument and the inner epidermis of the pericarp are already degenerating. Note the starch in the pericarp cells. Cells in the pericarp, nucellar epidermis, and integument appear different in the longitudinal section due to their orientation in the grain. The top longitudinal view in the right-hand panel shows a ventral section through the crease area; the bottom image shows a dorsal section. (Compare with Figs. 13.2 and 13.3 for different aspects of the projection.) After cellularization (panel c), only the two cell layers of the inner integument remain. The pericarp shows some degeneration close to the chlorenchyma. The vascular tissue is indicated by an arrow.

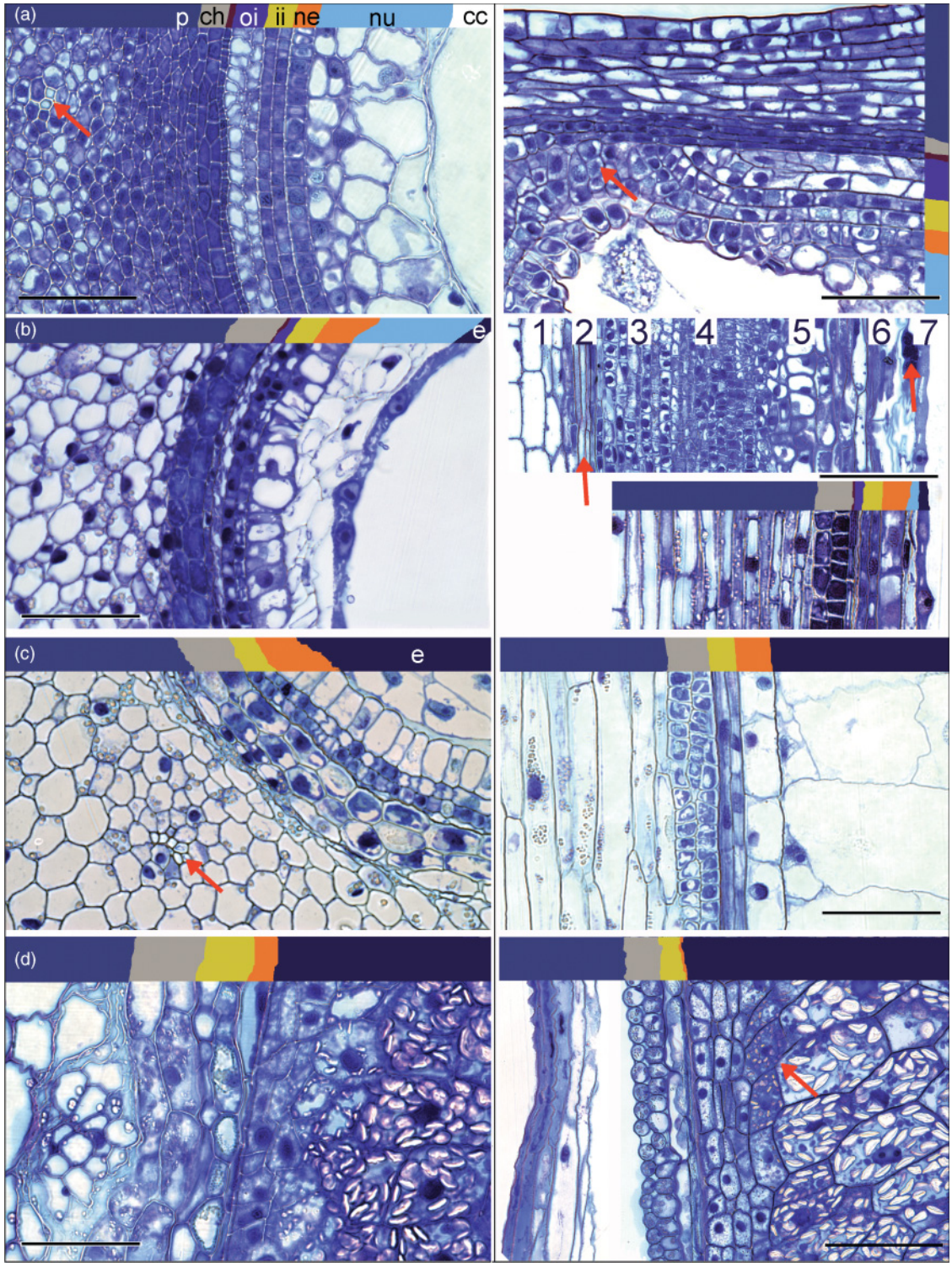
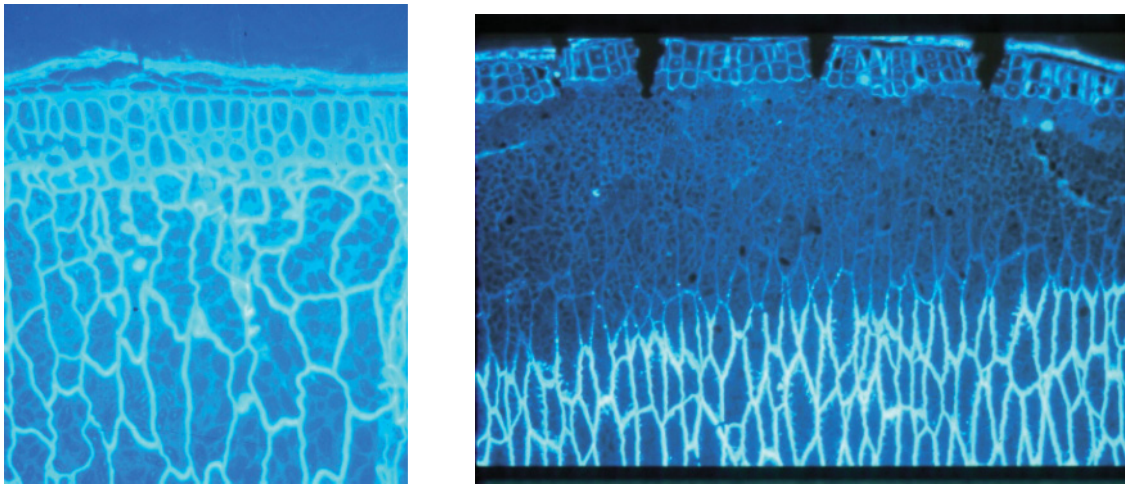


