Agnès Ricroch · Surinder Chopra
Shelby J. Fleischer *Editors*

# Plant Biotechnology

## Experience and Future Prospects

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ISBN 978-3-319-06891-6 ISBN 978-3-319-06892-3 (eBook)
DOI 10.1007/978-3-319-06892-3
Springer Cham Heidelberg New York Dordrecht London

Library of Congress Control Number: 2014944084

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# Foreword

Society embraces technology. We rush to update our mobile phones to the latest model and operating system. We marvel at thin lightweight tablets and cloud storage. We rely on automobiles that are smarter than we as drivers can be. Our household appliances manage our homes more efficiently than we do. We quickly complain about poor wireless coverage in shopping malls or on transcontinental airplane flights. We use life-saving medicines mass-produced with the tools of genetic engineering. And, if our part of the world does not yet have these technologies, we aspire to have them.

Yet we are concerned about continued use of technology in our food system. Why this disconnect?

"Continued use of technology" is exactly the right phrase, for humans have always applied the highest technologies available to improving our food supply. The earliest tools were, in hindsight, crude approaches leading to the first steps in domestication. The domestication process itself, of plants and animals, became the fodder through which our discoveries of the complexities of genetics and the evolutionary process were revealed.

Our conventional food sources have always been developed using a variety of – for their day – cutting edge technologies: manipulated hybridizations of different species that combined hundreds or thousands of genes that had evolved in separate lineages, mutagenizing chemicals, ionizing radiation. The development of genetic engineering capacity offered, to those of us in the biological sciences, a set of tools through which we could be much more precise in our work and, potentially, more impactful with our outcomes.

As an entomologist, not a sociologist, I hesitate to completely dissect this question, but I can provide empirical observations to the debate from the perspective of a decade and a half of agricultural administration experience.

Food is in the news. Food channels and programming on television, food blogs, and movies about food surround us in the media. Social media have exploded and have changed the communication paradigm – "viral" likely evinces reference to a YouTube video much more quickly than to disease processes in the minds of today's citizen. Despite the fact that crops produced with the tools of genetic engineering are among the most strictly regulated products in history, we recall the cases where regulation has failed to keep us safe in the past. Consumers often mix metaphors – the question of the product is frequently confused with opinions about the companies producing and selling the product. While much of the discussion is in developed countries, countries frequently dealing with an abundance of cheap, safe food, governments in developing countries have made far-reaching decisions about the acceptability of new products that impact perceptions of consumers in their countries.

Perhaps of greatest impact, those of us in the food system have too frequently been silent. Today's consumer in a developed country is far removed from the production of her or his food. Much of the plant science work is conducted by companies who, with good reason, do not widely share their techniques and approaches in order to maintain their business advantage. Scientists too frequently stay in the lab, greenhouse, and field and, too infrequently, invest time in talking to consumers or – surprisingly – students to answer questions and remove some of the perceived opacity from the process of developing new crop varieties.

This volume brings together broad perspectives and begins to close the gap between scientists talking to one another and scientists talking to our stakeholders – our neighbors – about the experiences we have had to date using a powerful new set of tools and the prospects for this approach to positively impact society.

Technology is necessary but not sufficient. We must develop all available tools if we're to meet the challenges of offering healthful food in the face of increased population, higher standards of living, and reductions in water, land, and energy. We must also, however, use the power of the social sciences to understand how society adopts new technologies and adapts them to fit local culture. This is particularly true of food, which has an almost mystical connection with our cultural stability. Most of all, we need to communicate; communicate with each other as scientists, with decision-makers, and with citizens around the globe. Only through this pathway will the tools we can develop to feed our neighbors and feed the world seem as acceptable and as necessary as the next smartphone model we simply must have.

The Ohio State University Dr. Bruce A.McPheron, Ph.D
Columbus, OH, USA

**Dr. Bruce A. McPheron Ph.D** is the Vice President for Agricultural Administration and Dean of the College of Food, Agricultural, and Environmental Sciences (Ohio State University), beginning his appointment in November, 2012. He joined Ohio State after serving for three and one-half years as Dean of Penn State's College of Agricultural Sciences. McPheron received his B.S. degree from The Ohio State University and his M.S. and Ph.D. degrees from the University of Illinois. He currently serves as chair of the Policy Board of Directors of Agriculture for the Association of Public and Land-Grant Universities. Dr. McPheron's research expertise has focused on the use of genetic tools to examine population structure in pest insects. His molecular diagnostic methods for source identification of Mediterranean fruit fly developed in his laboratory have been adopted by USDA-APHIS and several state and international agencies. He has taught a wide range of classes in entomology and has traveled internationally sharing his expertise.

# Contents

## Part I The Tools for Engineering Plants

## Part II Contributions to the Society

Part III Sustainable Management

Part IV Sustainable Environment

Part V Contributions to Food, Feed, and Health

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# Chapter 1
# Introduction: Biotechnological Interventions for Crop Improvement: Answers to Global Challenges

**William D. Dar**

The world population is about to reach seven billion and one in seven of these people is already hungry. 1.4 billion people (one in four) in the developing world lives in poverty; more than a quarter of the world's children are still malnourished. The projection is that the world population will reach eight billion by 2025, ten billion by 2083. This is an alarming time point for the whole society facing "the perfect storm" due to challenges such as climate change, energy crisis, food crisis, land degradation, loss of biodiversity, and increasing population that could lead to widespread food shortages and public unrest over the next few decades. To feed the growing world population, we have no option but to intensify crop production. The farming community, mostly composed of small and marginal farmers, is the most vulnerable and face unprecedented constraints due to combined effects of climate change and growing competition for land, water and energy. A recent scientific prediction states that worldwide drylands will expand by 11 % (FAO 2011), and that we will experience increased frequency and severity of droughts across the globe. The growing volatility in food prices and unexpected spikes in oil prices in past few decades no doubt affects the world's poor in worst way. The international financial crisis is looming large, especially in the developing countries. Amid all these, agricultural productivity, especially in developing countries, continues to drop, while degraded natural resources and climate change are increasingly affecting food production and prices.

The rapid developments in plant biotechnology have moved the agriculture from a resource-based to a science-based industry (Sharma and Ortiz 2000). The traditional plant-breeding methods along with biotechnological interventions may help to achieve productivity gains, strengthening of resistance to pest and diseases, reduction in pesticide use, improvement in crop tolerance to abiotic stress,

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A. Ricroch et al. (eds.), *Plant Biotechnology: Experience and Future Prospects*, DOI 10.1007/978-3-319-06892-3_1,

improvement in the nutritional value and enhancement of durability of products during harvesting and storage. Rise in productivity could increase smallholder farmer's incomes, reduce poverty, increase food access, reduce malnutrition, and improve the livelihoods of the poor.

## 1.1 Climate Change and Agricultural Prosperity

Climate change, a major threat to agricultural productivity, is mostly affecting the people who are bearing the brunt of the effects of climate change and is affecting those who can least afford to do so and who have done least to cause the problem. Development challenges are today compounded by the effects of increasingly extreme weather. Hence, we need to adopt a comprehensive approach involving all stakeholders, in identifying best adaptation and mitigation practices, and in prioritizing policy-relevant research across fields. More frequent and intense precipitation events, elevated temperatures, droughts, floods and other types of damaging weather, are all expected to take its tolls on crop yield and quality. This makes the challenge of feeding an estimated nine billion people in the world by 2050 exceedingly difficult. A recent study assessing rainfed cereal potential under different climate change scenarios revealed loss of rainfed production potential by 10–20 % (IIASA 2002). This is expected to affect 1–3 billion people by 2080 mostly in the tropical developing countries. The relationships between climate change, agriculture and food security are complex and dynamic. However, only by understanding this we can come up with solutions, open untapped opportunities to meet the challenge, and devise short-, medium- and long-term adaptation and mitigation strategies. Biotechnological interventions through molecular breeding approaches, Omics technology (genomics, transcriptomics, proteomics and metabolomics), and transgenics are the newer and stronger weapons to overcome the challenged situations of climate change.

## 1.2 Climate Changes and Nitrogen Fixation

The combustion of fossil fuels and other human activities, including deforestation and other changes in land use, has caused the $CO_2$ concentration in the atmosphere to increase from the preindustrial level of 270 μl l–1 to its current value of 375 μl l–1, and the concentration is expected to double from the preindustrial level later this century (IPCC 2001). Carbon dioxide is a potent greenhouse gas and its accumulation in the atmosphere is the primary driver for global warming. $CO_2$ along with water vapour, methane, $N_2O$, and other gasses maintains the habitable temperatures on earth. Recent estimates suggest that a doubling of atmospheric $CO_2$ will force a 1–6 °C increase in global mean temperature (IPCC 2001), and this warming may be accompanied by shifts in the distribution of precipitation at regional and continental scales (Rind et al. 1990). Changes in these three linked variables ($CO_2$, temperature, and water) will alter plant growth, biomass, and plant community composition at local, regional, and global scales. Plant responses, through increased carbon sequestration, can affect the variables driving climate change. Increasing atmospheric $CO_2$, as well as other components of climate change, has the potential to exert a strong influence on the productivity of symbiotic N2 – fixing organisms and the amounts of N contributed by these organisms to natural agroecosystems. Most biogeochemical models that incorporate future climate scenarios indicate that N limitations could exert considerable constraints on carbon sequestration by terrestrial ecosystems because N is frequently limiting to plant growth and decomposition (McMurtrie and Comins 1996; Rastetter et al. 1997; Nadelhoffer et al. 1999; Hungate et al. 2003).

## 1.3 Plant Breeding in Agricultural Productivity

Over the past century, conventional plant breeders and related scientists have worked diligently and skillfully to upgrade quality and yields of crop produce by employing various crop improvement techniques with commendable results. The Green Revolution, one of the greatest scientific achievements, has changed millions of lives by adoption of improved high yielding crop varieties of rice, wheat and maize, accompanied by the adoption of improved crop technologies including use of fertilizers, irrigation and improved management practices. Now, the technological challenge is to obtain improved agricultural productivity without destroying the global natural resource base. Agricultural biotechnology offers a responsible way to enhance agricultural crop productivity including potential reduction in input in terms of labour, machinery costs, harmful agrochemicals, and amount of land required for cultivation due to an increase in yield. The newly acquired ability to transfer genes between organisms without sexual crossing provides new opportunities to improve efficiency of production and to increase utility of agricultural crops.

## 1.4 Agricultural Biotechnology: A Weapon to Fight

Agricultural biotechnology provides modern ideas and techniques to complement agricultural research in plant breeding and traditional technologies by employing the tools of modern genetics to enhance the beneficial traits of plants, animals, and microorganisms for food production, making the future of agriculture more productive and sustainable. Since the limited variability in the available germplasm is a constraint to crop improvement, future breakthroughs depend on creating additional variability and inflow of desirable genes from related or unrelated species (Sharma and Ortiz 2000).

There are indications that global warming and climate change will lead to the emergence of more aggressive pest and pathogen populations, resulting in heavy losses by epidemics. Further, crops under abiotic stress are usually more susceptible to weeds, insects and diseases, which increase considerably the losses. Modern biotechnologies including molecular marker-aided technology and genetic engineering can be utilized to reduce pest-associated losses and the adverse effects of global warming and climate change.

## 1.5 Molecular Breeding in the Post-genomic Era

World crop production increased steadily and significantly after 1960s through "Green Revolution", development of agricultural techniques such as conventional breeding, pesticides, chemical fertilizers, and increased cultivated acreage through arable reclamation and irrigation. However, explosive improvements in traditional agricultural techniques and cultivated acreage increases can no longer be expected. The current productivity crisis in conventional plant breeding practices has prompted plant scientists to focus more on biotechnological approach and translational research (Gressel et al. 2004). It must, however, be emphasized that biotechnology is not the only solution to these and future problems and that it is part of a package of approaches that is available. Biotechnology has the potential to substantially increase the rates of return on investments in genetic improvement. Moreover, there are synergies between advances in DNA (deoxyribonucleic acid) sequencing, genome analysis, and bioinformatics. Information is a vital resource for farmers to take well-informed and timely decisions to make optimal use of available resources, together with new science tools such as GIS (geographical information systems) and modelling. Crop science draws from and is vastly enriched by molecular biology, cytology, systematics, physiology, pathology, entomology, chemistry, statistics (biometrics), even information technology. And at no other time have we been beset with a more staggering amount of data to analyse and assist us in our selection and hybridization challenges than now. Our scientists and plant breeders today need better ways to handle the massive amounts of data being generated by plant molecular studies, using approaches that should help speed up the development of improved crop varieties. But molecular tools require analyzing massive amounts of data, and important traits like drought tolerance and yield are the result of the combined actions of multiple genes, each with a small effect. These are called quantitative trait loci (QTLs), and the conventional Marker-Assisted Selection (MAS) approach to handling molecular data has limited power to detect small-effect QTLs and estimate their effects. This is where the next generation approaches come in to help more accurately predict trait values.

Applied genomics and molecular marker technologies are useful in assisting and speeding up with precision, the selection through conventional breeding. These are powerful methods for identifying the genetic basis of traits and are used to construct linkage maps to locate particular genes that determine beneficial traits. Using molecular markers, genetic maps with accurate detail have been developed for many crop species. These markers are particularly useful for analyzing the influence of complex traits like plant productivity and stress tolerance and are being employed to develop suitable cultivars of major crops. Cloning of QTLs is becoming increasingly feasible for manipulating quantitative traits by means of marker-assisted selection or genetic engineering (Salvi and Tuberosa 2005).

Apart from genomics approaches, transcriptome profiling has also been used to explore the cross-talk or the regulator of an altered trait. A number of studies in last few years have shown the ability of omics technology to characterize and assess several GM crops and to generate important information for the fulfilment of biosafety requirements. Similarly, proteomics and metabolic studies are also very useful to explore the changes of proteome or the metabolic networks, respectively. Thus, the system biology approach has helped not only to generate a vast amount of data but also for a better understanding of physiological changes. The fruit of this herculean datasets can be ripped by utilizing it for improvement in agricultural productivity either through molecular breeding or through gene transfer.

## 1.6 Gene Transfer Technology: Promises and Debates

The biotechnological approach in the form of GM Technology offers many benefits in terms of increased agricultural production and sustainable outcomes of research (Yonekura-Sakakibara and Saito 2006). The recent advancements in gene transfer technology has enabled scientists to find a better way to answer problems related to stress response, disease or herbicide resistance or development of tolerance against climate change. It is now feasible to introduce into crop plants, genes that have previously been inaccessible to the conventional plant breeder or which did not exist in the crop of interest. However, the availability of efficient transformation methods to introduce foreign DNA can be a substantial barrier to the application of recombinant DNA methods in some crop plants.

The application of modern biotechnology to agriculture has been underway for over 15 years, though discussion on genetically engineered foods has intensified within many countries more recently. There are those who recognise the potential benefits of agricultural biotechnology to society and advocate its rapid development and dissemination. Others urge the adoption of a slower, more cautious strategy, moving forward only as knowledge accumulates. Among the many issues arising from the use of genetic engineering in agriculture are consumer concerns about possible adverse effects on human and animal health and the impacts on the environment, particularly in the long term. For some there are also ethical concerns, regarding genetic manipulation. But it is needless to say that, transgenic technologies have enormous potential to improve crops of interest in a relatively precise way (Barampuram and Zhang 2011). Genes of interest are introduced, often by employing the cured strains of *Agrobacterium tumefaciens*, a gram negative soil bacterium, that in fact is a natural genetic engineer in its native form and causes crown galls in shrubs and trees. Genetic transformation of crop plants has emerged as a remarkable achievement in modern biotechnology. Transgenic plant varieties hold great promise for augmenting agricultural production and productivity when properly integrated into traditional agricultural research systems.

With the advent of gene transfer technology, there is hope for achieving higher productivity and better quality, including improved nutrition and storage properties of food. Several transgenic cultivars of major food crops, such as soybean, maize, canola, potato, and papaya, have been commercially released incorporating genes for resistance to herbicides, insects, and viruses. It is estimated that the global area planted with transgenic crops has risen from 1.7 million hectares in 1996 to 170.3 million hectares in 2012 (James 2013).

## 1.7 Biofortification: A Weapon to Flight Malnutrition

Another growing concern of the decade is the accelerating malnutrition. One of every three children in developing countries is malnourished. Malnutrition makes these children more susceptible to diseases, causes learning disabilities, mental retardation, poor health, blindness and even premature death. As a general rule, regions where the people consume less than 2,000 cal per day tend to have high rates of malnutrition (FAO 2013) Consuming this low amount of calories is an indicator that the people cannot afford to eat as much as they would like to eat. Keeping the only option of buying the cheapest foods available, which are starchy grains, roots and tubers. They cannot afford to eat enough meat and dairy products to avoid deficiencies of protein, fat and other nutrients in their diets.

Legumes which are called the “poor person’s meat” providing much cheaper protein than animal proteins can be a timely answer to this issue. While milk is still the best food for growing children, but when the poor cannot afford it, they need additional solutions. The oilseed legumes – soybean and groundnut – are rich in fat which is essential both for supplying energy to people who don’t eat enough calories, and also for the absorption of vitamins such as vitamin A in the human gut. Iron or vitamin fortified legumes are the best option to fight malnutrition which can be achieved by an intricate research in the area of biofortification. Biofortification is a nutrition-specific intervention designed to enhance the micronutrient content of foods through the use of agronomic practices and plant breeding. Unlike food fortification, which occurs during food processing, biofortification involves enriching the micronutrient content of plants. Biofortification can benefit farm households that produce primarily for their own consumption, as well as urban and rural households that purchase biofortified foods (Bouis et al. 2011). The biofortified products complement food diversification, food fortification and supplementation that are currently being employed to combat micronutrient malnutrition.

Biofortification through plant breeding can involve conventional varietal selection and breeding or more advanced molecular biology techniques such as marker-assisted selection or genetic engineering. Breeders can use the existing genetic diversity in a crop species and its wild relatives to identify, select and breed varieties that have higher nutritional content. Where a nutritional trait does not exist within the genome of the target crop, genetic engineering can be used to introduce the trait from another species. Biofortified crops can entail high start-up costs in the form of research, development and dissemination, but once biofortified staples are integrated into the food chain, they continue to provide micronutrient intervention with little additional input (Qaim et al. 2007). In 2008, biofortification interventions were ranked the fifth most costeffective development intervention by the Copenhagen Consensus (2008).

The best example of the successful translation of harnessing this technology is the development of Golden Rice, where a japonica variety of rice was genetically engineered to produce and store beta-carotene or pro-vitamin A. The incorporation of two specific genes allows the rice plant to modify certain metabolic pathways in its cells to produce precursors of vitamin A, which was previously not possible. This was considered a technical milestone, as most agronomic traits engineered to date have only required the introduction of a single gene. The same approach is taken in case of legumes to develop beta-carotene biofortified peanut through genetic engineering.

## 1.8 Risk Assessment and Biosafety Consideration

Modern biotechnology has the potential to lead to considerable advances in agriculture. Over the recent years, these technological advances have revolutionized our ability to introgress traits beyond the taxonomic family, which overcome natural physiological reproductive or recombination barriers, and which are not techniques used in traditional breeding and selection. Though it is impossible to demonstrate absolute safety or zero risk for any technology or activity (as all technologies and activities carry some risk), the risk assessment of the products of biotechnology must be strictly performed and precautionary principles should be considered in the decision-making process. Risk assessment comprises hazard identification, hazard characterization, exposure assessment, and risk characterization. Several approaches to address the risks are concerned with establishing good laboratory practices, the efficiency and security of the containment facilities, and the effects of genetically engineered organisms on human health and the environment. Several international organizations like OECD, UNIDO, and UNEP have invested considerably on developing biosafety information systems and harmonization of biosafety standards globally.

## 1.9 Ethical Issues and Intellectual Property Rights Related to Agribiotechnology

Agricultural biotechnology research and development represents one of such novel approaches with the capability of changing the face of agriculture so as to meet the increasing and varying needs (food, feed, fibre and fun) of people in the new millennium. This role has been acknowledged since its commercial introduction in 1996 (Penn 2003). Although, modern agricultural biotechnology has the potential to play a large role in more rapidly advancing agricultural productivity in developing countries while protecting the environment for future generations, the realization of benefits is linked with many challenges. The challenges involve issues of ethics, biosafety, and intellectual property rights (IPRs) in the use of new biotechnologies. This technology represents the latest scientific progress in the new millennium aimed toward fighting the persistent food crisis situations of many developing societies.

As biotechnologies were developed, a parallel strengthening of IPRs, particularly patent rights, took place due to TRIPS agreement of WTO. Emerging IPR regime is promoting privatization of scientific research in agricultural biotechnology, limiting the free access of knowledge and which might increase the gap of biotechnology know-how and its applications between various stakeholders (Serageldin 1999). The broad patents cover not only the biotech products, but also the techniques and tools used in developing those products such as patents on the vectors and genes, protocols, plant transformation systems, selectable markers, and gene expression technologies are of quite important for biotech products. The IPRs are also affecting the commercial development of new biotech products.

While agricultural biotechnology holds enormous promise for significantly increasing food production and relieving already strained land and water resources, it has become an emotional issue among some consumers, environmental groups and some societies. Handling these issues with a widened vision and with effective leadership are key areas to be attended.

The questions of safety, ethics, and welfare can be answered through taking adequate regulatory measures, public debate, human resource development and training, public-private sector collaboration, intellectual property management, and support from international development organizations.

The potential value of modern biotechnology in helping to achieve crop improvement and food security goals are being recognized by various stakeholders. Agricultural biotechnologies with its widened scope in various aspects have already proven to be a great weapon to fight against malnutrition, poverty and hunger through increasing productivity. The ethical questions, which are nevertheless to be said that, are often associated with the advent of any new technologies, are answerable, what is needed is precise follow up of the experimental results starting from field to laboratory and laboratory to market till commercialization of the technology. An open heart to welcome what is new, and beneficial to the society is the need of the time.

## 1.10 IMOD (Inclusive Market Oriented Development) Approach: A Key to Business Innovations

Modernizing the agro-food system can be a strong engine for direct and indirect growth and poverty reduction in the drylands. It is all about shared challenges, technology exchange, capacity building, and creating opportunities through partnerships. This is the essence of the Inclusive Market Oriented Development (IMOD) approach which is a dynamic progression from subsistence towards market-oriented agriculture. Since markets create demand for a wider diversity of higher-value foodstuffs and agro-industrial products, this pathway can reduce poverty as it involves value-addition of innovations (technical, policy, institutional and others) enabling the poor to capture larger rewards from markets, This also stimulates agro-enterprises that raise rural incomes and create opportunities beyond agriculture. By enabling farmers to become entrepreneurs and engage with the forward components of the agricultural value chain, will help in bringing an improvement in the productivity of the sector and improvement in the livelihood of the millions which depend on it. Once IMOD has been triggered for staple food crops, the stage is set to expand it into additional high-value cash crops, because the basic experiences and channels for input supply and output marketing would have been established.

## 1.11 Conclusions

Biotechnological advancements provide a very powerful weapon to irradicate hitherto non-curable diseases, to explore newer crop development tools, to find solutions of most of the problems related to "the perfect storm" of challenges such as climate change, energy crisis, food crisis, land degradation, loss of biodiversity, and increasing population. Hence it could help to solve the problems of widespread food shortages and public unrest over the next few decades. The challenge is to ensure the right direction to help the tropical dryland poor to find their way out of hunger and poverty, through bridging the gap between laboratory to field, and field to market. Different advancements in modern biotechnology discussed in different chapters of these books are nothing but to show the way-out to modern researchers and interested readers that the path of a secure future for the next generation is not a hard road to cross, only a focused vision and cumulative approach from different stratum of society is needed.

## References

Barampuram S, Zhang ZJ (2011) Recent advances in plant transformation. Methods Mol Biol 701:1–35

Bouis HE, Hotz C, McClafferty B, Meenakshi JV, Pfeiffer WH (2011) Biofortification: a new tool to reduce micronutrient malnutrition. Food Nutr Bull 32(1S):S31–S40

FAO (2011) Mountain Partnership Secretariat, UNCCD, SDC, CDE. Highlands and Drylands – mountains, a source of resilience in arid regions. FAO, UNCCD, Mountain Partnership, Swiss Agency for Development and Cooperation, and CDE, with the support of an international group of experts. Rome

FAO (2013) The state of food and agriculture 2013. Rome. (www.fao.org/docrep/018/i3300e/i3300e00.htm ISBN 9789251076712)

Gressel J, Hanafi A, Head G, Marasas W, Babatunde Obilana A, Ochanda J, Souissi T, Tzotzos G (2004) Major heretofore intractable biotic constraints to African food security that may be amenable to novel biotechnological solutions. Crop Prot 23:661–689

Hungate BA, Dukes JS, Shaw MR, Luo Y, Field CB (2003) Nitrogen and climate change. Science 302:1512–1513

IIASA (2002) Climate change and agricultural vulnerability. Special report for the UN World Summit on Sustainable Development, Johannesburg. (www.iiasa.ac.at/Research/LUC/JB-Report.pdf)

IPCC (2001) Intergovernmental panel on climate change, third assessment report. Cambridge University Press, Cambridge, UK. www.ipcc.ch

James C (2013) Highlights Global Status of Commercialized Biotech/GM Crops: 2012 ISAAA Brief 44-2012

McMurtrie RE, Comins HN (1996) The temporal response of forest ecosystems to doubled atmospheric CO2 concentration. Glob Change Biol 2:49–57

Nadelhoffer KJ, Emmett BA, Gunderson P, Kjønaas OJ, Koopmans CJ, Schleppi P, Tietema A, Wright RF (1999) Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. Nature 398:145–148

Penn JB (2003) Agricultural biotechnology and the developing world. Electron J US Dep State 8 (3). (www.usinfo.state.gov/journals/ites/0903/ijee/chassy.htm)

Qaim M, Stein AJ, Meenakshi JV (2007) Economics of biofortification. Agric Econ 37(s1):119–133

Rastetter EB, Agren GI, Shaver GR (1997) Responses of N-limited ecosystems to increased CO2: a balanced-nutrition, coupled-element-cycles model. Ecol Appl 7:444–460

Rind D, Suozzo R, Balachandran NK, Prather MJ (1990) Climate change and the middle atmosphere. 1. The doubled $CO_2$ climate. J Atmos Sci 47:475–494

Salvi S, Tuberosa R (2005) To clone or not to clone plant QTLs: present and future challenges. Trends Plant Sci 10:297–304

Serageldin I (1999) Biotechnology and food security in the 21st century. Science 285:387–389

Sharma KK, Ortiz R (2000) Program for the application of genetic transformation for crop improvement in the semiarid tropics. In Vitro Cell Dev Biol Plant 36:83–92

Yonekura-Sakakibara K, Saito K (2006) Review: genetically modified plants for the promotion of human health. Biotechnol Lett 28:1983–1991

# Part I
# The Tools for Engineering Plants

# Chapter 2
# The Evolution of Agriculture and Tools for Plant Innovation

Agnès Ricroch

**Abstract** Plants such as cereals and legumes on which humans depend on today were domesticated gradually and independently by ancient farmers in many different parts of the world over a few thousand years. Over time, ancient farmers converted hundreds wild species into cultivated crops (some of the world's most important crops). In the transition from foraging to farming 10,000 years ago, the wild forms of these plants mutated and were selected to result into new, domesticated species that were easier to harvest. This process continues today. Since the beginning of the twentieth century, innovation in plant genetic technologies has accelerated and produced better crops through increased resistance to pests and diseases, tolerance to drought and flooding. Dramatic and rapid progress has been made in our understanding and ability to alter gene expression in plants and in techniques for the identification, isolation and transfer of genes of interest. In many cases, this progress has been facilitated by the availability of efficient gene transfer methods. Geneticists use a wide variety of gene transfer methods to introduce foreign DNA (from microorganism, plant, animal) into plants. Plant genetic improvement offer an effective approach to increase food production and food security in order to support the world's growing population, especially in inhospitable climates. Plant innovations can also improve production of medicines for all.

**Keywords** Agriculture • Crop • Domestication • Breeding • Biotechnologies

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A. Ricroch et al. (eds.), *Plant Biotechnology: Experience and Future Prospects*, DOI 10.1007/978-3-319-06892-3_2,

> Genetically engineered crops are playing an increasingly important role in world agriculture, enabling scientists to reach across genera for useful genes to enhance tolerance to drought, heat, cold, and waterlogging, all likely consequences of global warming. I believe biotechnology will be essential to meet future food, feed, fiber, and biofuel demand. The battle to ensure food security for hundreds of millions of miserably poor people is far from won. We must increase world food supplies but also recognize the links between population growth, food production, and environmental sustainability. Without a better balance, efforts to halt global poverty will grind to a halt.
>
> Norman Borlaug – *Science*, 318: 359, 19 October 2007

## 2.1 Multiple Origins of Agriculture

Did you know that the oldest bee pollinators from 100 million-years ago were found in pieces of amber? Scientists also found evidence that human ancestors used fire one million years ago. According to fossils of starch grains from grinding stones and cooking pots found in archaeological sites, archaeologists stated that the history of plant breeding, and cultivation of major cereals started about 10,000 years ago.

### *2.1.1 Emergence of Agriculture*

The adaptation of crop plants to human needs and cultivation is a slow process evolving on a time scale of millennia. Wild cereals could have been cultivated for over one millennium before the emergence of domesticated landraces (Tanno and Willcox 2006). Human domestication of plants can be divided into three stages: "gathering," in which people gathered plants from wild stands; "cultivation," in which wild plants were systematically sown in fields of choice; and "domestication," in which mutant plants with desirable traits were raised (Weiss et al. 2006).

Based on recent DNA studies and radiocarbon dating of archaeobotanical remains, farming arose several times in several locations once the Ice Age had ended and climatic and environmental conditions were favourable for farming. Soon after humans adopted a sedentary existence agriculture arose (Tanno and Willcox 2006). These discoveries show the greatest revolution in human history: the transition from gathering foods from the wild to producing them on farms.

Foremost among the creations of ancient plant breeders are the cereals – rice, wheat, and maize, provide more than 50 % of the calories consumed by humans today. Agriculture happened first in the early villages of the Near East in the Fertile Crescent, a region from the Mediterranean Sea to Iran including modern-day Israel, Syria, Jordan northeastern Iraq and southeastern Turkey (Riehl et al. 2013). As early as 13,000 years ago, hunter-gatherers first began to gather and plant seeds from wild cereals and legumes, such as wheat, barley, and lentils and began their cultivating more than 11,500 years ago. Wild wheat persisted in cultivated fields as a weed in

Turkey. Plants were domesticated gradually and independently by people in many different parts of the world. Japonica rice, a subspecies of *Oryza sativa*, was bred about 10,000 years ago in the upstream region of the Yangtze River in China. Key crops such as rice and soybean originated in eastern Asia. This region is also the original home of several minor crops, such as certain types of millet. Maize eaten today by over one billion people was domesticated approximately 10,000 years ago in southwestern Mexico. For further information refer to the book "1491" by Charles C. Mann (2005).

## *2.1.2 The 'Domestication Syndrome'*

The dawn of agriculture, as well as of crop domestication, was a process of trials and errors. During domestication, humans subjected several key events to selection that make up the 'domestication syndrome'. During this process, ancient farmers, either consciously or unconsciously, saved seeds from plants with favoured characters to be sown the next year. Genetically-based traits that make up the 'domestication syndrome' include loss of seed falling (shattering), decreased dispersal, loss of seed dormancy, increased number of seeds, change in seed shape, compact growth habit (reduced branching, reduced plant size, dwarfism), increased size of fruits, adaptation of flowering time to local areas, and reduced toxic principles (safer food). Humans have also selected crops for disease-resistance.

The cereals – botanically a grass, from which the fruit which is called a caryopsis (grain) is harvested-, and most other crops, share a feature – a character or trait – central to domestication: their grains remain attached to the plant for harvest by humans rather than falling from the plant, as required by wild species to produce their next generation. For example, domestication of maize involved a plant architecture transformation from the wild ancestor (progenitor), *Zea mays* ssp. *parviglumis* resulting into an unbranched plant with seed attached to a cob, thereby making maize dependent on humans for cultivation. Subsequent to domestication, maize has been subject to intensive improvement efforts, culminating in the development of hybrid maize lines that are highly adapted to modern agricultural practices.

Understanding the origins and domestication of crops is of evolutionary interest. Understanding crop origins also allows the identification of useful genetic resources for crop improvement. Thus, domesticated plants provide a model system for studying adaptation of plants to their environment (the concept of adaptation is central in Darwin's work). Domestication shapes the genetic variation that is available to modern breeders as it influences diversity at the DNA level. Indeed, scientists today can follow how domestication proceeded at the level of DNA sequence change, from wild ancestors (progenitors) to cultivated crops. Insights into the domestication process reveal useful DNA information (at the gene level) for future crop breeding.

## 2.2 The Toolbox of Crop Improvement: Hybrids and First Biotechnologies

To accomplish the objectives for crop improvement plant breeders develop various tools and methods to broaden the possibilities for breeding new plant varieties: conventional breeding such as hybridization and mutation breeding, to advanced breeding techniques such as genetic modification.

The work of Charles Darwin (1859) and Gregor Mendel (1866) created the scientific foundation for plant breeding (Fedoroff 2004). The Austrian monk Gregory Mendel showed the importance of statistics in breeding experiments and the predictability in selective breeding. In 1866 he formulated the laws of inheritance on garden peas and discovered of unit factors (later defined as genes). Previously, the French family of the Vilmorins, who established the first seed company in 1727 in France (today part of the Limagrain Cooperative), introduced the pedigree method of breeding in 1830 (based on selected individual plants). The first seed company in North America was established by David Landreth in 1784. He published a catalog of vegetable seeds in 1799. The twentieth-century efforts were devoted to improving the productivity, reliability, and nutrition of crops: maize (George Beadle and Paul Mangelsdorf), fruits, vegetables, and ornamental flowers (Luther Burbank) to cite some. Indeed since the beginning of the twentieth-century the plant breeder’s toolbox has been developed to cause specific and permanent changes (genetic modifications): from first-generation hybrids (of maize and many other crops), wide-species crosses, mutation breeding, to genetic engineering. The new tools and methods are more and more rapid in their ability to create varieties with new and interesting traits.

### *2.2.1 Hybridization (Crosses Between Plants or Species)*

The transfer of traits between genetically distant or closely related species is not a new technique. Hybridization which is a cross between two parental plants which carry interesting traits has been achieved in numerous crops. It takes almost 15–20 years to create a new hybrid variety such as in sunflower, maize, oilseed rape, or new hybrid variety such as in sunflower, maize, or oilseed rape. In these wide crosses thousands of genes are affected while in transgenic plants one or six genes can be added (at the moment).

In 1919 in maize in Connecticut, Jones developed the double-cross method, which involved a cross between two single crosses (four inbred lines generated from the mating of parents who are closely related genetically are used). This technique made the commercial production of hybrid maize seed economical. In 1923 in Iowa, Wallace developed the first commercial hybrid maize. In 1926, he then founded the Hi-Bred Maize Company (today Pioneer Hi-Bred, a DuPont Company).

Hybrid seed technology generates heterozygous plants with improved yield and disease resistance by adding traits from two different parents. Average maize yields over the past 40 years have doubled in the USA, although not elsewhere.

### 2.2.2 Chemical- and Radiation-Induced Mutagenesis

Chemical- and radiation-induced mutagenesis (using Gamma-rays and X-rays since ca.1920) increases the frequency of genetic variations which can be used to create new mutant varieties. A mutant is a plant/organism in which a base-pair sequence change occurs within the DNA of a gene or chromosome resulting in the creation of a new character or trait. These mutations can be interesting for crop improvement, such as reducing the height of the plant, changing seed colour, or providing tolerance or resistance to abiotic (e.g. salinity and drought) and biotic (e.g. pests and diseases) stresses. In the UK, much of the beer was produced using a mutant variety of barley (the 'Golden Promise' variety, salt-tolerant spring barley with semi-dwarfness in stature). Wheat varieties developed through mutation breeding technique are used today for bread and pasta (e.g. induced mutability for yield). Many physiological and morphological mutants have been obtained (in cotton, date palm, rice, pear, pea, peppermint, sunflower, peanut, grapefruit, sesame, banana, cassava and sorghum . . . and also dahlia), refer to http://mvgs.iaea.org/Search.aspx. Over 2,900 crop and legume varieties developed through chemical or radiation induced mutagenesis have been released worldwide in more than 50 countries: for example the short height mutant of rice basmati '370' in Pakistan, the semi-dwarf mutant rice 'Calrose 76' in California, a mutated indica rice stain after exposure to 25 kR of Cobalt-60 gamma radiation, in Thailand. In organic agriculture farmers use the 'Calrose 76' strain of brown rice, also developed through mutagenesis. Lewis J. Stadler of the University of Missouri in the USA was the first to use X-rays on barley seeds in 1920s and ultraviolet radiation on maize pollen in 1936. Different kinds of mutagens are used in plant breeding, such as chemical mutagens like EMS (ethyl methanesulfonate) to generate mutants.

It takes more than 10 years to create a variety with such mutations, which will be then crossed with an elite variety adapted to local agronomical and climatic conditions. Such varieties carry a huge number of genes affected. The random results of this genetic technique illustrate how spontaneous mutations create the genetic diversity that drives evolution (one of the Darwin's concept), and the material upon which selective breeding can operate.

### 2.2.3 Other Techniques: In Vitro Techniques, Genome Sequencing and Gene Mapping

Other breeding techniques using *in vitro* tissue culture – micropropagation, and embryo rescue – permits the crossing of incompatible plants and allows the production of uniform plants.

Thanks to the knowledge at molecular (DNA) level and bioinformatics the latest step of innovation in plant breeding, dating from the 1980s, came from biotechnologies. Molecular marker–assisted selection (MAS) is now widely used to localize characters or traits on the genetic map of the crop and select commercially important characters or traits. In MAS for example, a DNA marker closely linked to a disease resistance locus can be used to predict whether a plant is likely to be resistant to that disease (Tester and Langridge 2010).

In 1944, DNA as the genetic material was discovered in pseudococcus by Oswald Avery, Colin MacLeod, and Maclyn McCarty, from the Rockefeller Institute in the USA. Then in 1953 James Watson, Francis Crick, Rosalind Franklin, and Maurice Wilkins determined the structure of DNA. Since the 50s DNA sequencing has seen rapid progress. The first sequencing of a natural gene from yeast was made in 1965 and took 2.5 years. In 1976, the first genome was sequenced (a bacteriophage). In 2008, the first human genome (six billion base pairs of DNA of James Watson's genome) was sequenced in 4 months and cost less than US$ 1.5 million. The price is dropping rapidly due to new DNA sequencing technologies. The National Human Genome Research Institute (USA) has set a target to be able to sequence a human-sized genome for US$1,000 by 2014.

A complete genome sequence is available for several crops since the late 1990s: apple, banana, bread wheat, cacao, cassava, chickpea, cotton, date palm, foxtail millet, grape, maize, papaya, peach, potato, rice, sorghum, soybean, strawberry, sweet orange . . . The latest sequenced genomes of 2014 are of loblolly pine, peanut, and pepper.

"Orphan" crops (i.e. Ethiopian mustard, finger millet, yam, baobab, roots and tubers, etc.) which are much less economically important on global markets, have been largely ignored by researchers and big seed companies. However, they will be bred and sequenced to meet needs of farmers in developing countries thanks to initiatives of foundations from both the public and the private sector. Indeed the Clinton Global Initiative in 2011 aims to sequence, assemble and annotate the genomes of 100 traditional African food crops in order to advance breeding that improves the nutrition, productivity and climatic adaptability of these crops. The data are publically available on the Internet to all researchers.

### *2.2.4 The Green Revolution*

Since 1940, foundations such as the Ford, the Rockefeller, the Howard Buffet or the Bill and Melinda Gates Foundations have played a major role in collaboration with governments for breeding of crops. The Green Revolution started in 1943 when the Mexican government and the Rockefeller Foundation co-sponsored a project, the Mexican Agricultural Program, to increase food production in Mexico, in particular wheat production. Using a double-concept (interdisciplinary approach and international team effort), the scientific team headed by an American wheat breeder at the Rockefeller Foundation, Norman E. Borlaug, started to assemble genetic resources (germplasm) of wheat from all over the world. The life and legacy of the father of the Green Revolution, Borlaug, who received the Nobel Peace Prize in 1970, is celebrated in 2014 for the 100th anniversary of his birth.

After the famine of 1961 in India, Borlaug advanced the development of high-yielding varieties such as IR8 – a semi-dwarf rice variety, along with expansion of irrigation infrastructure, and modernization of management techniques, distribution of hybrid seeds, fertilizers, and pesticides to farmers.

Today almost two billion people suffer from chronic hunger and malnutrition in developing countries. This makes agricultural development in developing countries a pressing need as they have the fastest population growth rate and they are also more at risk from resource shortages and the effects of climate change. Increasing food supply without deforestation or a net change in land use means increasing production. Modern agriculture – including biotechnology – has recently been kept out of Africa (Paarlberg 2009).

## 2.3 Advanced Breeding Techniques: Genetic Modification Technologies

In 1946 J. Lederberg and E.L. Tatum first discovered that DNA naturally transfers between organisms. Genetic engineering, also known as genetic modification (GM), exploits recombinant DNA technology as new tool for plant breeders. As a technique that is faster and able to deliver genetic changes that would never occur through conventional methods, GM is uniquely useful and one of tools of the plant breeder's toolbox.

Conventional breeding today encompasses all plant breeding techniques that do not fall under current regulations for GMOs. For example in Europe, the European legal framework defines GMOs and specifies various breeding techniques that are excluded from the GMO regulations (the European Directive 2001/18/EC on the deliberate release of GMOs into the environment). Excluded from this GMO Directive (and thus may be viewed as conventional breeding) are hybridization (cross breeding), in vitro fertilization, polyploidy induction, mutagenesis and fusion of protoplasts from sexually compatible plants.

### *2.3.1 Genetic Engineering Technologies*

Transgenic techniques provide genetic modification or genetic engineering of a recipient plant with one or more foreign genes. These foreign genes can come from plant or non-plant organisms. Transgenic plants are used for precise crop improvement because of transfer of limited genetic material as oppose to conventional breeding in which one half of the genome from each parental line is combined after hybridization. Genetic engineering also makes possible genetic changes, including between animals and plants, which would be highly unlikely or would never occur using mutagenesis or other conventional breeding techniques.

Advances in molecular biology in the 1970s made it possible to identify the specific gene responsible for a trait, isolate it, and transfer it, from any type of organism, to plant cells. Instead of making tens of thousands of genetic changes (cross or mutation breeding), with transgenesis a gene with a known single beneficial trait is inserted into the plant genome. Plant breeders embraced transgenesis because it offered this precision and a quicker way of obtaining a desired trait in a plant.

The original regulations on growing GM crops were instigated by scientists doing molecular biology research. The first GM experiment, published in 1972, described the insertion of bacteriophage genes into an animal viral DNA. Consequently scientists raised questions about potential risks of recombinant DNA to human health and organized the Asilomar Conference in 1975 in California in the USA, attended by scientists, lawyers and government officials to discuss the technology. They concluded that experiments could proceed under strict guidelines drawn up by the US National Institutes of Health (Berg et al. 1975).

There are several ways to genetically engineer plants, which are mainly: (i) infecting plant tissue by *Agrobacterium tumefaciens* will integrate the desired gene in its DNA, a mechanism of genetic engineering discovered by Marc Van Montagu and Jeff Schell (in Belgium) in 1977, or (ii) shooting plant tissue with a gun carrying tungsten or gold particles coated with the gene we want to transfer (also called biolistic particle delivery system) used since 1984 by John Sanford, Edward Wolf, and Nelson Allen in the USA. Introduced genes fall randomly amid the DNA strands. Plant mutation breeding (discussed above, 2.2) may induce more changes than transgene insertions through genetic engineering. It takes less than 5 years to create a variety with added transgenes. That variety will be then crossed with elite varieties adapted to specific agronomical and climatic conditions.

A special feature of genetic modification is that it allows the transfer into crop plants of one or a few genes from unrelated organisms (microorganisms such as bacteria, animal or human). Conventional breeding (hybridization between very distinct plants even from different genus) cannot form plants with genes coming from different kingdoms. Additional techniques of modern plant breeding are discussed in the Chap. 3 by Surinder Chopra.

### 2.3.2 Traits Expressed by the Genetic Engineering Technologies

The first GM plant produced was an antibiotic-resistant tobacco plant in 1982. The first commercialized GM crop was the FlavrSavr® tomato in 1994 in the USA. It contained a trait that suppressed early ripening in tomato to maintain flavor and taste. In the UK, a concentrated tomato paste using these GM tomatoes went on sale in 1996 (by Zeneca). It received an award in France for the best innovation. The earliest crops produced by transgenesis (insect-resistant and herbicide-tolerant varieties) have been commercially cultivated since 1995. A GM variety of maize developed to express a protein from *Bacillus thuringiensis* (‘*Bt* maize’) protects maize against the European maize borer and some other lepidopteran insects. *Bt*, originally discovered in 1911 in the province of Thuringia in Germany, has been used as a spray by organic farmers. The *Bt* genes produce CRY proteins are an alternative to chemical pesticides. These are introduced in more than a thousand elite varieties of maize, but also in cotton. *Bt* varieties of rice, soybean and poplar are not yet commercially available.

The global area cultivated with GM varieties was over 170 million hectares in 2012. Crops grown commercially today contain traits for herbicide tolerance, insect resistance, or both. These have been developed for commodity crops such as soybean, cotton, maize and oilseed rape. It is estimated that, for example, 88 % of the cotton grown in India is now GM due to its greater resistance to pests. The cultivation of GM insect-resistant crops, particularly varieties of cotton, in India and China, is also reducing the exposure of farmers to harmful organo-phosphate insecticides. There are a lot of products from GM crops in the food chain. In Europe it is estimated that 90 % of some animal feed (maize and soybean) is derived from GM varieties because of their low cost and large amount available.

The list of approved GM crop varieties is long: alfalfa, Argentine canola, bean, carnation, creeping bentgrass, flax, melon, papaya, petunia, plum, Polish canola, poplar, potato, rice, rose, squash, sugar beet, sugarcane, sweet pepper, tomato, wheat (for updated data visit http://www.isaaa.org/gmapprovaldatabase/default.asp).

Several genes of interest have been discovered including pest and disease (fungi, virus, bacterial) resistance genes, and new ones are being discovered at a rapid rate. Some of these genes have been incorporated into commercial varieties to breed for speciality traits and these include heat and drought tolerance, nitrogen use efficiency, modified alpha amylase, male sterility, modified amino acid, modified flower color (in dianthus), modified oil/fatty acid, and virus resistance. In Pamela Ronald's laboratory in UC Davis (USA) the discovery of the gene XA21 confers resistance to a bacterial disease, and the discovery of a gene of submergence tolerance of rice allows drowning weeds without drowning the rice, providing a method for weed management without relying on a herbicide (Ronald and Adamchak 2008).

Genetic technologies also aim to achieve more efficient industrial use (biofuels) for drastically reducing $CO_2$ emissions. Radical innovations concern nutritional benefits. Healthier vegetable oils with fewer trans-fats are being developed. Biofortifying key crops including cassava in Africa or rice in Asia illustrate the potential of genetic engineering to fight malnutrition. In developing countries, especially in Asia, vitamin-A deficiency causes childhood blindness. The most famous attempt to combat this deficiency is the development of 'Golden rice' by Ingo Potrykus in Switzerland and his colleagues (discussed in Chap. 19 by Robert Ziegler). They genetically transformed rice plants with carotenoid biosynthetic genes that result in more vitamin-A precursors. Today, geneticists are also trying to reduce allergens in foods using genetic engineering.