Fig. 13.1 (Continued) During grain filling (panel d), starch is accumulated in the endosperm cells. The aleurone and subaleurone (cells indicated by the arrow) are differentiated and the pericarp cells appear depleted and detached in the longitudinal view. The inner layer of the inner integument accumulating secondary plant products appears more prominent in the cross section. All sections are cut at about 3 µm with glass knives and are stained with toluidene blue. Bars: 55 µm for cross sections in panels a–d and for longitudinal sections in panels a and c. Bar: 110 µm for the longitudinal section in panel d. The bar between the two longitudinal sections in panel b indicates 110 µm for the upper image and 55 µm for the lower image. 1, pericarp; 2, vascular tissue, vessel indicated by arrow; 3, parenchyma cells; 4, chalazal zone at the base of the projection; 5 and 6, nucellar projection cells; 7, endosperm layer close to degenerating antipodal cell (arrow) in the nucellus.

Fig. 13.2 Early grain development in barley cv. Optic. Light micrographs of sections taken at anthesis (panels a–g) and during early coenocytic endosperm development (panels h–n). Cross section (b) lies at the height of the antipodal cells. Scheme of cross section (c) indicates the position of filaments (dark blue), lodicules (violet), lemma (light blue), and remnants of the palea (red). Longitudinal sections (a,d) show the egg cell and several antipodal cells in the embryo sac. The arrow in (d) indicates a vascular strand. Panels e–g are detailed views of a cross section (e) through the egg apparatus, a longitudinal section (f) of both polar nuclei and part of the egg apparatus, and a section (g) of pollen with vegetative and sperm nuclei. A cross section of caryopsis (i) at medium height shows a thin layer of developing endosperm, thicker on the projection side. For details of the endosperm over the nucellar projection, see (l). A corresponding longitudinal section (h) shows remnants of antipodal cells (arrows). The embryo in cross sections (k) and longitudinal sections (m) comprises only a few cells at this stage. A longitudinal section (n) shows part of the nucellar cap that is providing pollen tube guidance. The scheme in (h) indicates the relative sizes of the developing grain at anthesis and before cellularization of the endosperm. All sections are cut at about 3 µm with glass knives and are stained with toluidene blue. Bars: (a) 250 µm, (b), 225 µm, (d) 225 µm, (e) 55 µm, (f) 110 µm, (g) 55 µm, (h) 450 µm, (i) 225 µm, (k) 55 µm, (l) 55 µm, (m) 110 µm, (n) 110 µm..



(a) (b)

Fig. 14.1. Early stages of cell wall degradation in the starchy endosperm of germinated barley grain. The left panel (a) shows a low magnification micrograph of a section of ungerminated barley grain stained with Calcofluor White. The cell walls of the endosperm can be clearly seen, as can the starch granules in starchy endosperm cells. In the right panel (b), the degradation of walls progressively from the aleurone layer toward the center of the starchy endosperm can be seen. This degradation pattern reflects the diffusion of wall-degrading enzymes from the aleurone layer to the center of the grain. Photographs generously provided by Meredith Wallwork.