The ability to manipulate plant genes to produce certain human enzymes is not new. Interest in deriving pharmaceuticals from plants (known as 'bio-pharming') first took off in the 1990s after scientists showed that monoclonal antibodies could be made in tobacco plants. Plant-derived biologic treatments have proven successful in drugs given to animals in recent years and today in human patients suffering from Gaucher disease (discussed in Chap. 20 by Mary E. Mangan). This led to genetic engineering of plants to produce vaccines, antibodies and proteins for therapeutics.

### *2.3.3 Development of New Genetic Technologies*

In the past two decades, additional applications of biotech and molecular biology in plants have emerged, with the potential to further enlarge the plant breeder's toolbox. Several recently described techniques allow for site-directed mutagenesis of plant genes (to knock out or modify gene functions) and the targeted deletion or insertion of genes into plant genomes. Another innovative trend is the use of transgenes solely as a tool to facilitate the breeding process. In this application, transgenes are used in intermediate breeding steps and then selected for removal during later crosses, eliminating them from the final commercial variety. Among these tools are accelerated breeding techniques, where genes that promote early flowering are used to speed up breeding, and reverse breeding, a technique that produces homozygous parental lines from heterozygous elite plants (Lusser et al. 2012). New tools concern three techniques: cisgenesis, intragenesis, and the zinc finger nuclease 3 technique (ZFN-3). Cisgenesis is the genetic modification of a recipient organism with a gene from a crossable–sexually compatible organism (same species or closely related species). Intragenesis is a genetic modification of a recipient organism that leads to a combination of different gene fragments from donor organism(s) of the same or a sexually compatible species as the recipient. ZFN-3 allows the integration of gene(s) in a predefined insertion site in the genome of the recipient species.

## 2.4 How to Meet 70 % More Food by 2050?

Global population has risen from 2.6 billion in 1950 to around seven billion today, and is predicted to rise to a world population of near ten billion people by 2050. According to the Food and Agricultural Organization of the United Nations, the demand for food could rise by 70 % by 2050. To meet this goal an average annual increase in production of 44 million metric tons per year is required, representing a 38 % increase over historical increases in production, to be sustained for 40 years.

To feed the several billion people living on our planet, the yield and the production of high-quality food must increase with reduced inputs. This accomplishment will be particularly challenging in the face of global environmental change. The challenge for major changes in the global food system is that agriculture must meet the double challenge of feeding a growing population, with rising demand for meat and high-calorie diets, while simultaneously minimizing its global environmental impacts (Seufert et al. 2012).

Today farmers will have to hit targets for reducing greenhouse gas emissions, improving water use efficiency and meeting the demands of consumers for healthful food and high-value ingredients. In this context, all plant breeding techniques are needed to contribute improvements in crop productivity and sustainability.

New technologies must be developed to accelerate breeding through improving DNA methods and by increasing the available genetic diversity in breeding germplasm (collection of wild types and varieties). Scientists underline the importance of conserving and exploring traditional germplasm. Introgression of characters or traits (pest and disease resistances or adaptation to salinity, cold or heat temperatures for example) into locally adapted varieties is expected to considerably enhance productivity in protecting crops from pathogens and climate change and under abiotic stress conditions. The most gain will come from delivering these technologies in developing countries, but the technologies will have to be economically accessible and readily disseminated (discussed in Chap. 5 by Calestous Juma and Katherine Gordon).

With governments, the private sector, foundations, and development agencies faced with feeding a growing and hungry world, research to increase agricultural productivity and access to affordable and safe medicines is needed, along with the challenges of intellectual property rights and genetic resource preservation that now play major roles in the plant breeding enterprise. In this twenty-first-century we will assist with radical plant innovations.

## References

Berg P, Baltimore D, Brenner S, Roblin RO III, Singer MF (1975) Summary statement of the Asilomar Conference on recombinant DNA molecules. Proc Natl Acad Sci U S A 72: 1981–1984, also Science 188:991

Darwin C (1859) On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life, 1st edn. John Murray, London

Fedoroff N (2004) Mendel in the kitchen: a scientist's view of genetically modified food. The National Academies Press, Washington, DC

Mendel G (1866) "Versuche über Pflanzenhybriden". Verhandlungen des naturforschenden Vereines in Brünn, Bd. IV für das Jahr 1865, Abhandlungen, 3–47. For the English translation, see: Druery CT, Bateson W (1901) Experiments in plant hybridization. J Royal Hortic Soc 26:1–32

Lusser M, Parisi C, Plan D, Rodríguez-Cerezo E (2012) Deployment of new biotechnologies in plant breeding. Nat Biotech 30(3):231–239

Mann CC (2005) 1491. New revelations of the Americas before Columbus. Knopf, New York

Paarlberg R (2009) Starved for science. How biotechnology is being kept out of Africa. Harvard University Press, Cambridge, Forewords by Norman Borlaug and Jimmy Carter

Riehl S, Zeidi M, Conard NJ (2013) Emergence of agriculture in the foothills of the Zagros Mountains of Iran. Science 341(6141):65–67

Ronald PC, Adamchak RW (2008) Tomorrow's table: organic farming, genetics, and the future of food. Oxford University Press, New York

Seufert V, Ramankutty N, Foley JA (2012) Comparing the yields of organic and conventional agriculture. Nature 485:229–232

Tanno K, Willcox G (2006) How fast was wild wheat domesticated? Science 311:1886

Tester M, Langridge P (2010) Breeding technologies to increase crop production in a changing world. Science 327:818

Weiss E, Mordechai EK, Hartmann A (2006) Autonomous cultivation before domestication. Science 312:1608–1610

# Chapter 3
# Techniques and Tools of Modern Plant Breeding: Field Crops

**Surinder Chopra**

**Abstract** Field crops of maize, wheat, rice, sorghum, barley, sugarcane, soybean, etc. are primary source of food and feed. Field crops are also a rich source of cellulosic biomass and carbohydrates for ethanol production. One of the major challenges facing agriculture today is improving the productivity of field crops in an environmentally sustainable manner. Annual climate variation causes temperature extremes, floods, and droughts which all exacerbate the vulnerability of field crops to pests and diseases. Conventional plant breeding has evolved into a molecular breeding and these modern breeding methods have enhanced the pace of crop improvement work. Plant breeders now use molecular and genetic techniques to selectively identify phenotypes and genotypes that are associated with traits of interest. Such functional genomics studies help plant breeders efficiently utilize the germplasm. Cutting edge molecular tools are now available in economically important crops as well as model plant systems. Gene expression techniques have been combined with forward and reverse genetic methods to isolate and introgress desirable alleles into breeding populations that are used to develop elite hybrid crops. This chapter focuses on modern techniques and resources that field crop scientists use to generate genetic information and efficient breeding strategies.

**Keywords** Genetics • Genomics • Germplasm • Marker assisted selection • Transposon tagging

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A. Ricroch et al. (eds.), *Plant Biotechnology: Experience and Future Prospects*, DOI 10.1007/978-3-319-06892-3_3,

## 3.1 Plant Breeding and Plant Ideotypes

Plants are the primary source of food, feed and energy and without them life on earth cannot be imagined. With a tremendous increase in human population, dramatic variability in the climatic patterns from year to year-enhanced efforts are needed to breed efficient plants. Plant breeding specifies plant improvement which can be attained either via sexual or asexual methods of breeding. The goal of a plant breeding program is to facilitate the development of a variety or cultivar which ultimately helps the grower. Such a plant breeding process may involve domestication of a plant species from its wild native environment, modifying by crossing two different parental lines, developing inbreds, single cross or double cross breeding, and developing hybrids. Thus, plant breeding has been described both as an art and a science. The science of plant breeding relies on the principles of genetics, information on chemistry and physiology of metabolic pathways, and growth and development of the plant under scrutiny. On the other hand the art of plant breeding lies in the fact that the plant breeder has a special skill and an eye for selecting plants with morphological traits or features that conform to a preconceived ideotype. Different plant species offer a variety of plant parts/organs that can be utilized as food for human consumption. Since plants can-not move to defend themselves, a plant breeder also focuses on development of pest, disease and stress tolerant varieties. Thus depending upon the plant organ to be harvested and climatic conditions for growing that particular variety, the definition of an ideotype can be developed. Overall, development of an ideotype is based on the fact that an efficient plant type is needed which is capable of producing high yield with minimum inputs. Ideotype development is dictated by how efficiently a plant utilizes natural resources.

Modern plant breeders of field crops like maize, wheat, rice, sorghum, soybeans, etc., have several modern genetic tools and techniques available, which can be used to enhance the process of final product development.

## 3.2 Plant Breeding Exploits Phenotype and Genotype

In order to use the science of plant breeding, information on development of a phenotype is needed. Phenotype is the result of interaction of genes of a plant among themselves and with the environment in which the plant is growing. The biological processes involved in plant growth and development are complex and influenced by individual genes as well as a combination of several genes. Traits that are controlled by single genes give rise to qualitative variation while multigenic traits produce quantitative variation. Such quantitative traits exhibit complexity and are highly influenced by the environmental conditions. Plant domestication is one of the examples of phenotypic selection in which by growing a wild form ancient farmers have selected modern form. This is best exemplified by the domestication of teosinte into our modern maize. During the development of pre-industrial agriculture, phenotypic selections by farmers were also the basis of development of landraces. One can still find landraces of maize, wheat, rice, sorghum and other field crops as well as horticultural and ornamental plants, which represent selections made by farmers and breeders in a specific climatic condition in that geographical region. A plant breeder can now make use of sophisticated phenotyping tools to precisely measure the phenotypic effect of a trait.

Mendel's laws provided the genetic basis of segregation of traits (genes) and as the science of plant breeding evolved, a successful program understood the importance to combine traits from different germplasm sources achieved by hybridizations. After hybridization a plant breeder then grows the subsequent generations to select the best combinations. Cultivars were developed by the use of breeding methods like pure line selection in a self-pollinated crop like wheat. These pure lines were progenies of single plants that performed the best in a population. One drawback of pure breeding has been the genetic homogeneity, which caused instability especially during the growing season when a new race of a disease appeared. In open pollinated crops however, random mating of plants within a population followed by mass selection provided some advantage by selecting a population that performed better. Conventional plant breeding methods successfully utilized pedigree breeding. Pedigree breeding allowed a plant breeder to select parental plants, cross those and then from the progenies pedigree selections were done. Thus phenotypic selection used by conventional plant breeders and success of this art of selection has been well documented in the form of release of high yielding inbreds, hybrids and varieties.

## 3.3 Molecular Markers and Plant Breeding

In traditional plant breeding, genetic composition of the resulting population and progenies was not known. However, with the availability of information on DNA sequence for several crop plant genomes it is now possible to develop molecular markers. The power of these markers lies in the fact that parental lines and germplasm exhibit sequence variations in their genomes. Examples of commonly used markers include SSR (Simple Sequence Repeat) markers commonly known as satellite markers, and SNP (Single Nucleotide Polymorphic) markers. These reliable markers are based on PCR (Polymerase Chain Reaction) methodology. A plant breeder uses these markers in several ways. First and foremost, markers are used to enhance the process of breeding and this method is commonly called marker assisted selection (MAS). MAS is based on the polymorphisms between the two parental lines which are used in the hybridization. The segregating progenies from $F_2$ (second filial) generation onwards are then screened using the markers that are genetically linked with specific traits in one or the other parental line. Plant breeders also use molecular markers in mapping of genes. In rice, for example, the *Sub1* locus which provides tolerance to submergence, was introgressed from a landrace of Oryza sativa into rice cultivars by the use of a method known as marker-assisted backcrossing (MAB) (Septiningsih et al. 2009). Moreover, genes for salinity tolerance have been introgressed in wheat and rice by the use marker assisted breeding. Simple traits that are controlled by single genes can be mapped with relative ease by the use of molecular markers through backcross breeding. However, complex traits or quantitative traits are controlled by more than one gene and are thus mapped as quantitative traits locus (QTL). Using the DNA-gel-blot-based methods restriction fragment length polymorphic (RFLP) markers have been used previously in maize, sorghum, and barley to identify QTLs conferring tolerance to drought and diseases. In addition to gene and QTL mapping, molecular markers are used in association mapping studies at the single candidate gene level. There are several example of association of candidate gene and QTL with a given trait. In maize, genome wide association studies allowed the identification of loci that associate with leaf length, width and upper leaf angle. Flowering time variation analysis in maize has lead to the association of markers in *dwarf8* gene, provitamin A and molecular markers in the gene IcyE in maize, several agronomic traits in rice have been associated with markers based on single nucleotide polymorphism (SNP).

Technology innovations have led to modern genotyping platforms that have evolved from laborious gel-based methods. These innovations have also reduced the cost of DNA sequencing which in turn has improved the efficiency of generating new markers to assist with MAS. These modern genotyping techniques have been employed for field crops like rice maize, barley, and wheat. These re-sequencing efforts of diverse rice germplasm through the Rice SNP Consortium (http://www.ricesnp.org) have provided valuable information on millions of SNP markers.

## 3.4 Recombinant Inbred Lines for Plant Breeding

The process of plant breeding develops new genetic material with reduced genetic diversity because of selection of new plant types from a limited number of parental germplasm lines. Plant breeders thus rely on additional natural variability in order to exploit genetic diversity available in a crop plant. Molecular markers that are associated with specific traits are then used to identify diverse germplasm of economically important crops like maize, wheat, sorghum and soybeans. Plant scientists have developed resources to tap genetic diversity and these are used for genome wide association (GWAS) mapping studies. For example, in maize, nested association mapping (NAM) populations have been developed in this project http://www.panzea.org by crossing 25 diverse parental lines with B73, a common parental line. From each cross, 200 recombinant inbred lines (RILs) have been developed giving rise to 5,000 RILS in total. These 5,000 RILs capture approximately 136,000 recombination events. In addition to NAM populations, the IBM (inter-mated B73 × Mo17) population along with molecular markers has been developed and is now used by plant breeders for mapping maize loci of their interest. These and other plant breeding resources that capture natural variation or genetic diversity allow plant breeders to study the effect of different alleles that are present in diverse parents used for developing association mapping panels.

## 3.5 Availability of Sequenced Genomes of Field Crops

With the advent of modern DNA sequencing technologies, several plant genomes have been sequenced and are publicly available. Some of the plant genomes have been fully sequenced and these include plants with small to medium sized genomes like rice, sorghum and maize while field crops like wheat which have large genome sizes have been partially sequenced. Efforts are under way to complete sequencing of unfinished genomes as well as to sequence additional economically important plant species. A list as well as the sequence of several of these plant genomes can be found here http://www.gramene.org/info/about/species.html. These genome sequences provide tremendous opportunities of efficient crop improvement to a plant breeder. First of all, genome sequences are rich sources for developing molecular markers. As explained above, these molecular markers can exploit polymorphisms among different germplasm lines of that particular plant species. Plant breeder can then perform allele mining based on these sequence polymorphisms and use selected alleles in the breeding program. Secondly, plant breeders use these reference genome sequences to perform gene mapping of their traits of interest.

## 3.6 Plant Breeding and Gene Expression Techniques

Francis Crick in 1958 described the Central Dogma of molecular biology in which the genetic information from DNA is converted first into RNA, which is then translated into protein. Over the past four decades, the science of molecular biology has exploded because of innovations in technology as well as computational biology. Current focus of a crop improvement program is to develop strategies and decisions based on gene expression. These expression based techniques help identify, validate and use desirable genes in the breeding programs. Field crop scientists are now routinely using gene expression as a molecular marker to decide about the strength of an allele of the given gene. Gene expression technologies include expressed sequence tags (ESTs), which are short cDNA (complementary DNA) sequences that can provide information about the expression of genes. EST sequences available for different plant tissues can provide tissue-specific or tissue-preferred expression data. EST sequences are now being used to develop gene-specific markers of expressed genes that crop scientists use in MAS breeding projects. DNA microarray is a gene expression technique in which DNA of all the genes of a plant species is fixed on a slide or a support. These slides are then used to hybridize with RNA from the same tissue of different parental lines or different tissue of the same parental line. DNA microarrays thus provide RNA expression information (i.e. similarities and differences) among different breeding lines as well as tissue-specific changes of genes. RNA-seq is another gene expression analysis tool which generates large data sets from a high throughput sequencing platform. Bioinformatic techniques have been developed to statistically analyze large gene expression data sets. RNA-seq thus provides global gene expression from thousands of genes and this analysis can be extended to multiple breeding lines. The Illumina based sequencing platforms HiSeq and MiSeq can be used for multiplexing large number of RNA samples and these innovations provide huge data on expression of thousands of gene for hundreds of parental lines. These next generation sequencing (NGS) techniques have provided gene expression data for agronomically-important crops like maize, wheat, barley and rice. Gene expression information exploits allelic diversity and provides precise detail about the usefulness of a particular allele that can be then introgressed into the breeding material. Gene expression profiling has further revolutionized the characterization of complex traits, which are controlled by multiple genes and their effects have been mapped as QTL. The association between phenotype and genotype by the use of molecular markers is done during the identification of a QTL. Expression QTL (eQTL) utilizes the concept of traditional QTL mapping in concert with genotyping information available from transcription profiling data available. Agronomically important traits are complex traits and eQTL mapping offers an efficient breeding tool. These marker – trait associations have been further exploited by validating them in order use the relationship across different related species. For example, a major QTL identified in maize has been employed in sorghum to achieve virus and downy mildew resistance.

## 3.7 Forward Genetics for Plant Breeding

The goal of forward genetics techniques is to identify the genetic variation underlying a trait. Mutants or variants are either naturally existing or new mutants can be generated artificially. Newly occurring natural mutants are rare and occur at low frequency because these mutants are the direct result of the evolutionary process. Naturally occurring mutations represent the type that have adapted to a certain environment or a disease or insect pressure. All other negative or lethal mutations do not survive and become eradicated from the nature. Since naturally occurring mutations are not found for all traits, especially the traits of agronomic importance, plant breeders use artificial methods of generating mutations. Mutation breeding involves use of chemical, physical and insertion mutagens to generate new mutations and then identify plant phenotypes that are conferred by the DNA modifications. Commonly used chemical mutagens are methylnitrosourea (MNU) and ethyl methanesulfonate (EMS). EMS causes single base pair changes and can produce large number of mutations per kilobase pair of the DNA sequence. In maize, EMS populations are developed by treating the pollen grains followed by putting this EMS treated pollen on silks to self pollinate ears by the use of paint brush. In other field crops including sorghum and wheat chemical mutagens are used to treat the seeds. Plant obtained from pollinated ear or treated seeds represent $M_0$ generation. Dominant mutations are screened in the $M_0$ generation. However, selfed progeny of the $M_0$ plants give rise to $M_1$ where segregation of traits takes place and thus recessive mutations are then identified in the $M_1$ generation. Physical mutagens including gamma rays and fast neutrons have been used to generate deletions in chromosomes. In the forward breeding program, mutations are selected and introgressed to improve crops.

The third types of mutations are caused by insertion elements. Commonly used insertion elements are DNA transposable elements, commonly referred to as jumping genes. Barbara McClintock was awarded a Nobel Prize for her discovery of these jumping genes in maize (McClintock 1967). Transposable elements or transposons excise from one place in the genome and re-insert at another place randomly. When transposons jump into a region of the gene that encodes for a protein, the function of that gene is disrupted giving rise to a mutation. Today, crop scientists use transposable elements as genetic tags that can be used to clone the flanking gene sequence for which the mutation resides. Similar to the transposons, T-DNA (transfer DNA) has been used to develop insertion libraries of mutants. T-DNA is a plasmid DNA present in the engineered *Agrobacterium tumifaciens* and this type of T-DNA used as insertion tool carry certain marker and/or antibiotic or herbicide resistant genes. Public resources of maize, sorghum, brachypodium and rice are available for crop scientists to screen their desired mutations. Once a mutation is identified, a co-segregation analysis is performed in segregating generations to associate the insertion allele with the novel phenotype.

In forward genetics, once a mutation is identified, a mapping population is developed by crossing the mutant line with another parental line with large number of polymorphisms. In addition, the second parental line is chosen based on the availability of other genetic and genomic resources developed from this particular parental line. For example, the maize inbred line B73 has been used to develop the reference genome sequence, transposon insertion databases, and availability of transcriptome, proteome and metabolome resources and databases.

## 3.8 Reverse Genetics Tools

Plant breeding efforts have been enhanced by the availability of genome sequences. One of the challenges these days is to ascribe a function to a putative gene sequence. Forward genetics can identify a limited number of phenotypic mutations followed by the mapping of the underlying gene. Reverse genetics utilizes the available genic sequence to identify its function by developing gain of function or loss of function mutants. Reverse genetics tools thus allow crop scientists to dissect the function of a putative gene sequence. Several functional genomics techniques have been made available to perform reverse genetics and salient ones are described here.

Chemical mutagenesis via EMS has been advanced to the isolation of mutants by the use of a method known as TILLING (Targeting Induced Local Lesions in Genomes; Till et al. 2006). To perform TILLING, genomic DNA from pools of $M_2$ plants from EMS-induced population is screened using gene specific primers. To identify mutations, PCR products are denatured, annealed and digested with single strand specific nuclease CELI. CELI thus digests the PCR product of a heteroduplex and these digested PCR products are then identified by gel-based methods, cloned and sequenced. The corresponding $M_3$ seeds obtained from the $M_2$ progeny plants are grown to identify phenotypic mutations associated with the mutated DNA sequence. TILLING has been successfully used for maize, rice, wheat, barley and sorghum.

Insertional mutagenesis using transposons and T-DNA transgenes has been one of the popular reverse genetics techniques. Insertion elements are dispersed throughout the genome and such plant populations are then used to screen presence of insertion in the gene of interest. One gene specific primer and one insertion element specific primer is used to PCR amplify the product. Once an insertion within a gene is confirmed, seeds of the progeny segregating for the insertion are grown and mutant phenotype is visually identified. In general insertion mutagenesis identifies loss of function mutations. However, there are examples in maize where ‘gain of function’ mutations have been identified as well. Maize has very well developed public resources for the identification of insertion mutants by the use of transposons. These are Ac/Ds transposon tagging (http://www.plantgdb.org/prj/AcDsTagging/) and Mutator based reverse genetics database known as UniformMu (http://www.maizegdb.org/documentation/uniformmu/). Similarly, insertion databases in rice (https://tos.nias.affrc.go.jp) can also be screened to identify Tos17 insertions within genes of interest. Barley has an insertional mutagenesis resource based on the transgenic *Ds* element of maize while in sorghum insertion mutation library is under development.

RNA induced gene-silencing method that is also known as RNA interference (RNAi) is being used to study the function of a known sequence. In RNAi mutagenesis, plants are transformed with a vector which generates a double stranded RNA corresponding to the gene of interest. Synthesis of double stranded RNA in the plant cell triggers the cellular machinery to degrade the RNA produced from the gene of interest. Similar to RNAi, virus induced gene silencing (VIGS) has been used as a reverse genetics tool but compared to RNAi, VIGS is relatively quick because it does not involve development of transgenic plants. Thus VIGS produces transient phenotypes that are not heritable, while RNAi generated mutations are heritable and can be deleterious. To perform VIGS, the gene whose function has to be characterized is cloned in a DNA vector modified from an RNA virus. The construct is then used to transfect the plant. The construct produces a double-stranded RNA of the gene of interest and this double-stranded RNA is then degraded by the plant gene silencing machinery into 24 nucleotide size small interfering RNAs (siRNAs). These siRNAs then attack the RNA of the endogenous gene of interest causing a mutant phenotype.

## 3.9 Targeted Genome Editing Technology

Genome editing can be successful in enhancing the plant breeding process. Crossing two diverse parents to transfer segments of DNA carrying genes often has drastic effects because of the linkage drag. Backcrossing for several generations with the adapted parental line traditionally rectifies these deleterious defects but this process of backcross breeding is time consuming and use lots of resources. New technologies like Zinc finger nucleases (ZFN) and transcription activator-like effector nucleases (TALENS) have shown promise in genome editing (Bogdanove and Voytas 2011). In these methods, sequence-specific nucleases cleave targeted loci enabling entire sequence replacement of an allele, insertion of new DNA, and formation of indels (insertions and deletions).

## References

Bogdanove AJ, Voytas DF (2011) TAL effectors: customizable proteins for DNA targeting. Science 333(6051):1843–1846

McClintock B (1967) Regulation of pattern of gene expression by controlling elements in maize. Yearbook of the Carnegie Institution of Washington 1965–1966, pp 568–577

Septiningsih EM, Pamplona AM, Sanchez DL, Neeraja CN, Vergara GV, Heuer S, Ismail AM, Mackill DJ (2009) Development of submergence tolerant rice cultivars: the Sub1 locus and beyond. Ann Bot 103:151–160

Till BJ, Zerr T, Comai L, Henikoff S (2006) A protocol for TILLING and Ecotilling in plants and animals. Nat Protoc 1(5):2465–2477

# Chapter 4
# Genomic Methods for Improving Abiotic Stress Tolerance in Crops

**Dea-Wook Kim, Ganesh Kumar Agrawal, Randeep Rakwal, Shahid Ahmed, and Jai Singh Rohila**

**Abstract** The twentieth century has seen tremendous progress toward increased food production to feed the world's current population of 7.3 billion. However, natural climatic changes leading to extreme weather patterns and human-influenced changes, which include the growing population, are blamed for adversely affecting the environment and are putting enormous strain on food productions. In particular, the crop plants that feed the world and sustain human health are being exposed to diverse environmental stresses, resulting in reduced farm yields. The rising threat of low food security demands urgent action from the scientific community to counteract the abiotic and biotic stresses encountered by crop plants. In this chapter, we focus our attention on examining the approaches, especially the genetic/genomic methods, which are being utilized by the researchers to improve the crop standing in the field under abiotic stresses.

**Keywords** Abiotic stress • Plant • Crop • Breeding • Gene • Omics

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A. Ricroch et al. (eds.), *Plant Biotechnology: Experience and Future Prospects*, DOI 10.1007/978-3-319-06892-3_4,

## 4.1 Introduction

The global population is currently more than seven billion people, and it is estimated to increase to ten billion people by 2050. Thus, the global demand for agricultural products is projected to rise by at least 50 % over the next two decades (UN Millennium Project). Cereals are the most critical food sources for humans and animals furthermore it is estimated that more than one billion tonnes of cereals will be required to feed the world's population. The increased demand for food comes not only from the growing population but also from developing countries, such as China and India, where increased economic benefits have resulted in higher meat consumption. This demand has resulted in the shift of land use from growing food for people to cultivating feed crops for animals. Another factor is the trend for cultivation of crops for renewable energy. These biofuel crops shifting the food and feed equation further out of equilibrium. For example, food crops like corn and sugarcane are now being cultivated for biofuel production, thus reducing the net food productions.

Globally, there is a limited amount of the land available for crop cultivation, which cannot be increased by deforestation due to the need for preserving forests to safeguard our natural environment. Furthermore, the existing crop producing lands suffer from improper management techniques; this problem compounds by fluctuations in irrigation and soil fertility. Moreover, many arable lands are used for cultivating only one type of plant, such as cash crops e.g. coffee, that are more profitable to the farmers than food crops. Finally and most importantly, the use of marginal lands for food crop production is challenging due to the damage from abiotic stresses such as drought, salinity, flooding, extreme temperature, metal toxicity, and recently, the re-emerging threat of radioactive metal contaminants in the soil.

Even in normally arable land, the current climate change phenomenon (natural or influenced by human activity) has allowed abiotic stresses to severely damage the food crops. It is expected that such damage will be more frequent in the coming years because of variable climatic conditions. We do not wish to dwell on the cause(s) of global warming, but we are instead focusing on the fact that rising air temperatures affect weather patterns and that these changes drastically affect crop yields. However, it is now well-accepted that the climate change has been and continues to be a threat to agricultural production worldwide having the most severe effect in tropical and sub-tropical countries (Wheeler and von Braun 2013). Therefore, the damage from extreme weather has occurred and will continue to occur in the near future leading to instability in food production patterns. As a possible strategy for stable and sustainable food production, which is undoubtedly a current need, greater efforts to improve abiotic stress tolerance of food crops are critical. In the last century, people experienced the first green-revolution, which was a breakthrough in the productivity of major cereal crops such as wheat and rice. Since then, it has been a great challenge for breeders to achieve a second green revolution because the traditional breeding methodology has certain limitations.

However, with the recent advancements in genomics the breeders now have access to a huge amount of genomic information that they can use to manipulate existing crop varieties. Their aim is to improve crops not only for increased yield but also for protection against the rapidly changing environment and the stresses associated with it. The use of genomic tools can thus provide a new strategy to improve crop stress tolerance, which will result in our ability to meet the current and future demands for food.

## 4.2 The Limits of Conventional Breeding Methods Based on Phenotypic Evaluations of Tolerance

### *4.2.1 The Difficulty of Improving Abiotic Stress Tolerance in Crops*

Abiotic stress tolerance in crops is a complex trait that involves various biological and environmental factors. It is associated with the expression of multiple genes and is therefore called a quantitative trait. One key to success in breeding is to gather the optimum combination of genes associated with stress tolerance. In a traditional breeding process, breeders empirically select germplasm to cross with an elite cultivar and select a new line from the offspring based on their phenotypic performances. Because of the complex nature of abiotic stress tolerance, it is extremely difficult to improve this trait using only the empirical method of breeding.

In the first step of breeding for stress tolerance, it is essential to find a germplasm that has alleles associated with this trait (Fig. 4.1). Most crop species are well adapted to their stable cultivation environments. They usually have a narrow range of genetic diversity, meaning that they are not highly variable in either their genotype or their phenotype. In this case, wild relatives or land-race crop species could be the best choices of germplasm for breeding because they have evolved to survive under abiotic stress conditions.

After a genetic cross, progeny with stress tolerance can be selected, which may also have agriculturally important traits, such as high yield. Because the desired

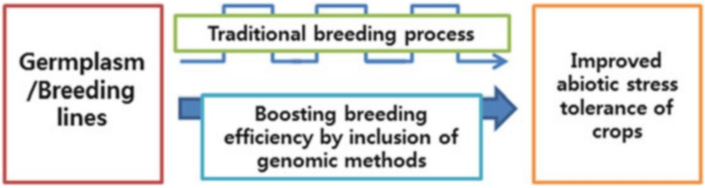

**Fig. 4.1** Schematic illustration to show the difference between conventional and genomics tools used in crop breeding programs for improving the stress tolerance

traits segregate among the progeny, repeated selection in each generation is needed to fix the traits of interest. Breeders select germplasm and progeny based on a phenotypic evaluation, which is costly and time-consuming process. Due to the complex nature of quantitative traits such as abiotic stress tolerance, using the empirical method is unlikely to result in plants with the optimal genetic makeup. Therefore, genetic markers have been developed to assist the phenotypic evaluation for more efficient selection of the breeding materials. The markers should identify the genotypic differences between individuals and between species. A molecular marker is a fragment of DNA located in a known region of the genome. It is useful because its nature and function is not affected by either environmental or developmental factors.

### *4.2.2 Some Basic Concept of QTL Analysis and MAS Performed at Gene Level*

Numerous types of molecular markers, including RFLPs, RAPDs, AFLPs, and SSRs, are being utilized by breeders. These markers, each with different advantages and disadvantages, have been used extensively for genetic studies and have been integrated into crop breeding efforts. With the availability of molecular markers, a new era has arrived for molecular breeding. Since the sequence information of genes was first identified, molecular markers have been employed to detect quantitative trait loci (QTLs), which are regions in the genome that control quantitative traits.

Breeders handle a large number of breeding materials during the screening of the germplasm for crosses and the selection of offspring from the segregating population. Therefore, selecting individual plants based on reliable genetic markers that are tightly linked to QTLs may greatly increase breeding efficiency. This diagnostic breeding tool, called marker assisted selection (MAS), enables breeders to distinguish among individuals that have the desired traits based on the differences in their DNA sequences but without phenotypic evaluation. MAS can substitute for the time-consuming phenotypic evaluation of a large number of breeding materials, which can be complicated by environmental factors. Moreover, plants carrying the desired traits can be selected at the seedling stage with specific markers prior to field tests and without phenotypic evaluations. Currently, most of the molecular markers are derived from differences in regions located near (linked to) a gene that controls the trait of interest, and the markers are not feasible to cover the whole genome. These factors may reduce the benefits of MAS for breeding by resulting in low selection accuracy.

Among the classical molecular marker technologies, simple sequence repeat (SSR) markers have been used most extensively to discover genetic variation in breeding materials. In recent years, because ofthe progress in next generation sequencing (NGS) and high-throughput marker detection technology, the highly abundant single nucleotide polymorphism (SNPs) markers have become the markers of choice in many breeding projects.

## 4.3 Genomic Methods Are Available for Gene Discovery and Increasing Breeding Efficiency

### *4.3.1 Next Generation Sequencing (NGS)*

The first DNA sequencing technology, called Sanger sequencing, was developed in the 1970s. This method was the primary sequencing tool for more than 30 years, and this time-consuming and costly technology was only applied to the genome sequencing of a few organisms such as humans, *Arabidopsis thaliana*, and rice. By 2007, an alternative type of sequencing technology, abbreviated NGS, had been developed. Using NGS, it became possible to rapidly analyze genome sequences of various organisms with a modest budget. Some may ask why we need the genome sequences of all crops or plants. The fully sequenced genomes of crop species facilitate the identification of the positional and functional information of genes that controls phenotypic expression. This tool accelerates genetics-based breeding for crop improvement. Since the first whole genome sequence of a crop species (*Oryza sativa* L.) was released, the genome sequences of other important food crops such as maize, soybean, and wheat have been completed or are near to complete, and genome sequences of other crop species and their wild relatives are expected to be unraveled in the coming years.

With the huge quantity of data generated by NGS and the related analyses at the level of the genome, researchers need bioinformatics tools to effectively use the genomic information for their breeding purposes. With such tools, computer algorithms, and software, genome sequence data can be adequately processed, assembled in order, and analyzed for their relevance to the biological properties of crops. The results of a bioinformatic analysis are stored as an easily accessible database that can be provided to the breeders.

The identification of genes involved in abiotic stress tolerance will allow the rapid screening of the germplasm for alleles that confer tolerance and will enable these alleles to be introgressed into cultivars using molecular markers. SNP discovery at a large scale has become more appealing by sequencing the parental genotypes of a mapping population by a high throughput technology such as NGS. This is feasible because of its high levels of automation and cost effectiveness. In the absence of a reference genome for minor crop species, the NGS data can be aligned with the transcript data from expressed sequence tag (EST) projects. In the case of a crop for which the reference genome sequence data are available, the detection of large-scale SNPs is possible, and subsequently can be employed as functional markers for crop breeding.

### *4.3.2 Association Analysis*

Association analysis is used to detect allelic variation associated with traits of interest in a population of genetically unrelated individuals based on linkage disequilibrium (LD), the non-random occurrence of allelic combinations. In the conventional method for QTL detection, a population of segregating progeny is developed for linkage analysis. A population of related individuals, such as a recombinant inbred line population, is prepared over a few generations and results in a limited number of recombination events. By using the natural germplasm instead of preparing a segregating population, association analysis can provide a much wider range of genetic diversity. Hence, association analysis is a time-saving approach that offers a better analysis of the multiple genetic variants in QTLs. Currently, two approaches have been employed for association mapping: candidate-gene association mapping and genome-wide association (GWA) studies.

By sequencing candidate genes using NGS technology, polymorphisms can be identified that are associated with the desired phenotypes. In the case of maize, the candidate genes *Dwarf8*, *Vgt1,* and *ZmRap2.7* were reported to be associated with flowering time (Pérez-de-Castro et al. 2012). In a GWA study, the large set of molecular markers derived from whole genome sequencing are used to detect the genetic variants associated with particular traits.

### *4.3.3 Genome-Wide Selection*

Complex quantitative traits such as abiotic stress tolerance are often controlled by several genes that individually have small effects. It is crucial and challenging to combine many genes with small effects into one plant for crop improvement. Due to the availability of genome-wide markers and their high-throughput detection, the genomic selection (GS) approach is appealing for its ability to combine multiple small-effect QTLs at the whole genome level (Heffner et al. 2009). Compared with traditional MAS, which requires the identification of markers associated with QTLs, GS can be conducted without this step. In the GS approach, a statistical model for determining genomic-estimated breeding values (GEBVs) is developed from the phenotype data and the genotype data, which are obtained using genome-wide markers, for the individuals in a reference population. Once the GEBV model is established, a plant (that has the desired traits) from the breeding population can be selected using the GEBV method with the genotypic data, and this process does not require a phenotypic evaluation. Despite having no requirement for previous marker selection, the information from this approach can be employed to detect QTLs that control the desired traits.

Nevertheless, even the identification of the QTLs responsible for a certain trait does not imply the identification of the specific gene(s) controlling that trait or an understanding of the mode of action. Models applied in genomic selection are useful to predict breeding values and, in some cases, to detect chromosomal regions associated with a trait; however, further work is necessary to identify the gene(s) responsible for the phenotypic variability observed. Therefore, we briefly introduce a collection of high-throughput technologies, called -omics (see below), that may be used to generate improved crop plants. The future exploitation of these strategies could facilitate the identification of candidate genes underlying the traits of interest and make MAS more meaningful and efficient.

### *4.3.4 -OMICS*

In the twenty-first century, the most prominent tools of functional genomics are the -omics technologies. As explained above, unraveling the numerous plant (including crop) species' genomes has brought a paradigm shift in the approaches to plant biology and crop breeding. The goal is very clear: crop improvement to meet the future global food demand. In this context, the identification and cataloging of genes, proteins and metabolites via high-throughput technologies such as – transcriptomics (the expression of genes), proteomics (the expression of proteins), and metabolomics (the levels of metabolites) have become powerful approaches that can be used to systematically reveal the function of each gene in the genome (Weckwerth 2011). How will -omics help achieve the goal of crop improvement? In addition to addressing fundamental biological questions, -omics tools will create new data on the molecular factors that are differentially expressed in plants that are tolerant versus those that are susceptible to abiotic stress. Their subsequent analysis through functional genomics could potentially be used to create new generations of plants that can withstand the adverse climatic conditions. With the development and increasing use of these -omics technologies, breeders will gain new strategies to search for candidate genes, proteins, and metabolites. In particular, the most important advantage for breeders is the potential to explore more genes than could ever have been found using single gene identification methods.

## 4.4 Conclusions

The above mentioned approaches namely, NGS, association mapping, GS, and -omics have the great genomic methods to generate biomarkers to help the modern plant breeders to select useful traits in their plant of interest. In particular, the -omics technologies have the advantage to identity the prospective candidate genes, proteins, and metabolites that can complement the biomarkers identified by the use of other genomic methods. However, in general speaking the -omics-based biomarker discovery program has not contributed much so far, and this technology require renewed efforts to screen large set of germplasm or selected germplasm resources based on the above three approaches; thereby increasing the chances of obtaining not only suitable and usable biomarkers for improving the crops but also for the inherently tolerant variety for further detailed genetic analyses. Other than the required large-scale screening, multiple abiotic (and biotic) stresses also have to be investigated using all the above mentioned approaches to link a potential biomarker to a particular stress, thereby increasing the chances to translate the newly generated information for the design of an abiotic stress-tolerant crop plant for commercial use by the farmers.

**Acknowledgments** DWK and JSR appreciate the financial support of Rural Development Administration (RDA), Republic of Korea. JSR acknowledges the support of Agriculture Experiment Station-South Dakota State University. RR acknowledges the great support of Professors Koji Nomura (Organization for Educational Initiatives, University of Tsukuba) and Yoshihiro Shiraiwa (Provost of Life and Environmental Sciences, University of Tsukuba), and Professor Seiji Shioda (Department of Anatomy I, Showa University School of Medicine) in promoting interdisciplinary research and unselfish encouragements. SA acknowledges financial help from Indian Council of Agricultural Research. Benjamin Benson was extremely helpful in reading this manuscript critically. Authors acknowledge the INPPO (www.inppo.com) platform in bringing together scientists across the countries in constructing this chapter.

## References

Heffner EL, Sorrells ME, Jannink J-L (2009) Genomic selection for crop improvement. Crop Sci 49:1–12

http://www.unmillenniumproject.org/

Pérez-de-Castro AM, Vilanova S, Cañizares J, Pascual L, Blanca JM, Díez MJ, Prohens J, Picó B (2012) Application of genomic tools in plant breeding. Curr Genomics 13:179–195

Weckwerth W (2011) Green systems biology-from single genomes, proteomes and metabolomes to ecosystems research and biotechnology. J Proteomics 75:284–305

Wheeler T, von Braun J (2013) Climate change impacts on global food security. Science 341:508–513

# Part II
# Contributions to the Society

# Chapter 5
# Transgenic Crops and Food Security

**Calestous Juma and Katherine Gordon**

**Abstract** This chapter provides an overview of the potential role of transgenic crops or genetically modified (GM) crops in enhancing food security. This chapter argues that although GM crops are still in their early states of adoption, emerging trends show their potential to contribute to food security. The crops have the potential to increase agricultural productivity on existing arable land; address issues of loss related to pests, disease, and drought; increase access to food through income gains; raise nutrition levels; and promote sustainable agriculture. But realizing the potential needs to be assessed in a non-deterministic, system-wide economic context. A key message is to view the role of GM technology as one of the many factors that influence food security whose contribution should be analyzed on a case-by-case basis.

**Keywords** Biotechnology • Environment • Food security • Genetic modification • Sustainable agriculture

## 5.1 Introduction

There is a need to feed a growing population of approximately nine billion by 2050 and address a surge in consumption, including a 70 % increase in the demand for food. Climate change and rising food prices will negatively impact developing countries the most. The challenge of feeding a growing population will include increasing production on existing arable land. One of the ways to combat this is by expanding the agricultural innovation toolkit, which includes genetically modified

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A. Ricroch et al. (eds.), *Plant Biotechnology: Experience and Future Prospects*, DOI 10.1007/978-3-319-06892-3_5,

(GM) crops. The aim of this chapter is to review the potential contributions of GM crops to food security, which is defined to include nutrition.

It argues that though GM crops are still in their early states of adoption, emerging trends show their potential to contribute to food security. This potential, however, should be viewed in a non-deterministic, system-wide economic context. Genetic modification is just one of the many technologies that constitute food security systems. Some of the examples in this chapter, especially in nutrition, are being pursued as proof of concept and their long-term impacts cannot be assessed at the moment. The chapter is divided into four sections. The first section summarizes the debate surrounding GM crops. The second section outlines trends in food security and biotechnology. This is followed by a section that examines some of the examples of the role of GM crops in enhancing food security. The final section reviews some of the major regulatory challenges associated with the adoption of GM crops.

## 5.2 Debating Biotechnology

There are many claims that biotechnology cannot contribute to solving food insecurity or benefit smallholder farmers. Critics argue that biotechnology is a red herring—that food insecurity is simply the result of poor infrastructure, distribution, and income level. GM crops are also criticized for being part of the agro-industrial complex. Critics link GMOs with increased pesticide use, monoculture, and industrialized farming at the expense of smallholder farmers. They argue that large agricultural corporations perpetuate food insecurity by selling expensive, unnecessary technology to poor farmers; preventing farmers from saving seeds; destroying plant diversity; and displacing millions of farmers. Critics claim that GM crops were developed with industrialized countries in mind; that they would never be adopted or accepted by developing countries; and that the technology continues to ignore the plight of smallholders because, for example, no drought-tolerant GM crop is commercially available yet (ISAAA 2013; Belay and Nyambura 2013).

These claims are driven by a wide range of concerns that tend to assert what has not been denied and deny what has not been asserted. GM crops have the potential to increase agricultural productivity on existing arable land; address issues of loss related to pests, disease, and drought; increase access to food through income gains; raise nutrition levels; and promote sustainable agriculture. But realizing the potential needs to be viewed in a wider food security context.

## 5.3 Food Security and Biotechnology Trends

Food security means different things to different people. At its root, the definition has evolved from the basic “right to food”—as codified in article 25 of the Universal Declaration on Human Rights—to a more complex understanding in 2009 when the FAO convened a World Summit on Food Security and determined that "food security exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food, which meets their dietary needs and food preferences for an active and healthy life" (FAO 2009, p. 1). Food security also depends on four interrelated factors: quantity of food, which translates into the need for increased agricultural productivity; access to food, which is determined both by income levels and quality of infrastructure; nutrition; and overall stability of the food system, such as resilience to shocks.

GM crops can benefit smallholder farmers in several major ways. First, they help farmers avoid both production and income loss due to pests, disease, and environmental factors such as drought or flooding. This results in greater productivity. Insect-resistant traits are found to have the greatest impact in warm, tropical places where pests are more prevalent and where insecticides and inputs are not widely used—namely in developing countries.

Furthermore, in areas where farmers face a variety of problems and extension agents are scarce, biotechnology can be successful at filling the void, as it can make farming less complex, "suggesting that farmers with less human capital may benefit the most" (Sexton and Zilberman 2011, p. 13). Most important, GM crops help farmers increase their income, which in turn provides them with increased ability to consume more nutritious food. Essentially, food security is about expanding ecologically sustainable agricultural practices as well as increasing access to nutritious food. The rest of this paper seeks to address how biotechnology can play a role in increasing agricultural productivity, income levels, nutrition, and stability and resilience of the food system to various shocks, thereby helping to increase food security at the global level but especially in developing countries.

Agricultural biotechnology, which was commercialized in 1996, refers to the application of scientific information and methods such as genetic modification of crops or animals to select certain traits that are more productive or desirable. Plant breeders have long sought to improve crops through traditional methods such as cross-breeding and hybridization, a time-consuming process that results in the presence of undesirable traits mixed in with desirable ones. Genetic modification is a significantly faster, more precise technology that is designed to achieve similar results as conventional plant breeding techniques by allowing the transfer of one specific gene to another plant. It has the potential to address a host of agricultural problems.

The major types of GM crops commercially available are herbicide-tolerant (HT) crops that are resistant to broad-spectrum herbicides such as glyphosate and gluphosinates; insect-resistant (IR) crops that include a specific bacterium, *Bacillus thuringiensis* (*Bt*), which is poisonous to certain insects; and/or crops with a combination of both (stacked trait). HT and IR traits help make weed and pest control more efficient, as crops need fewer applications of herbicides and/or eliminate the need for pesticides. HT crops are the most common, comprising more than half of the 175 million hectares of GM crops grown globally in 2013, followed by stacked-trait crops at 27 %, and IR crops at around 16 % (James 2014a, b).

Both first- and second-generation GM crops are produced commercially. First-generation crops typically have a single trait introduced. Newcomers, such as Burkina Faso, benefit most from adopting second-generation GM seeds, which contain two or more genes to resist specific pests or weeds. Monsanto's Genuity™ Bollgard II® cotton, for example, "work[s] against leaf-eating species such as armyworms, budworms, bollworms, and loopers . . . [and] cotton leaf perforators and saltmarsh caterpillars" (Juma 2011a, p. 37). Second-generation cotton is a superior technology because it takes longer for pests to develop resistance. First-generation GM technology is still beneficial but will break down sooner in terms of pest resistance.

Developing countries have seen clearly the potential of GM crops to increase agricultural productivity, income, and food security. Since their commercial introduction in 1996, GM crops have been one of the "fastest adopted crop technologies in recent history" (James 2014a). In 2013, "a record 175.2 million hectares of biotech crops were grown globally . . . at an annual growth rate of 3 %." (James 2014a). This is a 100-fold increase from 1996, when 1.7 million hectares were planted. Of the 28 countries that plant GM crops, 20 are developing countries. Finally, 90 % of those who grew biotech crops—that is, more than 16 million—were resource-poor smallholder farmers in developing countries (Ibid.). The impact of GM crops at the farm level has been significant. In 2011 alone, net economic benefits were $19.8 billion, and cumulative economic benefits amounted to $98.6 billion since 1996. The key point is that the "majority of these gains (51.2 %) went to farmers in developing countries" (Brookes and Barfoot 2013, p. 74).

Yet developing countries could benefit even more from adapting biotechnology to address local problems. The technology used to delay the ripening of tomatoes, for example, could be applied to tropical fruits, which ripen too quickly and end up going to waste due to lack of proper storage or transportation infrastructure. Another problem that is prevalent in tropical countries is soil acidity. "Acidic soils comprise about 3.95 billion ha . . . about 68 % of tropical America, 38 % of tropical Asia, and 27 % of tropical Africa. In spite of its global importance . . . problems that affect acid soils are investigated by only a handful of scientists in developed countries" (Herrera-Estrella 2000, p. 924). This problem is not limited to soil acidity. In fact, there is much scope for developing countries to invest in their own science and technology research institutes, which would allow local scientists to come up with solutions specific to local contexts.

## 5.4 Biotechnology's Contributions to Food Security

### *5.4.1 Agricultural Productivity*

Technology played an important role in generating significant increases in agricultural productivity during the Green Revolution. The combination of new, high-yielding crop varieties, agro-chemicals, and better irrigation techniques helped "raise food production to levels that no one would have dared predict . . . .farmers in the developing and developed countries nearly doubled their per-hectare output of cereal production, increasing yields during this time by 3.16 % annually" (Huang et al. 2002, p. 678). This led to a significant decline in poverty and hunger throughout much of Asia, because food levels rose, prices fell, and food trade and consumption increased.

However, the favorable conditions that led to the success of the Green Revolution have changed. Staple crops will be most affected by the "exhaustion of some past sources of growth [making] future yield expansion as great a challenge as in the past" (Ibid., p. 678). Overuse of fertilizers and chemical pesticides has led to pest and weed resistance and has contributed to environmental degradation; availability of arable land is declining; water resources are scarce; and climate change is causing significant changes in weather patterns, making it necessary to find alternatives to current production methods. Finally, the Green Revolution never addressed conditions specific to African agricultural productivity, such as soil depletion, lack of inputs, drought, and disease.

GM crops offer one alternative to addressing these challenges, as they are specifically designed to increase production while decreasing the use of pesticides and herbicides and addressing disease control. Increased production is necessary to feed a growing population and meet an ever-increasing demand for food. The genetically modified soybean enabled double-cropping in Argentina, which specifically helped to meet the huge increase in soy demand, driven primarily by an increased desire for meat in Asia, with only a limited effect on prices (Zilberman et al. 2010).

Although studies that examine production increases of GM crops have produced varying estimates, recent cotton studies in India and China confirmed earlier results: GM cotton production per hectare is demonstrably higher than non-GM cotton, especially in India. Other benefits include decreased pesticide use especially in China, and health benefits in both countries (Pray et al. 2011). Cotton was the most-adopted genetically engineered crop globally and saw the highest production increase, and the global price effects of planting *Bt* cotton are estimated at 10 % (Zilberman et al. 2010).

India had one of the lowest rates of cotton production in 2001–2002 (308 kg/ha). Aggregate levels of cotton increased substantially after the introduction of *Bt* cotton post-2002 (560 kg/ha) (Pray et al. 2011, p. 98). *Bt* cotton was adopted at a rate of 90 %, leading to "a 24 % increase in cotton yield per acre through reduced pest damage and a 50 % gain in cotton profit among smallholders. These benefits are stable; there are even indications that they have increased over time" (Kathage and Qaim 2012). Indian smallholder farmers who planted *Bt* cotton earned 50 % more from higher production due to reduced pest damage. With the extra income, farmers' consumption levels increased 18 % from 2006 to 2008 (Juma et al. 2014; Kathage and Qaim 2012).

In China, where surveys were conducted from 1999 to 2007, mean production of *Bt* cotton was higher than conventional cotton. One concern is that *Bt* cotton production levels will decline over time due to the development of bollworm resistance or as a result of being "backcrossed into more varieties by public- and private-sector plant breeders" (Pray et al. 2011, p. 93). Yet the data do not support these concerns. Indeed, "aggregate cotton yields continue to rise in China suggesting that *Bt* cotton also continues to do well" (Ibid.).

In developing countries more generally, where smallholder farmers use significantly fewer inputs than in developed countries, IR crops could have the greatest impact on production. By adapting the technology to local conditions, developing countries could also address the issue of yield drag, which occurs because companies typically modify generic seeds that are unspecific to a particular region. Developing countries could increase the production potential of GM crops by applying the technology to high-quality, local germplasm.

Higher production is not the only positive impact of GM crops. They also help reduce loss due to pests, weeds, and diseases. The potential of this technology lies in how it is adapted to meet specific, local needs in developing countries, which can range from combating diseases to improving indigenous crops.

Researchers in Uganda, for example, are using biotechnology to reverse the trend of *Xanthomonas* wilt, a bacterial disease that causes discoloration and early ripening of bananas and costs the Great Lakes region approximately $500 million annually. There is currently no treatment for the disease, and given its status as a staple crop in this region, solving this problem would directly increase food security and income (Juma et al. 2014; Juma 2011b). The most efficient method of containing the disease is by growing transgenic bananas instead of more labor-intensive methods. By transferring two genes from green peppers, scientists were able to grow highly resistant bananas.

In Nigeria the insect *Maruca vitrata* destroys nearly US$300 million worth of blackeyed peas—a major staple crop—and forces farmers to import pesticides worth US$500 million annually. To solve the problem, scientists at the Institute for Agricultural Research at Nigeria's Ahmadu Bello University have developed a pest-resistant, transgenic blackeyed pea variety using insecticide genes from the *Bacillus thuringiensis* bacterium.

These techniques have the potential to address a wide range of agricultural, health, and environmental issues in developing countries, leading to increased productivity and therefore contributing to increased food security.

### 5.4.2 Agricultural Incomes

Increasing production, reducing loss, and encouraging higher agricultural productivity among smallholder farmers has a significant effect on income and poverty. For one thing, growth in the agricultural sector is more effective at reducing poverty and increasing access to food than growth in any other sector. Since smallholder farmers comprise the majority of the workforce in sub-Saharan Africa, boosting their income levels through agricultural productivity would go a long way toward increasing food security.

The evidence from several long-term studies suggests that biotechnology is successful at helping smallholder farmers increase their income through costs savings. The last section showed how GM crops improve production and reduce loss. This translates into higher incomes at the farm level; indeed the income effect can be significant. A recent study explains how planting GM crops results in cost-savings up front, specifically with IR crops, which “require little capital and can substitute for chemical applications altogether” (Zilberman et al. 2010, p. 5). Not only were farmers able to reduce pesticide use, but they were also able to limit the related health risks.

Similarly, both IR and HT crops can reduce input expenses associated with pesticide use, such as machinery costs, fuel costs, and water use. Although seed prices for GM cotton were higher than for conventional seeds in India, these costs were “offset by reductions in expenditures on pesticides and labor, due in large part to reductions in number of required sprays” (Pray et al. 2011, p. 94). Overall production costs decreased, and net revenue increased. In fact, revenue from *Bt* cotton exceeded that of conventional cotton in every household surveyed in China (Ibid.). Results of *Bt* cotton studies in India also indicated that cost savings related to pesticide use, as well as higher production, offset the higher seed costs.[1]

When faced with less costs upfront, a reduction in crop loss, and more time available to pursue other income-generating activities, farmers have more income at their disposal, which also leads to greater consumption. So far, *Bt* cotton—which is the most widely adopted GM crop worldwide—has had the most impact on income. Approximately 15 million smallholder farmers in Burkina Faso, China, India, Pakistan, and a few other developing countries are growing *Bt* cotton. Several studies in India demonstrate the positive effects of *Bt* cotton on income, nutrition, and food security among poor farmers. Specifically, “*Bt* cotton adoption has raised consumption expenditures, a common measure of household living standard, by 18 % during the 2006–2008 period” (Kathage and Qaim 2012). In Burkina Faso, which grew 125,000 ha of Bt cotton in 2009, rural households saw production increases of approximately 18.2 % over those that grew conventional cotton; earning $39 per ha in profit. Although the seeds were more expensive, farmers saved money on inputs and labor (Vitale 2010).

Although *Bt* cotton does not directly contribute to better nutrition, it does indirectly contribute to food security by increasing household income levels and improving access to more nutritious food. This in turn increases the “purchasing power of farmers (and thus their exchange entitlements) and their access to food” (Juma et al. 2014). A recent study analyzes the impact of *Bt* cotton on caloric consumption and nutrition at the household level in four cotton-producing Indian states from 2003 to 2009. The authors find that households growing *Bt* cotton leads

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[1] Different studies used different methods for calculating income gain from Bt cotton, but all indicated significantly higher profit margins for Bt cotton farmers (Pray et al. 2011, pp. 99–100).

them to consume significantly more calories—specifically, “each ha of Bt cotton has increased total calorie consumption by 74 kcal per AE [adult equivalent] a day” (Qaim and Kouser 2013, p. 6).

Furthermore, a smaller proportion of households are food insecure (7.93 % of adopting *Bt* cotton households vs. 19.94 % of non-adopting households) (Ibid., table 2). The results also show that *Bt* adoption has led to consumption of more nutritious foods such as fruits, vegetables, and animal products. The authors estimate that if the households that do not currently grow *Bt* cotton switched, “the proportion of food insecure households would drop by 15–20 %” (Ibid., p. 6).

These findings indicate that increased income among smallholder farmer households that grow *Bt* cotton lead to greater food security and consumption of more nutritious food. But the results also demonstrate that farmers are the main beneficiaries of *Bt* cotton, rather than seed companies or biotechnology companies. This reinforces how plant biotechnology can be one important tool in addressing food insecurity.

Finally, farmers have seen their insurance costs decline as production risks stabilize. As a result, they will also gain access to better risk-management products. Given the increased production and income associated with *Bt* cotton, it can be extrapolated that further development of IR crops could “serve as an engine of rural economic growth that can contribute to the alleviation of poverty for the world’s small and resource-poor farmers” (James 2013).

### *5.4.3 Nutrition*

Biotechnology is also a useful technique for enhancing the nutrition in staple crops, specifically targeting low-income families. There are several bio-fortified crops that are currently available or being tested in developing countries. These include “Golden Rice,” which contains more beta carotene or Vitamin A, under evaluation in the Philippines and Bangladesh; and “Golden Bananas,” bio-fortified with Vitamin A and iron and developed by Ugandan researchers (Wamboga 2011). Nearly 15 million people either rely on bananas for their income or consumption, making it one of the most important crops in Uganda. It is estimated that the per capita consumption of bananas in Uganda is 0.7 kg per day. Scientists applied the pro-Vitamin A genes used in golden rice to a popular local crop to help solve a regional health issue. Addressing vitamin deficiencies would lead to lower healthcare costs and higher economic performance.

Drawbacks to bio-fortification include a long development process, enhancing micronutrient density at the expense of other traits such as drought or pest tolerance, and a lack of both biodiversity and competition because of a limited number of enhanced crop varieties produced by only a few companies. Realizing the potential of bio-fortification can be achieved through extensive collaboration between farmers, researchers, governments, NGOs, and nutritionists (Juma et al. 2014).

Nutritional enhancements through genetic modification are still in their infancy. Examples such as Golden Rice are important because they represent proof of concept. When confirmed, they will open a wide range of opportunities for related modifications in other crops as well as the use of new techniques to improve human nutrition.

### *5.4.4 Sustainability and Resilience*

It is well established that climate change will adversely affect agricultural productivity primarily in developing countries. Many regions are expected to suffer production loss due to "drought, flood, storms, rising sea levels, and warmer temperatures" (Goering 2012). In the past, these events were rare, and it was possible for farmers and regions to recover during the next growing season. Now it is imperative to determine ways of increasing the resilience and stability of food systems so that productivity is less affected by drought, flood, or both in the same season. Challenges include increasing productivity on existing land to conserve biodiversity and protect vulnerable land, as well as reducing agriculture's traditionally large environmental footprint.

GM crops, for example, are one of the better land-saving technologies available, as they are designed to increase production on existing plots, avoiding slash and burn agriculture often practiced in developing countries. Indeed, "if the 377 million tons of additional food, feed and fiber produced by biotech crops during the period 1996–2012 had been grown conventionally, it is estimated that an additional 123 million hectares . . . of conventional crops would have been required to produce the same tonnage" (James 2014a).

GM crops have succeeded in reducing the environmental impact of agriculture by reducing pesticide use (by an estimated 8.5 % in 2011 alone); and reducing fossil fuels and $CO_2$ emissions through less ploughing and less chemical spraying (saving approximately 1.9 billion kg of $CO_2$—the equivalent of removing 0.8 million cars from the road). The adoption of HT crops allows farmer to use a single broad-spectrum herbicide.

Limiting the practice of tilling, which is the use of mechanization for planting, weed control, and harvesting, is an important trend in sustainable agriculture. It refers to "direct planting into previous crop stubble without further soil disturbance" (Dill et al. 2008, p. 329). Farmers who practice conservation tillage aim to leave 30 % residue on the surface of the soil, which can help reduce soil erosion by 70 %.

Finally, several biotechnology tools, including tissue culture, diagnostics, genomics, and marker-assisted selection can be used collectively to isolate new traits such as drought or flood tolerance that can help mitigate the effects of climate change.

In 2012, drought wrecked havoc on maize production in the United States, highlighting what farmers in developing countries, especially in Africa, already know: drought is, "by far, the single most important constraint to increased productivity for crops worldwide." The development of drought-tolerant crops is arguably the most important GM trait that will occur in the next decade of commercialization (Edmeades 2013). The gene in question was isolated from a common soil bacterium known as *Bacillus subtilis*. It helps the plant cope better with stress caused by water shortages, allowing the plant to focus on filling the grains. The first drought-tolerant maize crop was set for commercial release in the United States in 2013, and it is hoped that it will be commercially available in sub-Saharan Africa by 2017.

In March 2008, a public-private partnership called 'Water Efficient Maize for Africa' (WEMA) was formed between Monsanto, which developed the drought-resistant technology; the African Agricultural Technology Foundation, which directs the partnership; the International Maize and Wheat Improvement Center; and five national agricultural research systems in East and Southern Africa (including Kenya, Mozambique, South Africa, Tanzania, and Uganda). WEMA is working to make the drought-resistant technology available to smallholder farmers through local and regional seed companies. The crop is being developed using conventional breeding, marker-assisted selection, and genetic modification to find the optimal crop for local conditions. Confined field trials thus far show 20–30 % higher production than conventional hybrids. Sites were selected specifically for their dry conditions. The five national research systems are coordinating the field trials. WEMA hopes to offer at least five "farmer-preferred" IR maize hybrids with and without the drought-tolerant gene by 2017, pending field trials and regulatory approval.

The 2008 food crisis demonstrated the effect of an increase in demand and a tightening of supply on the price of rice. After severe flooding in 2007 and 2008 decimated rice production in Southeast Asia, 12 countries including India and China responded by initiating export restrictions. Riots broke out in Haiti, Bangladesh, and Egypt. Although the food crisis affected all grains, a shortage of rice would prove disastrous. According to the International Rice Research Institute (IRRI), in 2005, rice comprised 20 % of global calories consumed; in Asia, 30 %. In addition, "two-thirds of the world's poor . . . subsist primarily on rice." With consumption and prices rising, production declining, and climate change effects expected to grow (e.g., Asia currently loses approximately $1 billion from flooding), IRRI estimates that "by 2015 the world must grow 50 million tons more rice per year than the 631.5 million tons grown in 2005. This will require boosting global average yields by more than 1.2 % per year, or about 12 % over the decade" (Normile 2008).

Furthermore, 25 % of the global rice supply comes from flood-prone regions. One solution has been to isolate the gene present in a variety of Indian rice that allows plants to survive after up to 3 weeks underwater. In collaboration with IRRI, researchers at the University of California at Davis used marker-assisted selection to breed this gene into locally important varieties. The result is a variety of rice that can tolerate flooding but which also retains the capability of high production. IRRI partnered with PhilRice, a nonprofit organization in the Philippines, to distribute the rice free of charge to seed growers and certain farmers who can disseminate further to other farmers. In 2011, over one million farmers in the Philippines, Bangladesh, and India planted the rice (Clayton 2009; Ronald n.d.).[2] So far, it has led to production increases of 1–3 tons after 10–15 days of flooding. Other varieties are also being studied, including drought tolerance, heat and cold tolerance, and salt tolerance. In Africa, IRRI is partnering with the Africa Rice Center (AfriRice) to develop rice that can tolerate poor soils.

## 5.5 Policy Implications and Future Directions

The claim that GM crops cannot benefit developing countries is clearly false. As population growth, climate change, and rising food prices become more important, it is imperative to consider all options for increasing agricultural productivity. GM crops offer one option in the agricultural innovation toolbox and must be considered as such. To be sure, GM crops are not without criticism. However, biotechnology is an important tool developing countries can use to address food security. Risks should be taken into account and the technology strengthened, but to deny farmers the right to grow GM crops would be irresponsible.

To fulfill the African Union's commitment to agricultural development, reforms are needed in country-level biotechnology R&D and risk analysis programs. To be successful, biotechnology must be embedded within a wider socioeconomic system. The policies needed to advance biotechnology are much broader than the implementation of biosafety laws. An overall policy framework is needed that promotes capacity building in local research institutes and universities (and links the two); promotes international technology cooperation; enhances knowledge management practices including intellectual property rights; and finally, addresses the safety aspect of biotechnology. For biotechnology to play a role in addressing food security, countries must not only set their priorities regarding agricultural innovation. They must also decide how science, technology, and innovation will be used in improving existing crops and agricultural productivity (Juma et al. 2014).

Developing countries must overcome strong regulatory barriers to adoption of GM crops. One of the biggest barriers to adoption is the controversy over the safety of GM crops, both in terms of human consumption and their effect on the environment. This is especially true in Africa. However, recent studies tend to support the safety of GM crops. For example, the European Commission funded more than 50 studies to evaluate this issue and found that "the use of biotechnology and of GE plants per se does not imply higher risks than classical breeding methods or production technologies" (Nicolia et al. 2013, p. 2). A literature review covering the last 10 years of GM crop safety and effects on biodiversity and human health concludes that "the scientific research conducted thus far has not detected any significant hazard directly connected with the use of GM crops" (Ibid.).

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[2] The three varieties planted in India, Bangladesh, and the Philippines include Swarna Sub1, Samba Mahsuri, and IR64-Sub1, respectively (IRRI n.d.)

Despite the growing body of scientific evidence, sub-Saharan Africa in particular follows a strict interpretation of the European regulatory model, which uses the precautionary principle to evaluate GM crops (as opposed to the United States, which evaluates the crop itself). Given the differences between U.S. and European regulatory systems, there is a lack of harmonization that hinders the adoption process. A final barrier to adoption is that farmers in sub-Saharan Africa have little political power and cannot make the case for adoption, despite comprising such a large percentage of the population. This is not always the case, however. South Africa, for example, has produced GM crops for the past 18 years and has a particularly effective biosafety regulatory framework and R&D investment. South Africa also trained farmers and scientists and embarked on a substantive public awareness campaign. In addition, farmers groups (including both large-scale and smallholder farmers) were supportive of the adoption of GM crops (Adenle et al. 2013).

## 5.6 Conclusions

This chapter has provided an overview of the potential role of GM in food security. The examples provided are indicative of emerging trends. A key message of the chapter is to view the role of GM technology as one of the many factors that influence food security. That it is only one factor does not mean its role is insignificant. To the contrary, genetic modification has also already demonstrated its transformative power and will continue to play an important role in food security.

The future of the role of GM crops in food security will be influenced greatly by advances in science and technology. New development in genomics, molecular biology, and other allied fields will expand technological options in ways that will address some of the current uncertainties. The growth in technological abundance will also play an important role in democratizing biotechnology and bringing more players into the field. This will go a long way in helping to spread the gains of biotechnology.

## References

Adenle AA, Morris EJ, Parayil G (2013) Status of development, regulation, and adoption of GM agriculture in Africa: view and positions of stakeholder groups. Food Policy 43:159–166

Belay M, Nyambura R (2013) GM crops won't help African farmers. Guardian Poverty Matters blog (blog).. http://www.theguardian.com/global-development/poverty-matters/2013/jun/24/gm-crops-african-farmers. Accessed 1 Nov 2013

Brookes G, Barfoot P (2013) The global income and production effects of genetically modified (GM) crops 1996–2011. GM Crops Food Biotechnol Agric Food Chain 4(1):74–83

Clayton S (2009) Filipino farmers welcome new rice varieties. IRRI (media release),4 June. http://irri.org/index.php?option=com_k2&view=item&id=8151&Itemid=100588&lang=en. Accessed 1 Nov 2013

Dill GM, Cajacob CA, Padgette SR (2008) Glyphosate-resistant crops: adoption, use and future considerations. Pest Manag Sci 64(4):326–331

Edmeades GO (2013) Progress in achieving and delivering drought tolerance in Maize—an update. ISAAA, Ithaca

Goering L (2012) FEATURE-'Green bullet' innovations aim to feed world of 9 billion. Reuters

Herrera-Estrella LR (2000) Genetically modified crops and developing countries. Plant Physiol 124(3):923–926

Huang J, Pray C, Rozelle S (2002) Enhancing the crops to feed the poor. Nature 418:678–684

International Rice Research Center (IRRI) (n.d) Climate change-ready rice (Online briefing). Available at http://irri.org/index.php?option=com_k2&view=item&id=9148&lang=en. Accessed 1 Nov 2013

International Service for the Acquisition of Agri-biotech Applications (ISAAA) (2013) Global biotech/GM crop plantings increase 100-fold from 1996; developing countries, including new adopters Sudan and Cuba, now dominate use of the technology. ISAAA brief no. 44 (press release), 20 February. http://www.isaaa.org/resources/publications/briefs/44/pressrelease/. Accessed 1 Nov 2013

James C (2013) Executive summary. In: Global status of commercialized biotech/GM Crops: 2012. ISAAA brief no. 44. ISAAA, Ithaca

James C (2014a) Executive summary. In: Global status of commercialized biotech/GM Crops: 2013, ISAAA brief no. 46. International Service for the Acquisition of Agri-Biotech Applications, Ithaca

James C (2014b) ISAAA brief 46-2013: slides & tables. 2013 ISAAA report on Global status of Biotech/GM Crops, February 2014. http://www.isaaa.org/resources/publications/briefs/46/pptslides/default.asp

Juma C (2011a) The new harvest: agricultural innovation in Africa. Oxford University Press, New York

Juma C (2011b) Preventing hunger: biotechnology is key. Nature (479)

Juma C, Conceição P, Levine S (2014) Biotechnology and food security. In: Smyth S, Phillips PWB, Castle D (eds) Handbook on agriculture, biotechnology and development. Edward Elgar, Cheltenham

Kathage J, Qaim M (2012) Economic impacts and impact dynamics of Bt (bacillus thuringiensis) cotton in India. Proc Natl Acad Sci 109(29):11652–11656

Nicolia A, Manzo A, Veronesi F, Rosellini D (2013) An overview of the last 10 years of genetically engineered crop safety research. Crit Rev Biotechnol (published early online 23 September). http://www.geneticliteracyproject.org/wp/wp-content/uploads/2013/10/Nicolia-20131.pdf. Accessed 1 Nov 2013

Normile D (2008) Reinventing rice to feed the world. Science 18:330–333

Pray CE, Nagarajan L, Huang J, Hu R, Ramaswami B (2011) Chapter 4: The impact of Bt cotton and the potential impact of biotechnology on other crops in China and India. In: Carter CA, Moschini G, Sheldon I (eds) Frontiers of economics and globalization, vol 10. Emerald, London

Qaim M, Kouser S (2013) Genetically modified crops and food security. PLoS One 8(6):1–7

Ronald P (n.d) New flood-tolerant rice offers relief for world's poorest farmers. Ronald Laboratory, University of California at Davis (press release). Available at http://indica.ucdavis.edu/news/new-flood-tolerant-rice-offers-relief-for-worlds. Accessed 1 Nov 2013

Sexton S, Zilberman D (2011) How agricultural biotechnology boosts food supply and accommodates biofuels. NBER working paper no. 16699. National Bureau of Economic Research, Cambridge, MA

United Nations Food and Agriculture Organization (FAO) (2009) Declaration of the World summit on food security. Rome, 16–18 November. ftp://ftp.fao.org/docrep/fao/Meeting/018/k6050e.pdf

Vitale JD (2010) The commercial application of GMO crops in Africa: Burkina Faso's decade of experience with Bt cotton. AgBioforum 13(4):320–332

Wamboga P (2011) Vitamin A and iron-rich bananas under trial in Uganda. Biovision 16 (newsletter). Available at http://www.biovisioneastafrica.com/publications/Biovision-16.pdf. Accessed 1 Nov 2013

Zilberman D, Sexton SE, Marra M, Fernandez-Cornejo J (2010) The economic impact of genetically engineered crops. Choices 25(2):1–25

# Chapter 6
# Intellectual Property Protection of Plant Innovation

**Bernard Le Buanec and Agnès Ricroch**

**Abstract** Plant innovation is expensive. It is therefore crucial to protect the intellectual property of the breeder as all plant varieties are living self-reproducible material. Intellectual property protection of plant innovation varies from country to country. Plant varieties are not patentable in Europe. Protection of biotechnological inventions is obtained by patent if the invention is new, has an inventive step and industrial applicability. The protection conferred by a patent to a biotechnological invention also applies to varieties in which it is inserted, namely transgenic varieties. In Europe the exceptions to the protection of conventional and transgenic varieties are the same, namely, under certain conditions, the possibility of using farm-saved seeds and access to the genetic diversity of the protected variety for breeding. The possibilities of protection depend on national laws and examples of various countries including developing countries growing transgenic varieties are presented.

**Keywords** Patent • Plant breeders right • Transgenic varieties • Farm-saved seed • Genetic diversity

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A. Ricroch et al. (eds.), *Plant Biotechnology: Experience and Future Prospects*, DOI 10.1007/978-3-319-06892-3_6,

## 6.1 Why Protect Intellectual Property?[1]

According to the historian Phylarque the first monopoly was granted to “inventor” around the seventh century BC in the south of Italy, then Greek, for new cooking recipes. The first comprehensive law on patents, known as *Parte Veneziana* was promulgated in the Republic of Venice in 1474. It was followed by the Statute of Monopoly passed by the British Parliament in 1623.

Under the influence of the ideas of the Enlightenment, the United States of America voted a law on patents on August 17, 1790, followed closely by France and its decree of January 7, 1791 stating that the inventors are owners of their inventions and providing to their benefit the grant of patents.

The US and French laws referred to the moral approach of the decision to grant intellectual property rights. This is also the approach of Article 27 of the Universal Declaration of Human Rights of 1948 which guarantees everyone “the right to the protection of the moral and material interests resulting from any scientific, literary or artistic production of which he is the author”.

The second approach is called “utilitarian”: the protection of intellectual property is granted not by moral obligation to reward the inventor but rather because the products he/she creates are useful to society, it is therefore necessary to encourage them to continue their research to foster innovation.

How to encourage innovation? Two approaches are possible: developing public research or encourage private research. These are political choices. Both approaches are not contradictory but complementary. However, in the current States budgetary context, increasing public spending seems difficult. It is therefore necessary to encourage private research and public-private partnerships and ensure a return on investment.

Research in plant biotechnology and plant breeding is expensive, around one million euros for a new variety. It is estimated that seed companies globally spend around € 3.5 billion € in research annually, or 10–12 % of the annual global turnover of the seed industry estimated at € 34 billion industry ISF (2013).

The table below presents an estimation of the 2012 research budgets of large seed companies worldwide (in million of euro):

| Monsanto | DuPont Pioneer | Syngenta | Limagrain | BASF | Bayer Crop Science | KWS | Florimond Desprez |
|---|---|---|---|---|---|---|---|
| 780 | 390 | 370 | 162 | 150 | 140 | 127 | 26 |

Source: Annual reports or personal communication

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[1] This chapter has been *partially* adapted from a text published in French (Le Buanec & Ricroch 2011. Comment protéger les innovations végétales. *In*: Biotechnologies végétales, environnement, alimentation, santé. Publisher Vuibert).

In addition it takes capital to produce a new variety and place it on the market. Then success is not necessarily guaranteed, either because the competition has developed equivalent or better products, or the market has changed since the theoretical design of the product. It takes a decade to develop a new variety and as for any applied research plant breeding is a risky business.

If the new variety fits the market, the breeder should be able to exploit it commercially under conditions allowing it to receive the benefits of its investments. Two problems arise: firstly competitors could capture the variety and sell seeds, either identical or with minor improvements, and at a lower price because they do not have to recoup the development costs; on the other hand the "invention" being often capable of self-reproduction (autogamous species or vegetative reproduction) users could reproduce it for their own use without paying the original developer.

Under these conditions, the innovator could not recover costs, it would have no incentive to continue his/her research efforts, and no genetic progress of varieties could be expected from the private sector. To avoid this situation, the breeder must be able to protect his new varieties.

It is therefore necessary to find practical and equitable solutions for the protection of intellectual property to encourage innovation in the context of a private enterprise. One possible solution is secrecy. But the protection of a secret is often difficult because it is ineffective in the context of living self-reproducible material.

Moreover, as the secret does not allow the disclosure of the method to get the results, it does not promote the incremental development of knowledge. Therefore, most countries have now put in place systems to protect intellectual property that are true social contracts between the inventor and society.

In particular, a patent is granted only if it has a description of the invention sufficiently clear and complete for a person in the art to reproduce it. Moreover, if the case arises, any description that may prove insufficient or inaccurate results de facto in the cancellation of the protection granted.

It is also important to be noted that, according to the Marrakesh Agreement of April 15, 1994 on Trade-Related Aspects of Intellectual Property Rights (TRIPS Agreement), all member countries of the World Trade Organization (WTO) must grant patents for any inventions of products or processes meeting the conditions of patentability.

However, for the particular case of plants and animals, each country can choose its protection system, if it is effective, as provided for in Article 27.3.b of the TRIPS Agreement. Thus, Member States may exclude from patentability "plants and animals other than micro-organisms, and essentially biological processes for the production of plants or animals [. . . ] However, Members shall provide for the protection of plant varieties by patents or by an effective *sui generis* system or by any combination thereof". This TRIPS article shows the difficulties encountered by legislators to grant intellectual property rights for living organisms. A first attempt was made in the Papal States by an edict of 1833 granting a monopoly of 5–15 years to a person who had discovered or introduced a new important type of agricultural plant. That edict is generally considered as the ancestor of the protection of new plant varieties. It was followed almost a century later by the Plant Patent Act in the USA; (see below).

Here we examine how it is possible to protect plant varieties and biotechnological innovations as well as the consequences of the protection on access to genetic resources for breeding and on the conditions under which the varieties can be multiplied by a farmer for its own use (production called “farm-saved seed”). As intellectual property rights are national or regional, and the exercise of the right is territorial and depending on judicial precedents, a global overview is not possible. We will examine in detail the case of Europe and United States of America and some references will be made to the situation in other countries.

## 6.2 Protection of Plant Innovation in Europe

### *6.2.1 Protection of Plant Varieties*

Europe does not allow the grant of a patent to a plant variety. The only way to protect a plant variety is the Plant Breeders Right (PBR) as defined by the UPOV convention. The granting of PBR is regulated by the Council Regulation (EC) No 2100/94 of 27 July 1994 on Community plant variety rights.

A plant variety protection certificate (PVP) may be obtained if the variety is new, that is to say if the variety constituents or harvested material of the variety have not been sold or otherwise disposed of to others by or with the consent of the breeder. In addition the variety must be:

- distinct of any other variety of common knowledge,
- subject to the variation that may be expected from the particular features of its propagation, sufficiently uniform in the expression of characteristics used for the variety description,
- stable, i.e. remaining unchanged after repeated propagation.

Moreover, the variety must be designated by a denomination

It must be noted that the protection of the harvested material of the protected variety (and possibly the protection of the products obtained directly from the harvested material) applies only if that harvested material was obtained through the unauthorized use of the variety constituents of the protected variety and unless the holder of the PVP certificate has had reasonable opportunity to exercise his right in relation to the said variety constituents. However the mode of implementation of that part of the rights has not yet been adopted.

For most of the species the duration of the protection is 25 years after the grant of the right. The scope of protection is as follows: in respect of varieties constituents or harvested material of the variety the acts listed hereinafter shall require the authorization of the rights’ holder: production or reproduction (multiplication), conditioning for the purpose of propagation, offering of sale, selling or other marketing, exporting from the Community, importing to the Community, stocking for any of the previous purposes, The holder may make his authorization subject to conditions and limitations.

The EC regulation provides exceptions to the breeders' right. Besides the classic exceptions for public order the Community plant variety rights do not extend to acts done privately and for non-commercial purposes, acts done for experimental purposes, acts done for the purpose of breeding or discovering other varieties, those other varieties being free of right expect if they are essentially derived from the initial protected variety (*c.f. infra*). This last exception is known as "the breeder's privilege" allowing access to plant genetic resources for further research and breeding.

In addition, in line with an optional exception of the 1991 act of the UPOV convention the EC Regulation explicitly authorizes, for certain species listed in an annex, the use of "farm saved seed", known as "the farmer privilege". That use is precisely regulated and a farmer using that privilege must pay a certain level of royalties, sensibly lower than the regular royalties on certified seed, to the variety owner. Small farmers, i.e. producing less than 92 t of cereal, are exempted. This provision of the UPOV 1991 act introducing an optional exception for farm saved seed is a compromise between the positions of UPOV members who did not wish to allow it at all, those who wanted that exception but with full royalty rate and those who wanted the authorization without limitations.

As indicated above one of the cornerstones of the breeders' right is the breeder privilege. This privilege, without limitation, could allow "plagiarism" of a protected variety in particular by a mere identification and selection of a mutant or of a somaclonal variant within the variety or by the introduction of a specific trait of interest achievable by different ways such as repeated backcrossing or transgenesis. To avoid that risk the EC regulation, also in line with the 1991 act of the UPOV convention, extends the right of the breeder to the varieties that are essentially derived from the protected variety, if that variety is not itself an essentially derived variety. A variety shall be deemed to be essentially derived from another variety called the initial variety when:

(a) it is predominantly derived from the initial variety, or from a variety that is itself predominantly derived from the initial variety;
(b) it is distinct from the initial variety; and
(c) except for the differences which result from the act of derivation, it conforms essentially to the initial variety in the expression of the characteristics that results from the genotype or combination of genotypes of the initial variety.

In fact the main motivation for the introduction of that concept was the development of genetic engineering. Indeed, without that concept the "simple" transfer by a third party of a patented gene in a variety would have allowed the appropriation of the transformed variety, distinct from the initial one, by that third party owner of the gene. This provision allows a balance between the PVP certificate and the patenting of genes of interest (see below protection of biotechnological invention).

### *6.2.2 Protection of Biotechnological Inventions*

Protection of innovations in plant breeding, based mostly on the provisions of the UPOV Convention, broadly satisfied the partners involved in the agricultural sector during the second half of the twentieth century. From the 1980s, new techniques used by breeders have emerged, namely genetic engineering and both structural and functional genomics, resulting in particular in the development of transgenic plants, the identification of genes of interest and molecular markers assisted selection. The debate on the protection scheme was launched again.

As we have just seen, the UPOV Convention was amended in 1991 and discussions on a European Directive on the legal protection of biotechnological inventions began in 1988. The debates were long and difficult and it took 10 years of work for a text to be adopted in 1998 (Directive 98/44/EC).

The first article of the directive stipulates that Member States shall protect biotechnological inventions under national patent law.

The general principles of patentability apply, namely novelty, inventive activity and industrial applicability even if the invention concerns a product consisting of or containing biological material or a process by means of which biological material is produced, processed or used. Any non-confidential publication of research results, both in writing or orally, destroys the novelty and prevents patenting an innovation.

The main provisions relating to plant breeding are as follows:

- plant varieties are not patentable, but the inventions which concern plants may be patented if the technical feasibility of the invention is not limited to a single variety. This is a complexity that is not always easy to understand. In Europe, transgenic plants carrying a patented event (transgenic trait) fall within the scope of the patent since this element is not limited to a single variety and at the same time a transgenic variety can also be individually protected by a PVP certificate.
- essentially biological processes for the production of plants and animals which exclusively use natural phenomena such as crossing or selection are not patentable,
- inventions relating to a product consisting wholly or partly of biological material or to a process by means of which biological material is processed or used. Any material containing genetic information and capable of reproducing itself or being reproduced in a biological system is regarded as biological material. For example a DNA fraction, a gene, a cell are patentable,
- the term of protection is 20 years from the date of filing of the application. Discussions are currently underway in Europe for the implementation of supplementary protection certificates (SPC) for biotechnological inventions, due to the length of examinations for obtaining the authorizations of putting a product on the market as for pharmaceutical and plant protection products patents.

The SPC extends the term of protection of the invention compensating the time needed to obtain the marketing authorization. It is granted for a period equal to the period between the filing date of the patent and the date of the marketing authorization. This additional time may in no case exceed 5 years.

- The protection conferred by a patent on a biological material possessing specific characteristics as the result of the invention shall extend to (apply) to any biological material derived from that biological material through propagation or multiplication and possessing those same properties. It is this provision which allows for real protection of biotechnological invention, noting that in the case of a self-replicating biological material the right exhaustion does not apply at the first sale.
- A patented biotechnological invention incorporated into a variety remains protected in this variety but by no means the variety itself is patented, which would be contrary to EU legislation prohibiting patenting of varieties. Thus the genome of this variety, when it no longer contains the patented biotechnological invention, is completely free of patent rights.

The latter provision was the subject of much debate during the adoption of the Directive, but it was necessary to give a meaning to the protection. Otherwise a patented characteristic introduced in a variety would have lost the benefit of the protection by enabling the creation of a new independent variety containing it. It is important to note that the protection of the patented characteristic is only valid if the genetic information related to the patent performs its function in the variety or the product of the variety.

A judgment on 6 July 2010 by the Grand Chamber of the European Court of Justice clarifies the point well and helps lift the burden of the risk of infringement that were running developing countries exporting to Europe agricultural products from unprotected GM varieties in the country of production. The judgment states that soybean meal imported into Europe, produced in Argentina from GM soybean tolerant to an herbicide and unprotected in this country, does not infringe a patent on soybean in Europe because the gene for tolerance to that herbicide does not exercise its function in the meal.

Authorization of the holder's right is required for making, using, offering for sale, selling or importing for this purposes the product covered by the patent. The scope of the right is substantially the same as that of a PVP certificate though a little less broad because it does not include export.

The rights do not extend to acts done privately and for non-commercial purposes or acts done for experimental purposes relating to the subject matter of the patented invention. In the case of patents of biotechnological invention two exceptions to the rights have been added:

(a) The sale or other form of commercialization of plant propagating material to a farmer by the holder of the patent or with his consent for agricultural use implies authorization for the farmer to use the product of his harvest for propagation or multiplication by him on his own farm, the extent and conditions of this derogation corresponding to those under Article 14 of Regulation (EC) No 2100/94 of 27 July 1994 establishing an EC plant variety right. This is the "farmer's privilege", which applies strictly identical to transgenic and conventional varieties.

(b) The recently adopted European unitary patent (December 2012), that applies in all EU countries except Italy and Spain and should enter into force on 1 January 2014 has also adopted the exception included in the French and German transpositions of the directive 98/44/EC stipulating that the rights do not extend to acts to breed or discover other varieties, these other varieties being free of rights if they do not express the characteristic of the patented invention. In fact it is the "breeder privilege" that exists in the case of the PVP certificate.

This is in line with the position of the International Seed Federation that reads "breeding with a commercialized plant variety comprising a patented gene or trait and non-patented genetic background, should not be considered an infringement of the respective patent on the gene or trait under the following conditions: If a new plant variety, resulting from that breeding, is outside the scope of the patent claims, it should be freely exploitable by its developer provided it is not an EDV. However, if the newly developed variety still falls under the scope of patent claims (i.e. if the patented gene express itself in the new variety, editor's note), no commercial acts (as defined in article 14 (1) of the UPOV 1991 Act) should be undertaken with the new variety without prior consent of the patent holder". (ISF view on Intellectual property (2012)).

It thus appears that, contrary to what is often said, in the field of plant varieties in most of the EU Member States, the scope of the law in case of patent or PVP certificate is very similar, with even a slightly greater extent in the case of PVP, as it also covers export.

### *6.2.3 The Balance Between the PVP and the Patent*

We have seen that in the case of PVP, the introduction of the concept of essentially derived variety establishes a balance between the rights of the holder of PVP certificate and the holder of "gene patent". The developer of a transgenic crop from a protected variety can obtain a PVP certificate for the new variety but this new variety cannot be exploited without the consent of the holder of the PVP certificate of the initial variety who has the right to subject his authorization to conditions and limitations.

The Directive on the protection of biotechnological inventions has also, to balance the rights between patents and PVP, introduced two provisions on cross licensing, compliant with article 31.l of the TRIPS agreement:

(a) Where a breeder cannot acquire or exploit a plant variety right without infringing a prior patent, he may apply for a compulsory license for non-exclusive use of the invention protected by the patent inasmuch as the license is necessary for the exploitation of the plant variety to be protected, subject to payment of an appropriate royalty. Member States shall provide that, where such a license is granted, the holder of the patent will be entitled to a cross-license on reasonable terms to use the protected variety.

(b) Where the holder of a patent concerning a biotechnological invention cannot exploit it without infringing a prior plant variety right, he may apply for a compulsory license for non-exclusive use of the plant variety protected by that right, subject to payment of an appropriate royalty. Member States shall provide that, where such a license is granted, the holder of the variety right will be entitled to a cross-license on reasonable terms to use the protected invention.

(c) Applicants for the licenses referred to in paragraphs 1 and 2 must demonstrate that:

  - they have applied unsuccessfully to the holder of the patent or of the plant variety right to obtain a contractual license;
  - the plant variety or the invention constitutes significant technical progress of considerable economic interest compared with the invention claimed in the patent or the protected plant variety.

Although not strictly parallel without that we understand well the reason, these two provisions allow, in principle, a balance between the two rights. Their implementation is however not obvious and will require the courts' decisions that should define what are a "*significant technical progress*" and a "*significant economic interest*".

## 6.3 The Protection of Plant Innovation in the USA

Contrary to most of the countries in the world it is possible to patent plant varieties in the US. Three main milestones have gradually developed the right to protect living organisms by patent.

The first milestone is the vote of the "Plant Patent Act" in 1930, allowing the patenting of asexually reproduces plants (except tuber crops).

The second determining milestone that confirmed the patentability of biological matter is the Supreme Court decision in 1980 confirming the patentability of a microorganism, namely a bacterium genetically modified to degrade hydrocarbons. In this decision (Diamond vs. Chakrabarty) the Court said that a patent may be obtained on "anything under the sun that is made by man" and that the patentee has produced a new bacterium with markedly different characteristics from any found in nature, and one having the potential for significant utility. His discovery is not nature's handiwork, but his own; accordingly it is patentable subject matter.

This development has paved the way for the patenting of biotechnological inventions as "Utility" patents without need to pass a new legislation as it has been the case in Europe (see *supra*). However, further to the grant of patents to genetic sequences without function indication and the ensuing debate, the USPTO (United States Patent and Trademark Office) published in 2001 new examination guidelines particularly relevant for gene related technology. Under these new Guidelines, the claimed invention must have "specific, substantial, and credible" utility. This is in line with the provisions of the European directive on the Protection of biotechnological inventions and actually is one of the very foundations of the patent. Recently (2013) the Supreme Court decision in the Myriad Genetics, Inc. case has invalided patents covering genetic sequences found in nature as ‘not made by human’.

The last step regarding patentability of living organisms was made in 1986 with the grant of a patent for a sexually reproduced variety, (a high tryptophan corn) based on the decision of the Board of Appeals and Interference of the USPTO. This possibility to patent sexually reproduced variety was confirmed in 2001 by the Supreme Court in the J.E.M. Ag Supply Inc. vs. Pioneer Hi-Bred International case.

However a question was still pending: is a patent an efficient protection for living material capable of self reproduction? The problem is well presented by Janis and Kesan (2002). Indeed, according to the patent exhaustion principle also known as the “implied license” the purchaser a patented product is allowed to use and resell it. When a patented seed grows and produces new seed, is the new seed a new “making” of the patented seed, and hence outside the implied license. Or is it an aspect of the original “using”, and hence within the scope of the implied license? In May 2013 the Supreme Court decides on that question in its decision Vernon Hugh Bowman *v*. Monsanto Company and decided unanimously that the new seed produced by a patented seed was a new making, thus outside the implied license. “Were the matter otherwise [ . . . ] patent would provide scant benefit [ . . . ]. The grower could multiply his initial purchase, and multiply that new creation, *ad infinitum*, each time profiting from the patented seed without compensating the inventor. [ . . . ]. The undiluted patent monopoly, it might be said, would extend not for 20 years (as the Parliament act promises), but for only one transaction. And that would result in less incentive for innovation than the congress wanted.” In fact that decision is in line with the European Directive which tackled that obvious issue at its very inception.

Parallel to the development of patents, the USA adopted in 1970 the “Plant Variety Protection Act” (PVPA) which is a system of UPOV-type protection for sexually propagated crops. However, the United States become member of UPOV only in 1981 after the 1978 revision of the Convention with the introduction of Article 37 providing for an exemption from the prohibition of plant variety protection in both forms of PVP certificate or patent, waiver made for them. In 1999 the USA ratified the 1991 Act of the UPOV Convention based on its 1997 PVPA act subsequently amended in 2005. The subject matter and the scope of the protection are quite similar to those of the European Regulation detailed above with two significant differences:

- In terms of subject matter the US PVPA covers any sexually reproduced or tuber propagated plant variety (other than fungi or bacteria) and not all genera and species as stipulated in the 1991 of the UOV Convention and in the EU Regulation.
- In terms of scope the US PVP certificate does not extend to “a person to save seed produced by the person from seed obtained, or descended from seed obtained, by authority of the owner of the variety for seeding purposes and use

> such saved seed in the production of a crop for use on the farm of the person, or for sale as provided in this section." In fact it is a broad farmer privilege, without "reasonable limits and subject to the safeguarding the legitimate interest of the breeder" as required par the UPOV convention. When discussing with the agricultural community in the USA it is rather ironic to hear that farmers would not accept a limitation of farm said seed for a PVP certificate when there is no possibility of farm saved seed at all, now confirmed by the Supreme Court, in case of patent.

In conclusion, in the USA, the plant breeder has the following options to protect his/her innovations:

(a) for biotechnological inventions the patent ("Utility Patent"), as in Europe, but with no specific exemptions for plant breeding ("breeder's privilege") or for farm-saved seed.
(b) for plant varieties different solutions are available:
    – for asexually propagated varieties the plant patent act with a scope of protection similar to the one of utility patent.
    – for sexually propagated and tuber propagated varieties either the PVPA, with the breeders exception and a broad farm-saved seed exception or the utility patent, with a limited research exception and no possibility for farm-saved seed.

Given the obvious imbalance of the scope of variety protection between PVP and patent, it is not surprising that in the USA breeders are massively applying for patent protection for their varieties. However, as in Europe, the development of transgenic varieties has no particular effect on the use of farm-saved seed and access to genetic diversity, the situation being the same for most the conventional varieties that are patented and transgenic varieties. Indeed, in both cases, farm-saved seed and access to transgenic varieties for further research and breeding are not allowed.