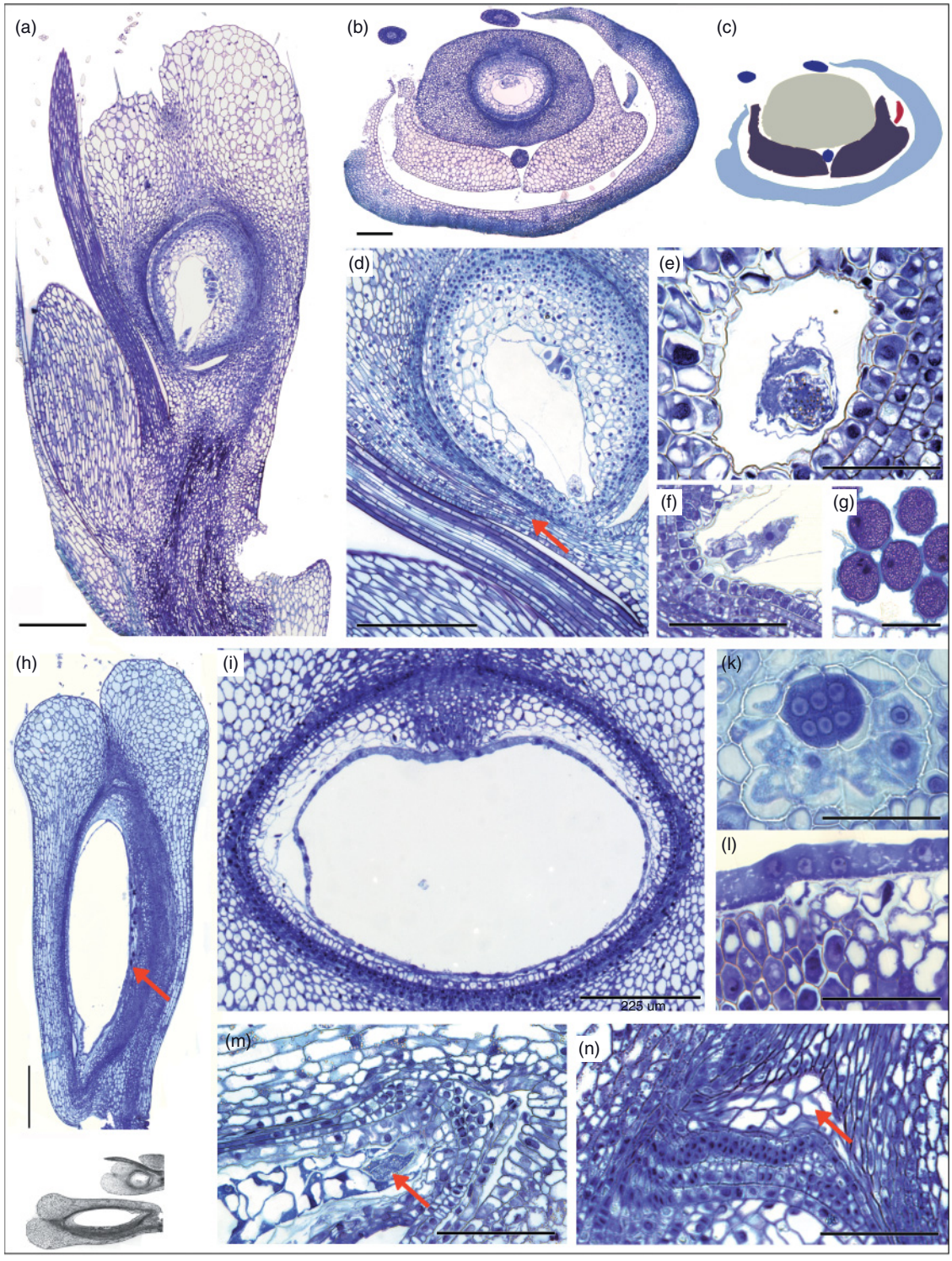

(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(k)
(l)
225 um
(m)
(n)

Fig. 13.3 Grain development in barley (cv. Optic) after cellularization of the endosperm. Panels a–f show the developing caryopsis at the onset of starch accumulation. (a) Longitudinal section through the upper part. (b) Median cross section with vascular tissue (va) indicated. (d) Longitudinal and (e) cross section views of the nucellar projection (np) and endospermal transfer cells (tr) at the border of the endosperm. (c) Cross and (f) longitudinal sections showing both the endosperm close to the embryo and the (white) cells of the starchy endosperm. Panels g–i show the developing caryopsis during starch accumulation. The cross sections in panels g and h and the longitudinal section in panel i show the pigment strand (pi) in the chalazal zone behind the nucellar projection. Panels k, m, n, and o are sections of ripe grain showing the endosperm (m), the crease region (n), and the organization of the outer wall layers (k) and aleurone (o). The scheme in (l) indicates the relative sizes of the three stages shown in Fig. 13.3. All sections are cut at about 3 µm with glass knives and are stained with toluidene blue. All images are light micrographs and panel (o) is color inverted. Bars: (a) 220 µm, (b,l) 450 µm, (c,g,o) 225 µm, (d,e,f,h,i,m) 110 µm, (k) 55 µm, (n) 80 µm.