## 6.4 An Overview of the Situation in Some Developing Countries

Utility patent is not allowed for plant varieties in developing countries. Having said that, it must be noted, that the level of protection of intellectual property depends on the technical, legal and socio-economic conditions of each country. As already indicated, intellectual property rights are national and their implementation is territorial. Protection depends on international treaties, their transposition that is not always entirely consistent in national laws, and on jurisprudence.

In addition, there are in many countries the opportunities to use legal mechanisms other than those provided by the legislation on the protection of intellectual property to protect the breeder's rights, such as the laws on contracts between seller and buyer. For example, the "shrink-wrap agreements", that is to say the tacit agreement on the conditions of use stated on the package when open, are increasingly used in many countries. It is not possible within the scope of this book to review all countries and in particular to analyze the situation in detail for each country. Only a few cases will therefore be presented briefly.

### *6.4.1 The Least Advanced Countries*

In general, the issue of protection of intellectual property arises less acutely in the least advanced countries, which do not have the administrative and legal structures to deal with this issue. In addition, the system of *Humanitarian Use Licenses* should enable the use by farmers producing for home consumption (subsistence) of widely patented technologies globally. Two examples illustrate this possibility further in the context of collaborative public/private sector.

The case of golden rice: it is a transgenic rice enriched with iron and provitamin A which should improve the diet of hundreds of millions of people in Southeast Asia. This rice, originally developed by Professor Ingo Potrykus at the University of Zurich, requires implementation of 70 patents and confidentiality agreements. In order to improve the product, a research project between the University of Zurich, the International Rice Research Institute and Syngenta was initiated.

Companies Bayer, Mogen, Monsanto, Novartis and Zeneca, and a Japanese company wishing to remain anonymous, gave free licenses necessary to launch the project. To enable small farmers in developing countries to benefit from the results in case of success, the company Syngenta has committed itself not to ask for royalties on seeds to farmers with an annual turnover of less than 10,000 dollars, that is all subsistence farmers.

The WEMA, Water Efficient Maize for Africa project, managed by the African Agricultural Technology Foundation (AATF) funded by the Bill & Melinda Gates Foundation, Howard G. Buffet, and by CIMMYT, Monsanto and the agricultural research systems of countries of Eastern and Southern Africa is a second example. The objective is to develop drought tolerant maize varieties using conventional breeding, marker-assisted breeding and transgenesis. These varieties with patented transgenes will be distributed to African seed companies without request of royalty's payment.

The situation of the other so-called "developing" countries is highly variable. It is not possible to present here a detailed situation especially as the protection of intellectual property depends on national laws, which vary widely from one country to another. We take a few examples of countries that have great importance in world agricultural production by answering two questions.

## *6.4.2 Some Other Developing Countries*

There is no precise definition of developing countries some of them being also called emerging countries such as the examples below.

### 6.4.2.1 Argentina

Argentina is a member of UPOV since 1994 (Act of 1978) and the WTO since 1995.

(a) Can farmers use farm-saved seed? Yes, without limitation with respect to the non-transgenic varieties under the law of protection of plant varieties; however, to obtain payment of a royalty on farm-saved seed for their new varieties, breeders use the contracts law according to a system known under the name of "Extended Royalties System (ERS)." For transgenic varieties the situation is not clear, according to patent law the answer is no, but the seed law allows the use of farm seeds of any protected variety, conventional or transgenic. We need to wait until some jurisprudence to have a definitive answer.

(b) Does the breeder's privilege exist? Yes for non-transgenic varieties. Once again the answer is not clear for transgenic varieties, depending on whether it refers to the seed law or that the patent law. The answer is positive in the first case, negative in the second one.

A new seed law is being prepared. The law, if passed, would clarify the situation by allowing farm saved-seed of all varieties against compensation of the breeder, with the exception of small farmers who would be exempted and establishing the breeder's privilege for all varieties including transgenic varieties.

### 6.4.2.2 Brazil

Brazil is a member of UPOV since 1999 (Act of 1978) and the WTO since 1995.

(a) Can farmers use farm-saved seed? Yes, at no charge for conventional varieties, with the exception of sugar cane cuttings, unless they wish to ask for a bank loan before sowing for the next agricultural campaign. In this case, the bank requires the purchase of certified seeds. For transgenic varieties, farmers can use farm-saved seed but must pay a fee for the patented technology, fee that is retained at the end point delivery.

(b) Does the breeder's privilege exist? Yes for conventional varieties; in addition the concept of "essentially derived variety" applies. It would be possible to use a transgenic variety in a breeding program, but the new variety could be freely marketed only if the transgenic trait is not expressed or if expressed, at the expiration of the patent. Here again we have to wait for law cases to have a definitive answer.

#### 6.4.2.3 China

China is a member of UPOV since 1999 (Act of 1978) and the WTO since 2001. It is difficult to have a clear view of the situation due to the lack of wide dissemination of laws, the importance of Regulations at the provincial level, the rather general lack of enforcement of intellectual property, although situation improves significantly.

(a) Can farmers use farm-saved seed? Yes for conventional varieties without payment of royalties. However, some provincial governments provide subsidies to encourage farmers to buy commercial quality seeds for major species such as cereals and oilseeds. Regarding transgenic varieties, mainly cotton, it seems that the use of farm-saved seed is significantly tolerated.
(b) Does the breeder's privilege exist? Yes for all varieties. Transgenic varieties, after marketing authorization, must obtain a PVP certificate as plant varieties are not patentable as such.

#### 6.4.2.4 India

India is a member of the WTO since 1995. It is not a member of UPOV despite a membership application, its PVP law being too far away from some basic principles of UPOV.

(a) Can farmers use farm-saved seed? Yes with no limit for all varieties, whether or not transgenic. They can even exchange, share and sell farm-saved seed as long as the sale does not take place under the name of the variety. However, the transgenic varieties are also subject to other laws, especially on the release into the environment. Farmers must respect those other laws, which may limit their ability to use farm-saved seed of such varieties.
(b) Does the breeder's privilege exist? Yes, in principle, regardless of the type of variety. As for farm seeds, in case of transgenic varieties, other laws and restrictions may apply.

## 6.5 Conclusions

The research in plant biotechnology and the development of a new variety are risky and expensive. The protection of plant breeders is therefore a necessity. Legal instruments for the protection of plant innovations have been implemented through successive conventions of UPOV, the TRIPS Agreement and various regional and national legislations. While maintaining effective protection of biotechnological inventions and new varieties, we must ensure that these instruments allow enriching the genetic diversity available to breeders, ensure access to transgenic varieties for research and creation of new varieties and stimulate public and/or private critical research for future generations.

## References

ISF (2013) Estimated Value of the Domestic Seed Market in Selected Countries for the year 2012

Janis MD, Kesan KP (2002) Intellectual property protection for plant innovation: unsolved issues after J.E.M. v Pioneer. Nat Biotechnol 20:1161–1164

# Chapter 7
# Prospects for Agricultural Biotechnology to 2030

**David B. Sawaya**

**Abstract** Though not a silver bullet, agricultural biotechnologies represent an important tool for meeting future food demand. Transgenic and non-transgenic biotechnologies are used in agriculture in developed and developing countries. Non-transgenic techniques are pervasive and will likely permeate some part of the development or production of nearly all commercial crops in the medium term. Transgenic technologies, at present, have been primarily used to impart herbicide tolerance (HT) and pest resistance (PR) in crops used for processed foods, cloth production and animal feed. Analyses of research pipelines indicate that in the near future, transgenic crops will be commercialized with agronomic traits (e.g. increased yield and environmental stress tolerance). This development has the potential to increase efficiency of agricultural production and decrease intensive agriculture's environmental impacts. Realizing the full potential will require addressing non-technical challenges related to business models, regulations and public opinion. If productivity increases outpace demand, agricultural products could supply the world's food needs and become an even more important sustainable primary material for the production of a variety of materials and products.

**Keywords** Agricultural biotechnology • Biotechnology forecasting • Biotechnology regulation • Concentration in agriculture • Agronomic traits

## 7.1 Introduction

A convergence of global trends has increased interest in agricultural production. An increasing and more affluent population which has boosted competition for scarce resources, along with looming production challenges such as climate change,

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A. Ricroch et al. (eds.), *Plant Biotechnology: Experience and Future Prospects*, DOI 10.1007/978-3-319-06892-3_7,

portend that this trend will continue into the future. Though they are not the only solution, and they are not a silver bullet, agricultural biotechnologies are an important tool in the search for ensuring supply and meeting demand.

Agricultural biotechnologies, both transgenic and non-transgenic, have seen strong uptake in modern agricultural systems and have contributed to the improvement of plant varieties of numerous species. These technologies can, when used correctly, reduce pesticide use, encourage preservation of soil and water quality, and provide an economic benefit to farmers.[1] Potential future benefits are substantial as new varieties of plants which provide higher yields and environmental stress tolerances are under development. These could allow more food to be produced on less land or on lands that are today considered marginal.

Realizing the full promise of these technologies hinges, however, upon properly addressing a number of existing challenges. Public opinion has had a major impact on the uptake of these technologies, driving some countries to reject their cultivation and limit their use. In part, public opinion has been soured by perceived health and environmental issues related to the consumption of GM foods. Another issue which can negatively impact opinion relates to the economic and business practices of multi-national agribusiness firms which have driven concentration in the sector and which the public can view as a threat to food security and cultural traditions.

Robust regulatory frameworks have been established in most areas in an attempt to address some of these issues. At present, public attention and regulations have been focused mainly on transgenic technologies, but as the use of biotechnologies become more pervasive in agriculture, governments are also turning their attention towards non-transgenic technologies to determine whether or not specific regulatory measures are required. If not properly addressed public opinion, regulations and increasing concentration of R&D capacity in a limited number actors could have a negative impact on the future development of innovative agricultural biotechnologies that can be used to address global agricultural challenges.

If technology development keeps pace with current projections, agricultural biotechnologies will represent an important tool in helping society meet future food demand. Towards the middle of the twenty-first century as population growth slows, agricultural production could begin to outpace demand leaving the door open to renewed interest in the use of agricultural products as a sustainable feedstock for the industrial production of fuels, chemicals and materials.

## 7.2 Global Trends Affecting Agriculture

It is expected that demand for agricultural products will increase substantially over the next several decades. The UN Population Division estimates that global population will rise from 7.2 billion today to around 9.6 billion in 2050. Over 95 %

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[1] See for example National Academies of Sciences 2010.

of this population growth is expected to occur in less developed regions where substantial economic growth and increases in personal incomes are likely. As a result people will change their consumption habits to increase caloric consumption and, in particular, meat and dairy-based calories whose production are resource intensive as compared to the direct consumption of grains.[2]

Population growth after 2050 is expected to continue but fertility rates will decline resulting in slower growth. If increases in agricultural productivity meet demand and are sustained, this population growth slowdown raises the specter of agricultural production outpacing demand. This could usher in an era of surpluses of agricultural products and byproducts which can provide a supply of sustainable feedstock for biofuel, biochemical and biomaterials.

Further future demand for agricultural products is also likely as a result of biofuel production. Governments in both developed and developing countries have put in place ambitious objectives and strong incentives for the production of biofuels. The long term ambition is for biofuels to be produced by microbes or algae, or from feedstock that is either unrelated to or is a byproduct of food production (e.g. biofuels produced from grasses or wastes). Today, however much biofuel production is based on feedstock that can also be used as foodstuffs or animal feed. While the debate over the full impact on price and demand of this diversion of these materials from food to fuel continues, it is clear that biofuel production increases demand.

Climate change and other environmental issues such as water quality and scarcity, soil quality, and fertilizer availability also loom as potential challenges to future agricultural supply.

Existing agricultural biotechnologies and those in development can help address these challenges. Herbicide tolerant plant varieties that are currently under cultivation have allowed farmers to use pesticides that are less harmful to water quality and pest resistant varieties have contributed to reducing pesticide use. New "second generation" biotech plant varieties that are currently under development aim to impart agronomic and product quality traits. These traits can improve yields, increase the area available to cultivation, and allow for tailoring plants (e.g. by increasing oil or starch content) to improve the production efficiency of specific fuels, chemicals and materials.

## 7.3 Current Use of Biotechnologies

Both transgenic (genetically modified, GM) and non-transgenic crops varieties are used in modern agriculture. The economic and environmental effects of new crop varieties are due to the characteristics of the trait that is included in the plant variety.

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[2] For example, producing 1 kg of chicken meat requires about 2 kg of grain while 1 kg of beef requires roughly 8 kg of grain and ten times the water required to produce 1 kg of grain.

Both GM and non-GM commercial research programs focus on one or more of the following traits[3]:

- *Herbicide tolerance* (*HT*) allows plants to resist the effects of specific herbicides (e.g. glyphosate). HT has primarily been commercialized using transgenesis technology but other breeding techniques can also be used.
- *Pest resistance* improves the ability of the plant to resist harmful insects, viruses, bacteria, fungi and nematodes. The most common form of GM pest resistance uses a gene from bacteria (*Bacillus thuringiensis*, or Bt) to emit an organic toxin that kills some insect species. GM pest resistance has also been successfully used to develop a variety of papaya resistant to the ring spot virus.
- *Agronomic traits* can improve yields and provide resistance to environmental stresses that can reduce yields, such as heat, cold, drought and salinity.
- *Product quality* characteristics include modified flavor or color, modified starch or oil composition that improves nutritional value or processing characteristics, and the production of medical and industrial compounds.

The adoption of biotechnology in the agricultural sector varies by crop variety. Non-transgenic techniques are used in some part of the development or production of many commercially grown crops while transgenic technologies, at present, have been primarily used to impart herbicide tolerance (HT) and pest resistance (PR) in crops used for processed foods, cloth production and animal feed.

### *7.3.1 Private and Public Research Actors*

The private and public sectors are both active in the development of agricultural biotechnologies. Given the strong commercial incentives associated with commercialized agricultural biotechnology products, the private sector accounts for the majority of research and development activity. The field is dominated by a handful of large multinational seed firms that focus primarily on large market crops. There has been substantial consolidation in the sector and the effect this concentration can have on future biotechnology development is discussed below.

In OECD countries, the public research sector (including universities, research institutes and private non-profit institutions) in the late 2000s, accounted for approximately one fifth to one quarter of the GM field trials and biotechnology plant patent applications. While this level of activity is nearly ten-fold the public

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[3] In addition to the categories listed here, biotechnology research can focus on *technical traits*, such as molecular markers. These traits are useful to improving breeding and knowledge about crop improvement but are not considered “commercial” as they do not have substantial direct value for seed developers.

research sector in other industries,[4] it has declined from a peak of over 30 % in the late 1990s. The reason for this decline is not entirely clear but it may be related to a conscious decision not to patent agricultural biotechnology innovations developed by public research.

The public sector's research targets differ from that of the private sector. The share of public sector research aimed at trials for agronomic and product quality traits – the so-called "second generation" traits – as well as technical traits is much higher than that of the private sector. For instance, the share of public sector trials for technical traits is nearly 2.5 times that of private firms. The public sector is also more focused on small market crops. Between 1987 and 2008, the public sector conducted 39.9 % of its GM field trials on small market crops, over twice the 17.6 % share of private sector trials for small market crops. These shares are roughly stable over time (Arundel and Sawaya 2009a).

### *7.3.2 Transgenic Plant Varieties*

Transgenic plant varieties (or GM plant varieties) refer to the development of a plant genome through the insertion of a gene or genes from a species that cannot interbreed with the target plant under normal conditions. Quantitative data related to the amount, species and variety of GM crops under cultivation are readily available due to the strict regulatory regimes surrounding their use. Information about varieties under development is also available because prior to commercialization GM crops are required to undergo regulated field trials. Successful plant varieties generally remain in field trials between 2 and 5 years prior to commercialization.

Field trials have been conducted for GM traits in more than 130 species, but the 25 most researched species account for nearly 95 % of all trials. Only 13 species of plant have GM varieties approved for commercial use anywhere in the world. Table 7.1 lists these species along with the number of varieties that have received regulatory approval somewhere in the world. Four plant species – canola (rapeseed), cotton, corn (maize), and soybean – account for nearly 70 % of all of the approved GM plant varieties, with corn having nearly 40 % of all approvals. Potatoes and tomatoes also have a substantial number of approved varieties, combined the two species account for approximately 13 % of all approvals. Both of these species had GM varieties available commercially at one time, but cultivation ceased in the late 1990s.

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[4] Graff et al. (2003) estimated the public sector contribution at only 2.7 % of all types of patents granted by USPTO between 1981 and 2000.

**Table 7.1** Number and share of GM plant varieties having received regulatory approval in the world, by species

| Crop | Number of approved varieties | Share of all approvals % |
|---|---|---|
| **Alfalfa** | 3 | 0.91 |
| **Argentine Canola** | 30 | 9.12 |
| **Bean** | 1 | 0.30 |
| **Carnation** | 15 | 4.56 |
| **Chicory** | 3 | 0.91 |
| **Cotton** | 49 | 14.89 |
| **Creeping Bentgrass** | 1 | 0.30 |
| **Flax** | 1 | 0.30 |
| **Maize (corn)** | 125 | 37.99 |
| **Melon** | 2 | 0.61 |
| **Papaya** | 4 | 1.22 |
| **Petunia** | 1 | 0.30 |
| **Plum** | 1 | 0.30 |
| **Polish canola** | 4 | 1.22 |
| **Poplar** | 2 | 0.61 |
| **Potato** | 31 | 9.42 |
| **Rice** | 7 | 2.13 |
| **Rose** | 2 | 0.61 |
| **Soybean** | 24 | 7.29 |
| **Squash** | 2 | 0.61 |
| **Sugar Beet** | 3 | 0.91 |
| **Sugarcane** | 3 | 0.91 |
| **Sweet pepper** | 1 | 0.30 |
| **Tobacco** | 2 | 0.61 |
| **Tomato** | 11 | 3.34 |
| **Wheat** | 1 | 0.30 |
| **Total** | **329** | **100.00** |

Source: Author, based on ISAAA's GM Approval Database. http://www.isaaa.org/gmapprovaldatabase/, accessed September 30, 2013

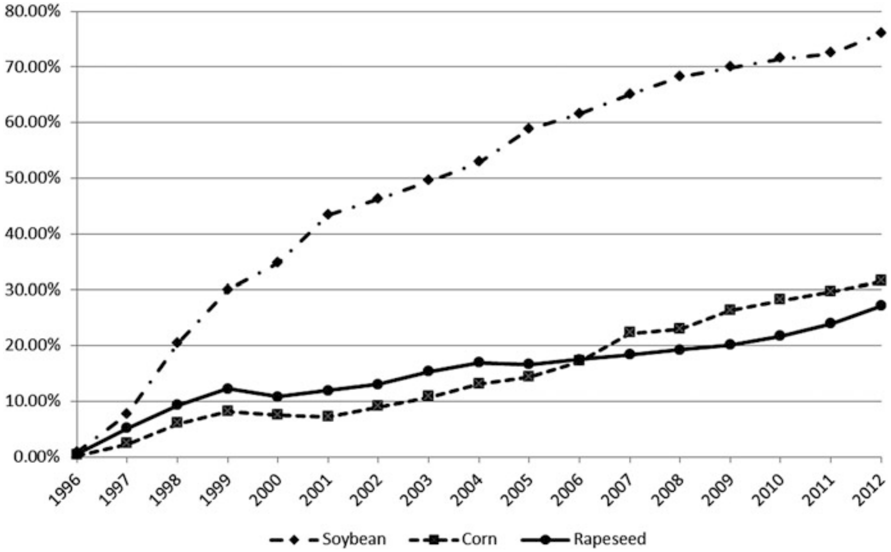

**Fig. 7.1** Global GM share of total hectares planted, by species (Source: Author, based on world hectare data from the FAO's FAOSTAT database (2013) and GM plantings from James, various years)

Transgenic crops were first used commercially in 1996 and have seen rapid uptake. In 2013, 175.2 million ha of biotech crops were grown globally. This is a 3 % increase over 2012 and, following 17 years of consecutive growth, a 100-fold increase since their introduction (James 2014).

The four crops which have received the largest number of regulatory approvals (canola, cotton, corn, and soybean) also account for the large majority of all hectares of GM cultivation globally. Figure 7.1 shows the GM share of global hectares planted for three of these four main GM crops since 1996, the year of their introduction. The share of all three species has continued to rise over time. In 2012, GM plantings accounted for around 30 % of global hectares planted for both rapeseed and corn. The growth in GM soybean has dramatically outpaced other crops with over 75 % of global hectares being planted to GM. Uptake in the United States has been even faster than the rest of the globe. In 2013, 90 % of all corn and cotton and 93 % of all soybean planted in the United States were GM varieties (USDA Economic Research Service 2013).

In 2013, 27 countries planted GM crop varieties, and for the second consecutive year, the number of hectares planted in developing countries surpassed that of developed countries, planting 54 % of all transgenic crops. Eleven of these 27 countries cultivated over 1 million ha (James 2014). While the number of countries planting GM crops has increased on a relatively consistent basis over time, growth has been variable by region. Both North and South America have seen very rapid approval and uptake and account for the vast majority of all regulatory approvals and hectares planted globally. Pakistan, India and China account for nearly all other hectares planted with a combined 18 million ha of GM cotton.[5] African countries were slow to approve the planting of transgenic crops, but South Africa has become a major planter of corn, soybean and cotton and Burkina Faso and Sudan have small areas of GM cotton under cultivation.

Regulatory approvals for GM crops have been much more challenging in Europe where only five countries allow GM cultivation and only one, Spain; plant more than 50,000 ha of any transgenic variety (Bt corn). Numerous European countries have put in place bans on the cultivation of GM crops or specific GM crop varieties[6], and the removal of GM varieties from the market due to commercial reasons (e.g. the Amflora potato that BASF removed from the market) has halted cultivation of GM crops even in some countries where they are allowed. Despite not allowing the cultivation of GM crops, most European and many African countries do permit the import of approved GM crops and grains, primarily for use as animal feed. None the less, tolerance for the import of unapproved varieties is quite low.

### *7.3.3 Other (Non-transgenic) Biotechnology Varieties*

Non-transgenic breeding methods include a broad range of techniques (e.g. marker assisted selection, MAS) that are used to speed up conventional breeding, increase genetic variety or have plants express beneficial traits, similar to those targeted by transgenic research. Notably, it does not use interspecies gene transfer, as with GM.

Due to a less strict regulatory regime, quantitative data is scarce for non-transgenic plant varieties. Non-transgenic technologies have been used in commercial agriculture since before the introduction of transgenic varieties in the mid-1990s and they have impacted many commercially available plant varieties and species. Measured as such, their impact on modern agriculture has been much broader than that of transgenesis technologies. The firms and research institutions using non-transgenic technologies also far outnumber those using transgenesis. Interviews and publicly available information on seed firm research activities indicate that, with the exception of small seed firms active in breeding vegetable varieties, almost all seed firms currently use some form of non-transgenic biotech in at least some of their plant breeding programs (Arundel and Sawaya 2009a). There is also evidence that the intensity of research into these areas have increased substantially in recent

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[5] China also plants some GM varieties of small market crops such as papaya and sweet pepper, but these are considered negligible in the hectare figures presented here.

[6] For detailed information on the status of GM crop regulations in European countries see http://greenbiotech.eu/eu-gm-crops/

years. A 2010 study identified a clear upward trend from 1998 to 2010 in both the number of patents and the number of scientific publications of a selection of seven non-transgenic biotechnologies (Lusser et al. 2011).[7]

## 7.4 Prospects for Future Developments in Agricultural Biotechnology

Understanding the role that agricultural biotechnologies will play in the future necessitates an understanding of how the technology and adoption landscapes are expected to develop. Significant shifts are expected to occur in the near- to medium-term related to the types of biotech plant varieties being commercialized as well as the geographic range of research and uptake.

### *7.4.1 Developments in Plant Breeding*

#### 7.4.1.1 Transgenic Technologies

The pace of development in technology is always difficult to predict. Naysayers and critics downplay prospects while the excitement surrounding agricultural biotechnologies as a tool for addressing future agricultural needs can lead to wildly ambitious predictions that amount to boosterism. A more moderate and data-based approach can provide a view of what types of agricultural biotechnologies can realistically be expected to receive commercial approval in the next 3–5 years.

GM crops are required to undergo field trials prior to regulatory approval. These trials provide a robust data set that includes information on the species of plants and characteristics targeted, and success rates. As a result, field trial data can be used as leading indicators of the types of GM plant varieties and traits likely to reach the market in the next few years, as well as indicators of research trends. This type of analysis was conducted by the author and a colleague while at the OECD[8] and, along with an examination of more recent field trial data, provides the basis for the expected technological developments provided here.

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[7] The selected technologies examined were zinc finger nuclease (ZFN) technology, oligonucleotide directed mutagenesis (ODM), cisgenesis and intragenesis, RNA-dependent DNA methylation (RdDM), grafting (on GM rootstock), reverse breeding, and agro-infiltration (agro-infiltration "sensu stricto", agro-inoculation, floral dip).

[8] See Arundel and Sawaya (2009a, pp 38–47). The publication also includes a second analysis on the annual reports of four of the world's largest seed firms which corroborated the conclusions derived from the GM field trial data.

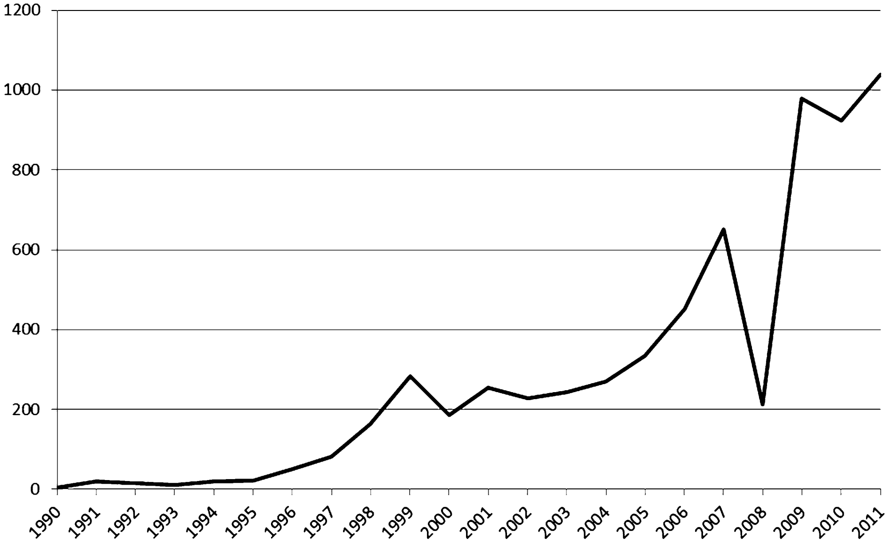

**Fig. 7.2** Number of GM plant field trials for agronomic traits, by year (Source: Arundel and Bowen-Butchart, MERIT-AIRC field trial database 2013)

In the near-term, new commercialized GM varieties are most likely to appear in the species that have received the vast majority of all regulatory approvals and research funding to date: corn, soybean, cotton, and rapeseed. Potatoes and tomatoes have also received regulatory successes and are the targets of currently research but have been less successful commercially. Other crops that have received substantial research attention include barley, peanuts, peas and sugarcane and new varieties of these species are possible over the next few years.

Due to a shift in research efforts, it is expected that there will be a change in the traits receiving commercial approval in the near term. An analysis of all field trial data in OECD countries from 1990 to 2011 shows that throughout the 1990s pest resistance (PR) traits were a major focus of research activity accounting for around 35 % of all trials per year. In the early 2000s however the PR share of all trials began a sustained decline and PR trials only accounted for around 10 % of trials from 2009 to 2011. Over the same time, the number and share of agronomic trait trials has increased dramatically (see Fig. 7.2). These traits accounted for less than 10 % of trials until 2001, reached 20 % of trials in 2006 and accounted for around 35 % of all trials from 2009 to 2011. The share of all field trials focusing on herbicide tolerance and product quality traits have remained relatively stable at around 30–35 % and 10–15 %, respectively, since 1990.

Due to the number of trials, past successes and familiarity of regulators, this data indicates that over the next few years, most new commercial transgenic varieties will be, as in the past, herbicide tolerant. It is also likely that the number of pest resistance approvals will decline as compared to the past. The data for product quality traits are less telling as these consistently represent a fairly small share of all trials. This may indicate that research has been more challenging than in other areas, or it may indicate that developers have concerns related to consumer acceptance of these types of traits. Furthermore, it is probable that the largest value of product quality traits (e.g. increased oil or starch content and reduced lignin) is for the use of these materials in fuel, chemical or material production. These are areas that are likely to increase in importance in the future buy may not be seen as a priority in a period of sustained high food prices. Agronomic varieties can be expected to be approved as research matures and field trials conclude. These are most likely in the main target GM crops of corn, cotton, rapeseed, and soybean. The forecast analysis conducted a few years ago predicted a slightly quicker shift to these agronomic traits than has materialized. None the less, the research trends continue to point towards agronomic traits quickly becoming available and the first agronomic variety of corn, which is resistant to drought, was released by Monsanto in the United States in 2013. This shift towards agronomic traits is expected to have the largest impact on the future of agriculture.

#### 7.4.1.2 Non-transgenic Technologies

Prospects for non-transgenic technologies are much more difficult to determine due to a lack of quantitative data. None the less, the analysis of transgenic R&D can be used as a proxy for non-transgenic R&D because many of these technologies form the foundation of GM research. Along with current literature on the subject, this can be used to formulate a view of the future prospects for the development and uptake of non-transgenic agricultural biotechnologies.

Non-transgenic technologies are also expected to see substantial advances. It is highly likely that in the medium-term – probably before 2030 – that some form of biotechnology will be used at some stage of the development of nearly every new large market commercial plant variety. The use of MAS and related technologies that improve the efficiency of breeding programs will become much easier to use and less expensive following from major advances and cost decreases in platform biotechnologies like gene sequencing and synthesis and bioinformatics. Other non-transgenic techniques that have a direct impact on gene expression will also see growth. As research continues these techniques hold the promise of contributing to an increase in agricultural yields as plant varieties have improved stress tolerance and become even better adapted to local conditions.

### *7.4.2 The Role of Developing Countries*

Developing countries are poised to take a leadership role in agricultural biotechnology in the near future, providing a substantial increase to R&D as more actors become involved and the technologies are adapted and applied to new regions and local crops. Agricultural biotechnologies have the potential not only to contribute significantly to reducing hunger and malnutrition which remain significant problems in developing regions, but also to help satisfy increasing demand for agricultural products due to increasing populations and income levels.

Agriculture is also of major importance to developing economies as it accounts for a much larger share of GDP and employment than in developed countries. Agriculture accounts for over 13 % of the GDP of many developing countries compared to less than 2 % of GDP for developed countries. The number and share of agricultural workers, is also much larger in the developing world than it is in developed countries. While high-income countries have 38 million agricultural workers (about 3 % of the world total), developing countries have over 1.1 billion.

Developing countries have taken the lead in terms of the adoption of biotech crops and they are also taking a leadership position in approvals of new transgenic crop varieties. China, for instance, is the only country in the world to have issued commercial approval for a transgenic tree, a pest resistant variety of local poplar. Brazil has already approved the first soybean variety developed by seed firm Monsanto specifically for a non-US market. The Intacta RR2 PRO™ soybean, which is being cultivated in 2013, includes both pest resistance and herbicide tolerance traits (Monsanto 2013).

R&D expenditures and capabilities in developing countries are also approaching that of developed economies and public research institutions in these countries have seen notable successes. For example Brazil, which is the world's second largest adopter of GM crops, provides an annual budget (not only for GM research) of around 1 billion USD to Embrapa, the country's agricultural and livestock research Institute (Embrapa 2010). Embrapa has developed and received Brazilian regulatory approval for a GM bean which is resistant to the golden mosaic virus (Embrapa 2011).

There is little consistent data on private sector agricultural biotechnology R&D in developing countries. However, other evidence suggests that the research capabilities these countries private firms could increase rapidly. The number of doctoral degrees awarded annually in China in agricultural sciences increased from less than 500 in 2000 to nearly 2,000 in 2008 (National Science Foundation 2012), providing an increasingly large pool of trained scientists for both the public and private sectors.

In the near term, many developing countries will continue to rely on development assistance and innovations from developed countries as well as technology partnerships and joint ventures with companies from developed countries that are looking for access to large developing markets. However, as research capacities increase, public sector institutes and private firms in developing countries are likely to develop new biotech crops on their own. In the not too-distant future, agricultural biotech research could surpass that in developed countries, in the same way that production already has.

## 7.5 Non-technological Issues

Ultimately both the development of agricultural biotechnologies and their utility towards meeting future socio-economic goals and challenges will not only rely on the success of R&D programs. Harnessing the full potential of agricultural biotechnology will require addressing a number of non-technical challenges in a way that does not unduly impinge upon development and deployment of useful technologies.

### *7.5.1 The Effect of Public Opinion and Activism*

Public opinion has proven a major issue in the adoption of GM crops. This has been particularly notable in Europe where cultivation bans exist in some countries, no countries have planted GM crops on a large scale, and several countries who had once planted modest amounts have stopped plantings altogether. None the less, Europe is a major importer of GM products (nearly all for use in animal feed) but few varieties have been approved for import and the tolerance for trace amounts of non-approved varieties is very low. Opposition is not limited to Europe, however; even in the United States, the world's largest adopter of GM crops, debate is ongoing in some areas over the location of field trials and labeling of GM products.

This reluctance towards the use of GM foods is driven by concerns over perceived health impacts which persist despite numerous studies by respected scientists and scientific organizations across the globe finding no evidence of adverse health effects.[9] Public concern has also focused on the effect agricultural biotechnologies can have on the environment, such as the potential for gene flow to non-target species and plants. Regulations, including buffer zones around GM fields, have been put in place to address these issues.

The ways in which attitudes towards the use of GM will change in the future are unclear. Public opinion is influenced by numerous factors including access to information or misinformation, commercial actions by corporations, moral and ethical beliefs, and perceptions of personal benefit from the technology. It is clear, however, that the way in which public attitudes evolve will have a major impact on future technology. Staunch negative attitudes towards these products can reduce private firms' willingness to invest in expensive R&D activities and will influence research targets. Today, very few GM crops are directly consumed by humans; most GM products are used for animal feed. Concerns about public perception of directly

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[9] See for example Nicolia et al. 2013 which reviewed 1,783 scientific records produced from 2002 to 2012 related to the environmental and health impacts of GM crops. The report concludes that the "the scientific research conducted so far has not detected any significant hazards directly connected with the use of GE crops; however, the debate is still intense."

consuming GM products were a major cause of removing GM potato[10] and tomato varieties from the market in the 1990s. Should concerns of this nature persist, it is likely that large seed firms R&D efforts will continue to focus on crops that are used in processed foods and as animal feed.

Negative public opinion has also given way to extreme acts of anti-GM activism that have a direct negative impact on innovation and increase the cost of research. A 2012 report detailed about 80 acts of vandalism against and destruction of GM research in six European countries.[11] In addition to the research setbacks that these acts represent, they have contributed to the reduction in the number of field trials conducted in Europe (discussed in the "Regulations" section below). Furthermore, vandalism can substantially increase the cost of research due to the need for security measures. "Data obtained in Switzerland indicate that for each euro spent on GMO field research, an additional 78 cents were spent on security," and in the UK nearly 20 % of a GM wheat trial's £912,000 budget was dedicated to security (Kuntz 2012).

### 7.5.2 *Concentration*

The agricultural biotechnology industry has seen increasing concentration of actors due to companies ceasing activity and mergers and acquisitions. As shown in Table 7.2, the top firms, as measured by the number of field trials conducted, have increased their share of R&D activity consistently since the mid-1990s. Between 1995 and 1999 the top ten firms accounted for 72 % of all field trials while between 2009 and 2012 the top ten firms conducted over 92 %. The top firm, which was Monsanto in all three periods shown in the table, increased its share of field trials from 31 to 56 %. The same trend of concentration is apparent in the share of all agricultural biotechnology patents over time, although the number of firms applying for or receiving patents increased (Arundel and Sawaya 2009a). Concentration has also occurred geographically with American firms increasing their share of all field trials from 64 % of all trials between 1995 and 1999 to 82 % from 2004 to 2008.

Increasing concentration in the agricultural biotechnology industry could be of concern to future technology development because the large seed firms' activity has been focused on a limited number of large market crops in large markets. The apparent inability of other smaller firms to bring agricultural biotechnology research

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[10] Destruction and vandalism of GM crops in Europe was, for example, a major factor in the German firm BASF's decision to stop commercialization in the EU of the Amflora potato which was cultivated in the Czech Republic, Germany and Sweden from 2010 to 2012.

[11] The six European countries examined were Belgium, France, Germany, Italy, Switzerland and the United Kingdom. In addition to the 80 acts of vandalism detailed in the article, many more were identified. For instance over 100 acts of vandalism against GM crop research were identified in Germany alone.

**Table 7.2** Percent of GM plant field trial applications by leading firms in OECD countries

| | 1995–1999 | 2004–2008 | 2009–2012 |
|---|---|---|---|
| | 6,091 field trials (%) | 5,029 field trials (%) | 5,084 field trials (%) |
| Top firm[a] | 31.7 | 47.2 | 56.4 |
| Top 5 firms[b] | 60.8 | 79.4 | 83.5 |
| Top 10 firms | 72.1 | 90.3 | 92.4 |
| Top 20 firms | 82.3 | 95.7 | 97.0 |
| Top 25 firms | 84.9 | 97.4 | 98.1 |

Source: Author, based on data from A. Arundel and D. Bowen-Butchart, MERIT-AIRC field trial database 2013

[a] "Top firm" as measured by number of field trials conducted. The periods considered are not identical

[b] The top firm in all periods was Monsanto. The top five firms between 1995 and 1999 were Monsanto, Hoechst, Pioneer, Dekalb and DuPont. From 2004 to 2008 the top five firms were Monsanto, Targeted Growth, DuPont Pioneer Hi-Bred, Syngenta, and Bayer CropScience. From 2009 to 2012 the top five firms were Monsanto, DuPont Pioneer Hi-Bred, Dow, Syngenta, and Bayer CropScience

to commercialization does not bode well for the technology's application in other areas. However, the increasingly important role of developing countries in the sector, and their interest in applying biotechnology to locally important plant varieties, could in the future counteract some effects of concentration.

### *7.5.3 Regulations*

The use of transgenic plants is highly regulated. Field trials are required prior to the approval of any new GM variety. Estimates of the cost of these trials and the regulatory burdens associated with them vary from around 500,000 USD, if the cost of proving environmental and health safety are excluded, to over 13 million USD if these are included.

Regulations can impact upon the development of technologies in two ways. First, high regulatory costs can provide a competitive advantage to large firms which can drive concentration in a sector (see above). Second, stringent regulations can reduce the competitiveness of specific countries or regions and drive out or reduce R&D activity.

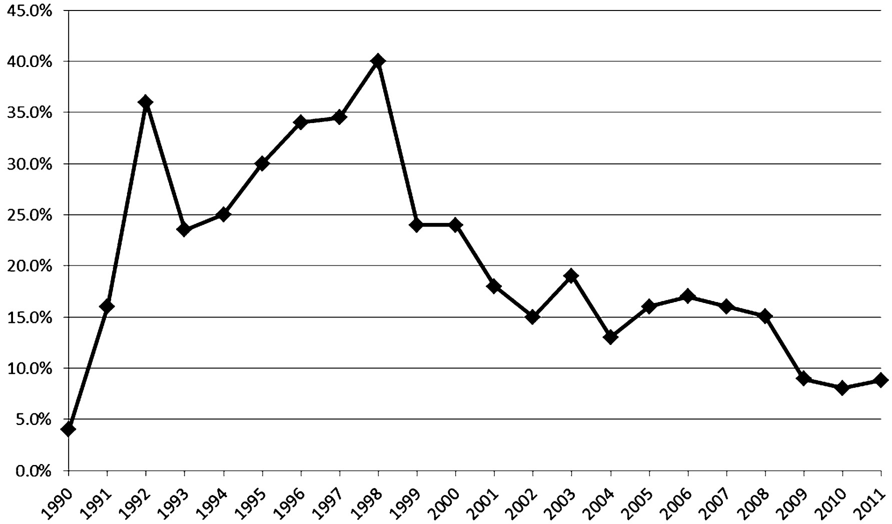

**Fig. 7.3** Share of private sector field trials of GM plants conducted by European firms (Source: Arundel and Bowen-Butchart, MERIT-AIRC field trial database 2013)

An example of this latter impact is the *de facto* moratorium introduced in 1999 by the European Union on the commercial use of GM crops. While this did not prohibit GM field trials, the commercial restriction and mediatization of the issue caused a major drop in European field trials. As shown in Fig. 7.3, the European private sector's share of field trials dropped sharply at the time of the moratorium from a peak of over 40 % of all field trials to less than 10 % per year from 2009 to 2011. The European public research sector was also adversely affected by the moratorium with the number of trials they conducted decreasing by 60 % from 50 trials in 1999 to below 20 after 2004. At the same time, the number of trials conducted by public institutions in North America increased (Arundel and Sawaya 2009b).

As the use of non-transgenic techniques expands and the technologies become more powerful, some regulatory institutions have taken notice. A recent review of related regulatory regimes in seven countries (Argentina, Australia, Canada, EU, Japan, South Africa and the United States) found that "legislation, definitions and regulatory approaches for biotechnology derived crops differ significantly between these countries," and that progress in developing regulatory regimes has been variable between the countries with some nearly concluding the process and others only beginning preliminary discussions (Lusser and Davies 2013).

The question as to whether or not regulations are required for these non-transgenic techniques and what form they should take is an important one. Overzealous or poorly crafted regulations could have a decidedly negative impact on the use of these techniques in plant breeding. In countries where transgenic technologies have taken hold, this prospect seems unlikely. However, as discussed above, transgenic regulations have halted cultivation and stymied plant research in Europe and concerns exist as to whether or not this could also occur for non-transgenic biotechnologies.

Although no decisions have been made on how, if at all, to regulate these non-transgenic techniques in Europe, the European Commission asked the European Food Safety Authority (EFSA) to address the safety assessment of plants developed through three non-transgenic techniques: cisgenesis, intragenesis, and the zinc finger nuclease 3 technique (ZFN-3). EFSA's results stated that the same guidance documents related to the risk assessments of transgenic plants are applicable to these techniques, but that it could be possible on a case-by-case basis to require less event specific data. It also concluded that the potential hazards associated with cigenesis were similar to that of conventionally bred plants while novel hazards can be associated with intragenic, ZFN-3 and transgenic plants (EFSA 2012a, b). While these published opinions do not constitute a legal decision, should the Commission base their legal decision on EFSA's opinion, it is likely that at least some non-transgenic plant breeding techniques will be subject to similarly strict regulation in Europe as transgenic plant varieties.

## 7.6 Conclusions

The uptake of agricultural biotechnologies, both transgenic and non-transgenic, has been very fast, impacting numerous plant species and many millions of hectares of agriculture globally. Given these technologies potential to address increasing global demand and environmental challenges, and the active role that developing countries are playing both as consumers and developers, this trend is likely to continue. Furthermore, an examination of current R&D pipelines indicates that plant varieties with agronomic traits that increase yields and resistance to environmental stresses are nearing commercialization. These will provide larger benefits to both consumers and farmers than the existing herbicide tolerance and pest resistance traits and will allow more food to be produced on less land, or on land that was previously considered sub-optimal. The pervasiveness of biotechnologies is such that it can be expected that, by around 2030, nearly every new large market crop variety in the world will be impacted at some stage of its development by biotechnology. Ensuring that these technologies live up to expectations will however require addressing challenges that could slow, limit, or halt research and development including negative public opinion, sectoral concentration, and regulations. Should these challenges be met, and productivity gains from agriculture research keep pace, agricultural production could begin to outpace demand as population growth slows after the middle of the century possibly ushering in a more important role for agricultural products as a sustainable feedstock for industrial production.

## References

Arundel A, Bowen-Butchart D (2013) MERIT-AIRC field trial database. Maastricht Economic and Social Research Institute on Innovation and Technology, Maastricht

Arundel A, Sawaya D (2009a) Trends in the application of biotechnology to agriculture and related natural resources to 2015. OECD J 3:7–112, http://www.oecd.org/futures/bioeconomy/2015. Accessed 19 Feb 2014

Arundel A, Sawaya D (2009b) The Bioeconomy to 2030: designing a policy agenda. OECD, Paris

EFSA (European Food Safety Authority) (2012a) Scientific opinion addressing the safety assessment of plants developed through cisgenesis and intragenesis. http://www.efsa.europa.eu/en/efsajournal/pub/2561.htm. Accessed 19 Feb 2014

EFSA (European Food Safety Authority) (2012b) Scientific opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function. http://www.efsa.europa.eu/en/efsajournal/pub/2943.htm. Accessed 19 Feb 2014

Embrapa (2010) Embrapa: an overview. http://www.cnpmf.embrapa.br/destaques/Embrapa_an_overview.pdf. Accessed 19 Feb 2014

Embrapa (2011) Transgenic bean developed by Embrapa is approved in Brazil. http://www.cenargen.embrapa.br/_comunicacao/2011/cenargenda/cenargenda62_en_2011.html. Accessed 19 Feb 2014

FAO (2013) FAOSTAT Database. FAO, Rome

Graff GD, Cullen SE, Bradford KJ, Zilberman D, Bennet AB (2003) The public-private structure of intellectual property ownership in agricultural biotechnology. Nat Biotechnol 21:989–995

James C (2014) Global status of commercialized biotech/GM crops: 2013 – executive summary, vol. 46. The International Service for the Acquisition of Agri-biotech Applications (ISAAA). http://www.isaaa.org/resources/publications/briefs/46/executivesummary/default.asp. Accessed 19 Feb 2014

Kuntz M (2012) Destruction of public and governmental experiments of GMO in Europe. GM Crops Food Biotechnol Agric Food Chain 3:1–7

Lusser M, Davies H (2013) Comparative regulatory approaches for groups of new plant breeding techniques. New Biotechnol 30:437–444

Lusser M, Parisi C, Plan D, Rodríguez-Cerezo E (2011) New plant breeding techniques state-of-the-art and prospects for commercial development. European Commission Joint Research Centre Institute for Prospective Technological Studies, Seville, http://ftp.jrc.es/EURdoc/JRC63971.pdf

Monsanto (2013) Monsanto Company receives final key regulatory approval for Intacta RR2 PRO™ Soybeans, Setting Up Commercial Launch In Brazil. http://news.monsanto.com/press-release/products/monsanto-company-receives-final-key-regulatory-approval-intacta-rr2-pro-soybe. Accessed 19 Feb 2014

National Academies of Sciences (2010) Impact of genetically engineered crops on farm sustainability in the United States. NAS Press, Washington, DC

National Science Foundation (2012) Science and engineering indicators 2012: table 2–38. http://www.nsf.gov/statistics/seind12/appendix.htm#c2. Accessed 19 Feb 2014

Nicolia A, Manzo A, Veronesi F, Rosellini D (2013) An overview of the last 10 years of genetically engineered crop safety research. Crit Rev Biotechnol: 1–12. http://www.geneticliteracyproject.org/wp/wp-content/uploads/2013/10/Nicolia-20131.pdf. Accessed 19 Feb 2014

USDA Economic Research Service (2013) Adoption of genetically engineered crops in the US. http://www.ers.usda.gov/data-products/adoption-of-genetically-engineered-crops-in-the-us.aspx. Accessed 19 Feb 2014

# Chapter 8
# Genetically Engineered Crops and Rural Society

**Leland Glenna and Kristal Jones**

**Abstract** This paper seeks to contribute to the genetically engineered crop debates by exploring some social dimensions of new agricultural technologies. After assessing the social dimensions of current GE crops as they relate to agricultural research and development, we examine issues related to farmer adoption of GE crops. We conclude with a discussion on obstacles to socially equitable agricultural innovation and potential policy solutions. We contend that failing to consider these social dimensions of technology development and diffusion are likely to generate unforeseen problems and unsustainable technological developments.