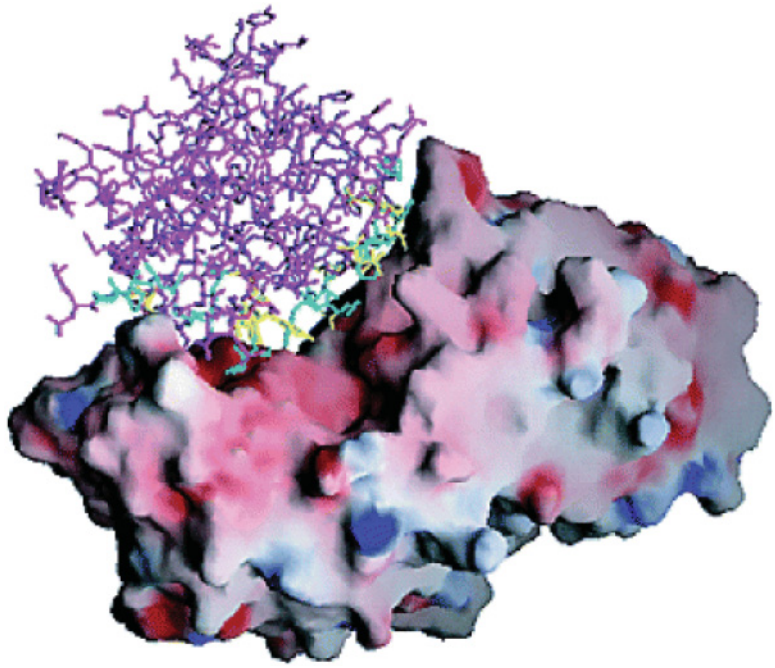
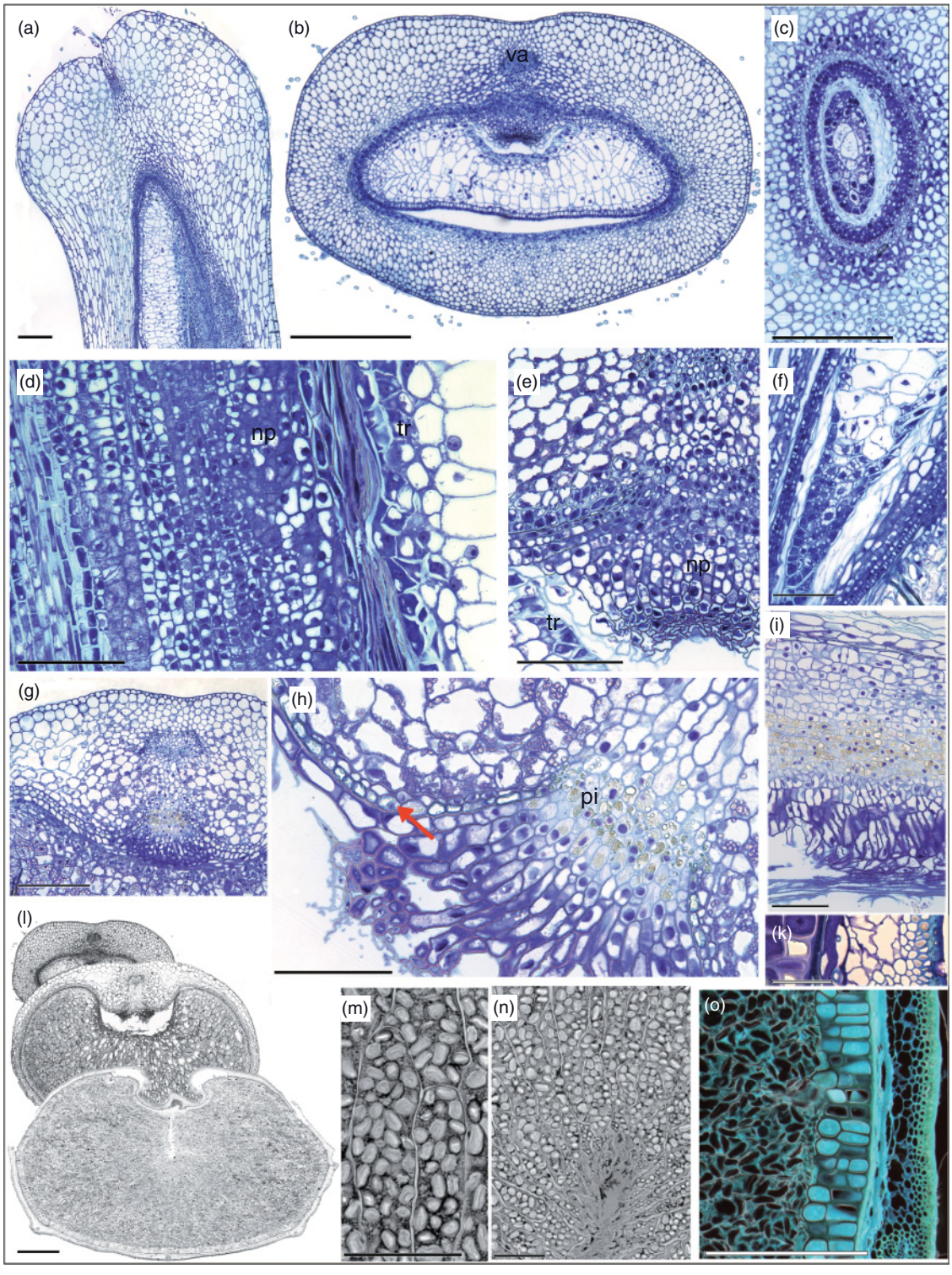