**Keywords** Technology diffusion • Science norms • Public-interest research

## 8.1 Introduction

One of the challenges to creating genuine dialogue about genetically engineered (GE) crops is that competing arguments tend to be grounded in radically different assumptions about the relationship between scientific discovery and social change (Stone 2002). A useful way of conceptualizing the research, development, and diffusion process is to distinguish between invention and innovation. Invention is the creation of a new technique or technology, whereas innovation occurs when that invention is put to use in a novel way. Some proponents of GE crops tend to assume (or hope) that the invention-innovation process is a linear and direct one, so that the

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A. Ricroch et al. (eds.), *Plant Biotechnology: Experience and Future Prospects*, DOI 10.1007/978-3-319-06892-3_8,

development of more efficient technologies will lead to adoption and optimal use. Much agricultural R&D also functions within this framework, where there is an assumption that solving an agronomic problem will lead to solutions to economic and social problems, and where solving agronomic problems, such as increasing crop yields, is an unmitigated public good. There seems to be little consideration given to the idea that agricultural innovations might create economic and social problems as well as solve them, or that innovations bring both benefits and costs, and those benefits and costs are seldom distributed equally.

Those who are skeptical of the promises of GE crops are not all opposed to the technology *per se*, but they do tend to find flaws in the assumed linear relationship between the genetic modification of a plant and agronomic, economic, and social impacts. Kinchy (2012), for example, documents the complex effects of the use of GE seeds in Mexico and Canada, arguing that genes are the foundation for not only agricultural production, but also for market opportunities and cultural heritage. Other skeptics tend to point to the potentially inequitable social dimensions of the processes that shape the development, distribution, and impacts of technologies. Their arguments that GE crops are exclusionary or exploitative might at times also lack empirical support. However, just as research is being done and arguments made on the production and ecosystem impacts of GE crops, consideration of the social dimensions of their creation and diffusion is necessary to gain a comprehensive understanding of impacts.

When we discuss the social dimensions of technology, we are referring to a range of legal, political, social, and economic conditions that influence and are influenced by the invention and innovation process. We cannot touch upon all of these issues in such a brief paper. However, we can explore relevant dimensions within two broad topical areas. First, we assess the social dimensions of current GE crops as they relate to agricultural research and development. We then examine issues related to farmer adoption of GE crops. We conclude with a discussion on obstacles to socially equitable agricultural innovation and potential policy solutions. Failing to consider these social dimensions of technology development and diffusion will likely generate unforeseen problems and unsustainable technological developments.

Although our assessments are primarily focused on experiences in the United States and other industrialized countries, these are the settings in which most agricultural research and development is being conducted (Fuglie et al. 2012). And although adoption rates are increasing rapidly in developing countries, industrialized countries account for a very large fraction of GE crop adoption on an acreage basis, and usually have the longest time frame over which adoption has occurred (James 2012). Focusing on the areas where the research is conducted and where the crops have been adopted over the longest time frame enables us to develop insights from past agricultural research, development, and applications.

## 8.2 Social Dimensions of GE Crops on Agricultural and Food System R&D

A 2008 article in *Science* on the subject of the International Assessment of Agricultural Science and Technology for Development referenced dueling visions for meeting the world's food security needs (Stokstad 2008: 1474). One vision may be summarized as favoring high technology and global sourcing, treating nature as something that can be standardized with inputs, and emphasizing efficiency and profit. The other vision emphasizes natural processes and local sourcing, with community building and serving human needs as underlying motivations. To be sure, these are ideal-type categories that are not necessarily mutually exclusive and may not capture the range of perspectives among scientists, policy makers, or the general public. For example, a scientist may promote a high-technology cultivar in a production system that emphasizes natural processes. However, it is a useful heuristic device for categorizing the GE crop debate since many people are likely to align more closely with one of the two visions.

Proponents from either perspective may genuinely believe that their approach will best feed hungry people and also address social, economic, and ecological sustainability issues. But the mechanisms for achieving those goals and recognition of possible negative side effects are dramatically different. One favors the private sector, with global agribusinesses diffusing commercialized high technology, to solve problems, while the other recognizes a central role for the public sector and may even harbor substantial concerns about the likelihood that agribusiness, with its fiduciary responsibilities to shareholders, can deliver the products that will improve long term ecological health, economic vitality, and social well-being.

Some might argue that public-private partnerships are a way to bridge the two visions. However, Kleinman and Vallas (2001) point out that such private-public partnerships lead to an institutional convergence, and that convergence favors the private sector. This suggests that efforts around the world to promote public-private partnerships ultimately favor the vision that the private sector, with global agribusinesses diffusing commercialized high technology, is the way to solve problems, and offer a diminished role for the public-sector vision.

We argue that the two visions for how best to feed the world reflect different norms of agricultural science and technology. Science norms can be defined as commonly held ideas about how research, development, and diffusion *should* be done. For much of the latter half of the twentieth century in the industrialized world, social scientists and policy makers generally operated on a theory that some combination of public and private sector institutions, which are governed by different science norms, was needed to promote public welfare. The foundation of that theory is that public (university and government) research institutions and private (industry) research institutions have distinct and different incentives, missions, and goals that lead individual scientists to hold different values and to generate different research outputs. Although there are important exceptions, public-sector scientists have tended to produce basic and non-proprietary research outputs, while private-sector scientists have tended to produce more applied and proprietary outputs (Glenna et al. 2011). The United States' 1980 Bayh-Dole Act, which promoted the conversion of university research from non-proprietary to proprietary goods, along with subsequent policies, is often portrayed as a watershed event in promoting the emergence of a commercial-science orientation in the public-sector research institutions. The emergence of commercial science has led many scholars to question whether the distinct institutional research cultures might be converging, and that public-sector research norms that guide academic and government scientists are being replaced by private-sector norms (Glenna et al. 2011).

Two equations help to illustrate the transition from public-sector research norms to commercial science. The first equation reflects the assumption that the public good is considered the sum of public and private goods (8.1):

$$\textit{public goods} + \textit{private goods} = \textit{the public good} \tag{8.1}$$

The second equation reflects the assumption that public goods must be converted into proprietary outputs to contribute to social welfare (8.2):

$$\textit{public goods} \to \textit{private goods} \gg \textit{the public good} \tag{8.2}$$

The Bayh-Dole Act and subsequent policies served to apply the second equation to university research, has shifted expectations of public research agendas. Universities are still expected to conduct basic research and other types of research that may not have immediate commercial applications. However, universities now have an additional expectation of generating intellectual property and developing university-industry research collaboration to enhance their revenue base and explore commercial opportunities (Glenna et al. 2011).

Again, it is important to point out here that, although this effort to model the shift towards the commercialization of science was conducted in the United States, the trend is happening globally. Fuglie et al. (2012: 1031) assert that "by 2000, 54 % of total food and agricultural R&D in OECD countries and 39 % globally was private." Public-private partnerships in the international agricultural research for development context reflect a similar, if less extensive, shift in public-sector work toward the incorporation of proprietary technology or knowledge into the public research for development process.

A survey of agricultural biotechnology scientists at 60 research universities in the United States reveals those scientists' science norms. The scientists were asked to answer on a scale of 1 (strongly agree) to 5 (strongly disagree) the extent of their commitment to a set of normative statements. A low mean score means that scientists are generally more in favor of one norm over another. Table 8.1 presents the mean scores for several questions measuring science norms. We grouped the norms into three categories that we consider to be meaningful distinctions, and which refer to the social groups and institutions that the scientists believe their research is serving and their stance on what the best mechanisms are for extending the scientific results.

**Table 8.1** Norms of science in agricultural biotechnology research ($N = 672^a$)

| | Mean |
|---|---|
| Category I: focus on common goods | |
| Public scientists should focus on producing knowledge or technologies with public (non-excludable) benefits | 2.0319 |
| Citizen groups should play a central role in influencing public research scientists' agendas | 3.7604 |
| Hunger is primarily a wealth distribution problem | 3.9878 |
| Category II: focus on expertise | |
| Public scientists should focus on producing knowledge or technologies that advance their field or discipline | 1.3445 |
| Scientist panels are the most appropriate vehicles for setting the research agendas of public research scientists | 2.4538 |
| Trained scientists are the most accurate arbiters of the relative social value of new technology | 3.0013 |
| Hunger is primarily a production problem that will be solved through advances in scientific expertise | 3.6760 |
| Category III: focus on private goods | |
| Public scientists should focus on producing knowledge or technologies with market potential | 3.3543 |
| Industry should play a central role, in influencing public research scientists' agendas | 4.0387 |
| The market is the most accurate arbiter of the relative social value of new technology | 3.7149 |
| Hunger is best solved by greater reliance on market forces and the private sector | 3.6109 |

Respondents asked to answer on a scale of 1–5, with 1 = Strongly agree and 5 = Strongly disagree

[a] The survey conducted was conducted between October 2003 and March 2004. Although there were 912 respondents, not all respondents answered every question. The statistical program deleted those missing cases through a listwise deletion procedure, yielding the final sample of 672 for this table

These scientists tend to agree with the statement that they should focus on advancing knowledge and technologies within their field and discipline (1.34), and they tend to think that they should produce non-proprietary outputs (2.03). They tend to disagree strongly with the idea that industry should be involved in shaping research agendas (4.04). However, when looking only at the norms related to issues of world hunger, we find that the notion that hunger might best be solved through a reliance on market forces and the private sector has more support (3.61) than the notion that hunger can best be solved through the application of scientific expertise (3.68) or that hunger is a wealth distribution problem (3.99).

These findings should raise concerns for at least two reasons. First, although the distinctions between the scores may seem slight, these value orientations have a statistically significant influence on research outputs. Glenna et al.'s (2011) analysis of a nation-wide survey of plant and animal biotechnology scientists at 60 United States research universities reveals that scientists' value orientations affect the amount of industry funding they receive, the proprietary nature of their discoveries, and the percentage of basic science research conducted in their laboratories. This means that a scientist's perspective on these scales is likely to influence their research practices.

Second, if university scientists and universities more generally are becoming more reliant on the private sector to address problems of world hunger, they might begin to contribute less to one of the university's key social functions—namely, research on orphan crops. Research in the United States already indicates reason for concern. Welsh and Glenna's (2006) study of university research on transgenic crops explains that traditional expectations for university agriculture and food research have been manifested in public goods research. Specifically, agribusiness has little incentive to study minor (or orphan) crops, because those crops offer limited opportunity for return on investment. Therefore, public investment in university research has traditionally brought with it an expectation that universities will contribute appreciably to minor and orphan crops research. Although university research in major crops is also expected, the rationale is that private sector research facilities already have an incentive, and greater infrastructure, to concentrate on major crops because of the potential for profit. However, society also needs research on minor and orphan crops (see NRC 2010). Non-governmental organizations, such as the Clinton Global Initiative, are helping meet the research need (see Ricroch, this volume (Chap. 2), about genomic resources for orphan crops).

Welsh and Glenna (2006) tested this theory of universities as the repository of public-interest research by comparing industry and university applications to conduct research on GE crops. They hypothesize that if university researchers are expected to focus on public-interest research, then agricultural crop research at universities would primarily be conducted on minor crops and minor traits. Private-sector research, by contrast, would focus on major crops and major traits. What Welsh and Glenna (2006) found, however, is that university research on GE crops over time increasingly mirrors the research done in the private sector. Although they are not able to assign a causal connection, their evidence supports arguments on the converging trends of GE crop research and the decline in public-interest research.

In the case of the United States, the foundational rationale for the Bayh-Dole Act was that university inventions would create a revenue stream that could reduce reliance on public funding for agricultural research. However, this makes public research institutions more dependent on private-interest funding sources. A similar phenomenon is occurring in public institutions designed to serve developing countries. Insofar as seeds and varieties can be seen both as a basic livelihood safeguard and a commercial product, both types of research must be supported by funding and regulatory agencies to ensure a diversity of research and outputs.

These findings suggest that strong incentives for public-science research along with adequate public-research funds to preserve the university's vital role in conducting basic and non-proprietary research are needed to complement private-sector research investments at universities. Fuglie et al. (2012) document a trend of increasing private-sector investments in agricultural research and development, led particularly in the biotechnology sector, while the public sector's investments have stagnated, throughout the Organization for Economic Cooperation and Development (OECD) countries. There are positive examples of increased public expenditures on agricultural R&D in places like China, India, and Brazil. However, as Pardey and Alston (2010) observe, there are dramatic disparities in the education and skills of the scientific workforce and overall R&D budgets between industrialized and developing nations. They point out that the investment in time and finances, even for emerging countries like India, would need to be immense to catch up with industrialized nations. That is not to say that this would not be a worthy goal, just that there is likely to be a lag before these investments pay off.

## 8.3 Social Dimensions of Farmer Adoption of GE Crops

James (2012) documents how dramatically GE crops have been adopted around the globe, with an annual growth rate of about 6 % over the past 17 years. His research shows that 17.3 million farmers, 90 % of whom are resource-poor and living in developing countries, have adopted one or more GE crops. And he speculates that this trend is likely to continue as more Asian and African countries adopt legal and regulatory measures that support GE crops.

These impressive numbers need to be put in context, however. First, when small farmers in some developing countries planted GE crops, journalists documented that they often did so in violation of their nation's laws and international intellectual property laws (Ghosh 2003; Tatge 2004). Current laws in Argentina, Brazil, China and India (LeBuanec and Ricroch chapter in this book) have been designed to address this issue. Second, over 40 % of that GE crop acreage is in the United States alone. And nearly 90 % of the crop acreage is located in just five countries (United States, Brazil, Argentina, Canada, and India). Furthermore, the vast majority of the GE crops planted are herbicide-tolerant major commodity crops. A few other pest-resistant major crops and crops with stacked traits (multiple improved characteristics) make up the bulk of the remaining GE crops in production (James 2012). The vast majority of major crops, whether GE or non-GE, is produced on a relatively small number of large farms. According to a recent analysis of United States Census of Agriculture, around 12 % of large farms (annual sales exceeding $250,000) account for 84 % of the value of all agricultural production. Therefore, although James's (2012) claim that 17.3 million farmers, including many small and resource poor farmers, around the world might be adopting GE crops, a large share of the acreage is planted on a relatively small number of large farms in the United States.

Analyzing the spatial distribution of GE crops is relevant to assessing the social implications of adoption of GE crops because of what is commonly referred to as the technology treadmill. As the metaphor implies, technology adoption for farmers is like running on a treadmill. The early adopters are often able to secure benefits from it. But later adopters are compelled to do so merely to keep up. For example, early adopters of a new seed variety may gain some yield increase. Higher production is likely to lower commodity prices, which means that other farmers have to adopt the higher yielding varieties to stay in business. Farmers slowly shift to purchasing their inputs and competing with other farmers to sell their outputs in increasingly oligopolistic commodity markets. And the introduction and expansion of GE crops has coincided with the trend towards a tighter cost-price squeeze and uncompetitive markets. Indeed, the consolidation in the agricultural input markets has been driven by agribusiness efforts to secure intellectual property in the GE-crop sector. Just three firms control 85 % of GE corn plants and almost 70 % of GE non-corn plants (Glenna and Cahoy 2009). Social scientists tend to define a market shifting from competitive to monopolistic or oligopolistic when four or fewer firms gain control of more than 40 % of the market. Oligopolistic control of a market is prone to unfair pricing.

In a book documenting the legal struggles between the Monsanto Corporation and farmers in Canada and the United States, Pechlaner (2012) describes how farmers in a highly competitive market and with tight margins have chosen to adopt GE corn, canola, soybeans, and cotton because of small yield increases or because of labor-saving benefits. However, as more farmers have adopted the GE crops, non-GE seeds have become less available, and prices for the seeds have risen (see also NRC 2010). In some cases, the seed price includes savings on various inputs. When prices for that technology rise to inaccessible levels, the farmers' are left with little to do but harbor resentment and file lawsuits.

Two points about these intellectual property cases needs to be made here. The first is that the cases are about more than settling whether there was unintentional gene transfer or whether farmers intentionally saved and replanted seeds. The issue is about traditional farming methods being expropriated from farmers. In Supreme Court cases in Canada and the United States, Monsanto has succeeded in getting contradictory decisions. On the one hand they have been granted patent protection as though the trait and the germplasm are distinct, since the germplasm is naturally occurring and cannot be patented. However, a farmer cannot save seed, even after paying a royalty for the trait, because the courts agree with Monsanto that the trait and the germplasm are inseparable (Pechlaner 2012).

The second point that needs to be addressed is that existing intellectual property laws and legal precedents are subject to change. The LeBuanec and Ricroch chapter in this volume describes the EC directive and the International Union for the Protection of New Varieties of Plants (the UPOV), that allows farmers to sow seeds from previous cultures, whether they are GE crops or non-GE crops. However, currently, there is only one GE crop grown in Europe, a type of corn resistant to some insects. The World Trade Organization's Trade-Related Aspects of Intellectual Property Rights (TRIPS) was established in 1994 to regulate global intellectual property use and infringements, including GE crops. Those policies tend to favor agricultural biotechnology firm interests over broad social interests. The United States' very strict intellectual property protection policies are the standard for TRIPS (Stein 2005). The World Trade Organization is called upon to settle disputes between international trading partners that have different regulations, such as intellectual property protections for GE crops. It seems reasonable to suggest that a dispute might arise over Europe's intellectual property policies governing GE crops if Europe were to allow the production of more GE crops.

One need only look at the case of an Indian seed company selling GE seed prior to commercial approval. The practice was deemed illegal (due to the selling of seed prior to governmental approval in this case, as opposed to an IPR violation), and Monsanto's partner in India asked for the government to step in and prohibit seed piracy (Ghosh 2003). Eventually, Monsanto worked with the Indian government to legalize the planting of GE crops and to enforce patents by charging farmers a small fee. In Brazil, Monsanto came up with an innovative strategy to recoup lost revenue. The company made an alliance with grain elevators to test grain and charge farmers a fee if the grain tests positive as a Monsanto soybean in Brazil (Tatge 2004). As Qaim (2009: 677) points out, when countries have lax intellectual property policies or fail to enforce the ones they have, it is "difficult for companies to capture innovation rents." Although regulations are addressing this issue (see LeBuanec and Ricroch, this volume), as noted by Dar (introductory chapter, this volume), intellectual property rights continue to be one of the key challenges.

We do not include this information to give a bad impression of seed companies producing GE seeds. They are in a competitive global market place and the company is simply exercising its legal rights. Furthermore, the patents in the US expire in 20 years. The reason we include this discussion is to disabuse people of the notion that the great majority of GE crops in use today are being generated by non-profit charity organizations (we recognize the important cases where they are being generated by NGOs, such as described in Zeigler chapter in this book). We also highlight these examples to offer evidence that seed companies are capable of getting legal and legislative outcomes that secure their intellectual property rights in other countries, just as Pechlaner (2012) describes how Monsanto secures the outcomes it seeks in the United States and Canada. Stein (2005) explains how agricultural biotechnology firms marshalled economic and political influence on the formation and enforcement of intellectual property policies in the United States and how these became dominant around the world through the World Trade Organization's TRIPS. Scholars have questioned whether these policies are benefiting the private sector and limiting social welfare benefits (Stein 2005; Qaim 2009). The European intellectual property policies (see the LeBuanec and Ricroch chapter, describing UPOV rules) governing GE crops may represent a model more favorable to social welfare, and it is not clear that these UPOV rules would change with large scale GM cultivation. However, that matter would likely need to be resolved through a dispute resolution process in the World Trade Organization.

There is evidence to suggest that the technology treadmill and pricing that may be associated with oligopolistic market control, and the subsequent consolidation of other resources like landholdings, occurs in the developing countries, as well as in industrialized countries. After a thorough assessment of the Green Revolution, Evenson and Gollin (2003) conclude that large farmers disproportionately benefitted, while smallholder farmers tended to face negative social and economic impacts. This is likely because the Green Revolution focused on chemically intensive technology best suited to large-scale, capital intensive production. Even if the Green Revolution technologies increased yields over the short or medium term, many farmers did not have economic access to the optimal set of inputs or to the ideal production environment. These disadvantaged farmers were still, however, affected by the broader economic impacts of the Green Revolution, like lower prices and more output competition, so that the physical technology at times created greater inequalities rather than increasing incomes and well-being for all. This is consistent with the way the technological treadmill has progressed in industrialized nations and suggests that the Green Revolution was an example of the United States agricultural innovation model being exported to the developing world.

We offer these illustrations not to suggest that there are inherent flaws in agricultural innovations or to impugn the intentions of the inventors and proponents of such innovations. Rather, the point is to highlight the political, legal, economic, and social dimensions of characteristics of invention and innovation, as well as their subsequent impacts. Many social scientists now argue that the current efforts to promote GE crops are likely to reproduce some of the same negative outcomes as the Green Revolution, because of the similar emphasis on production agriculture and the consolidation of proprietary, commercialized knowledge. It might be wise to consider policies that would minimize such negative outcomes. Important factors distinguish some GE crops that could minimize negative outcomes. For example, insect-resistant crops tend to be scale-neutral, and not capital-intensive. In India, insect-resistant crops have helped reduce food security and human poisonings (see Fleischer et al. Chap. 10).

The example of Golden Rice, a product of a public-private partnership, may present an innovative model that is bridging intellectual property and funding challenges needed to avoid the negative outcomes associated with the first Green Revolution (Potrykus 2001). However, in some ways, the story of Golden Rice illustrates the very point we are trying to make. To develop Golden Rice to meet humanitarian needs, which has been the goal from the outset, the developers of Golden Rice needed to secure free licenses related to 70 intellectual property protections held by 32 different companies and universities. This has enabled developers to claim Golden Rice as a success and an example of a GE crop that addresses challenges raised by earlier opposition to GE crops (Potrykus 2001). Indeed, Golden Rice may be a success story that should be promoted. However, Potrykus (2001) may be overreaching to suggest that this could be a model for other GE crops. First, it is incredible to think that developers of new crops will have the time and resources needed to secure free licenses from all the companies and universities for each new crop. Second, it is just as incredible to think that agricultural biotechnology companies would stay in business very long if they were to give away their licenses only for humanitarian purposes (see Qaim 2009; Stein 2005). However, many companies have this willingness to donate or share some licenses, they have done so for various public-private partnerships, and the intellectual property protections timeline is limited to 20 years.

## 8.4 Obstacles to Agricultural Innovation

Some go so far as to suggest that one thing standing in the way of feeding the world in an environmentally sustainable way is excessive government regulations that prevent GE crops from being developed and diffused (see Potrykus 2010).

GE crops may indeed be a useful tool for solving specific challenges, such as meeting nutritional needs (see Zeigler chapter, this volume). And we recognize that regulatory processes that differentiate based on the method of breeding is not scientifically sound, and government agencies could become more effective and efficient in managing the applications for approving GE crops. However, such bold proclamations of the promises of GE crops and the roadblocks created by undue regulation often rely on assessments of hypothetical yield increases based in a productivist paradigm. A National Research Council (2010) study addressing the situation in the US found no peer-reviewed studies documenting evidence that regulations are providing an undue burden on the development of GE crops. That assertion would not apply to nations like Europe, or other parts of the world, as the NRC study was reviewing the status in the US. However, the decline in rate of growth of public funding for agricultural research in the industrialized world and the consolidation and concentration of the input and commodity processing sector has been highlighted as a factor in the decline in agricultural innovation and food production (Fuglie et al. 2012).

Fuglie et al. (2012) observe that the decline in the rate of growth in public spending on agricultural R&D in OECD nations has coincided with the decline in crop yield and the rise in food prices around the world. They are not able to demonstrate causation, but the relationship is worth exploring in future research. They also note that private investment in agricultural R&D has increased to more than three times public investment in R&D in OECD countries, and they question whether this may be a substitute for the drop in public investment. We would argue that Welsh and Glenna's (2006) study indicates that private investment is not a substitute for a decline in public investment. Indeed, the National Research Council (2010) panel recommended increased support for university and government research to develop public-good applications from crop biotechnology innovations.

Another important strategy for feeding the hungry around the world is to get money into the hands of poor people. Too often, proposed plans to feed people overlook the basic distinction between hunger and demand for food. Hunger occurs when people lack sufficient food. Demand for food refers to the ability to participate in a market transaction. A hungry person who lacks access to money is unable to make a market demand for food. Therefore, counter to what many agricultural biotechnology scientists in the United States believe (see Table 8.1), hunger is a wealth distribution problem. Money needs to be transferred to poor people to enable them to demand food.

Some may question whether it is wishful thinking to propose wealth redistribution as a solution to the problem of poverty and hunger. However, it is no more wishful thinking than suggesting that a technology can solve a social problem. One simply needs to ponder where smallholder farmers are going to find the money to be able to purchase these new technologies. Certainly, GE crops can meet the needs of some smallholder farmers, since there are increasing numbers of cases where poor farmers can and will use GE technology, and access may increase. However, as we discussed earlier, the business model that is driving the development of GE crops is one of charging farmers for seeds each year. That business model is not likely to change. If GE crops are going to be beneficial to smallholder farmers, those farmers must get access to the wealth and the social infrastructure necessary to purchase and market them (Qaim 2009).

One of the more intriguing ideas for promoting economic growth to emerge in recent years is the United Nation's Environment Programme's Global Green New Deal. The idea is based on United States' President Franklin Roosevelt's "New Deal," which provided large public investments to stimulate economic growth during the Great Depression of the 1930s. This welfare-state approach to economic development was adopted by nations in Europe and East Asia (Chang 2008). And it is important to note that many developing nations were using the welfare-state strategy to promote economic growth until the World Bank's Berg Report initiated an austerity program for developing nations (Mkandawire 2001). The UNEP's addition of "Global" to the old idea of the "New Deal" expands the scale of the project and use of the term "Green" places an emphasis on the need for ecologically sustainable strategies. But its emphasis on investing in public goods and people is consistent with the original New Deal. And there is a special section dedicated to the need for investment in sustainable agricultural production in developing nations.

Such policy approaches are important because policy makers over the past three decades have imposed a kind of recession on developing nations. These policies have had very negative consequences on the social and physical infrastructure necessary to increase agricultural production and to feed hungry people (Moseley et al. 2010). A Global Green New Deal offers the potential to transfer money, technology, and skills to smallholder farmers to increase agricultural production and to feed themselves and others. Furthermore, substantial investments in global agricultural R&D could help to narrow the science gap that Pardey and Alston (2010) highlight between industrialized and developing nations, and which they contend limits the ability of developing nations to address problems even when they do initiate public investments in agricultural R&D. If GE crops were promoted as part of such a program, the potential social, economic, and ecological benefits are likely to be more equitable and enduring than if they were introduced in the current political and economic context.

The greatest obstacle to innovations needed to solve global hunger and food problems may be the tendency of key policy makers, organizations, and scientists involved in agricultural R&D to ignore these social dimensions of agricultural innovation. Once that obstacle is cleared, new ways of thinking about global agricultural challenges become evident. For example, recent research suggests that crop yields could be improved through a focus on enhancing smallholder access to agro-ecological intensification techniques (De Schutter and Vanloqueren 2011). GE crop researchers genuinely interested in solving problems of world hunger may want to consider how this insight might be harnessed to promote innovation that maximizes social benefits and reduces negative outcomes.

## References

Chang H (2008) Bad Samaritans: the myth of free trade and the secret history of capitalism. Bloomsbury Press, New York

De Schutter O, Vanloqueren G (2011) The new green revolution: how twenty-first century science can feed the world. Solutions 2(4):33–44

Evenson RE, Gollin D (2003) Assessing the impact of the green revolution. Science 300(5620):758–762

Fuglie K, Heisey P, King J, Pray CE, Schimmelpfennig D (2012) The contribution of private industry to agricultural innovation. Science 338(6110):1031–1032

Ghosh P (2003) India's GM seed piracy. BBC News, 17 June 2003 (Downloaded 5 Dec 2013: http://news.bbc.co.uk/2/hi/science/nature/2998150.stm)

Glenna LL, Cahoy DR (2009) Agribusiness concentration, intellectual property, and the prospects for rural economic benefits from the emerging biofuel economy. South Rural Sociol 24(2):111–129

Glenna LL, Welsh R, Ervin D, Lacy WB, Biscotti D (2011) Commercial science, scientists' values, and university biotechnology research agenda. Res Policy 40(7):957–968

James C (2012) Global status of commercialized biotech/GM crops: 2012. ISAAA briefs no. 44. International Service for the Acquisition of Agri-biotech Applications, Ithaca

Kinchy A (2012) Seeds, science and struggle: the global politics of transgenic crops. The MIT Press, Cambridge, MA

Kleinman DL, Vallas SP (2001) Science, capitalism, and the rise of the 'Knowledge Worker': the changing structure of knowledge production in the United States. Theory Soc 30:451–492

Mkandawire T (2001) Thinking about developmental states in Africa. Camb J Econ 25(3):289–313

Moseley WG, Carney J, Becker L (2010) Neoliberal policy, rural livelihoods, and urban food security in West Africa: a comparative study of the Gambia, Cote d/Ivoire, and Mali. Proc Natl Acad Sci USA 107(13):5774–5779

National Research Council (NRC) (2010) Impacts of genetically-engineered crops on farm sustainability in the United States. The National Academies Press, Washington, DC

Pardey PG, Alston JM (2010) U.S. agricultural research in a Global Food Security setting. Center for Strategic and International Studies, Washington, DC

Pechlaner G (2012) Corporate crops: biotechnology, agriculture, and the struggle for control. University of Texas Press, Austin

Potrykus I (2001) Golden rice and beyond. Plant Physiol 125(3):1157–1161

Potrykus I (2010) Regulation must be revolutionized. Nature 466:561

Qaim M (2009) The economics of genetically modified crops. Annu Rev Resour Econ 1:665–694

Stein H (2005) Intellectual property and genetically modified seeds: the United States, trade, and the developing world. Northwest J Technol Intellect Prop 3(2):160–178

Stokstad E (2008) Dueling visions for a hungry world. Science 319:1474–1476

Stone GD (2002) Both sides now: fallacies in the genetic-modification wars, implications for developing countries, and anthropological perspectives. Curr Anthropol 43(4):611–630

Tatge M (2004) Piracy on the high plains. Forbes, 4 Dec 2004 (Downloaded 6 Dec 2013: http://www.forbes.com/forbes/2004/0412/135.html)

Welsh R, Glenna LL (2006) Considering the role of the university in conducting research on agri-biotechnologies. Soc Stud Sci 36(6):929–942

# Chapter 9
# Is It Possible to Overcome the GMO Controversy? Some Elements for a Philosophical Perspective

**Marcel Kuntz**

**Abstract** The main belief systems that express themselves over GMOs are summarized. The existence of these different modes of thought (termed modernism, postmodernism, environmentalism and religious views) partially explains the reason why it has not been possible to overcome the public controversy despite the accumulation of scientific data. In addition, the divergent views on GMOs often reflect more general value judgments on the free market economy and on the integration of agriculture and food production in a globalized economy. In this context, it has proven difficult for most people to distinguish genuine scientific controversies from political ones.

**Keywords** Modernism • Postmodernism • Environmentalism • Political dispute • Scientific controversy

## 9.1 A Dispute That Is Not Just a Scientific Controversy

As indicated by the rapid adoption rate of GMOs in countries where their cultivation is permitted, the current transgenic varieties appear to match farmers' needs. In addition, as shown in Chap. 2, agricultural biotechnology holds numerous promises. Furthermore, a significant number of scientific publications are now available related to the debated potential risks linked to the commercial use of transgenic varieties. Nevertheless, the use of GMOs is still fiercely opposed by certain organizations. Obviously, views on agricultural biotechnology do not converge towards a consensus in the media, or internet etc., despite the accumulation of scientific knowledge. This suggests that this controversy is not primarily a

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A. Ricroch et al. (eds.), *Plant Biotechnology: Experience and Future Prospects*, DOI 10.1007/978-3-319-06892-3_9,

scientific one. Controversies in biology do not normally last more than 15 years. For example, the Monarch butterfly controversy ignited by a 1999 scientific paper suggesting that Bt corn pollen can harm this butterfly was largely extinguished by a series of six papers published in 2001 (see Minorsky 2001). Scientific debates on potential effects of GMOs on butterflies then moved, for example, towards the impact of *Bt* crops on rare butterflies or the effects of herbicide-tolerant GMOs on the availability of host plants for butterflies. In contrast to the normal evolution of scientific research (new data will often open new questions) illustrated here by the GMO/butterfly case, the public controversy appears highly entrenched. To understand the real nature of the dispute, it is important to examine the various belief systems that express themselves over GMOs.

## 9.2 An Overview of Various Modes of Thought

### *9.2.1 The 'Modern' Thought*

This term is used here for the school of thought inherited from the Enlightment. This rational view of the world has actually been built over millennia (starting with Ancient Greek philosophers) to access objective reality. It is the traditional rational basis of scientific activities; many? often? Often, scientists will support an evidence-based judgment and a case-by-case assessment of GMOs (and other technologies) and consider that risk will be reduced, and even appropriately managed, by increasing scientific knowledge. It should be emphasized that the 'modernist' attitude has profoundly changed since the philosophical views termed 'scientism' or 'positivism' during the end of nineteenth and early twentieth centuries; nowadays very few 'modernists' still believe that science and technology will *necessarily* lead to social improvement. Rather, they generally consider that *without* science and technology, social (and environmental) progress would be impossible in the context of a growing human population (and climate change).

### *9.2.2 The 'Environmentalist' Thought*

The dominant 'environmentalist' views (this term is used here in a philosophical sense) are that human technologies are now so powerful that they can cause not only local damage but potentially the destruction of the planet Earth. 'Environmentalism' has growing support since the 1970s because of the awareness of the impact of human activities on the environment, the arising of a different attitude towards nature and a distrust of artificial processes and products by many consumers. The view that GMOs are 'unnatural' has had a profoundly negative impact on their acceptance and ignores the fact that no conventional crop variety is actually natural, but rather has been subject to an artificial (human) selection process (many crop species, notably corn, rice, wheat, would not have existed without human intervention). Despite the fact that the ‘unnatural’ argument against GMOs is not unanimously shared amongst environmentalists, it remains important for many and for consumers (we tend to consider as ‘natural’ something we are familiar with, although, strictly speaking, it is often artificial).

To ‘save the planet’, environmentalism has adopted a strategy which is often denounced as ‘fear mongering’ by their detractors. The philosopher Hans Jonas (1984) provided the theoretical background for this strategy in his ‘Heuristics of Fear’ (where fear is considered to be a better motivator than positive incitement).

### *9.2.3 The ‘Postmodern’ Thought*

‘Postmodern’ philosophy attempts to deconstruct the foundation of the Western (modern) philosophy and its tendency to promote universal values. Within this postmodern movement, the ‘science studies’ school of thought (see Barnes et al. 1996) claims that scientific truth is merely a ‘cultural construction’ of truth by a scientific community bound together by allegiance to a shared paradigm. This social science movement also criticizes the scientific method and its universality. It exerts a strong influence on academic thought in the Western world, despite being often criticized as representing a form of relativism.

Postmodern sociologists consider that public distrust of some technologies is not due to a lack of knowledge (the ‘deficit model’) but to the fact that the public was not involved in discussion on the technology and decision-making (they advocate an ‘upstream public engagement model’). Thus, to deal with ‘controversies’ related to technologies (GMOs, nanotechnology, synthetic biology), postmodernists recommend ‘citizen participation’ in science and ‘coproduction’ of scientific programs (often with opponents). They are also critical of the scientific risk assessment of these technologies and its separation from the socio-political world. A criticism of this approach is that there is currently no convincing evidence that controversies over technologies, especially over GMOs (Kuntz 2012a), have actually been appeased by following these recommendations. Nevertheless, public policies are often embedded in a postmodern (‘participative’) doctrine.

### *9.2.4 Religious Views on GMOs*

There is no clear consensus view on GMOs among Christian, Jewish or Islamic religious leaders.

A 2009 study on GMOs sponsored by the Pontifical Academy of Sciences concluded favorably on the technology, viewing it to be praiseworthy for improving the living conditions of the poor (see GMO Compass 2010). However, the Popes’ positions were more ambivalent. In 2000, Pope John Paul II stated that "*application of biotechnology* [ . . . ] *cannot be evaluated solely on the basis of immediate economic interests*". Pope Benedict XVI's position has been diversely interpreted. The same social concern is summarized by Glenna and Jussaume (2010) as follows: "*we argue that the development and adoption of GE* [*genetically engineered*] *technology has taken place in the context of an agricultural system that is economically and socially inequitable. A greater focus on social equity may help to break down barriers between GE researchers and sustainable agriculture groups*."

## 9.3 Why Is There No Consensus on GMOs?

At first glance, not all the views summarized above seem to be incompatible. It is difficult to envisage a GMO that is more "socially equitable" than Golden Rice (see Chap. 18). However, this humanitarian rice is targeted by radical opponents in the same way as Monsanto seeds are. Academic and governmental research projects on GMOs have been subject to around 80 acts of destruction in Europe alone (Kuntz 2012b). Most of these experiments were designed to assess the safety of GMOs. It is evident that even these trials are not acceptable to those who argue that GMOs have not been tested enough for their potential effects. Therefore, it can be suggested that further improvement in the safety of GMOs and their social benefits, or addressing sensible questions such as diversification in agriculture, is unlikely to lead to a change of mind for the most determined opponents, simply because their primary motivation may well not lie there.

It is relatively easy to find statements by opponents which are of clear political nature. For example, Bruno Rebelle, a spokesman for Greenpeace in France, explained on 2nd February 2002 during an official audition by a State Council: "*we are not afraid of GMOs. We are just convinced it is the wrong solution* [ . . . ] *GMOs may be a wonderful solution for a certain type of society project. But it is precisely this type of society we do not want*" (translated from French). Thus, since the divergent views on GMOs appear to reflect more general value judgments on the free market economy and on the integration of agriculture and food production in a globalized economy, it is difficult to envisage how a consensus could actually be reached.

Therefore, it can be predicted that plant biotechnology will remain a battlefield for the divergent visions of good and evil. Industrialized countries will remain divided on the topic with poor countries having to choose one side. However, the possibility that some may change position (in either direction) cannot be ruled out if the political balance of power changes.

One may also wonder why the accumulation of sound scientific data on GMOs does not overcome political views. In fact, scientists, scientific risk assessment and even science itself are dragged into this political battle. The widespread postmodern view contributes to the idea that science may be considered as an opinion among many other opinions, which needs to be debated by 'stakeholders' with divergent agendas. Thus, it has proven difficult for most people to distinguish genuine scientific controversies from political ones.

An example of a genuine scientific question is gene flow and its consequences in terms of agronomy or biodiversity, while views on ‘purity’ of corn landraces for example tend to be a matter of cultural ‘identity’ for some Mexican farmers. In an interesting article, Bellon and Berthaud (2004) distinguished scientific questions from value judgments on this topic. However, such considerations will rarely be used in the ‘debate’.

Others topics such as for example lifestyle choices among farmers or economical protection of domestic markets by some governments are quite distinct from environmental or food safety issues. However, the latter are often used to justify restrictions on GMO marketing.

## References

Barnes B, Bloor D, Henry J (1996) Scientific knowledge: a sociological analysis. University of Chicago Press, Chicago

Bellon MR, Berthaud J (2004) Transgenic maize and the evolution of landrace diversity in Mexico. The importance of farmers’ behavior. Plant Physiol 134:883–888

Glenna LL, Jussaume RA Jr (2010) Social equity and the genetically engineered crops controversy. Choices 2nd Quarter 25(2)

GMO Compass (2010) Vatican scientists see “moral imperative” in GMO. http://www.gmo-compass.org/eng/news/547.docu.html

Jonas H (1984) The imperative of responsibility: In search of ethics for the technological age (trans: Das Prinzip Verantwortung). University of Chicago Press, Chicago

Kuntz M (2012a) The postmodern assault on science. If all truths are equal, who cares what science has to say? EMBO Rep 13:885–889

Kuntz M (2012b) Destruction of public and governmental experiments of GMO in Europe. GM Crops Food 3:1–7

Minorsky PV (2001) The monarch butterfly controversy. Plant Physiol 127: 709–710. http://www.plantphysiol.org/content/127/3/709.full

# Part III
# Sustainable Management

# Chapter 10
# Sustainable Management of Insect-Resistant Crops

**Shelby J. Fleischer, William D. Hutchison, and Steven E. Naranjo**

**Abstract** Sustainability is a goal-oriented process that advances based on new knowledge. We discuss factors relevant to insect-resistant crops and sustainability: adoption patterns, insecticide use patterns and their influence on humans, biological control, areawide effects, and evolution of populations resistant to the transgenic crop. Genetically engineered insect-resistant crops were introduced at a time when insecticide options and use patterns were changing. Management of lepidopteran and coleopteran pests has been achieved through constitutive expression of proteins derived from the crystalline spore and the vegetative stage of various strains of *Bacillus thuringiensis*. Management to aphid-transmitted viruses has been achieved through expression of viral coat proteins. Adoption patterns have been rapid where use is allowed. Areawide reductions in pest populations have occurred in cotton and maize in multiple parts of the world, enabled eradication programs, and conferred significant economic benefits to crops that are not transgenic. Insecticide use has decreased dramatically in cotton, leading to improved biological control, reductions in pesticide poisonings, and changes in species composition that achieve pest status. Pro-active resistance management programs, the first to be deployed in all of agriculture, has slowed but not stopped the evolution of resistant populations. Five insect pest species have evolved resistance. Future constructs may provide induced or tissue-specific expression, or use RNAi to deliver protection from

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A. Ricroch et al. (eds.), *Plant Biotechnology: Experience and Future Prospects*, DOI 10.1007/978-3-319-06892-3_10,

insect pests. Constructs that alter plant metabolism, to achieve drought tolerance, nitrogen-utilization, or biomass conversion efficiency, may also affect insect populations and communities. Sustainable management of insect-resistant transgenic crops requires consideration of regional effects of both densities and genetics of mobile target insect populations. The underlying assumption of IPM, that multiple and diverse management tactics are more sustainable, continues to be highly relevant, and necessary, to maintain the utility of transgenic crops, to manage the wider community of species relevant to agroecosystems, and to enable agriculture to adapt to change.

**Keywords** IPM • Areawide • *Bacillus thuringiensis* • Insecticide • Resistance

## 10.1 Introduction

Genetically engineered crops with resistance to insects or insect-vectored viruses have been used on over a billion acres worldwide since 1996. Commercial plantings include cotton, maize, potato, papaya and squash; potential commercial lines also exist for broccoli, eggplant, rice, and plum. Genes have targeted above- and below-ground herbivores from two taxonomic orders of insects, and successfully managed aphid-transmitted viruses. All of these examples—indeed, any change in plant phenotype—affect both the cropping system and the insect populations and communities that utilize those crops. The practice of Integrated Pest Management (IPM), rooted in the science of applied ecology and entomology, provides our context for describing effects on insect populations and communities. Here, we briefly summarize IPM and applied entomology concepts and existing transgenic crops, then discuss opportunities and challenges for their sustainable management at field, landscape, and regional scales.

### *10.1.1 Insect Resistance Traits*

Insect resistance has been categorized as conferring antibiosis, antixenosis, or tolerance. Antibiosis traits directly reduce fitness of the insect, such as decreasing survivorship, prolonging development, or reducing fecundity. Plant expression may be continuous or induced (expressed in response to specific stimuli). Current commercially deployed transgenic crops that express proteins from *Bacillus thuringiensis* express constitutive antibiosis. The concentration of these proteins, however, varies within the plant, through time as the plant develops and senesces, and across the landscape depending on adoption patterns. The interaction of the protein concentration with the degree to which it affects insect fitness is critical to both effectiveness and sustainability of insect resistant crops. Engineering crops with induced antibiosis may be deployed in the future. Induced proteins would affect the spatio-temporal dynamics of insect exposure, and thus the selective pressure for resistance.

The additional categories—antixenosis and tolerance—also affect insect populations. Antixenosis refers to phenotypic traits that affect insect behavior, and tolerance refers to traits that affect the way in which the plant allocates resources to compensate for pest attack: for example, compared to older cultivars, modern cultivars of maize may produce higher grain yields in the presence of low to moderate amounts of stem-boring by lepidopterans (=caterpillars) due to a wide range of structural and biochemical traits that compensate for damage. Both the transgene that, for example, reduces survivorship of an herbivore, and other phenotypic traits that influence insect behavior and plant resource allocation, are integrated into elite hybrids during modern plant breeding. In addition, when considering insect-resistant crops in the future, it is important to realize that traits that may not be directly targeting insects, such as drought-tolerance or nutritional content, may also affect insect populations and communities through their affects on insect behavior and fitness.

### *10.1.2 Insecticides and Their Integration into IPM*

Genetically engineered plants with insect resistant traits were commercialized while the types and availability of commercial insecticides were changing rapidly. Advances in insect physiology, toxicology, and formulation technology led to improved targeting and delivery of insecticidal molecules. Increased ecological and human safety is achieved, in part, through development of selective insecticides. Today's insecticides are classified into 26 chemical classes, and multiple subclasses, on the basis of their modes-of-action, defined globally.[1]

Insecticides made from the microbe *Bacillus thuringiensis* (*B.t.*) achieve high levels of selectivity. This microbe produces biodegradable protein crystals (termed Cry proteins), with typically three components (termed domains) during sporulation; some strains also produce additional insecticidal proteins during vegetative growth (termed vegetative insecticidal proteins, or VIPs). The Cry proteins separate into their domain subunits in the micro-environmental conditions of the insect gut, and subunits bind to protein receptors on the microvilli of the insect midgut lining. Effective binding results in pore formation and osmotic shock, which is followed by septicemia of the insect, probably involving microbes beyond the *B.t.* species. Selectivity is achieved through specificity of micro-environmental conditions, and binding properties of specific Cry proteins with specific receptor proteins, all associated with the insect gut. While the degree of selectivity varies, and thus some non-target species can be affected, high degrees of selectivity are common, often at the species level. Thus, a given Cry protein may be effective on one species of caterpillar but not a related species of caterpillar. Furthermore, effectiveness often

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[1] www.irac-online.org

varies with the life stage of an insect. Many *B.t.* materials need to be acquired by immature (larval) life stages, and are viewed as larvicides. Selectivity is further achieved through the requirements needed to deliver the protein to the target site: acquisition must be through ingestion, in contrast to modes-of-action that can be delivered through contact.

Over 250 insecticidal proteins have been recognized from *B.t.* The Cry proteins are classified by their amino acid sequence, with 67 major groups (Cry1 through Cry67), and additional subgroups defined by their evolutionary similarities.[2] For example, Cry1Ab, commonly used in agriculture, refers to category 1, subgroup A, and an additional subgroup b within A. Sprayable formulations of a few *B.t.* groups have been used for over 70 years in agricultural production, protection of stored grain, and mosquito control. Agriculturally relevant formulations have been derived from *B.t. kurstaki* (isolates produce CryIAb, Cry1Ac, or Cry2Aa,); *B.t. aizawai* (isolates produce Cry1Aa, Cry1B, Cry1Ca, or Cry1Da); *B.t. san diego* or *tenebrionis* (isolates produce Cry3Aa), and *B.t. kumamotoensis* (isolates produce Cry3Bb1). When *B.t.* is used as sprayable formulations, typically produced in fermentation culture, the *B.t.* insecticides require precise targeting because microbes can be sensitive to solar irradiation and they require ingestion by early insect life stages. By 1987 transgenic plants had been created that produced Cry proteins. This enabled efficient targeting of insects through ingestion by immature insect life stages. Commercial lines were first available in 1995.

The integration of insecticides with other tactics for insect management, notably biological control, driven by problems of resistance and additional species achieving pest status, was a primary basis for the emergence of Integrated Pest Management (IPM) programs during the last half of the twentieth century. An underlying assumption is that multiple and diverse management tactics are more sustainable when applied as a package than any one would be when relied on in isolation. A classic IPM pyramid (Fig. 10.1) shows a base, designed to minimize the effect of a pest upon the crop, built from knowledge of pest biology and ecology, biological control, host plant resistance, cultural control, and landscape factors. Monitoring, decision-making, and the use of insecticides in response to economically threatening population densities that have developed despite avoidance tactics are used in IPM. Insect resistance management (IRM) —efforts to delay the evolution of resistance— is also now formally integrated into IPM programs. Management may be at the field scale, or at larger geographic scales. Areawide management programs strive to remove, reduce, or slow the geographic expansion of pest populations at wide geographic scales. One way to focus the debate about the use of *B.t.*-transgenes is to ask if they represent host-plant resistance, or pro-active deployment of an insecticide. Both are true.

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[2] www.lifesci.sussex.ac.uk/home/Neil_Crickmore/B.t.

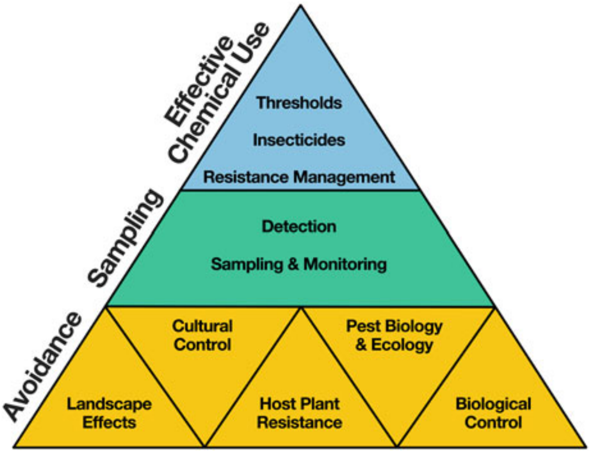

**Fig. 10.1** Classic IPM pyramid (From Naranjo 2011, with permission)

## 10.2 The Emergence of Insect-Resistant Crops, Pyramids, Stacks, and Coupled Technologies

During the breeding process that leads to transgenic insecticidal plants, genes are isolated, connected to markers, and inserted into plants. The final construct after successful insertion is called an event, and government registrations are issued for specific events. Early constructs, and those still in use in some crops today, include a single event which codes for a single protein, such as Cry1Ab or Cry1Ac. Constructs can also be pyramided with multiple genes targeting the same pest (or a slightly overlapping group of related pest species), to broaden its activity and reduce the likelihood of resistance, or stacked with other traits such as herbicide tolerance. Pyramided constructs generally have different modes-of-action targeting the same species, and are replacing single gene plants because of their increased effectiveness. For example, the MON89034 event is a pyramided stack which codes for two Cry proteins, Cry1A.105 and Cry2Ab, targeting a group of lepidoterans. Increasingly, the insecticidal trait, conferred with either a single gene or through a pyramided event, is being stacked with events that confer herbicide tolerance.

Sweet corn provides a simple example. In the U.S., cultivars with the Bt11 event that codes for Cry1Ab have been in use since 1996. By 2012, cultivars became available with vector stacks that code for the Cry3Bb protein which confers resistance to rootworm larvae, plus CP4 which provides tolerance to glyphosate herbicide. Also, within the same cultivar, pyramided vector stacks became available in 2012 that produce Cry1A.105 and Cry2Ab, which provide resistance to several additional caterpillar species through different modes-of-action, and cultivars that include expression of VIPs are projected for commercial deployment soon.

Field maize presents a much wider array of transgenic cultivars. A summary in 2010 showed sources from 5 commercial enterprises provided 22 trait groups, some of which involve licensing agreements among several companies. All but one of these cultivars stacked insect resistance with herbicide tolerance. Insect resistance was conferred with nine different proteins, either singly, stacked, or pyramided in varying combinations, ranging up to five proteins aimed at insect pests from two different taxonomic orders. The range of Cry proteins expressed includes several from the Cry1A group, at least one Cry1F and one Cry2, several from the Cry3 group, and cultivars that express VIPs. Early cultivars provided resistance to moths in the family Crambidae. Newer stacks add resistance to several moth species in the family Noctuidae, and/or larval stages of beetles in the family Chrysomelidae with Cry3 proteins. Cry3 proteins had been introduced earlier, in potatoes in the mid-1990s, to control another Chrysomelidae species (Colorado potato beetle), and later potato cultivars included traits that conferred resistance to several aphid transmitted viruses, however these cultivars are not currently in use.

Simpler stacked and pyramided constructs are found in cotton, primarily because they target only lepidopteran pests. The initially introduced events were grown in a number of countries, and expressed a single Cry protein (Cry1Ac). Cry1Ac was then pyramided with either Cry1F or Cry2Ab2 to provide for better resistance management and to enhance the spectrum of efficacy within the lepidopteran group. China and India have cultivated a few unique events including a Cry1Ab + Cry1Ac pyramid and a pyramid involving a fusion protein (Cry1A) combined with a cowpea trypsin inhibitor. The addition of VIPs to several current constructs is underway by several companies.

Notable among insect-resistant crops beyond maize and cotton, China is developing insect-resistant rice with stacked and pyramided constructs, reviewed in Chen et al. (2011). As with maize and cotton, many of the insect-resistant events have been stacked with constructs conferring tolerance to several herbicides.

In addition to constructs modeled from proteins derived from *B.t.*, insect management is influenced by constructs derived from viral coat proteins (see Chap. 13). Expression of those coat proteins result in activation of a plant immune response, mediated by small RNA molecules, providing protection against infection by the virus of origin. This strategy has been used to achieve control of papaya ringspot virus in papaya since 1997, and one or more strains of four viruses in squash or zucchini since 1994. Aphids transmit these viruses by first acquiring them from an infected host. The virions adhere to receptor proteins in the needle-like mouthparts of the aphid. In subsequent feeding probes by the aphid, the virions are injected into a new plant. Where aphids pose a threat of pathogen transmission, tolerance of aphid populations by farmers is very low, resulting in a higher incidence of insecticide use. In contrast, when aphids do not pose a threat of pathogen transmission, tolerance of aphid feeding itself can be very high, and aphid management tends to rely primarily on biological control through natural enemies and entomopathogenic fungi. There are multiple other plants for which insect-transmitted plant-pathogenic viruses or bacteria can be controlled using transgenic methods, including apple, potato, and plum, but these have not moved into commercial production.

In addition to management of insect-transmitted pathogens, RNA-mediated processes are being developed to target insects that are direct pests of the plant, and mites that are parasites of honeybees. These involve a different mode-of-action than achieved with proteins. They can result in high degrees of specificity and can interfere with expression of specific genes in the insect (they are termed RNAi, for RNA-interference). Insect-resistant cultivars are also under development that stacks Cry or VIP proteins along with RNAi.

## 10.3 Sustainable Management of Insect-Resistant Crops

The agroecosystems in which transgenic crops are introduced are dynamic, and components do not operate independently. Transgenic crops often involve coupled technologies, including stacks or pyramids of insect-resistant genes and stacks of herbicide-tolerant genes. Transgenic as well as some non-transgenic cultivars are increasingly (currently almost always in the U.S.) being coupled with systemic insecticidal seed treatments, and may include seed coatings to help with mechanized planting, to protect against soil-borne pathogens, or as biostimulants that aim to induce up-regulation of resistance genes. Sustainability, which we recognize as a process with inherent goals and values, is affected by all of these technologies and their interactions with socioeconomic factors. Here, we illustrate factors particularly relevant to insect-resistant crops and the broad definition of sustainability: adoption patterns, insecticide use patterns and their influence on human welfare and biological control, areawide effects, and evolution of insect populations that are resistant to transgenic crops.

### *10.3.1 Adoption Patterns*

Adoption patterns are defined overwhelmingly by social, political and economic factors. In maize and cotton, adoption rates are among the highest for any agricultural technology in countries where they are allowed. By 2011, adoption rates in five of the six largest cotton producing countries exceeded 70 % and *B.t.*-cotton comprised over 60 % of the world's production. *B.t.*-maize comprised 67 % of the U.S. crop in 2012.[3] Adoption of *B.t.*-sweet corn has been estimated at between 18 and 25 % among crops destined for fresh-market, but figures have not been available for the processing market. Adoption of transgenic papaya reached about 80 % in Hawaii, and 12 % of the squash in the U.S. utilized transgenes in 2005 (NRC 2010). In contrast, commercial sales of *B.t.*-potato in the U.S. were halted after about 6 years. These cultivars had resistance to Colorado potato beetle and several viruses, but processors declined to accept market risk, and growers tended to adopt systemic neonicotinoid insecticides that were introduced at the same time (NRC 2010). Neonicotinoid insecticides controlled a much broader array of insects and

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[3] www.ers.usda.gov/data/biotechcrops/

thus were easier to use with a much wider array of potato cultivars. *B.t.*-eggplant for use in India to control stem borers has been developed into commercially useful lines, completed regulatory reviews, and is projected to dramatically reduce insecticide use. Multiple *B.t.*-crucifer crops have advanced to commercially relevant lines, within the context of international public-private partnerships, although to date none are being produced commercially (see Shelton et al. 2008 for a good review of transgenic vegetables and fruit relevant to insect management). Market forces, political and business decisions, prohibitions, and labeling requirements, are among the primary factors slowing or stopping the commercialization of insect-resistant transgenes in vegetable and fruit crops. Insect-resistance achieved with genetic engineering techniques is totally prohibited in certified organic production for any crop, although the same *B.t.* proteins can be sprayed onto the plant. In cotton and maize, adoption rates are being influenced by the interest growers place on stacked traits such as herbicide-tolerance or traits aimed at multiple insect species. Adoption is also being influenced by the availability of seed: in some examples, seed without transgenic traits, or without stacked traits, may be hard to obtain. In the future, adoption rates may decline in response to decreasing pest populations resulting from areawide effects, discussed below.

### *10.3.2 Insecticide Use*

Where insecticide inputs have been low on a per-acre basis prior to the adoption of transgenic cultivars, as in maize, changes in insecticide inputs are less clear, and may increase, in part due to the coupling of neonicotinoid seed treatments with transgenic crops. This coupling is a common, but not an inherent property of transgenic technology. Changes in use patterns are often driven by market factors interacting with factors driving the intensification of agriculture.

Insecticide use dramatically declined in cropping systems that were heavily dependent on insecticides prior to the introduction of transgenic crops, such as cotton. Reductions due to *B.t.*-cotton have been profound (Naranjo 2011). Debates that consider values inherent in sustainability are incomplete if they ignore these reductions and their implications. *B.t.*-cotton has reduced insecticide active ingredient use by 170.5 million kilograms between 1996 and 2010, with an associated 26 % reduction in the environmental impact quotient (a measure of the pesticide's impact on the environment and human health) (Brookes and Barfoot 2012). This has led to improved biological control of several pest species. Gains to human health can be dramatic when adoption of insect-resistant genotypes reduces insecticide use. Examples are well-documented in small-holder production systems. In India, where pesticide applications were reduced by 50 %, with larger reductions of the more toxic materials, *B.t.*-cotton is decreasing the incidence of pesticide poisonings by several million cases per year (Kouser and Qaim 2011). Studies also document fewer pesticide poisoning events in China and South Africa. Reductions of insecticide exposure to farm-workers and insecticide poisoning are consistent with values embedded in the process of sustainability. Socio-economic studies, controlled for other factors, have also documented improved dietary quality and caloric value, along with reduced food insecurity, among smallholder households that adopted *B.t.*-cotton (Qaim and Kouser 2013). The per-acre insecticide load is highest in vegetable and fruit crops, where manual labor is much more prevalent and insecticide problems related to human safety tend to be the most dramatic. Ironically, this is where market and regulatory forces are slowing development or adoption of insect-resistant transgenic cultivars. In a recent study in five US states across multiple years, *B.t.* sweet corn performed better and required fewer sprays than conventional sweet corn to meet market standards, thus reducing hazards to farm workers and the environment (Shelton et al. 2013). Unfortunately, debates about sustainability or desirability of transgenic crops rarely elaborate on effects on farm-workers.

### *10.3.3 Areawide Effects*

If females deposit eggs equally among cultivars, and the transgenic cultivar reduces survivorship, then the transgenic cultivar acts as a population sink. The degree to which it drives down populations depends on rates of insect dispersal and adoption of the transgenic cultivar. For pink bollworm, a specialist herbivore, Carriere et al. (2003) showed adoption rates of about 65 % would drive down regional populations. Regional reductions have also occurred with polyphagous species, including *Heliothis virescens*, and to a lesser extent *Helicoverpa zea*, in cotton in the eastern U.S. In China, transgenic cotton dramatically reduced *Helicoverpa armigera* populations both in the cotton crop, and in the surrounding matrix of vegetable, corn, peanut, and soybean (Wu et al. 2008). Even in the presence of complex cyclic dynamics, Hutchison et al. (2010) documented how *B.t.*-maize reduced population growth rates of European corn borer, driving populations to historically low levels in large and multiple areas of the Midwestern U.S.

The areawide affects of *B.t.* plants are influencing IPM in ways relevant to values associated with sustainability. For pink bollworm, transgenic cultivars led to an organized eradication program that integrates transgenic cultivars with mating disruption via pheromone technologies, sterile insect release, cultural controls, and insecticides. Pink bollworm has been essentially eliminated from the U.S., and greatly reduced in bordering states of Mexico. While reductions in insecticides due to *B.t.*-cotton enabled other species (mirid bugs) to emerge into pest status, it also significantly increased populations of beneficial arthropod predators, which reduced herbivorous (aphid) prey populations, both in the cotton crop and surrounding maize, peanut and soybean crops in China (Lu et al. 2012). In combination with other IPM tactics, *B.t.* cotton in the western U.S. has dramatically enabled biological control of non-lepidopteran pests such as whiteflies and mirids and driven overall insecticide use down by nearly 90 %. In the Midwestern U.S., economic analyses considered effects to both land planted to *B.t.*-maize, and to the land planted to non-*B.t.* cultivars. Cumulative benefits were $3.2 billion in three states, with a surprisingly high percentage (75 %) accruing to non-*B.t.*-maize growers because the non-*B.t.* acreage did not carry the additional expense of the *B.t.*-seed. Similar high returns, and high fractions accruing to non-*B.t.* maize growers, occurred in an additional two states. Clearly, areawide effects—including eradication programs, reductions in insecticide use, increases in biocontrol, and economic savings—extend well beyond the boundaries of the planted crop.

Adoption patterns in the future could also be influenced by areawide effects. Theoretically, as populations decline, growers could shift to non-transgenic cultivars if they are available as elite hybrids, thus saving the appreciable cost of *B.t.*-seed, although some question if the non-*B.t.* hybrids will be available at a wide scale. Theoretically, both resistance management (discussed below) and maintenance of low populations could be achieved through spatio-temporal dynamics in adoption patterns at landscape and regional scales.

### 10.3.4 Evolution of Populations Resistant to the Transgenic Crop

Deployment of insecticides or insect-resistant germplasm has never been static. For example, to manage Hessian fly, over 60 wheat cultivars have been released with antibiosis resistance. The pest, in turn, has evolved over 16 biotypes that can overcome antibiosis, and management programs include variable spatial deployments of resistant germplasm. Insects are incredibly adaptable, and 550 species include populations with resistance to one or more insecticides. Sole reliance on antibiosis traits, regardless of the plant-breeding technology or insecticide mode-of-action, often creates a "treadmill": a race between evolution of resistance and new trait development and deployment. Models to help manage this evolutionary process were established prior to the deployment of transgenic crops. These models estimate time to acquire resistance, defined as an increase in the frequency of a resistant allele, as a function of life history, fitness, and population genetics. Simulations and experiments considered varying deployment options, and how they affected the time to acquire resistance.

Insect-resistant transgenic crops were deployed in the U.S. only after a resistance management plan was defined and accepted by the U.S. Environmental Protection Agency. Although heavily critiqued, and often lacking enforcement, to our knowledge this is the first, and only, regulatory-mandated use of resistance management plans prior to deployment of any technology in agriculture. These plans typically rely on refuges of non-*B.t.* hosts, and assume that alleles conferring resistance are rare, so that very few individuals survive on the *B.t.*-crop. The non-*B.t.* hosts provide a relatively large population of susceptible individuals, and the plans assume the rare survivor on the *B.t.*-crop will have a much higher probability of mating with a susceptible individual, resulting in individuals that are heterozygous for the resistant allele. Expression of *B.t.* is typically targeted sufficiently high to kill the heterozygote offspring. This is termed the "high-dose refuge" strategy. Additional assumptions inherent to the high-dose refuge strategy include random mating and single alleles conferring resistance. Additional factors that can contribute to delayed resistance include lower fitness or competitive abilities of individuals that manage to develop on the *B.t.*-crop. In the U.S. there have been many variations of refuge design, in terms of the percent of the crop ("structured refuge"), or non-crop alternative host ("unstructured refuge"), which serves as a source of susceptible individuals, and their spatial placement. The area required for planting to non-*B.t.* maize has varied from 5 to 50 %. For cotton, structured refuge has varied from 5 to 20 %. Spatial placements of structured refuges have varied from nearby blocks to seed mixes termed "refuge-in-a-bag". Refuge requirements for pink bollworm were suspended as the eradication program was deployed, with the assumption that sterile male releases were providing susceptible phenotypes. In one case for *H. zea*, carbon-isotope studies documented that non-crop plants were providing susceptible individuals, leading to inclusion of "non-structured refuges" in resistant management plans under certain circumstances. Work with *B.t.*-crucifer crops as a model system demonstrated that deployment of pyramided constructs prior to the deployment of single constructs delays resistance, and pyramided deployments are becoming more common. For certain cotton cultivars planted east of west Texas, where unstructured refuges contributed susceptible phenotypes and the cultivars included pyramided resistant genes, the structured refuge requirement has dropped to 0 %. Stacked constructs aimed at multiple insect species require refuge designs appropriate to each of the targeted species, which can be difficult due to their differing behaviors (e.g., dispersal patterns and how that influences mating probabilities). Different life stages of the insect may have different susceptibilities to the resistant trait, which may also be expressed at variable levels within the plant or during the plant's development, all of which affect the ability to consistently achieve a dose that kills heterozygous individuals. Thus, refuge designs change as new transgenic cultivars become commercialized, often with considerable debate among parties with conflicting interests.

Tabashnik et al. (2013) suggested that field-evolved resistance has been delayed when the allele conferring resistance has a low initial frequency, refuges are abundant, and pyramided toxins are used. They define resistance as the "... genetically based decrease in susceptibility of a population ... caused by exposure to the toxin in the field", regardless of whether there are reductions in expected levels of control, or whether the insect was a pest that was expected to be controlled. By this definition, five insect species now include populations in specific locations that are resistant to *B.t.* crops. The degree to which this has affected pest control varies among populations and species. In maize, three species now exhibit sufficiently high resistance to a single protein in some populations to affect control. In the case of *B.t.* corn targeting corn rootworm larvae, the dose is not sufficiently high to meet criteria typically assumed to be necessary to achieve the "high-dose refuge" strategy, mating may not be random, resistance appears to be caused by more than a single allele and some of these may not be rare, and frequencies of fields with unexpected damage has recently been increasing. In cotton, two species have evolved a level of resistance to result in significant field damage in specific locations of the world and to specific events. In all five cases, plants with pyramided *B.t.* proteins are currently still effective, although the reduced selection process that should be conferred by separate modes-of-action may be compromised when the efficacy of one of the proteins is compromised There are additional cases where the frequency of resistant alleles has increased, but not at a level that has affected pest control as of 2013. Various tactics have been implemented to manage resistant populations when resistance resulted in significantly reduced field control. In the first clear case of resistance resulting in field failure, one case of resistance on a Caribbean island, the transgenic cultivar was removed from the market in Puerto Rico. In another, there has been increased emphasis on crop rotation, and rotation among cultivars that express different *cry* genes. There has also been increased emphasis on development and deployment of pyramided constructs and adherence to established refuge requirements.

## 10.4 Summary

Transgenic crops affect population densities of pest and beneficial insect species, biological control services, insecticide use patterns, pesticide poisoning of humans, and economics. Values relevant to discussions about sustainability exist for deployment of insect-resistant genetically engineered crops. Many examples document environmental and human health benefits in the first 17 years of adoption, which has been remarkably rapid where the technology has been allowed. The need for resistance management makes it clear that we are also dealing with effects on population genetics. The effects often occur at scales that transcend the land planted to the transgenic crop. Missing from many discussions, at least for vegetable and fruit crops, are effect on farm-workers. Sustainable management of insect-resistant transgenic crops requires consideration and management of regional effects of both densities and genetics of mobile insect populations.

The underlying assumption of IPM, that multiple and diverse management tactics are necessary to be more sustainable, continues to be highly relevant. Widescale adoption and over-reliance on only host plant resistance, especially when conferred via a single protein, creates exceptionally strong selection pressure, and insects have and will adapt with heritable changes in their genotypes and phenotypes. Insect resistance management (IRM), a component of IPM, is an integral part of deployment of transgenic cultivars. Sole reliance on a treadmill strategy with transgenic traits is not a sustainable strategy. Integration of insect-resistant traits with diverse pest management methods, through IPM, enables agriculture to also adapt and evolve, for management of the species targeted by the transgene(s), but also for the wider community of pest and beneficial species in the agroecosystems, and in the wider realm of changing markets, policies, and social and economic structures in which farmers operate.

**Acknowledgements** The authors thank T. Sappington and A. Shelton for valuable reviews which improved the manuscript.

## References

Brookes G, Barfoot P (2012) GM crops: global socio-economic and environmental impacts 1996–2010. PG Economics Ltd., Dorchester, 187 pp

Carriere Y, Ellers-Kirk C, Sisteron M, Antilla L, Whitlow M, Dennehy TJ, Tabashnik B (2003) Long-term regional suppression of pink bollworm by *Bacillus thuringiensis* cotton. Proc Natl Acad Sci 100:1519–1523

Chen M, Shelton A, Gong-yin Y (2011) Insect-resistant genetically modified rice in China: from research to commercialization. Annu Rev Entomol 56:81–101

Hutchison WD, Burkness EC, Mitchell PD, Moon RD, Leslie TW, Fleischer SJ, Abrahamson M, Hamilton KL, Steffey KL, Gray ME, Hellmich RL, Kaster LV, Hunt TE, Wright RJ, Pecinovsky K, Rabaey TL, Flood BR, Raun ES (2010) Areawide suppression of European corn borer with *B.t.* maize reaps savings to non-*B.t.* growers. Science 330:222–225

Kouser S, Qaim M (2011) Impact of *B.t.* cotton on pesticide poisoning in smallholder agriculture: a panel data analysis. Ecol Econ 70:2105–2113

Lu Y, Wu K, Jiang Y, Guo Y, Desneux N (2012) Widespread adoption of *B.t.* cotton and insecticide decreases promote biocontrol services. Nature 487:362–365

Naranjo SE (2011) Impacts of *B.t.* transgenic cotton on Integrated Pest Management. J Agric Food Chem 59:5842–5851

National Research Council (NRC) (2010) The impact of genetically engineered crops on farm sustainability in the United States. The National Academies Press, Washington, DC

Qaim M, Kouser S (2013) Genetically modified crops and food security. PLoS One 8(6):e64879. doi:10.1371/journal.pone.0064879

Shelton AM, Fuchs M, Shotkoski FA (2008) Transgenic vegetables and fruits for control of insects and insect-vectored pathogens. In: Romeis J, Shelton AM, Kennedy GG (eds) Integration of insect-resistant genetically modified crops within IPM programs. Springer, Dordrecht

Shelton AM, Olmstead DL, Burkness EC, Hutchison WD, Dively G, Welty C, Sparks AN (2013) Multi-state trials of *B.t.* sweet corn varieties for control of the corn earworm. J Econ Entomol 106:2151–2159

Tabashnik BE, Brevault T, Carriere Y (2013) Insect resistance to *B.t.* crops: lessons from the first billion acres. Nat Biotechnol 31:510–521

Wu KM, Lu YH, Feng HQ, Jiang YY, Zhao JZ (2008) Suppression of cotton bollworm in multiple crops in China in areas with *B.t.*-toxin-containing cotton. Science 321:1676–1678

# Chapter 11
# Effects of GM Crops on Non-target Organisms

**Steven E. Naranjo**

**Abstract** Genetically modified (GM) crops have now been part of the agricultural landscape for 17 years and have become important tools in crop production and Integrated Pest Management (IPM) in many countries. There has been considerable research addressing many associated issues including environmental and food safety, as well as economic and social impacts. In particular, extensive laboratory and field research has been generated relative to the assessment of non-target effect in transgenic Bt crops that produce the insecticidal proteins of a ubiquitous bacterium, *Bacillus thuringeinsis*. This body of evidence and the quantitative and qualitative syntheses of the data through meta-analysis and other compilations generally indicate a lack of direct impacts of Bt crops on non-target macro-invertebrates. The data also clearly show that Bt crops are much safer to non-target organisms than the alternative use of traditional insecticides for control of the pests targeted by the Bt proteins. Some indirect effects on arthropod natural enemies associated with reduced abundance or quality of Bt target herbivores have been shown, but the ramifications of these effects are unclear. As one tactic in the IPM toolbox, Bt crops have contributed to large reductions in insecticide use. While reduced insecticide use may be involved in precipitating new pest problems in Bt crops it also has broadened opportunities for deployment of another IPM tactic, biological control.

**Keywords** Transgenic Bt crops • Risk assessment • Meta-analysis • Ecological guilds • Biological control

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A. Ricroch et al. (eds.), *Plant Biotechnology: Experience and Future Prospects*, DOI 10.1007/978-3-319-06892-3_11,

## 11.1 Introduction

Genetically modified (GM) crops have now been part of the agricultural landscape for 17 years and their geographic scope and trait breadth continues to advance. By 2012, over 170 million hectares of GM crops were cultivated in 28 countries, with 20 of these developing nations. The primary GM crops currently under cultivation involve those that have been engineered to either display tolerance to several broad-spectrum herbicides or selective resistance to specific insect pest groups. GM cotton and maize often include varieties that offer both traits. Major GM crops include soybean, maize, cotton and canola (oilseed rape), grown in many adopting countries, with much smaller plantings of herbicide-tolerant alfalfa and sugar beets, and virus-resistant papaya and other minor crops in the USA and Canada. Consistent with this 17-year adoption and implementation, there has been considerable research addressing many associated issues including environmental and food safety, economic and social impacts, and effects on crop production and protection. The potential negative effects of GM crop technology has been perhaps most visible and controversial in the areas of environmental and food safety.

### *11.1.1 Bt Crops*

Insect resistant crops that produce one or more of the crystal proteins of a ubiquitous gram-positive bacterium, *Bacillus thuringiensis* (Bt), comprise about 41 % of all GM crops produced globally. The insecticidal properties of this bacterium have been known for more than 100 years and commercial products based on this organism have been available since the 1940s. Bt spray products currently occupy >90 % of the bio-pesticide market and are an important tool for pest control, particularly in organic farming. Bt cotton and Bt maize are the dominant forms of transgenic, insect-pest control technology at present, although Bt soybean was approved for production in Brazil in 2012 and several countries are evaluating Bt rice for potential production in the near future. Bt eggplant was initially granted approval for cultivation in India in 2009, but a governmental moratorium was imposed shortly thereafter citing the need for more testing and evaluation. Recently, Bt eggplant was approved for cultivation in Bangladesh.

### *11.1.2 The IPM Context*

The breadth and scope of GM crop technology is undeniably large on the world stage as are the potential solutions they contribute to agriculture in the face of a rapidly growing human population. However, it is important to keep their role in focus when thinking about crop productivity and crop protection, especially with

Bt crops. Whether one considers Bt crops to be a form of host plant resistance or alternatively a convenient method for the delivery of an insecticide, they represent only a single tactic within the integrated pest management (IPM) toolbox. Effective and sustainable crop protection must include multiple tactics that are carefully integrated to manage multiple pests within agricultural landscapes. Nonetheless, some global compilations based on the adoption of Bt cotton and maize suggests that they have contributed significantly to economic and environmental gains. For example, Brookes and Barfoot (2012) estimate that Bt cotton and Bt maize have increased global farm level incomes by $24.4 M and $14.1 M, respectively, from the period 1996–2010. In part, this has been accomplished by large reductions in the use of insecticides (170.5 Mkg and 42.9 Mkg of active ingredient in Bt cotton and maize, respectively, over this same period). These insecticide reductions have paid dividends in some systems by facilitating improved biological control of non-target pests (see Fleischer et al., this volume (Chap. 10)). In contrast, the nearly ubiquitous use of insecticide seed treatments for maize (Bt and non-Bt) in countries like the USA have reversed some of the gains in insecticide reduction afforded by the elimination of treatments for Bt targeted pests. Recent problems with resistance of corn rootworms to Bt maize may also erase some of these gains in insecticide reduction for this crop.

### *11.1.3 What Is a Non-target Organism?*

The focus of this chapter is to consider what we know about the effects of GM crops on non-target organisms. What is a non-target organism? Very simply, a non-target organism is broadly defined as any organism that the transgenic technology was not intended to control. Given that the intended targets of Bt crops are quite narrow, for example, several species of corn rootworm beetles (*Diabrotica* spp.) for Cry3 Bt maize, and several dozen species of caterpillars (various bollworms, defoliators and stalk borers) for Cry1 and Cry2 Bt maize and cotton, the list of non-targets is potentially quite extensive. In Bt crops, non-targets include other arthropod crop pests that are not susceptible to Bt proteins and a wide range of organisms, many of which provide important ecosystem services such as biological control, pollination and decomposition. Much of the research focus has been placed on arthropods and other invertebrates, but some attention has been placed on vertebrates and it is common for regulatory agencies to require testing on a wide range of organisms including birds, mammals, fish and multiple invertebrate groups as part of the registration process for Bt crops. For instance, the US-Environmental Protection Agency considers Bt engineered into crops to be plant-incorporated-protectants (so-called PIPs), and subjects such crops to a process similar to that required for pesticides. This process often involves the use of surrogate species in a tiered testing system (see below) starting with laboratory experiments under worst-case exposure conditions but is increasingly emphasizing more extensive evaluations on non-target organisms in crop fields.

This chapter will focus primarily on the effects of Bt crops on non-target arthropods. These organisms are often among the most abundant and important residents of agricultural fields where they serve a wide variety of ecosystem functions and represent a significant portion of agroecosystem biodiversity. The focus here on insect resistant Bt crops stems from the fact that much of the non-target research conducted has focused on these crops. It is recognized that Bt proteins engineered into crops represent a different risk to non-target organisms and overall biodiversity than Bt proteins applied as foliar spray treatments. For example, Bt proteins are continually produced in Bt crop plants and these proteins are protected from the environmental degradation that would occur in sprayable products applied to the plant surface. To date, well over 500 scientific studies have been completed to assess effects of Bt crops on non-target invertebrates in both the laboratory and in the field. A compilation of these data for meta-analyses in 2008 (Naranjo 2009) showed the wide coverage, including 2 dozen individual and pyramided (2 proteins) Bt proteins, 8 Bt crops in 20 countries, and over 300 species in 3 Phyla (Arthropoda, Annelida, Mollusca) examined in laboratory and/or field studies. These collective data have been the subject of dozens of review articles. The data have also been used in more quantitative synthetic studies called meta-analyses, which is simply a way to enhance the rigor and power of testing for non-target effects by statistically combining the results of multiple studies (see Naranjo 2009). A summary of these meta-analyses will be presented and discussed. For coverage of the other environmental risk issues associated with GM crops, the reader is directed to recent reviews on gene flow (Chandler and Dunwell 2008) and soil ecosystem effects (Icoz and Stotzky 2008).

## 11.2 Effects on Non-target Organisms

### *11.2.1 How to Characterize Risk*

Debate continues over the most appropriate approach for assessing risk to non-target organisms in transgenic crops. In the scientific literature and popular press readers will find reference to essentially two competing approaches for assessing risk, the ecological method and the ecotoxicological method. Briefly, the ecological model may employ a tiered approach, but focuses on representative species belonging to functional groups that have both ecological and anthropocentric relevance, and attempts to measure longer-term fitness parameters based on potential routes of exposure to toxins. It also focuses more on trying to understand complex ecological interactions. The ecotoxicology model, which is most often associated with the tiered system used by many regulatory bodies, focuses more on acute toxicity in a similar manner to testing of pesticides. Tier testing involves directly exposing the non-target organism to doses of the toxin that are often 10× or greater than the concentration that would be found in the environment. The goal is to establish the potential for harm toxicologically. Higher tier testing (more realistic exposure, semi-field or field) would only proceed if harm was demonstrated in this first tier or if results were inconclusive. Recently, Romeis et al. (2008) brought together scientists and regulators from Europe and the USA to develop a process for risk assessment that capitalizes on the positive elements of both ecotoxicological and ecological approaches. Their framework seeks to characterize risk sufficiently, but not unduly hamper the introduction of important pest management technologies. The approach uses a problem formulation process to assess current knowledge and identify areas of concern or uncertainty. Through this, risk hypotheses are developed and subsequently tested. As in conventional tier testing, escalation through more complex and realistic tiers only proceeds if the null hypothesis of no risk is rejected or other uncertainties exist. Important functional groups of non-target organisms are the focus, but representative surrogate species are used as needed to aid practicality. It also is critical that studies that are conducted in support of risk assessment are rigorous and can meet minimum quality standards (Romeis et al. 2013). In the end, the ecological and ecotoxicological approaches are more alike than dissimilar with both recognizing the need for a tiered approach and both accepting the need for practicality in implementation. Regardless of the process it is ultimately up to decision-making bodies to determine the balance of risks and benefits to society as a whole.

### 11.2.2 General Findings

Although the topic area of non-target effects of Bt crops has enjoyed its share of controversy and debate, the extant body of research generally supports the conclusion that these crops have minimal negative effects on non-target organisms and certainly less impact that the alternative use of insecticides to control the same target pests. Three broad meta-analyses have so far been completed beginning with Marvier et al. (2007). Based on funding from the US-EPA, this group developed the first database that attempted to compile the global English-language published research on the effects of Bt crops and Bt proteins on non-target organisms (primarily Arthropods but also including Annelida and Mollusca) in 2005. The database included studies conducted in both the laboratory and the field, although the Marvier et al. (2007) study examined only field studies. Their analyses showed that the abundance of all non-target invertebrates combined was slightly lower in Bt maize and cotton compared with non-Bt crops, but that abundances were much higher in Bt crops compared with non-Bt crops that had been treated with insecticides to suppress Bt targeted pests. They further concluded that taxonomic affiliation did not alter these general findings and that it was unclear if the observed reductions of abundance in Bt crops were due to direct toxicity or indirect effects causes by lowered target prey/host availability in the case of natural enemies.

Several subsequent and more detailed meta-analyses were conducted, including Naranjo (2009), the most recent and based on updated literature through 2008.

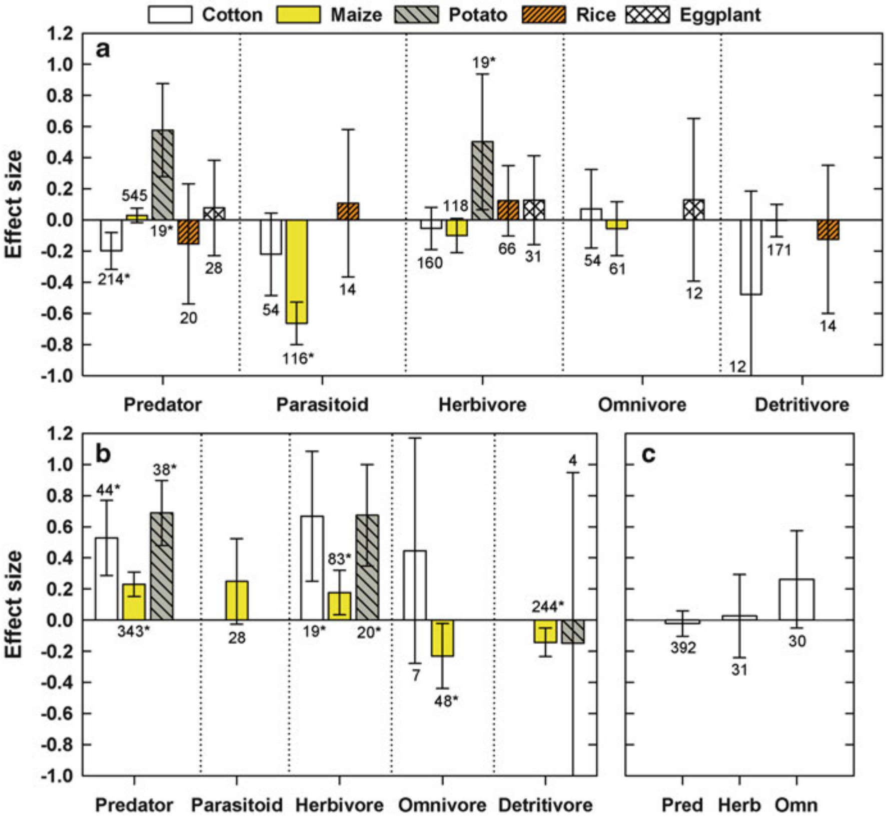

**Fig. 11.1** Meta-analyses of field studies that examined the comparative abundance of non-target invertebrates in Bt and non-Bt crops. Meta-analysis quantitatively combines the results of multiple studies using a metric called the effect size that takes into account the variability, sample sizes and the magnitude of differences in individual comparative studies. The data are plotted such that a negative effect size denotes a lower abundance in the Bt crop compared with the non-Bt crop; a positive effect size denotes the opposite. Here the data are parsed into ecological guilds that represent different ecosystem functions. (**a**) Neither the Bt or non-Bt crop received any insecticide treatments. These analyses test the hypothesis that the Bt protein or any other differences in the Bt plant affected non-target abundance either directly or indirectly. (**b**) Here the non-Bt crop was sprayed with insecticide and these analyses test the hypothesis that the method used to control the Bt targeted pest affects non-target abundance. (**c**) Finally, both the Bt and non-Bt crop are treated with insecticides to control both target and non-target pests and these analyses test the realistic hypothesis that management of pests in both Bt and non-Bt crops affect non-target abundance. The numbers above or below the bars denote sample size and the asterisks denote statistical significance of the effect size, i.e. significantly lower or higher than zero (Figure reproduced from Naranjo (2009) with permission from CABI)

Results from field studies based on this recent meta-analysis are summarized in Fig. 11.1. In general, the analyses found that predator abundance was slightly reduced in Bt cotton compared with non-Bt cotton without any insecticide use (Fig. 11.1a). This pattern was largely driven by the reduced abundance of one group of predators (Nabids) that tend to prey on caterpillars targeted by the Bt crop. The elimination of Nabids in the analysis resulted in equal abundance of predators overall in Bt and non-Bt crops. Laboratory studies have shown that one species of Nabid was unaffected after ingesting Bt proteins. Thus, this reduction in abundance is likely an indirect ecological effect and not a direct toxicological one. Analyses also showed that parasitoids as a group were much less abundant in Bt maize compared with non-Bt maize when both were untreated with insecticides. This pattern was found to be entirely due to reduced populations of a specialist exotic parasitoid that attacks the European corn borer, the primary target of Bt maize in the USA. This provides another example of an indirect ecological effect – the parasitoid requires its host. It should be noted that in both of these examples of indirect ecological effects, any tactic that lowers the Bt targeted pest (the goal of management) would likely result in the same reduction in specific natural enemies. Another result of note is the effect of Bt potato on predators and herbivores (Fig. 11.1a). Here, the abundance of both groups was higher in the Bt crop. This is yet another example of an indirect ecological effect in which higher herbivore populations in Bt potato, primarily sucking insects (insects that feed by inserting their straw-like mouthparts into plant parts) led to a corresponding increase in predators responding to higher prey availability. The reason for increased sucking insect populations has not been studied but it could be related to the lack of induced plant defenses in Bt potato when its primary targeted defoliator is controlled, or the lack of collateral control previously provided by insecticides (see Non-target Pests below). Other functional guilds (herbivores, omnivores and detritivores) were unaffected in Bt maize, cotton or potato in comparison with untreated non-Bt controls. For Bt rice and eggplant, where many fewer studies have been conducted, there was no indication of effects of Bt crops on any ecological guild (Fig. 11.1a).

Most ecological guilds were more abundant in Bt maize, cotton and potato when the comparative non-Bt crop was treated with a variety of insecticides for control of target pests (Fig. 11.1b). The results for detritivores in Bt maize provides another example of indirect ecological effects wherein springtails, the primary detritivores in the system, were released from control by soil dwelling predatory beetles when insecticides were applied. When insecticides are used in both the Bt and non-Bt crops, a common situation in cotton, which harbors multiple pests, the abundance of the ecological guilds available for analyses were the same in both crops. While different pest complexes would have been targeted in Bt and non-Bt cotton, both systems currently rely on relatively broad-spectrum insecticides for non-target pest control, albeit generally fewer applications are needed for the Bt crop (Fig. 11.1c).

Finally, meta-analyses were conducted to examine the relationship between laboratory and field studies. As noted above, many agencies that regulate GM crops used a tiered system to test for safety and assess risk. Very often, field studies are conducted regardless of the outcome of early tier testing, specifically by academic and public research organizations. Field studies are also increasingly being requested of industry by regulatory authorities as part of the registration process. Thus, there are robust datasets from both the laboratory and the field that allows a way to test the validity of the tier system. One such study found that “laboratory studies of transgenic insecticidal crops show effects that are either consistent with, or more conservative than, those found in field studies” (Duan et al. 2010). These findings suggest that the tier system can function to identify harm or the lack thereof in the environment.

### *11.2.3 Non-target Pests*

The unique physiological effect of Bt proteins currently found in GM crops, a characteristic governed by the specific receptors and conditions in a caterpillar’s or beetle’s gut allowing activation of the Bt proteins, limits their activity to relatively few arthropod pests of crops. Thus, there are often a wide range of other insect and mite pests not affected by Bt crops, particularly in long season crops like cotton grown in lower latitudes. Many of these pest species are managed much as they were before the advent of Bt crops and represent an equal threat to Bt as well as conventional non-Bt crops. It is these pests that force a greater focus on the principles of IPM, which calls for a suite of integrated tactics to provide effective crop protection.

In general, meta-analyses of field abundance studies in Bt cotton, maize, rice and eggplant shows that non-target herbivores, that would include non-target pests are no more abundant in Bt crops compared with non-Bt crops when no insecticides are used (Fig. 11.1a). Thus, it does not appear there is anything specific about Bt crops themselves that would alter herbivore communities. This conclusion is further supported by meta-analyses of laboratory studies, which clearly show a lack of toxicity of Bt crops to non-target pests (Naranjo 2009). Alternatively, when insecticides are used in non-Bt crops and then compared to untreated Bt crops, then herbivores, again including non-target pests are considerably more abundant in Bt crops (Fig. 11.1b). An analysis focused just on non-target pests showed the same result (Naranjo 2009). This does not mean that all these pests are necessarily more problematic in Bt crops but that additional management is required to suppress their numbers as discussed above.

However, some non-target pests (in this case secondary or induced pests) have become more problematic in Bt crops in some production systems. Some of the most visible examples have arisen within sucking insects. Some examples include plant bugs in China, Australia, and the USA in cotton. One of the most well known cases comes from China, where multiple species of plant bugs have become more pestiferous in cotton but also in a number of other crops cultivated in the same region (see Fleischer et al., this volume (Chap. 10)). Both plant bugs and stink bugs also have risen in importance as pests of cotton in parts of the mid-southern and southeastern USA. The causes for these increases are not completely known, but in some areas like China and the USA, the problem appears to be ironically associated with the general reduction in broad-spectrum insecticides that were once used to manage caterpillar pests now effectively controlled by Bt cotton. These insecticides would often provide collateral control of these non-target pests. Similarly, in Australia it is thought that reduced insecticide use for bollworms has allowed plant bugs, stink bugs, leafhoppers and thrips to become more prominent. Sprays now applied to these pests have in turn disrupted a complex of natural enemies and lead to secondary outbreaks of pests such as spider mites, aphids and whiteflies. Growers in India are facing similar issues with mealy bugs, thrips and leafhoppers.

The increased emergence of non-target pests in Bt maize has been relatively minor in comparison to the situation with Bt cotton. Bean cutworm (a caterpillar but not sensitive to single gene Cry1Ab Bt maize) has been less problematic with the introduction of pyramided cultivars producing at least two Bt proteins. In part, the increased intensity of this pest is also thought to have been associated with the increase adoption of no-till production systems afforded by herbicide tolerant maize. Issues with minor pests like wireworms and grubs (beetles) have been addressed mainly through insecticide-treated seed, a now common practice in the USA.

As noted, the rising importance of some non-target pests in Bt crops is likely associated with the large reductions in insecticides previously applied to control Bt targeted pests. The fact that reduced insecticides and associated conservation of natural enemies did not enhance control of these non-target pests suggests that biological control does not strongly operate for these pests. The induction of natural plant defenses is another factor that may play a role. Both maize and cotton are known to produce defensive compounds in response to certain types of herbivory. For instance, it is well known for maize and cotton that caterpillar feeding leads to the release of volatile compounds that act as attractants for natural enemies (parasitoids in particular), thus facilitating biological control. In addition to such volatile signaling, herbivory, particularly by chewing herbivores, also induces plants to produce defensive compounds that can have negative effects on other herbivores feeding on the plant. In Bt crops this induction by chewing herbivores (caterpillars) is lessened significantly. A recent study in cotton showed that a group of chemicals called terpenoids have lower levels of induction in Bt cotton compared with non-Bt cotton in the presence of caterpillar feeding. This difference allows better survival and growth in other pests such as aphids in Bt cotton. Reduced competition from target pests also may play a role and allow non-target pests to perform better in Bt crops.

### 11.2.4 Valued Non-target Organisms

While all non-target organisms could be considered valuable for multiple reasons, there are several groups that hold special significance because of the way they are valued by the general public. Such groups include pollinators (e.g., honey bees), charismatic butterflies (e.g., Monarchs) and moths of special economic value (e.g., silk moths). Natural enemies that provide biological control services would also fall into this group, but they will be discussed separately below due to the key roles they potentially play in crop protection. One charismatic insect came to represent the debate about the safety of GM crops more than all others, the Monarch butterfly. This species is a well-known resident of North America but also found in many other parts of the world due to its ability to move long distances. In 1999, a study in the prominent science journal Nature suggested that pollen and anthers from an unidentified event of Bt maize could cause larval mortality when applied in large quantities to the surface of the butterfly's milkweed host plant. In addition to lots of popular press coverage, this study also precipitated a large research effort by multiple scientific groups in the mid-western USA to examine many aspects of this issue. Data from these studies and others was then used to construct a robust risk assessment that took into account many variables including factors relative to hazard (toxicity) and exposure. Ultimately, hazard was found to be low, especially with the primary Bt maize events under production that contained very little Bt in their pollen. This coupled with the very low potential for exposure (timing and extent of pollen dispersal from corn, proportion of Bt maize in the butterfly's breeding habitat, etc.) to the toxin in the butterfly's habitat ultimately led to a conclusion of negligible risk in the field. Interestingly, this was the same conclusion reached by the US-EPA during the registration process prior to 1996. The susceptibility of the Monarch to Bt proteins was never doubted given its taxonomic affinity with target caterpillars and a meta-analysis of laboratory studies on Monarch and other valued Lepidoptera showed this to be true (Naranjo 2009). The larger focus today is on the widespread use of glyphosate and other herbicides on herbicide-tolerant maize leading to potential destruction of the butterfly's host plant within and bordering maize fields.