Fig. 14.4 The three-dimensional structure of AMY2–BASI. BASI is shown in stick representation with highlighted residues participating in hydrogen bond interactions (yellow) or in other contacts up to 3.9 Å (blue) with AMY2. The AMY2 surface electrostatic potential is color coded from negative (red) to positive (blue). From Vallée et al. (1994).


(a)
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va
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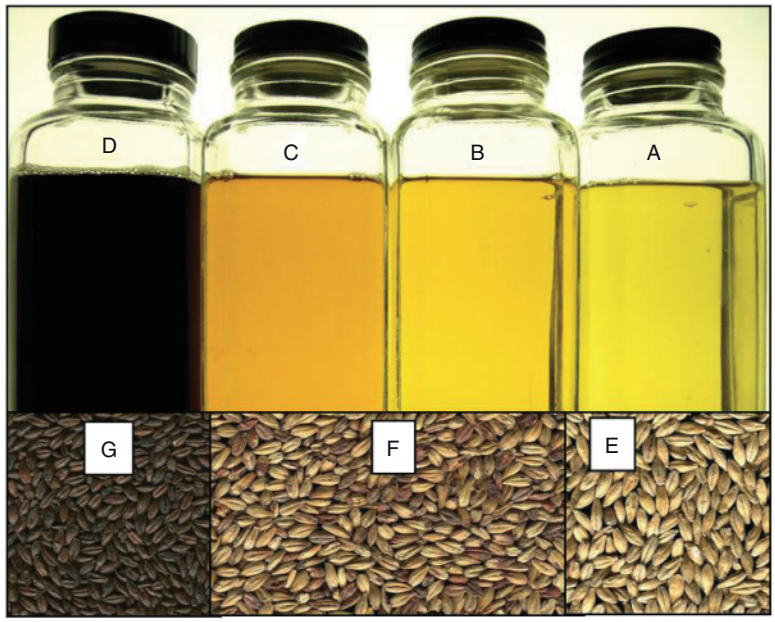

Fig. 15.4. Congress worts prepared from 100% brewer's malt (a), brewers malt and 5% caramel-60 (b), brewers malt and 10% caramel-60 (c), and brewers malt and 5% black malt (d). Brewer's malt, caramel-60 and black malt are shown in E–G, respectively.

Fig. 17.1 Barley food products: barley bob (cooked barley and rice mixture, upper left), barley steam bun (upper right), waxy barley walnut-cookie (bottom left), and waxy barley pancake (bottom right). Steam bun reprinted with permission from Bobo's Jeju (Jeju.com) corporation (Seoul, Korea); barley walnut-cookie and waxy barley pancake with azuki paste filling reprinted with permission from Mac corporation (Anyang, Korea).

Fig. 17.2 Whole, pearled, and rolled barley grain, and flake. *Source*: http://www.sakthifoundation.org/kitchen_breakfast.htm.

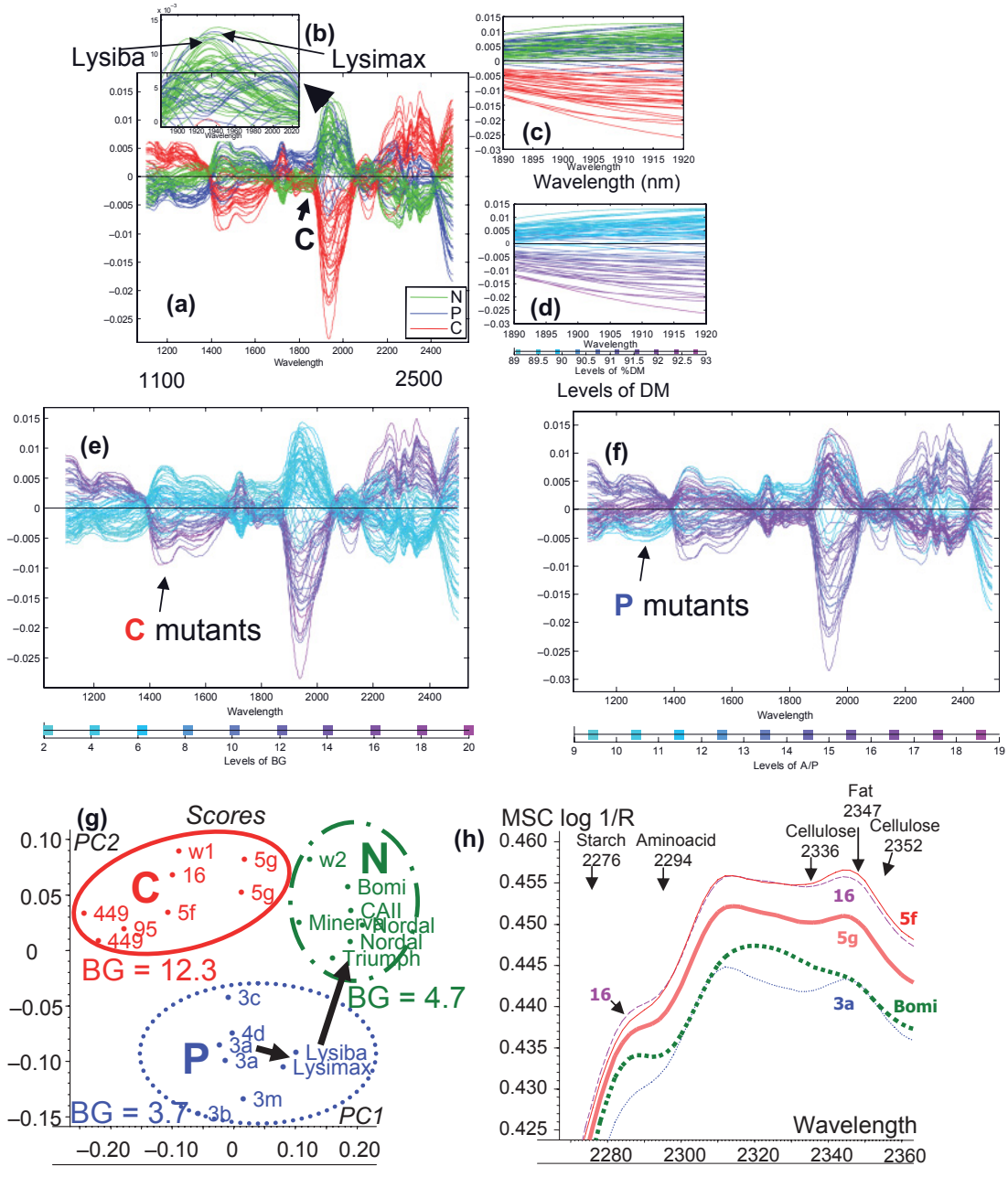

Fig. 18.1. (a) Mean centered log 1/R from 92 barley seed samples. Green, normal barley (N); blue, protein (high lysine, P); and red, carbotrydrate (C) mutants. (b) Enlargement of peak at 1935 nm; P outliers Lysimax and Lysiba. (c) Interval 1890–1920 nm from (a). (d) Marking of spectra from (c) for dry matter 89%–93% (e) Coloring for β-glucan (2%–20%) of the spectra in (a). (f) The same for amide/protein index (9–19). (g) PCA classification of 23 field barley NIR spectra (1100–2500 nm) for normal barley (N) and protein (P) and carbohydrate mutants (C). BG β-glucan % DM. (h) NIR spectra (2270–2360 nm) of Bomí (N) and its P lys3a and C mutants lys5f, lys5g, and 16 grown in the greenhouse. (a–d) and (g–h) (Munck, 2007) by permission from Wiley & Sons Ltd.