Pollinators are another important non-target group, and awareness has been heightened even more with the current issues surrounding declining honey bee health and colony collapse. A meta-analysis based on 25 laboratory studies showed that survival of neither adult or larval stage honey bees was affected by Bt proteins targeting either caterpillar or beetle pests. An independent meta-analysis that included honey bees as well as bumble bees reached the same conclusion based on both survival and development in the laboratory (Naranjo 2009). Relatively few field studies have examined pollinators in general, but laboratory studies on a few species of bees indicate a lack of hazard from the current suite of commercial Bt proteins.

#### 11.2.4.1 Non-target Effects on Arthropod Natural Enemies

Arthropod natural enemies represent another valuable group of organisms that require consideration in assessing risks from GM crops. They can potentially provide biological control services critical to controlling target and non-target pests, may help to ameliorate the evolution of resistance to Bt crops, and represent important members of communities in natural and managed habitats in general. Due to their importance there has been considerable research in assessing the impact of Bt crops on biological traits (e.g., survival, development, reproduction), abundance, and to a more limited degree, biological control function. Species of arthropod predators and parasitoids can potentially be exposed to Bt proteins through at least two routes. First, most natural enemies feed on plants or plant products such as nectar and pollen; some species such as lacewings and hover flies feed exclusively on nectar and pollen (and often insect honeydew) as adults. Such an exposure route is frequently called bi-trophic—plant to natural enemy. Secondly, they can be

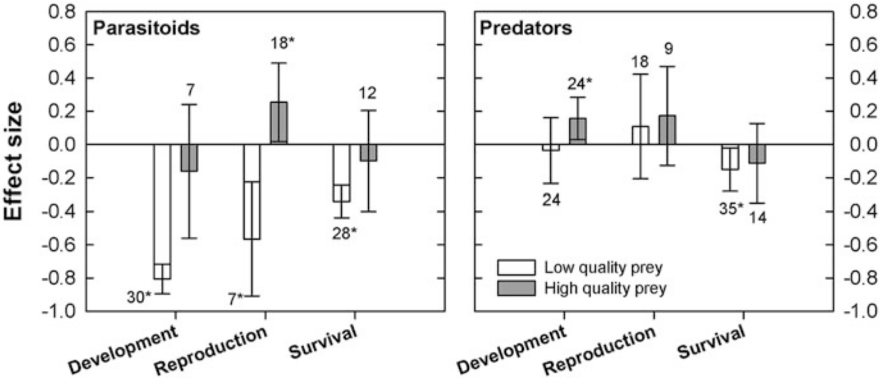

**Fig. 11.2** Meta-analyses of laboratory studies that examined the non-target effects of Bt crops on arthropod natural enemies exposed to Bt proteins via their prey or host that had fed on Bt plants or diets (tri-trophic exposure). Bt susceptible prey often suffer sub-lethal effects that degrade their quality as food for natural enemies (low quality prey) while non-susceptible or resistant prey are normal (high quality prey). The data are plotted such that a negative effect size would denote a negative impact on performance in the Bt crop compared with the non-Bt crop; a positive effect size denotes the opposite. The numbers above or below the bars denote sample size and the asterisks denote statistical significance of the effect size, i.e. significantly lower or higher than zero (Figure reproduced from Naranjo (2009) with permission from CABI)

exposed to Bt proteins through their prey, which have feed directly on the plant. This route is referred to as tri-trophic—plant to prey to natural enemy. Many studies have examined both pathways in a number of species in the laboratory. Meta-analyses have shown that bi-trophic exposure, or direct feeding on either the plant or Bt containing artificial diets (e.g., honey water) has no effect on important biological parameters such as development/growth, survival or reproduction (Naranjo 2009).

Interpreting the results from exposure studies examining tri-trophic interactions, or feeding on prey that have ingested Bt proteins, has been more problematic. An issue that has not always been factored into the interpretation of study results is that prey that are susceptible to Bt proteins (e.g., target caterpillars) are frequently affected by this feeding even if they do not die from the exposure. Those that survive are typically smaller and grow slower, a sign of sublethal effects from the Bt protein. Natural enemies that in turn use these compromised preys often suffer as well. The question of whether this is a direct or an indirect effect of the Bt protein is important but sometimes muddled. In order to establish that effects are direct, i.e., toxicological, it is necessary to control for prey quality effects. Two approaches have been used including the use of prey that are not susceptible to Bt proteins because they are unrelated taxonomically to the target insects, or the use of target insects that have been selected to be resistant to Bt proteins. Both of these strategies have been used effectively to eliminate prey quality effects and enable a clear testing of direct effects of Bt proteins. Meta-analyses have compared studies where prey quality effects were apparent or were eliminated using non-susceptible or resistant prey (Fig. 11.2, Naranjo 2009). Analyses show that the use of susceptible prey results in slower development and reduced reproduction and survival in parasitoids and lower survival in predators. If the effects of prey quality are removed by using non-susceptible or Bt resistant prey then these parameters were either not affected or were affected positively (i.e., better performance on prey containing Bt proteins). These results demonstrate that Bt proteins do not by themselves have any toxicological effects on the arthropod natural enemies so far examined. However, much like the field based results discussed above there are indirect effects because biological attributes can be negatively affected if natural enemies use compromised prey. The impacts of these indirect effects in the environment are not clear and they are not limited to cases in which Bt crops are being deployed. Any tactic that affects the target prey (previous parasitism, insecticides, other host plant resistance factors, etc.) would likely yield the same indirect effect on the associated natural enemy. It is also unclear if such effects would have any ramification for the services provided by natural enemy populations as will be discussed below.

Issues with differing interpretations of data from tri-trophic studies have created debates in the scientific community. One of the most widely known cases involves the green lacewing, a common and important predator found in many cropping systems. In the late 1990s a group showed that certain biological attributes of green lacewing larvae were negatively affected when feeding on caterpillar prey that have been exposed to certain Bt proteins. They also showed that bi-trophic exposure routes resulted in negative biological effects. Numerous issues with experimental design were identified in these studies but work conducted in the same laboratory and many others since this initial report have failed to duplicate any of these direct negative findings for several Bt proteins. Another more recent debate involved a laboratory-based meta-analysis (Lövei et al. 2009) that reported direct negative effects on arthropod predators and parasitoid by various Bt proteins. This result was surprising and not consistent with many other reviews and meta-analyses, including those discussed here. The rebuttal identified a number of statistical and logical issues with the Lövei et al. (2009) study but one of the overriding factors was that these authors failed to account for prey quality issues when examining tri-trophic studies (Shelton et al. 2009). The data presented in Fig. 11.2 shows how different the results can be when prey-mediated effects are not taken into account.

#### 11.2.4.2 Effects on Biological Control Function

The impacts of Bt crops on arthropod natural enemies have already been discussed (see Fig. 11.1). Cases where abundance was reduced were associated with indirect ecological effects such as prey scarcity or possibly with the indirect effects resulting from preying on compromised, Bt susceptible prey. While measures of abundance and general biodiversity are a simple means to gauge non-target effects in the field, the more critical question for natural enemies is whether or not the biological control services they provide have been compromised. Compared with abundance studies relatively few studies have examined some measure of function. Such studies have used a variety of techniques including simple measures of parasitism from field samples, and measurement of predation or parasitism rates on prey artificially placed in the field, to more comprehensive life tables quantifying predation and parasitism rates on natural prey populations. Except for a few cases in which parasitism by specialist parasitoids attacking target pests have been reduced, there is no evidence that biological control capacity differs between Bt and non-Bt crop fields. Even in cases were natural enemies might be less abundant in the Bt crop there is no evidence that biological control services are reduced. For example, a long-term study in Bt cotton showed that a group of five common predators were reduced by about 20 % in the Bt crop, but rates of predation on a key whitefly pest remained unchanged compared to the non-Bt crop. Overall, opportunities for enhanced biological control in Bt crops have been demonstrated in several systems (see Fleischer et al., this volume (Chap. 10)).

## 11.3 Conclusions

GM crops have become important tools in crop production and protection in many countries and contribute significantly to overall IPM programs. Extensive laboratory and field data have been generated relative to the assessment of ecological risk in these crops, particularly for non-target organisms in Bt crops. This body of evidence and the quantitative and qualitative syntheses of the data through meta-analysis and other compilations generally indicate a lack of direct impacts of Bt crops and the insecticidal proteins they produce on non-target invertebrates. The data also clearly show that Bt crops are much better than the alternative use of traditional insecticides for control of the pests targeted by Bt crops. Some indirect effects on natural enemies associated with reduced abundance or quality of Bt target herbivores have been shown, but the ramifications of these effects are unclear. As one tactic in the IPM toolbox, Bt crops have had a profound effect on insecticide use patterns. While reduced insecticide use may be involved in precipitating new pest problems in Bt crops it also has broadened opportunities for deployment of another IPM tactic, biological control.

**Acknowledgements** I thank J. Romeis and R. Hellmich for their reviews of my manuscript.

## References

Brookes G, Barfoot P (2012) GM crops: global socio-economic and environmental impacts 1996–2010. PG Economics Ltd., Dorchester, 187 pp

Chandler S, Dunwell JM (2008) Gene flow, risk assessment and the environmental release of transgenic plants. Crit Rev Plant Sci 27:25–49

Duan JJ, Lundgren JG, Naranjo SE, Marvier M (2010) Extrapolating non-target risk of Bt crops from laboratory to field. Biol Lett 6:74–77

Icoz I, Stotzky G (2008) Fate and effects of insect-resistant Bt crops in soil ecosystems. Soil Biol Biochem 40:559–586

Lövei GL, Andow DA, Arpaia S (2009) Transgenic insecticidal crops and natural enemies: a detailed review of laboratory studies. Environ Entomol 38:293–306

Marvier M, McCreedy C, Regetz J, Kareiva P (2007) A meta-analysis of effects of Bt cotton and maize on nontarget invertebrates. Science 316:1475–1477

Naranjo SE (2009) Impacts of Bt crops on non-target organisms and insecticide use patterns. CAB reviews: perspectives in agriculture, veterinary science, nutrition and natural resources 4, No. 011. doi:10.1079/PAVSNNR20094011

Romeis J, Bartsch D, Bigler F, Candolfi MP, Gielkens MMC, Hartley SE, Hellmich RL, Huesing JE, Jepson PC, Layton R, Quemada H, Raybould A, Rose RI, Schiemann J, Sears MK, Shelton AM, Sweet J, Vaituzis Z, Wolt JD (2008) Assessment of risk of insect-resistant transgenic crops to nontarget arthropods. Nat Biotechnol 26:203–208

Romeis J, McLean M, Shelton A (2013) When bad science makes good headlines: Bt maize and regulatory bans. Nat Biotechnol 31:386–387

Shelton AM, Naranjo SE, Romeis J, Hellmich RL, Wolt JD, Federici BA, Albajes R, Bigler F, Burgess EPJ, Dively GP, Gatehouse AMR, Malone LA, Roush R, Sears M, Sehnal F, Ferry N, Bell HA (2009) Appropriate analytical methods are necessary to assess nontarget effects of insecticidal proteins in Gm crops through meta-analysis (Response to Andow et al. 2009). Environ Entomol 38:1533–1538

# Chapter 12
# Herbicide-Resistant Crop Biotechnology: Potential and Pitfalls

**J. Franklin Egan**

**Abstract** Herbicide-resistant crops are an important agricultural biotechnology that can enable farmers to effectively control weeds without harming their crops. Glyphosate-resistant (i.e. Roundup Ready) crops have been the most commercially successful varieties of herbicide-resistant crops and have been planted on millions of hectares globally. These crops have brought substantial benefits including greater flexibility and efficiency in weed management operations, reduced environmental pollution from herbicides, and increased adoption of conservation tillage or no-tillage cropping systems. At the same time, an over reliance on the herbicide glyphosate has led to extensive problems with weed species that have evolved resistance to this herbicide. The commercial success of herbicide-resistant crops has also been correlated with consolidation in the agrichemical and seed industries, with declining investment in public agriculture research and extension, and with increasing farm size and specialization. These three interrelated socio-economic trends have made it increasingly difficult for farmers to counter herbicide-resistant weed challenges with integrated weed management practices that combine chemical, mechanical, and cultural techniques. Experience thus far therefore demonstrates that while herbicide-resistant crops can have substantial benefits for agriculture and the environment, it will be difficult to embrace this technology without foreclosing other options for sustainable weed management.

**Keywords** Weeds • Herbicide-resistance • Glyphosate • Conservation-tillage • Integrated weed management

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A. Ricroch et al. (eds.), *Plant Biotechnology: Experience and Future Prospects*, DOI 10.1007/978-3-319-06892-3_12,

## 12.1 Weed Management and Herbicide-Resistant Crops in Context

Weeds can be broadly defined as plants that interfere with human activity, and they are a ubiquitous part of all agricultural systems. In any given region, agriculture begins when humans clear away mature established vegetation like forests or grasslands to make space for growing crops. Globally, an impressive diversity of fast-growing plant species have evolved to disperse into such disturbed areas and compete with crop plants for newly available light, water, and soil nutrient resources. Because of this resource competition, weeds can drastically reduce crop yield and quality, and they must be managed to maintain agricultural productivity. Once established, weeds can persist in crop fields through seeds or vegetative propagules (e.g. tissues such as tubers, rhizomes, and stolons) for many years. Because the majority of the human food supply is based on staple crops like cereals, pulses, and tubers that develop over an annual life cycle, this pattern of weed growth and weed management must be repeated year after year, over millions of hectares of cropland globally. Much of the energy, labor, and environmental costs involved in agricultural systems are therefore somehow related to weed management.

Over the centuries, agriculturalists have continually developed new approaches and technologies for controlling weeds. Metal implements including plows and cultivators can be pulled across the soil to prevent weed seeds from emerging or physically dislodge growing plants. Different crops can be rotated from year to year to disrupt the timing of weed life cycles and prevent problematic species from becoming dominant. Beginning in the 1940s, compounds specifically toxic to plants—herbicides—were discovered that could be applied to soils and leaf surfaces to kill growing or emerging weeds. Herbicides have proven to be a powerful weed control approach, but until relatively recently, this technology was limited by the fact that many weed species closely resemble crop species taxonomically, biochemically, and ecologically. Therefore it could be difficult for farmers to find an herbicide that would effectively control their weeds without also killing or significantly injuring their crops. Herbicides could still be an important tool, but farmers were tasked to carefully match a precise combination of herbicide active ingredient and timing of application such that they could control weeds without also harming the crop. Often, this meant that at least a few weed species would escape control, such that multiple combinations of different herbicides, mechanical tools, and cultural practices were all necessary to successfully control weeds.

The development of herbicide-resistant (HR) crops has significantly relaxed these constraints. Most herbicides work by binding to specific enzymes that play a key role in the metabolism of healthy plant cells and thereby disrupting the normal functions of that enzyme. Various plant breeding techniques have been used to generate HR crops that possess genes that encode either a new version of the target enzyme that is insensitive to the herbicide or novel enzymes that are capable of metabolizing the herbicide (Green and Owen 2011). HR crops have been produced using mutagenesis approaches, in which a chemical mutagen is used to produce large numbers of spontaneous mutations in a collection of pollen, seeds, or other plant tissue, and this collection is then screened for individuals that happen to possess new resistant traits. HR crops can also be produced through genetic-engineering techniques. In this approach, scientists look for naturally occurring microbes that are insensitive to an herbicide, isolate DNA sequences in the microbial genome that encode an insensitive target enzyme or herbicide-degrading enzymes, and then transfer these DNA sequences into the genome of a crop plant. Numerous HR crops have been developed to date, including imidazolinone-resistant corn, canola, wheat, rice, and sunflower (through mutagenesis techniques), glufosinate-resistant corn, cotton and canola (through genetic engineering techniques), and glyphosate-resistant varieties in corn, cotton, canola, soybean, sugarbeet, and alfalfa (also through genetic engineering techniques).

## 12.2 Potential

Of these, glyphosate-resistant (GR) crops have been by far the most successful (Dill et al. 2008), and they have even been described as the most rapidly adopted technology in the history of agriculture. The Monsanto Company first commercialized GR soybean in the US in 1996, and by 2000 just over 54 % of the U.S. soybean crop was planted to GR varieties. GR corn and cotton were subsequently released and were also adopted by growers with extreme enthusiasm. By 2012, nearly 85, 82, and 93 % of the corn, cotton, and soybean planted in the U.S. were GR, respectively (ERS 2013). Similar trajectories also occurred in the soybean industries of Brazil and Argentina, with GR adoption jumping to over 90 % in Argentina within 4 years of commercialization. Australian cotton growers and Canadian canola growers have also enthusiastically planted GR varieties. As of 2012, farmers planted genetically-engineered HR varieties of soybean, cotton, canola, sugar beet, and alfalfa on 144 million hectares globally, with the large majority of these being GR varieties (James 2012).

GR technologies have no direct impact on crop yields, but farmers embraced the technology because of marked improvements in the efficacy, consistency, and efficiency of weed management (NRC 2010). Glyphosate is a broad spectrum herbicide that is toxic to almost all weed species across many different plant families. By protecting the crop against this powerful compound, GR crops allowed farmers to control most species of weeds often with a single herbicide application. Initially, this enabled growers to dramatically cut the time, fuel, and total herbicide amounts involved in weed control. And although the GR technology came at a significant economic cost to farmers (seeds carrying GR traits are significantly more expensive than comparable non-HR varieties), many growers found that their bottom line significantly improved from savings in labor, fuel, and machinery depreciation. Because the resistance traits also broadened the time window during which growers could apply the broad-spectrum glyphosate herbicide, GR weed control programs were remarkably flexible and appeared to free farmers from factors involving weather and timing that complicate traditional herbicide and mechanical weed management practices. During the rising crest of popularity of GR crops in the U.S. in the early 2000s, agricultural weed management was probably simpler, cheaper, and easier than it had ever been before in human history.

In addition to the economic benefits captured by some farmers, many have argued that GR technology led to important progress in reducing the environmental impact of agriculture. This progress has occurred in two ways.

First, in many respects, glyphosate is environmentally a relatively benign pesticide. It has low acute toxicity to humans and most wildlife groups (other than plants), degrades relatively rapidly in the soil, and does not easily leach into ground water (Duke and Powles 2008). Thus, glyphosate substantially reduced the toxicological burden of agriculture relative to weed control systems that relied heavily on older more problematic compounds such as atrazine or 2,4-D. To this end, glyphosate accounted for only 4 % of U.S. acre treatments in 1995 but increased to 23 % by 2001 and 45 % by 2010 (Osteen and Fernandez-Cornejo 2013). Moreover, the potential to control weeds with a single application often tended to reduce the total quantity of herbicides applied to cropland. In the U.S. during the period 1996–2001, herbicide applications (in kg total active ingredient applied per hectare) decline by 15 % in corn, 12 % in cotton, and 17 % in soybean (Benbrook 2009). While part of the reductions during this period also came from the commercialization of high potency active ingredients that are applied in low amounts, especially the sulfonylurea herbicides, GR crops were an important factor.

The second benefit to the environment was related to the expansion of no-tillage or conservation tillage farming systems. Tillage involves disturbing or inverting soil surfaces using tractor-pulled implements such as a moldboard plow, a disc plow, or a chisel cultivator. Various forms of these implements have been used by farmers for centuries to mechanically control weeds or to prepare a seed bed for planting. When used in the same field season after season, tillage and cultivation can lead to serious erosion and soil quality problems. For instance, repeated moldboard plowing of fragile semi-arid soils was a primary cause of the disastrous dust storms experienced in the U.S. Central Plains during the "dust bowl" years of the 1930s. Because pulling heavy metal implements across a field requires substantial amounts of diesel fuel, mechanical weed control can also have negative implications for climate change. Moreover, disturbing soil stimulates the activity of soil microbes and can lead to a flush of carbon dioxide into the atmosphere, thereby increasing the global warming contributions of farming.

Beginning in the late 1950s farmers and scientists began developing a series of innovations that enabled farmers to raise annual crops with very little soil disturbance. Engineers developed new planting equipment that "drilled" grain seeds directly into the ground with minimal disruption of the soil surface. An expanding list of herbicide options was also key in enabling farmers to control weeds without physically disrupting the soil. However, through the early 90s conservation tillage farming spread modestly, partially because it remained difficult to apply mixtures of herbicides that would effectively control weeds without damaging crops. Thus, some combination of chemical and mechanical weed control remained essential to consistently control weeds.

With the rapid proliferation of GR resistant crop varieties, the weed management constraints on conservation tillage appeared to be eliminated, and adoption of conservation tillage rapidly increased. For instance, from 1996 to 2008, conservation tillage in the U.S. increased from 13.7 to 18.6 million hectares in soybean and from 0.6 to 1.2 million hectares in cotton (NRC 2010). Similar expansion of conservation tillage also occurred in soybean production in Argentina and Brazil and in several crops in Australia and Canada (Derpsch et al. 2010). However, farmers who embraced conservation tillage did not necessarily also plant GR varieties, and a suite of other factors besides GR technology facilitated the expansion of conservation tillage during this period. For instance, beginning with the 1986 U.S. Farm Bill, farmers who wished to receive federal crop subsidies were obligated to cross-comply with environmental guidelines for soil conservation. This policy certainly encouraged farmers to embrace conservation tillage, and indeed conservation tillage in U.S. corn began to plateau at about 13 million hectares by 1996, the same year GR technology was first commercialized (NRC 2010). Moreover, by combining soil-building practices like cover crops, judicious use of tillage, and alternative herbicides, it is also certainly possible to capture some of the soil quality benefits of conservation tillage without using GR crops. However, given the global trends that have been observed since the mid 1990s, HR technologies, and GR varieties in particular, have clearly facilitated the expansion of conservation tillage, with significant benefits for agriculture and the environment.

## 12.3 Pitfalls

Even before GR crops were widely commercialized, some scientists and environmental or agricultural advocacy groups expressed concerns about the technology that would turn out to be highly prescient. Namely, skeptics argued that over-reliance on glyphosate and GR crops would (1) lead to significant problems with herbicide-resistant weed species and (2) erode important sources of diversity and adaptability in modern agricultural systems.

Like all organisms, weedy plants can evolve and adapt to changing environmental conditions. Farmers attracted to the ease and simplicity of the GR crops applied glyphosate over large areas, year after year, often as their only weed management practice. This behavior created intense selection pressure for the evolution of new traits that conferred resistance to glyphosate in weed populations. As of 2013, globally a total of 24 weed species have evolved resistance to glyphosate. Resistant populations have appeared nearly everywhere GR crops are grown, and in the most problematic instances, resistance populations now infest millions of hectares of cropland.

Weed species have developed resistance through a variety of fascinating and often unexpected mechanisms. For instance, Palmer amaranth (*Amaranthus* *palmeri*) is one of the most economically significant weeds in cotton and soybean production in the southeastern U.S., and at one time, glyphosate was an easy and effective way to control this plant (Price et al. 2011). Glyphosate affects plants by interfering with EPSPS, an enzyme that plays an essential role in the synthesis of amino acids that are the building blocks for proteins (Duke and Powles 2008). In plant cells, the instructions on how to make the EPSPS enzyme are encoded by a specific region of DNA. Around 2004, in Georgia, U.S., a few *A. palmerii* seedlings emerged that happened to possess a random genetic mutation such that the section of DNA encoding the EPSPS enzyme had been duplicated 5–160 times (Gaines et al. 2010). This mutation enabled its cells to produce a surplus of EPSPS enzyme, such that if it absorbed a normal weed-killing dose of glyphosate, some proportion of the EPSPS enzyme might be rendered inactive but there would still be more than enough unaffected enzyme available to carry on normal metabolic activities. Relative to susceptible pigweed plants, these resistant mutants experienced a tremendous competitive advantage, and the resistance trait proliferated rapidly through Palmer amaranth populations in Georgia and throughout the southeast U.S. GR Palmer amaranth populations now infest an estimated two million hectares and have become virtually impossible to control with glyphosate.

By 2010, the widespread emergence of glyphosate resistant weeds had reversed much of the environmental and agronomic progress initially accredited to GR crop technologies. When resistant weed problems first started appearing, farmers responded by increasing the rates or number of glyphosate applications. Where this approach was not effective, growers began combining glyphosate with additional herbicides sequentially or in mixtures, often including older, more toxic active ingredients. Thus, by 2010 a promising trend of decreasing herbicide use in the U.S. had completely reversed course, with 173 million kg applied in 2010 relative to 146.2 million kg applied in 1995, the year before the commercialization of GR crops (Osteen and Fernandez-Cornejo 2013). Moreover, in areas where infestations were severe and unmanageable with available mixtures of herbicides, farmers that had previously embraced conservation tillage practices began reincorporating tillage and cultivation into their cropping systems. For instance, in cotton producing areas where glyphosate resistant Palmer amaranth had become severe, growers often found that aggressive moldboard plowing became necessary to knock back established populations (Price et al. 2011). Moldboard plowing involves cutting and fully inverting the upper 15 cm of topsoil and can be extremely damaging to the heavy clay and drought-prone soils of the southeastern U.S.

Herbicide-resistant weed problems are not unique to glyphosate and glyphosate-resistant cropping systems. Indeed, weeds have evolved resistance to nearly every type of herbicide that has been used repeatedly in a specific region for a prolonged period of time. Weed species have evolved resistance to all 21 major groups of herbicides, or herbicide modes of action, and hundreds of different resistant populations occur globally (Heap 2013). These resistant populations display a very wide array of biochemical, morphological, and ecological adaptations and represent fascinating examples of evolution by natural selection in real-time action. However, weedy plants are resilient in many ways and can even evolve adaptations that confer tolerance to mechanical or cultural management practices. For instance, in rice-paddies in Asia that are traditionally managed by small-holder farmers using hand tools, the weed early watergrass (*Echinochloa oryzoides*) has evolved to look more like rice plants, such that it may go unnoticed and avoid being dislodged by weeding. However, what is unique about glyphosate is the speed and severity with which resistant weed populations came to dominate cropping systems. The conditions for this epidemic were clearly facilitated by the deliberate coupling of a single broad-spectrum herbicide with a HR trait such that growers were encouraged to apply the same herbicide year after year across vast stretches of farmland.

In addition to new herbicide-resistant weed problems, the unprecedented popularity of GR crops has also had more systemic impacts on the diversity and adaptability of agricultural systems. Genetic diversity resources in our staple crops enable farmers in different regions to plant varieties that are optimized to local conditions and to switch to new varieties as weather and environmental challenges fluctuate. Each year since 2007, more than 90 % of the soybeans planted in the U.S. have been GR varieties (ERS 2013), with the genetics ultimately patented by the Monsanto Company. This powerful market share controlled by a single firm means that it has become increasingly difficult for farmers to find high quality soybean varieties that do not include GR traits. Effectively, U.S. soybean growers in this situation lack a meaningful choice except to plant Roundup Ready varieties, and having paid fees for the technology, encounter an incentive to also purchase and use glyphosate herbicide. To a somewhat lesser extent, a similar situation has developed in the U.S. corn, sugar beet, and cotton industries, although the nearly complete takeover of cotton crops by glyphosate-resistant Palmer amaranth has led to a rapidly increasing market share of glufosinate-resistant varieties purveyed by Bayer Crop Science. During the rising period of GR crops, Monsanto acquired and absorbed many smaller agronomic and horticultural seed companies, and increasingly the U.S. seed industry has become dominated by a few very large multinational corporations (NRC 2010). Little research has been conducted on this topic, but during this period of corporate consolidation, it is likely that the total genetic diversity present in U.S. commodity crops has dramatically bottlenecked. As growers scramble to respond and adapt to resistant weed challenges, this has left the agricultural sector with only a few seed and technology purveyors to work with for potential solutions.

The success of GR crops may have also facilitated homogenization of U.S. agriculture at the farm scale. One of the key benefits of GR crops for producers was the opportunity to decrease time, labor, and fuel costs involved in weed management. These benefits were not captured equally by all firms but were instead most easily captured by larger and well-capitalized operations (NRC 2010). This pattern was particularly true during the early phase of adoption when the financial rewards from using new technologies are most abundant. In these conditions of falling production costs, larger farms were able to expand in size and efficiency by buying out or absorbing smaller operations. To this point, during the period 1982–2007, the midpoint area of U.S. farms increased from 500 to 1,071 ha (Macdonald et al. 2013). Most of this increase was due to the growth of very large farms; the largest farms (800 ha or larger) managed 24.1 % of cropland in 2001 but managed 34.4 % of cropland by 2011. Of course, the trend towards larger, more capitalized farms is a long-standing trend in the U.S. and other countries with a modernized agricultural sector, and there are many complex causes beyond any single technology. However, given the well-documented benefits of lowering the time and labor requirements for weed management, it seems clear that GR crops have contributed to the consolidation of U.S. farms (Macdonald et al. 2013; NRC 2010). Operations that now manage thousands of hectares with a small labor force require weed management solutions that are cheap, simple, and can be applied over large areas in a small window of time. Through much of the 2000s, GR technology fit those requirements perfectly, but now that resistant weeds have compromised the efficacy of glyphosate, large-scale growers are dependent on solutions that are equally convenient. Thus, the ubiquity of GR crops has locked U.S. agriculture into a singular weed management paradigm and narrowed the option space of ready-to-use solutions.

## 12.4 Moving Forward?

To address the problem of glyphosate-resistant weeds, several multinational seed and agrichemical companies are currently developing new genetically engineered varieties with resistance to additional herbicide chemistries (Waltz 2010). In the U.S., several of these traits are already under review by the U.S. Department of Agriculture and commercialization is likely by 2015. As problems with glyphosate-resistant weeds prolong and intensify, many large-scale growers are anxiously awaiting the arrival of these technologies.

In the short term, new HR crops will likely provide a workable solution to glyphosate-resistant weeds that is compatible with the production systems of modern industrial agriculture. Many glyphosate-resistant weed species remain susceptible to one or more of the additional compounds. Thus, the combination of glyphosate and an additional herbicide would enable growers to control the most problematic resistant species while also making use of glyphosate's potency on the many species that have not yet developed resistance. Early trials of these herbicide mixtures applied to HR crops indicate that the technology will offer effective and convenient weed control. However, unless significant changes occur in the way farmers combine herbicides and HR crop technologies, this success is likely to be short-lived.

While most herbicide-resistant weed populations are resistant to only a single type of herbicide, there are a variety of mechanisms through which weeds can develop resistance to multiple different herbicides. In some cases, weeds develop independent mutations conferring resistance to two different herbicides, and repeated use of the same combinations of herbicides leads to an increasing frequency of both resistant traits in a population. In other cases, plants can develop enzymes that are capable of breaking down or degrading several distinct herbicide compounds. In South Australia, a population of the rigid ryegrass (*Lolium rigridum*) has evolved the ability to metabolize seven different herbicide groups, making it extremely difficult to control with available chemistry. Examples of weeds with multiple resistance are appearing with increasing frequency, and 45 % of the 65 species with resistance to two or more active ingredients have appeared just since 2005 (Heap 2013). Consequently, if the new herbicide resistant crops are adopted with anywhere near the enthusiasm of the original glyphosate traits, we can expect significant multiple resistant weed problems to appear within 5-10 years (Mortensen et al. 2012).

This cycle can be avoided: new combinations of herbicides and HR crops are by no means the only available or practical solution to GR weed challenges. Growers and scientists have already been working for decades to develop a robust, diverse set of management practices that could be implemented to address resistant-weed issues.

Integrated weed management (IWM) use a diversity of techniques that are based on agroecological principles (Liebman et al. 2001). IWM integrates tactics, including crop rotation, cover crops, competitive crop cultivars, limited use of tillage, and targeted herbicide applications to reduce weed populations and the selection pressures that drive the evolution of resistant weeds. Instead of relying exclusively on glyphosate year after year, a grain farmer using an IWM approach could use mechanical soil disturbance such as inter-row cultivation combined with banded herbicide applications in a soybean crop 1 year, and then rotate to a different crop, integrating different practices, the next year (Fig. 12.1). In fact, several long-term experiments have demonstrated that cropping systems using IWM can achieve competitive yields and profit margins that are comparable to systems relying chiefly on herbicides. In one study, herbicides were reduced by 94 % and profits were similar to a conventional system (Liebman et al. 2008).

The ideas behind IWM connect to broader principles underlying sustainable agriculture in general, and IWM practices can therefore have multiple benefits for farmers and the environment. Crop cultivars bred specifically for their competiveness against weed species can be a valuable component of an IWM plan, and demand for these traits could provide seed dealers with an incentive to add genetic diversity back into commercial crop populations. Longer, more diverse crop rotations can help suppress problematic weeds and can also work to disrupt the development cycles of destructive insects, nematodes, and fungal pathogens. Incorporating perennial forages into grain rotations can reduce the soil seed banks of annual weeds while also building soil organic matter and creating habitat for birds and other wildlife. Winter cover crops can be grown as a weed-suppressing mulch and can also work to prevent erosion and hold plant nutrients in soil.

Yet despite these multiple benefits, important constraints currently discourage broad-scale adoption of more sustainable weed management techniques. First, IWM methods are definitely more management intensive, and even where the bottom-line profitability of these approaches may be favorable, they are difficult to integrate into very large farms where time and labor are scarce. In this way, the ongoing trajectory towards larger and more specialized farms has compromised the potential to pursue

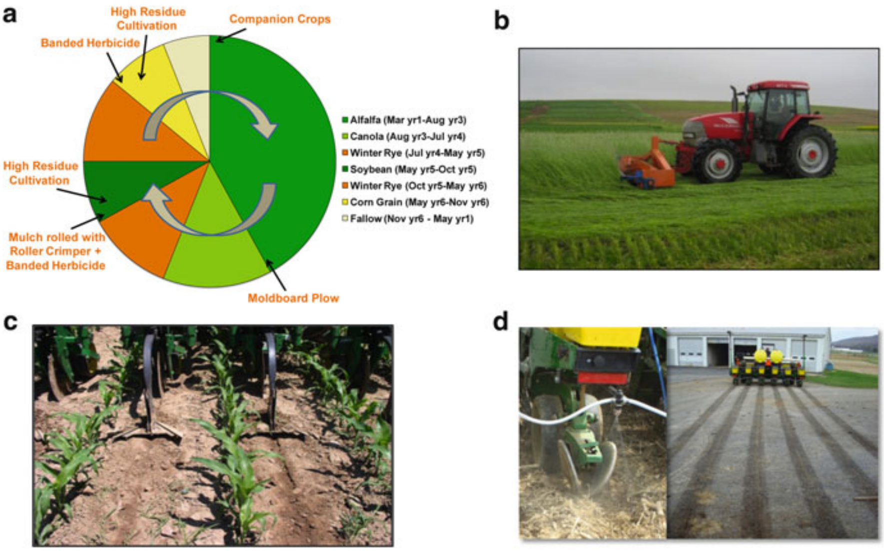

**Fig. 12.1** Example of an integrated weed management plan as implemented in Penn State University's Sustainable Dairy Cropping Systems Experiment (Snyder 2013). In (**a**), a complex, 6 year crop rotation combines a perennial forage (alfalfa) established using companion crops (triticale and peas), a winter annual grain crop (canola), a winter annual cover crop (winter rye), and two summer annual grain crops (soybean and corn). By combining crops with different establishment times and growth patterns, weed life cycles are continuously disrupted over the 6 year period. A diversity of weed management practices are applied including: crushing the winter rye with a roller crimper to create a weed-suppressing mulch (**b**), mechanically controlling weeds within the corn and soybean rows using a high residue cultivator (**c**), and banded application of herbicides applied over the crop rows (**d**). The photo in the right-hand side of (**d**) demonstrates the substantial reduction in herbicide quantity achieved through a banded application (Figure and photo credits: Eli Snyder and Bill Curran, Penn State University)

sustainable weed management. Second, unlike HR crops, which generate enormous revenues for multinational companies, IWM technologies lack a clear market mechanism to push their research and development. IWM involves connecting locally adapted practices with evolving agronomic and ecological knowledge and therefore requires public investment in research and extension. Total U.S. federal investment in agricultural research has remained essentially constant since 1980, while the expenditures of private agrichemical firms have continually increased (NRC 2010). Investment in university or cooperative agricultural extension has correspondingly declined substantially over recent years, creating a situation where most growers are dependent on private consultants or industry for information and advice. These factors create a situation where it is very difficult to advance weed management ideas that are not tied to the sales of agrichemicals or biotech seeds. Consequently, promoting IWM and its connections to sustainable agriculture will likely require both reinvesting in public research and extension and in creating policies and incentives that support smaller, more-diversified farming systems.

## 12.5 Concluding Thoughts

Weedy plants will always create challenges for agriculture. The advent in the mid 1990s of genetically-engineered, HR crops, especially GR varieties, enabled important changes in modern farming systems. Initially, GR technologies provided farmers with an easy and effective means to control weeds without harming their crops, reduced total herbicide use, and also facilitated the adoption of soil-enhancing conservation tillage practices. These positive trends have been recently compromised by a global outbreak of aggressive glyphosate-resistant weed species. Agrichemical companies are currently pursuing HR crops that are resistant to multiple herbicides, but resistant weeds could also be addressed with more sustainable, integrated weed management practices. However, adoption of IWM practices is constrained by interrelated trends towards larger, more specialized farms and consolidated corporate interests in the seed and agrichemical industries. Although in principle, there is no reason that HR crops and IWM approaches cannot be freely combined, to date HR crops have clearly come at the opportunity cost of the advancement of IWM. Thus the recent experience with these technologies raises challenging questions about the intersection of science, agriculture, and public policy. Namely, what is a society's vision for what a sustainable agricultural system should look like, and to what extent can HR crops (or other biotechnologies) be adopted without constraining alternative strategies for the pursuit of that vision?

## References

Benbrook C (2009) Impacts of genetically engineered crops on pesticide use: the first thirteen years, The Organic Center, Washington, DC

Derpsch R, Friedrich T, Kassam A, Hongwen L, Consultant F (2010) Current status of adoption of no-till farming in the world and some of its main benefits. Int J Agric Biol Eng 3:1–26

Dill GM, Cajacob CA, Padgette SR (2008) Glyphosate-resistant crops: adoption, use and future considerations. Pest Manag Sci 64:326–331

Duke SO, Powles SB (2008) Glyphosate: a once-in-a-century herbicide. Pest Manag Sci 64:319–325

ERS (2013) Adoption of genetically engineered crops in the U.S. [WWW Document]. http://www.ers.usda.gov/data-products/. Accessed 14 Oct 2013

Gaines TA, Zhang W, Wang D, Bukun B, Chisholm ST, Shaner DL, Nissen SJ, Patzoldt WL, Tranel PJ, Culpepper AS, Grey TL, Webster TM, Vencill WK, Sammons RD, Jiang J, Preston C, Leach JE, Westra P (2010) Gene amplification confers glyphosate resistance in Amaranthus palmeri. Proc Natl Acad Sci U S A 107:1029–1034

Green JM, Owen MDK (2011) Herbicide-resistant crops: utilities and limitations for herbicide-resistant weed management. J Agric Food Chem 59:5819–5829

Heap I (2013) International survey of herbicide resistant weeds [WWW Document]. www.weedscience.org. Accessed 14 Oct 2013

James C (2012) Global status of commercialized biotech/GM Crops: 2012. International Service for the Acquisition of Agri-Biotech Applications, Ithaca, NY

Liebman M, Mohler CL, Staver CP (2001) Ecological management of agricultural weeds. Cambridge University Press, Cambridge, UK

Liebman M, Gibson LR, Sundberg DN, Heggenstaller AH, Westerman PR, Chase CA, Hartzler RG, Menalled FD, Davis AS, Dixon PM (2008) Agronomic and economic performance characteristics of conventional and low-external-input cropping systems in the central corn belt. Agron J 100:600–610

Macdonald JM, Korb P, Hoppe RA (2013) Farm size and the organization of U.S. crop farming. USDA Economic Research Service, Washington, DC

Mortensen DA, Egan JF, Maxwell BD, Ryan MR, Smith RG (2012) Navigating a critical juncture for sustainable weed management. Bioscience 62:75–84

National Research Council (NRC) (2010) The impact of genetically engineered crops on farm sustainability. U.S. National Academies Press, Washington, DC

Osteen CD, Fernandez-Cornejo J (2013) Economic and policy issues of U.S. agricultural pesticide use trends. Pest Manag Sci 69:1001–1025

Price AJ, Balkcom KS, Culpepper SA, Kelton JA, Nichols RL, Schomberg H (2011) Glyphosate-resistant Palmer amaranth: a threat to conservation tillage. J Soil Water Conserv 66:265–275

Snyder EM (2013) Evaluating strategies for reducing herbicide use in a no-till dairy cropping system. Masters thesis, The Pennsylvania State University, University Park

Waltz E (2010) Glyphosate resistance threatens Roundup hegemony. Nat Biotechnol 28:537–538

# Chapter 13
# Virus-Resistant Crops and Trees

**Cristina Rosa and Bryce W. Falk**

**Abstract** Plant viral and other plant microbial diseases cause significant economic losses every year and limit food supplies worldwide. To control viral diseases, we can use a variety of strategies, including various forms of genetic resistance. Genetic resistance can be manipulated to control viruses by exploiting a natural eukaryotic defense system called RNA interference or gene silencing. This system can be additionally exploited to control insect vectors of viruses, broadening the impact of transgenic technologies. An overview on plant defense, plant viruses and integration of transgenic technologies in virus resistant crops is given in this chapter.

**Keywords** RNA interference • Gene silencing • Plant viruses • Plant defenses • Viral induced gene silencing

## 13.1 Introduction

In the natural environment, plants are colonized by a microbiota (a microbial community) composed of various organisms including viruses, bacteria and fungi. The majority are not pathogenic and do not harm their host. On the contrary, they are necessary for plant wellness and participate in a mutualistic interaction beneficial to themselves and their plant host. For instance, some plant viruses confer tolerance to stress, such as heat or drought stresses (Marquez et al. 2007;

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A. Ricroch et al. (eds.), *Plant Biotechnology: Experience and Future Prospects*, DOI 10.1007/978-3-319-06892-3_13,

Xu et al. 2008), allowing the infected plants to colonize extreme environments (such as the extremely hot soil in the Yellowstone Park, US) and to survive sudden changes in their habitat (such as water fluctuations due to tide surges).

Viruses are ancient microbes and shape the evolution of all living organisms by their host:virus interactions and by transferring genetic material among species. Today many viruses are used for beneficial applications including in medicine to fight bacterial infections, in forest ecosystems to kill defoliating caterpillars, and in nano/biotechnology as carriers for genetic information, delivering drugs into the right type of cells or expressing proteins in plants, insect cells or bacteria. In contrast, and this is true especially in the agricultural landscape, some plant viruses, as well as bacteria and fungi, can cause diseases that become eventually lethal to their hosts, and most importantly can cause a substantial yield reduction in crops that are important for food and fiber. Deleterious viruses seem to have evolved as consequence of the advent of agriculture, and are relatively evolutionarily new, compared to viruses found in natural landscapes. *The negative impact of plant viral diseases, even to the point of limiting food supply, is the reason we are concerned about growing healthy crops*. It is estimated that plant diseases in general cause up to a 14 % loss in total crop production every year, a percentage that equals hundreds of billions of US dollars in lost revenues. Ten to 15 % of this loss can be attributed to viruses, but for specific crops and in specific locations, like in the Asian and African continents where food supplies are already limited, the losses can have severe direct effects on human health. To control viral diseases, we routinely use a variety of strategies, ranging from the application of pesticides aimed to reduce the number of insect vectors that spread viruses, the adoption of Integrated Pest Management (a system designed to use multiple pest management practices in an environmentally sound manner), the establishment of plant and animal quarantine areas, the use of certified germplasm material (a collection of genetic resources, for instance seeds and tubers) and various forms of genetic resistance.

## 13.2 What Are Plant Viruses, and Do Plant Viruses Differ from Animal Viruses?

Plant viruses are generally smaller and less complex than animal and bacterial viruses. The sizes of the viral particles range from 20 to 200 nm, and their chemical composition is generally simple. Virus particles typically have an outer shell made of proteins arranged in a geometrical form, either in a rod-shaped or in an isometric "coat" (Fig. 13.1).

The viral shell is composed of repetitive small protein subunits and for some plant viruses the shell is surrounded by a lipid (fatty) membrane derived from the host cell. The viral genome is protected inside the shell, and can be made of either RNA or DNA (Fig. 13.2).

**Fig. 13.1** Representative plant virus particles purified from infected leaves. (**a**) *Tobacco streak virus (isometric).* (**b**) *Pea seed-borne mosaic virus (rod shaped)*

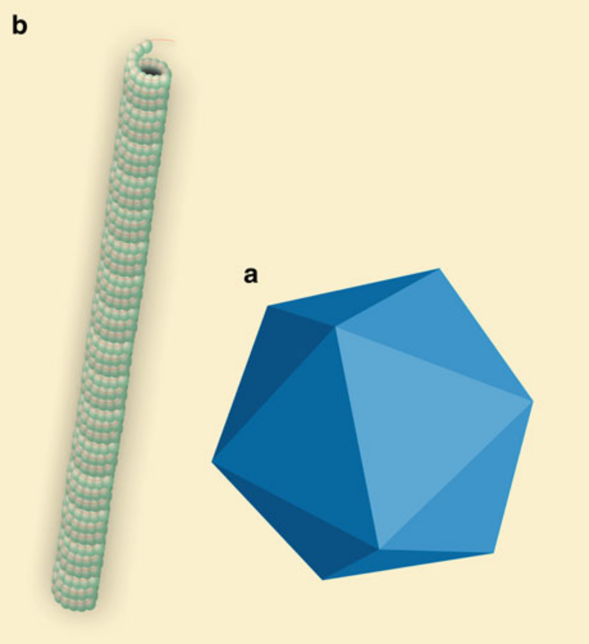
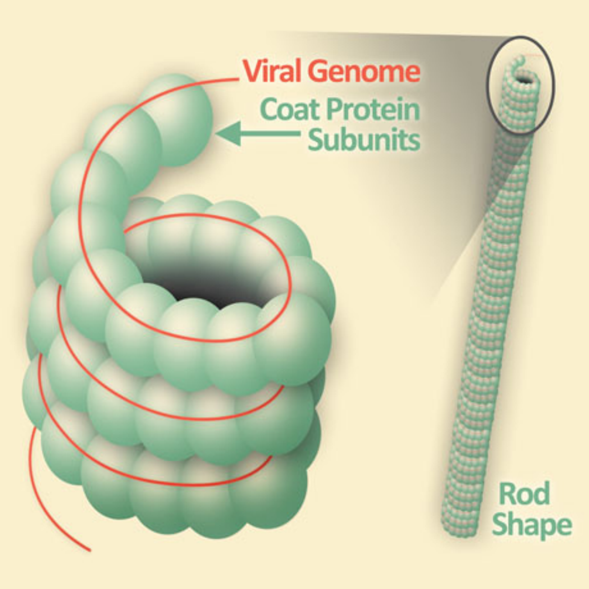


**Fig. 13.2** (1) *The capsid* is the protein shell that encloses the nucleic acid. It is built of structural subunits. (2) *Coat protein subunits* are the smallest functional equivalent building units of the *capsid.* (3) *The capsid* with its nucleic acid is called the *nucleocapsid.* (4) *The nucleocapsid* may be protected by an *envelope.* (5) *The virion is the infective virus particle*



The viral genome can be circular or linear, can be composed of one or more "chromosomes" and can be contained in single or multiple viral particles or virions. The plant viral genome size is generally small, usually ranging from 3,000 to 30,000 nucleotides (blocks that build RNA and DNA), while animal virus genomes can consist of 800 thousand nucleotides and encode for up to a few hundred proteins. The virions of a few viruses may contain viral proteins necessary to initiate viral replication or multiplication, but viruses are unable to replicate without the host's cellular machinery; they are intracellular molecular obligate parasites. They exploit and highjack the host cell to multiply and they cannot perform this outside living cells. Since plant viruses are so small, they can express only a few proteins, and are thus amazing in their ability to replicate and respond to plant defenses, considering their limited genomic arsenal. Viruses rely on multifunctional proteins, protein modifications and on timely regulated protein expression and genomic replication to successfully complete their life cycle.

### *13.2.1 Can Plants Defend Themselves Against Viruses?*

Let's have a first look at how plant defenses function, and how they differ from animal defenses.

Plants are multicellular organisms and their cells are interconnected by channels called plasmodesmata. Plasmodesmata serve as freeways that allow small molecules to travel between cells, or even throughout the entire plant. Plant viruses take advantage of the plasmodesmata to spread within the infected plant (Fig. 13.3).

Plants possess constitutive and inducible defenses against insects, microbes and other stressors (Dangl and Jones 2001; Howe and Jander 2008). Constitutive defenses are always present; they include physical barriers such as modified cell wall composition or the presence of leaf trichomes (hairs or appendages), and chemical barriers such as production of chemical deterrents. Inducible defenses are those that are 'turned on' only as consequence of pathogen attack, when the plant cell's innate immune system recognizes pathogen-encoded effectors that are signature molecules specific of a class of organisms. Hypersensitive Response, Reactive Oxygen Species release and Programmed Cell Death are the names given to plant reactions linked to Resistance (R)-gene mediated resistance. Here upon recognizing the pathogen, plant cells release chemical defenses and "commit suicide", in the attempt to contain infections from spreading into healthy tissues. The leaves of plants expressing these reactions show necrotic spots, corresponding to the points in which the attack started (Fig. 13.4).

Signals originated from the site of infection then stimulate the Jasmonic Acid, Ethylene and/or Salicylic Acid dependent or independent defense pathways in distal parts of the plants (Fig. 13.5). These pathways are sequential events that turn on and off other genes and their products to help the plant in its fight against pathogens. Viruses and other plant pathogens have evolved means to counteract

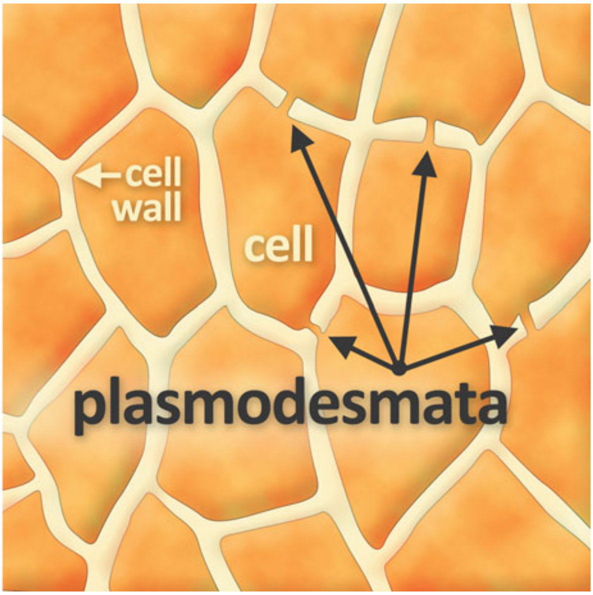


**Fig. 13.3** Plant cells are protected by cell walls, and connected by *plasmodesmata*

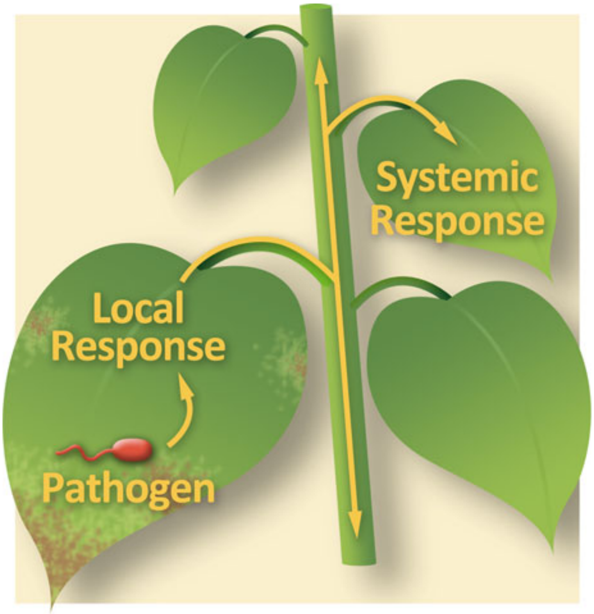
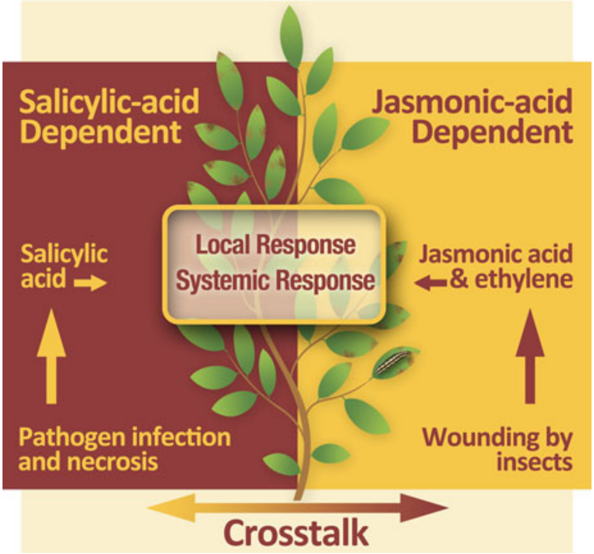


**Fig. 13.4** Pathogens are perceived by plants and activate local responses as well as systemic responses in the attacked plant

plant defeneses, thus plant resistance and pathogen counter defense represent an ongoing evolutionary "arms race".

Finally, plants lack the somatic adaptive immune system (antibodies) typical of animals and do not possess lymphocytes (type of white blood cells).

**Fig. 13.5** Pathogen infection, cell necrosis, or insect wounding activate the *salicylic acid* or *the jasmonic acid and ethylene pathways,* leading to local and systemic plant responses. These defense pathways are interconnected



## 13.2.2 Are Cultivated Plants More Susceptible to Viruses Than Their Wild Relatives?

Individual plants in a natural population are not genetically identical and usually differ in their resistance to pathogens. By contrast agricultural species grown in monocultures (where all the plants belong to the same species and are essentially identical) are equally susceptible or resistant to specific pathogens. Thus, if wild relatives that show degrees of resistance or tolerance to the same pathogen can be identified, these can serve as sources of genetic resistance for cultivated crop plants. Resistance can be conferred by expression of a single host gene (R-gene), or by multiple genes, and plant breeders try routinely to introgress (move) genes for resistance found in wild plants into cultivated varieties via traditional breeding, or via transgenesis.

## 13.2.3 Examples of Natural Resistance

Many plants show R-gene mediated natural resistance to viruses. For example, in many *Arabidopsis thaliana* accessions (a collection of plants from the same location), some proteins produced by the plant called the Restricted Tobacco etch virus Movement RTM1, RTM2 and RTM3 restrict long distance movement of plant viruses called potyviruses. This resistance is not present in *A. thaliana* accessions showing amino acid changes in their RTM proteins. Since the viral coat protein is the effector recognized by the RTM proteins, potyviruses that naturally show changes in their outer shell structure are not recognized by *A. thaliana* without changes in their RTM proteins and can infect plants with *Rtm* genes.

Many tomato *(Solanum)* species show natural resistance to viruses called tospoviruses, as well as to other plant pathogens. *S. peruvianum*, S. *chilense*, S. *habrochaites* and S. *pimpinellifolium* are used as source of resistance genes to be introgressed into cultivated tomatoes *(Solanum lycopersicum)* that are susceptible to tospoviruses. A single gene called *Sw-5b* introgressed from S. *peruvianum* into S. *lycopersicum*cultivar Stevens shows broad-spectrum resistance to tospoviruses. The *Sw-5b* gene belongs to a particular class of plant resistance genes and is similar to other resistance genes such as the tomato nematode and aphid resistance gene *Mi*. The resistance conferred by *Sw-5b* can elicit a hypersensitive response in virus inoculated tissues, where it blocks the spread of the virus, but the resistance is absence in tomato fruits.

Some plant proteins called Endosomal Sorting Complex Required for Transport (ESCRT) are involved in endosome maturation. Endosomes are cellular vesicles that help in transporting proteins to different destinations in the cells and to or from the cell surface. Most viruses exploit the ESCRT system during their replication and movement, and impairment in ESCRT interferes directly with the ability of the viruses to replicate and move. In fact, *Arabidopsis* plants modified to lack ESCRT show inhibited viral replication and infection for a group of viruses called tombusviruses.

The resistance to a virus called *Tobacco mosaic virus* in the plant *Nicotiana glutinosa* is due to the 'Necrotic-type response to infection with TMV' *N* gene. This gene product also interferes with viral replication and as the name suggests induces plant cells to necrotize (commit suicide) in order to stop viral infection.

### *13.2.4 Examples of Transgenic Resistance*

In absence of natural resistance, today we can sometimes use transgenesis to incorporate viral resistance traits into crop plants. In some instances, we can move natural R-genes from one crop to another crop, but this is not always effective, probably due in part to the genetic background of the recipient crop plant. In the late 1980s, scientists started exploring the idea that inserting viral genes into plants could trigger the transgenic plants to become 'immune' to viruses, in a kind of self-perpetuating plant vaccination. In 1986 Powell-Abel et al. successfully generated the first transgenic tobacco plants expressing the TMV coat protein. In 1988 Nelson et al. engineered whole transgenic tomatoes to express the TMV coat protein. Some of the transgenic lines were partially resistant to TMV infection. Since then, many plants (barley, canola, corn, oat, rice, wheat, chrysanthemum, dendrobium, gladiolus, grapefruit, grapevine, lime, melon, papaya, pineapple, plum, raspberry, strawberry, tamarillo, walnut, watermelon, alfalfa, sugarcane, bean, clover, groundnut, pea, peanut, soybean, lettuce, pepper, potato, squash, sugar beet, sweet potato, and tomato) have been transformed to be resistant to one or more viruses. However, of all the plants that have been generated and tested in laboratory or greenhouse settings for their viral resistance, few have reached the market.

The transgenic summer squash line ZW-20 resistant to *Watermelon mosaic virus* and *Zucchini mosaic virus*, and the transgenic squash line CZW-3 resistant to *Cucumber mosaic virus*, *Watermelon mosaic virus* and *Zucchini mosaic virus* were commercially released in the US in 1994 and 1996, respectively (Tricoli et al. 1995). A transgenic papaya (Gonsalves et al. 1997) resistant to *Papaya ringspot virus* (PRV) was released in 1998, while transgenic green pepper varieties and tomato resistant to *Cucumber mosaic virus* are today released in the People's Republic of China. Two potato lines resistant to *Potato virus Y* were deregulated in Canada (1998) and the US (1999) but were later abandoned because of the extremely negative public opinion (Kaniweski and Thomas 2004). Of the transgenic plants released to the market, the most successful story comes from the use of PRV transgenic papaya in Hawaii. This event saved the papaya industry in Hawaii from complete destruction due to PRV, allowed the cultivation of non-transgenic papaya cultivars in between transgenic fields, and increased the cultivar diversity in the islands. Today transgenic papaya cultivars resistant to local PRV strains have been developed in Thailand, Jamaica, Brazil, and Venezuela and are at different stages of deregulation.

The latest virus resistant transgenic plant that has been deregulated (2011) in the US is the *Plum pox virus* resistant 'HoneySweet' plum. Research to establish the safety and characteristics of this plum variety took more than 20 years, and it has been particularly important since there is no high level of PPV resistance known in *Prunus domestica, P. spinosa and P. insitita.* Only *P. cerasifera* offers a cultivar that is hypersensitive to PPV inoculation, and young plants of this cultivar naturally die when exposed to the virus. 'HoneySweet' plums score high in fruit quality and yield, and are today crossed with other plum varieties since they can transmit the dominant resistant trait as a single locus. Scorza et al. in 2013 wrote a highly remarkable review on the process of deregulation on HoneySweet.

The mechanism that allows these transgenic plants to be resistant to viruses is not always known, but in most of the cases the resistance is due to a host natural defense system called gene silencing or RNA interference.

## 13.3 RNAi: A Newly Discovered, Nucleic Acid Sequence-Based Inducible Defense Mechanism

The eukaryotic inducible defense mechanism that evolved specifically against viruses, called RNA interference (RNAi), or gene silencing (Voinnet 2001; Waterhouse et al. 2001), works against specific nucleotide sequences, and it is thus atypical when compared to the classic effector-mediated plant defense system described above.

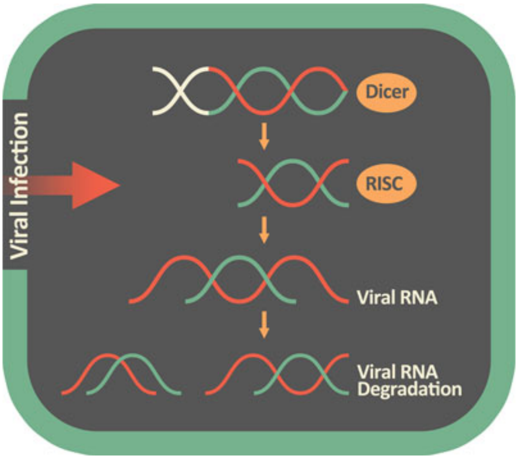


**Fig. 13.6** After viral infection, *viral dsRNA* is produced in eukaryotic cells. The *dsRNA* is recognized by the *cell enzyme Dicer* and processed into 21 nucleotide long *siRNA molecules* that are incorporated into the cell *RISC complex* and used to search for complementary viral RNA sequences for their degradation. *Red and blue colors are used to show complementary and opposite RNA polarities*

### *13.3.1 How Does RNAi Work?*

When viruses replicate in eukaryotic cells, a double-stranded RNA (dsRNA) form of the viral genome is produced by the viral enzyme RNA dependent RNA polymerase that uses the RNA genome from the virus as a template, and copies it in an RNA molecule of opposite polarity. Since the two RNA molecules are complementary, they can anneal to each other and form a double stranded RNA helix. DsRNA can also be generated from the pairing of complementary stretches of RNA on the same molecule, and is not always linked to viral replication. Large dsRNA molecules, such as those generated during virus infections or viral replication, are not found in healthy cells and their presence is recognized as foreign and serves as the trigger of the eukaryotes' (e.g. plants) RNAi pathway.

When dsRNA is found in a healthy plant cell, a plant enzyme called Dicer cleaves the dsRNA into small duplex fragments that are 21 nucleotides long, called small interfering RNAs (siRNAs). Dicer has a pocket that is exactly 65 Å in size, the distance that equals 21 nucleotides (Fig. 13.6). This pocket serves as molecular ruler. The siRNA duplex is dissociated into two strands by Dicer and one of the two strands (the sense or passenger strand) is degraded by the same enzyme. The second strand (the antisense or guide strand) gets incorporated into an enzymatic complex called the RNA Induced Silencing Complex, or RISC. RISC uses the antisense strand as template to find and hybridize with RNA from viruses and with a complementary nucleotide sequence (more viral RNA), and to degrade it. In this way, cells are able to find and destroy viral RNA and to distinguish it from other cellular *messenger RNA* (mRNA).

Messenger RNA (mRNA) is the nucleic acid that is transcribed using DNA as template. RNA leaves the nucleus and is translated into proteins in the cell cytoplasm.

While animals have evolved other defenses such as an interferon based signals to alert healthy cells about a virus attack, and antibodies to recognize specific viruses and other microbes, plants especially, but also insects, rely heavily on the RNAi pathway to defend themselves against viruses.

### *13.3.2 How Can We Manipulate RNAi to Induce Virus Resistance in Plants?*

If we genetically transform plants to express double-stranded RNAs, the plant RNAi machinery will recognize the dsRNA and initiate a response. If the engineered plant expresses a plant virus double-stranded RNA sequence, the plant RNAi response will recognize that viral RNA. This strategy has been used to develop virus "immune" plants: plants that recognize viral nucleic acids produced during viral infection or replication, degrade those viruses, and thus stop the infection or viral replication process.

So, going back to the examples of the transgenic plants expressing virus sequences such as those encoding for the viral coat protein, those plants are very efficient in recognizing and destroying the invading virus RNAs. If we analyze the genome of those transgenic plants, we can find the inserted viral sequence, and the corresponding 21 nucleotide siRNAs derived from the inserted sequence but resulting from the Dicer activity (Fig. 13.7).

Other transgenic plants produced in laboratory trials where RNAi against viruses has been exploited are: walnut, ryegrass, tomato, tobacco, sweet potato, soybean, potato, rice, poplar, opium, maize, ornamental crops, and apple.

### *13.3.3 Does RNAi ALWAYS Involve the Use of Transgenic Plants?*

In addition to transgenic methods to enable plants to utilize RNAi, 'Viral induced gene silencing', or VIGS is used to introduce specific nucleotide sequences that can induce RNAi effects in plants via non-lethal recombinant viruses. These viruses are used as carriers to express the nucleotide sequence complementary to the one that

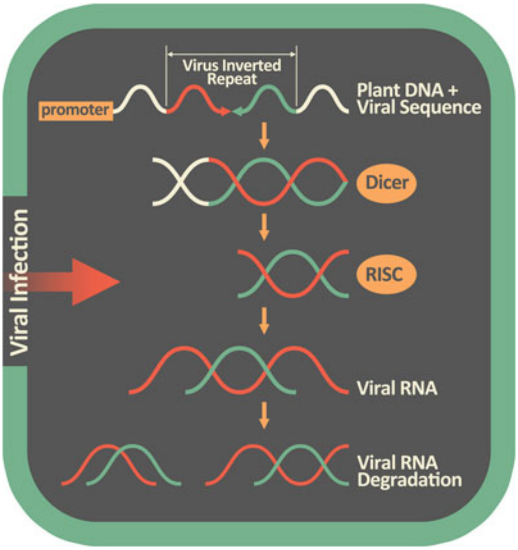


**Fig. 13.7** Transgenic plants can be made by inserting a part of a viral sequence and its complementary sequence, under the control of a plant or virus promoter. The plant will express a *double stranded form of the viral sequence* that will trigger the *plant RNAi pathway* against those specific viral sequence. If the plant is challenged by the target virus also containing that particular sequence, the plant will use the primed *RNAi pathway* to destroy the viral RNA and halt infection

we wish to target via RNAi, but since these viruses do not integrate into the plant genome, the resulting plants are not transgenic. These viruses are usually inoculated (or transferred) mechanically into their host plants. A type of VIGS is for example used in Brazil, Australia, South Africa and Japan to fight *Citrus tristeza virus* (CTV) in citrus orchards. There, a mild strain of CTV is inoculated in the trees, and serves as vaccine against severe CTV strains (Costa and Muller 1980) (Fig. 13.8).

### 13.3.4 Issues Linked with VIGS and RNAi

There are a few issues associated with the use of RNAi against plant viruses, due to the ability of plant viruses to evade the plants RNAi response. Plant viruses evolve rapidly and can mutate their nucleotide sequences. If their nucleotide sequence changes compared to the one targeted by RNAi, it may not be recognized anymore by the RISC complex. New strains of viruses with differences in the RNAi target region are always emerging, and would not be affected by RNAi. Third, plant viruses encode for proteins that can allow the virus to evade the RNAi machinery. These proteins are called ‘suppressors of gene silencing’ (Qu and Morris 2005), and other viruses expressing potent suppressors of gene silencing can sometimes protect the viruses that are the RNAi target, if they co-infect the transgenic plant. Mixed infections, where multiple viruses infect the same plants, are common in nature.

**Fig. 13.8** Plant on the far left is a healthy citrus plant. Plant on the far right is infected with a severe CTV strain *(depicted by a blue leaf)* and plant in the middle left is infected with a mild CTV strain *(red leaf).* If a plant is inoculated first with a mild CTV strain *(middle right)* and then by a severe CTV strain *(red and blue leaf respectively),* the plant will be partially protected and will grow better than plants infected by the severe CTV strain

### *13.3.5 Modification of the RNAi Strategy: RNAi, or Gene Silencing, Can Be Used, for Instance, to Affect Insect Vector Performance*

Viruses in nature can be either vertically transmitted via infected pollen and seeds, or horizontally transmitted by virus vectors, most commonly insects (Nault 1997). Some viruses can be transmitted in both ways, but often agriculturally significant viruses are transmitted mainly by their insect vectors. Viruses are picked up by insects when they feed on virus-infected plants and then are introduced into healthy plants when the insects move from plant to plant and eject saliva containing viruses in the newly encountered plants, while feeding.

Novel research focuses on provoking the plant to control insect vectors and their associated viral diseases using RNAi, either through genetic transformation or VIGS. Many studies suggest that RNAi effects can be induced in insect cells, and even in whole insects that feed on such plants (plants expressing the RNAi sequences by transformation or VIGS). Artificial dsRNAs can be used to trigger the RNAi pathway. If the artificial dsRNA nucleotide sequence is synthesized (artificially assembled) to be identical to a specific insect mRNA, then that mRNA becomes the target of the RNAi machinery for destruction, effectively ‘silencing’ the corresponding gene and stopping protein translation. For instance, if a plant is transformed to produce a dsRNA molecule whose sequence corresponds to an insect gene, the plant will produce siRNAs that will target the insect RNA target, and the plant might become insect and virus resistant. How? When insects feed on the transgenic plants, they ingest the plant-produced siRNAs, and since insects also have a RNAi machinery, their defense system will use the ingested siRNAs to find and destroy the corresponding target RNA sequence, in this case an insect RNA!

### *13.3.6 Modification of the RNAi Strategy: RNAi, or Gene Silencing, Can Be Used, for Instance, to Affect Insect Vector Performance*

A recent study (Baum et al. 2007) has reported the use of RNAi in corn roots to control the western corn rootworm. The transgenic corn plants express in its roots the dsRNAs against the western corn rootworm ATP-ase mRNA, (ATP-ase is expressed in the insect gut and necessary for many vital processes) and these plants have been shown to be highly resistant to rootworm damage. This technology can be used, for instance to increase the durability of transgenic corn using the Bt (*Bacillus thuringiensis*) resistance.

## 13.4 Future Perspectives

Transgenesis in plants and the use of RNAi technologies is a subject of hot debates. Recently, scientists have discredited (Dickinson et al. 2013) a study published by Zhang et al. in 2012 where the authors reported plant microRNA168 in blood of mammals (humans and mice) fed on rice, and that this microRNA regulated mammalian gene expression in the liver. MicroRNAs belong to a class of small RNA molecules very similar to the one composed by siRNAs. MicroRNAs are produced by every organism and are used to regulate gene expression, especially during the organism’s growth and development. Since the structure of miRNAs is similar to the one of siRNAs, this study indirectly poses a question mark on the stability across the mammalian digestive system of siRNA generated by transgenic plants and, not surprisingly, is the subject of intense debate. SiRNAs seem to be stable in the digestive tract of arthropods and siRNAs generated by plants and ingested by insects in some cases have been shown to affect distant organs, but no study has proven the same stability in mammalians. At the same time, we are exposed every day to our own miRNAs and ingest miRNAs produced by plants, other animals and even by microorganisms in large amounts. Further studies are needed to examine the stability and potential effects of miRNAs in the mammalian digestive system.

**Acknowledgements** We thank Nick Sloff for the illustrations.

# References

Abel PP, Nelson RS, De B, Hoffman N, Rogers SG, Fraley RT, Beachy RN (1986) Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. Science 232:738–743

Baum JA, Bogaert T, Clinton W, Heck GR, Feldman P, Oliver I, Scott J, Geert P, Tichafa M, Michael P, Ty V, James R (2007) Control of coleopteran insect pests through RNA interference. Nat Biotechnol 25:1322–1326

Costa AS, Muller GW (1980) Tristeza control by cross protection: a U.S.-Brazil cooperative success. Plant Dis 64:538–541

Dangl JL, Jones JDG (2001) Plant pathogens and integrated defence responses to infection. Nature 411:826–833

Dickinson B, Zhang Y, Petrick JS, Heck G, Ivashuta S, Marshal WS (2013) Lack of detectable oral bioavailability of plant microRNAs after feeding in mice. Nat Biotechnol 31:965–967

Gonsalves C, Cai W, Tennant P, Gonsalves D (1997) Efficient production of virus resistant transgenic papaya plants containing the untranslatable coat protein gene of papaya ringspot virus. Phytopathology 87:S34

Howe GA, Jander G (2008) Plant immunity to insect herbivores. Annu Rev Plant Biol 59:41–66

Kaniweski WK, Thomas PE (2004) The potato story. Agric Biol Forum 7:41–46

Márquez LM, Redman RS, Rodriguez R, Roossinck MJ (2007) A virus in a fungus in a plant: three-way symbiosis required for thermal tolerance. Science 315:513–515

Nault LR (1997) Arthropod transmission of plant viruses: a new synthesis. Ann Entomol Soc Am 90:521–541

Nelson RS, McCormick SM, Delannay X, Dubé P, Layton J, Anderson EJ, Kaniewska M, Proksch RK, Horsch RB, Stephen RG, Fraley RT, Beachy RN (1988) Virus tolerance, plant performance of transgenic tomato plants expressing coat protein from tobacco mosaic virus. Nat Biotechnol 6:403–409

Qu F, Morris TJ (2005) Suppressors of RNA silencing encoded by plant viruses and their role in viral infections. FEBS Lett 579:5958–5964

Scorza R, Callahan A, Dardick C, Ravelonandro M, Polak J, Malinowski T, Zagrai I, Cambra M, Kamenova I (2013) Genetic engineering of plum pox virus resistance: ‘HoneySweet’ plum – from concept to product. Plant Cell Tissue Organ Cult (PCTOC) 115:1–12

Tricoli DM, Carney KJ, Russell PF, McMaster JR, Groff DW, Hadden KC, Himmel PT, Hubbard JP, Boeshore ML, Quemada HD (1995) Field evaluation of transgenic squash containing single or multiple virus coat protein gene constructs for resistance to cucumber mosaic virus, watermelon mosaic virus 2, and Zucchini yellow mosaic virus. Bio Technol 13:1458–1465

Voinnet O (2001) RNA silencing as a plant immune system against viruses. Trends Genet 17:449–459

Waterhouse PM, Wang MB, Lough T (2001) Gene silencing as an adaptive defence against viruses. Nature 411:834–842

Xu P, Chen F, Mannas JP, Feldman T, Sumner LW, Roossinck MJ (2008) Virus infection improves drought tolerance. New Phytol 180:911–921

# Chapter 14
# Role of Biotechnology to Produce Plants Resistant to Fungal Pathogens

**Iffa Gaffoor and Surinder Chopra**

**Abstract** Fungal pathogens produce a wide variety of chemically distinct compounds, some of which facilitate their invasion and infections, making fungi infection formidable opponents. In order to fight fungal diseases, extensive research is being carried out in laboratories worldwide to identify suitable plant genes that function in fungal resistance. This pursuit is increasingly possible due to rapid advances in technology that has led to affordable and faster methods for next generation sequencing of DNA, RNA, and proteins of both host plants and pathogens. Under controlled conditions, it has been demonstrated that modifying the expression of genes in transgenic plants has improved resistance against fungal pathogens. However, most of this research has not progressed beyond the laboratory. This is in part due to the high costs of producing and deregulating transgenic plants that can be grown by the farmer. Another reason why fungal resistant transgenic plants are lagging is because fungi are more complex pathogens requiring more sophisticated strategies. But there are a few promising examples where transgenic plants have been tested under field conditions over several years and it is possible that we may see them commercially in the near future. We also expect that the advances in sequencing technology will aid in the understanding of the interactions between plant and pathogen, thereby broadening the pool of candidate genes that can be used to produce transgenic plants resistant to fungal diseases.

**Keywords** 3-deoxyanthocyanidins • Flavan-4-ols • Fungus resistance • Phytoalexins • Powdery mildew

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A. Ricroch et al. (eds.), *Plant Biotechnology: Experience and Future Prospects*, DOI 10.1007/978-3-319-06892-3_14,

## 14.1 Introduction to Fungi

Fungi are an extremely diverse group of organisms that belong to their own kingdom, as they possess several unique features that set them apart from plants, animals, protists and bacteria. Throughout history there has been much debate about their classification as belonging to either the animal or plant kingdom. This is because they possess several characteristics thought to be unique to plants and some others thought to be unique to animals. Like animals they are heterotrophic; i.e., they have to obtain the energy required to grow and reproduce from an external source such as decomposing plant and animal matter, and they are unable to produce it themselves like most plants are able to do. They also have cell walls that are composed of chitin – the same material that makes up the exoskeletons of insects. This provides the fungal organism with structural support and protection. Although plants and bacteria have cell walls, these walls are composed of cellulose in plants and a variety of materials in bacteria. Being eukaryotic, their cells contain nuclei and other organelles such as mitochondria, chloroplasts and the Golgi apparatus enclosed within membranes. Furthermore, their genetic material (DNA) is organized within chromosomes. Therefore, they have complex cellular processes similar to plants and animals.

Fungi are diverse in their form, ranging from the unicellular yeasts to the multicellular mushrooms that we are all familiar with. Since fungi are heterotrophic, they employ a wide diversity of strategies to obtain their requisite nutrition. Some fungi are mycorrhizal. This is a symbiotic relationship that they form with the roots of higher land plants. It is estimated that up to 90 % of land plants form these associations as the fungal partner provides the plant with an increased water and nutrient absorption capability, especially in soils that are of poor quality. In return the fungus obtains sustenance from the host plant. Some other fungi employ an endophytic lifestyle where they live within the host plant without giving rise to disease symptoms. Although our understanding of the exact nature of this relationship is limited, it is believed that the plant provides the fungus with shelter and nutrients while the fungus improves the plant's ability to tolerate abiotic stresses such as drought and biotic stresses such as herbivory. Still other fungi are saprophytic – they live on dead plant and animal matter by decomposing it. This releases the mineral nutrients from the tissues so that they can be reutilized by plants. If not for this important role played by fungi, we will be inundated with plant and animal matter and the soil will be depleted of nutrients. A proportion of fungal species, albeit a small portion, may also be pathogens of plants and animals; however, they are the number one cause of crop loss worldwide.

### *14.1.1 Plant Disease Caused by Fungi*

Fungi that are pathogenic on a species may cause devastating epidemics resulting in economic losses and in some instances famines. Some of the losses are because the fungi infect the leaf tissue reducing the amount of energy the plant is able to convert by photosynthesis. This will result in lower yields during harvest. The annual losses due to the rice blast disease caused by *Magnaporthe oryzae* are between 10 and 30 % of the rice harvest. In some years there can be epidemics similar to that of the one that occurred in Bhutan in 1995 where losses were 100 % in some fields. Since approximately 50 % of the world's population depend on rice as a staple, these losses are particularly severe. In most cases farmers grow rice in the most productive fields and more hardy crops like millet in land unsuited for rice cultivation as a backup for when the rice crop fails. Since *M. oryzae* is able to cause disease on these crops as well, epidemics can be catastrophic. Sometimes the fungi infect the plant part that is being harvested such as the fruit. This will result in direct losses especially as the fungi continue to spoil the harvested product during storage. Gray mold, caused by *Botrytis cinerea* is one such devastating disease. This disease is economically important since it is known to affect over 200 plant species and can cause losses at all stages of production from seedlings to product meant for consumption. Other pathogenic fungi are important because of the toxic compounds they produce as they infect plant tissue. Losses due to fusarium head blight caused by *Fusarium graminearum* are compounded by the mycotoxins produced by this fungus as it infects the developing grain. Mycotoxins are compounds produced by fungi that are toxic to humans and animals. Therefore these toxins make the grain unfit for human and animal consumption. Fungi can also cause disease epidemics on tree species. The American chestnut (*Castanea dentata*) was once the dominant tree species in the forests of the eastern part of North America. In addition to several industrial uses such as posts for fences, telephones etc., the nuts were a major source of food for animals that inhabited these forests. Chestnut blight, caused by the fungus *Cryphonectria parasitica* was inadvertently introduced into the US from Asia. Since the American chestnut was extremely susceptible to this exotic pathogen, the disease spread rapidly and causing the near extinction of this species. The repercussions were felt higher up in the food chain as the animals depended on shelter provided by these enormous trees and the nutrient rich nuts for sustenance especially through the winter months. There are several devastating diseases caused by a group of similar organisms called the oömycetes, the most notable being the potato famines of Europe in the 1840s. Potato late blight is caused by *Phytophthora infestans*. The food shortages caused by the failure of the potato crop over successive years resulted in heavy death tolls across Europe, most famously in Ireland, where over a million people died. Many other countries were also affected, especially Belgium and Prussia. In addition to mortality, the famine also resulted in widespread migration out of Europe, with over two million from Ireland alone. These factors in addition to the reduced birthrates resulted in a declining population in Europe.

### 14.1.2 Traditional Methods Used to Control Fungal Diseases

There are a variety of strategies used to control fungal diseases. The most commonly used methods include the use of clean seed, the avoidance of the pathogen by crop rotation, management or sanitation, elimination of alternate hosts, fungicides, biocontrol and host resistance. Crop rotation exploits the fact that most pathogens have a specific host plant species and are unable to cause disease on other plant species, especially if they are not closely related. For example, the fungus *F. graminearum* infects cereals such as wheat and maize but not soybeans. Therefore, planting wheat on a field where maize was planted the previous season will increase the disease incidence, while this can be avoided by planting soybeans in that field. Although much success has been achieved by crop rotation, in recent years the infection of non-cereal crop species with *F. graminearum* has been reported. Another useful method of controlling most fungal pathogens is to plough under the infected crop residue in order to eliminate the overwintering inoculum, especially in the temperate regions. However, this practice causes soil compaction and results in the degradation of soil aggregates, loss of organic matter and the loss of beneficial soil microbes, among a host of other deleterious effects. Therefore, it is a less desirable option.

Some fungi such as the rust fungi need an alternate host to complete their life cycle. If the alternate host is not the crop species, eliminating it would prevent the fungus from completing its lifecycle, thereby reducing the inoculum available to cause disease when the crop plant is growing. A classic example of this tactic is the barberry plant that acts as the alternate host for the stem rust fungus *Puccinia graminis*. To prevent the spread of stem rust, the growing of barberry has been outlawed in wheat growing regions such as Rouen, France, since 1660.

Fungicides have been widely used to control disease but are accompanied by a host of problems, including the high cost of the chemicals and their application. Another problem with fungicide use is their toxicity to humans and animals. They may be exposed to the compounds either when they come in contact with treated plants or when they consume produce that contains traces of it. Some fungicides such as those containing mercury may accumulate in the treated plants and therefore build up in the animals feeding on these plants, making the animals toxic, too. These fungicidal compounds will also eliminate the fungi that are beneficial to the plant, making them more susceptible to biotic and abiotic stresses and less productive. The most concerning problem about fungicide use is that the pathogenic fungi develop resistance to the chemicals after a relatively short period of use. Although the effective life span of a fungus can be increased by the use of resistance management strategies where no one fungicide is used over a prolonged period, the effective lifespan still remains limited.

Biocontrol is the practice of using living organisms to control the proliferation of disease causing organisms. When an option, this method is desirable as the negative environmental effects of fungicide use are not a problem. *Clonostachys rosea* ‘IK726’ is a fungal species isolated from barley roots and is effective in controlling several seed borne fungal diseases in crops including cereals, carrots, cabbage and sugar beets. However, the possibility of introducing invasive organisms that may create greater ecological disasters or the evolution into organisms that can cause disease instead of protecting the plant from it are some of the negative aspects of this option. The use of fungus resistant crops does not carry as many of these risks. For this reason, the development of fungus resistant crops is a viable, long-term option.

Resistance can be classified in two broad groups – horizontal and vertical. Horizontal resistance describes plants that are resistant against a broad range of pathogens. It is determined by a large number of genes. While the degree of resistance may not be very high, it tends to be very stable and cannot be easily overcome by the pathogen. Breeding this type of resistance in a crop involves several generations of selection for the most resistant plants and can take a number of years. However, new techniques such as marker-assisted selection permit the screening of a larger number of plants at the seedling stage, thereby shortening the time required to produce the new variety. Vertical resistance describes plants that are resistant to a specific pathogen species or in some cases a particular race within a species. It is determined by either one or a few genes. Although it is able to provide near absolute protection to the plant, the pathogen can readily evolve to overcome this resistance. Breeding for vertical resistance requires a sound understanding of the interaction between the plant and pathogen and the availability of resistance genes either in the crop species or the wild relatives of those species so that they can be transferred into commercial varieties of the crop. Traditionally, breeders would introduce this gene to the commercial variety by crossing. Sometimes these crosses are not successful, especially if the plant with the resistance gene is a wild relative of the crop plant. Even when successful, several other genes will be introduced to the commercial variety along with the resistance gene that may lead to reduction in the quality or quantity of yield. However, these problems can be overcome by the use of transgenic technologies, as it is now possible to transfer specific genes into the plant. Since it is now possible to transfer genes from unrelated species, it also broadens the source of resistance genes.

Although each individual method described above has its innate problems, when several of these methods are used in concert, they can have greater efficacy, reduce the use of harmful chemicals, and prolong the useful life of both resistance genes and fungicides.

### *14.1.3 What Strategies Do Fungi Employ to Successfully Invade Their Host Plant?*

The strategies fungi use to invade and colonize plants can be broadly classified into two groups – the necrotrophs and the biotrophs. Necrotrophs are those fungi that produce toxins to kill the host cells and then feed off the dead cells. The biotrophs are fungi that use ‘stealth technology’ to invade the plant without tripping any of the plant defenses. They require live host tissue for survival and obtain nutrients from the plant cells through a specialized structure called a haustorium. There are also some that fall in between these two extremes. They are the hemi-biotrophs – they start out biotrophic and then convert to a necrotrophic lifestyle. It is important to have a good understanding of these processes before selecting candidate genes for use in producing resistant plants.

## 14.2 How Do Plants Resist Fungal Pathogens in Nature?

Plants have numerous innate mechanisms that help them to resist invasion by the countless fungal pathogens surrounding them. These mechanisms can be broadly categorized as being either constitutive or induced. The constitutive mechanisms are those that are expressed constantly regardless of the level of threat from pathogens. These include structural features such as thicker cuticles or cell walls that act as a barrier to the invading pathogen. Apple scab is caused by the fungus *Venturia inequalis*. When one of these spores lands on a leaf, it germinates to form a germ tube that penetrates the cuticle. The fungus is then able to spread within the leaf to cause disease (Gevens and Nicholson 2000). However, the fungus is able to penetrate only the cuticle of juvenile leaves when it is relatively thin. As the leaf matures the cuticle thickens becoming less penetrable to the fungus.

Some plants are able to produce antifungal compounds that prevent the fungus from being able to proliferate within the plant tissue and cause disease. The saponins are one such group of compounds that have been identified in about 100 plant families. The toxicity of these compounds is attributed to their ability to form complexes with components of the fungal cell membrane, making it porous (Osbourn et al. 1994). This is deleterious to the fungus as the cellular contents will leak out. Some fungi produce compounds that are toxic to the plant host and aid the fungus in colonizing the plant to cause disease. *Helminthosporium carbonum*, a virulent maize pathogen produces HC-toxin (*Helminthosporium carbonum*-toxin), a compound that affects the DNA of host cells. Maize varieties that are able to produce the enzyme (carbonyl reductase) that detoxifies HC-toxin by modifying the structure of the toxin are immune to the effects of this compound.

Plants are also able to mount a gamut of induced responses when under attack. Detection of the invader sets off a series of reactions that culminate in the expression of an array of responses both locally and systemically. These responses include the production of compounds that are toxic to the pathogen, such as reactive oxygen species (ROS) or phytoalexins and enzymes that are able to degrade the pathogen. ROS are free radicals of oxygen that are produced as a burst immediately after infection. In addition to being harmful to the invading pathogen, they are also the signal that alerts the surrounding cells to mount a response to the pathogen. Phytoalexins are a group of chemically diverse, antimicrobial compounds that are produced by plants immediately after the pathogen attacks. Some of the well-known phytoalexins include resveratrol in grapes, pisatin in peas and 3-deoxyanthocyanidins in sorghum and sugarcane. The 3-deoxyanthocyanidins are a group of flavonoid compounds that have potent antifungal activity. In our laboratory we study the role these compounds play in making the plant resistant to fungal pathogens. Sorghum plants that are able to produce these compounds are better able to resist invasion by fungal pathogen *Colletotrichum sublineolum*. The release of these compounds results in the death of the fungal cells and the plant cells surrounding the invading pathogen. In this way the pathogen is contained and disease is prevented. In similar plants that are not able to produce these compounds the fungal pathogens are able to proliferate in the leaf tissue resulting in the premature death of the leaf (Ibraheem et al. 2010). The plant also produces enzymes such as chitinases – enzymes that degrade chitin. Since chitin is unique to the fungal cell wall, these enzymes will be harmful to fungal cells only.

The most drastic response is the phenomenon known as the hypersensitive response, a form of programmed cell death where the host cells in the immediate vicinity of the invader are 'killed off' to restrict the spread of infection. This phenomenon is mediated by some of the toxic compounds produced to inhibit the pathogen as they are also toxic to the plant cells producing it. The infected cells also produce signaling molecules such as jasmonic and salicylic acids that inform both the adjacent cells and the rest of the plant of the pathogen attack. These cells are then able to mount a defense in anticipation of the pathogen attack. Although this is the general *modus operandi* of how plants defend themselves against fungal pathogens, the specifics of the fungal-plant interaction have at least a few unique aspects that are determined by the individuals involved in the interaction. Therefore, it is important to have a detailed understanding of the specific interaction when selecting candidate genes for use in engineering plants resistant to fungi.

### 14.2.1 Use of Transgene Technology to Produce Plants Resistant to Fungal Pathogens

To date, no transgenic crops resistant to fungal diseases have been released. However, there are several examples of transgenic plants that have been developed to be resistant to fungal diseases. In many instances, these transgenic plants are resistant to the disease when tested in the laboratory but when the trials are extended to the field, the results have not been conclusive. However, some of them are in the advanced stages of testing and have undergone several successful years of field trials. There are several strategies employed when selecting candidate genes for transformation. Much success has been achieved by transforming the plant with genes coding for compounds that have antimicrobial activity such as the thionins, chitinases, glucanases, etc. The other option is to transform the plants with resistance genes, or R genes as they are more commonly known. These genes code for molecules that are able to recognize a fungal molecule coded by an avirulence gene (*avr* gene) and set off a series of events that result in the expression of resistance mechanisms described above. This includes the production of the ROS burst in the short-term and long-term responses, such as the production of antimicrobial compounds. Although this strategy is very effective, as it can provide total resistance and can be achieved by the transfer of a single gene, the downside to it is that it is very specific to a fungal species and in some cases to a race within that species. Therefore, the plant will continue to be susceptible to other races of the pathogen. The other problem is that the fungi are able to evolve rapidly such that the avr gene product cannot be recognized by the plant, leading to a breakdown in the resistance. However, the lifespan of an R gene can be extended by producing plants with multiple R genes or by deploying a mixture of R genes within the crop.

### *14.2.2 Some Examples of Transgenic Plants Shown to Be Resistant to Fungal Disease*

Apple scab is one of the main diseases encountered in commercial apple production, but it requires several sprayings of fungicide during the growing season. Since apple trees have a relatively long generation time of 6–8 years and they do not readily self-pollinate, breeding for resistance using traditional methods is even less feasible.

Two varieties of transgenic plants containing the type 1 α-hordothionin gene from barley were produced and tested extensively both in the greenhouse and in the field over four years. They were able to identify four lines that showed reduced symptoms compared to the controls (Krens et al. 2011). The α-thionins have been shown to be antimicrobial. It is believed that they act by making the fungal membrane more permeable leading to the loss of cellular contents.

In wheat, one of the most damaging fungal diseases is powdery mildew. Over 40 powdery mildew R genes (*Pm*) have been identified. Several transgenic wheat lines were produced by transforming them with different versions of the *Pm3* R gene. Extensive testing including 2 years of field trials have shown that the plants containing the *Pm3* gene were more resistant to powdery mildew than the non-transgenic control plants (Brunner et al. 2012).

Golden Promise, a variety of barley that is highly susceptible to stem rust was transformed with the *Rpg1* gene to produce a variety highly resistant to the disease. The transgenic plants showed very few symptoms and produced far fewer fungal spores, thereby reducing the amount of inoculum available for subsequent infections. To date the performance of these transgenic plants has not been tested in the field. *Rpg1*, an R gene has been shown to be a receptor kinase – a molecule that is able to recognize the presence of the fungus and signal the cellular machinery to mount a defense response (Horvath et al. 2003).

The chestnut blight fungus secretes several toxic compounds like oxalic acid which lowers the pH of the surrounding plant tissue. This causes the death of the infected tissue. Plants transformed with an oxalate oxidase gene from wheat are able to detoxify the oxalic acid thereby starving the fungus and restricting it to the bark. These plants have been shown to be tolerant to the disease and have undergone rigorous testing in the laboratory and several years of successful field trials.

Late blight of potato is one of the most devastating diseases and is caused by a pathogen similar to fungi. The most common control method in use today consists of fungicide applications, sometimes as frequently as once in 4 days. In addition to the fungicide being costly and environmentally unsuitable, the pathogen is able to evolve resistance to the chemicals rapidly. Therefore, several lines of transgenic potato containing R genes identified in wild potato species have been produced. Since these R genes were identified in wild potato species, the use of transgene technology facilitated the rapid transfer of these genes into cultivated potato varieties. These plants have been shown to be resistant to late blight in two years of field tests (Bradeen et al. 2009; Foster et al. 2009).

The examples cited above are only a minute fraction of the transgenic plants showing resistance to fungal diseases. However, they are the most extensively tested and closest to deployment.

## References

Bradeen JM, Iorizzo M, Mollov DS, Raasch J, Kramer LC, Millett BP, Austin-Phillips S, Jiang J, Carputo D (2009) Higher copy numbers of the potato *RB* transgene correspond to enhanced transcript and late blight resistance levels. Mol Plant-Microbe Interact 22:437–446

Brunner S, Stirnweis D, Diaz Quijano C, Buesing G, Herren G, Parlange F, Barret P, Tassy C, Sautter C, Winzeler M (2012) Transgenic *Pm3* multilines of wheat show increased powdery mildew resistance in the field. Plant Biotechnol J 10:398–409

Foster SJ, Park T-H, Pel M, Brigneti G, Śliwka J, Jagger L, van der Vossen E, Jones JD (2009) *Rpi-vnt1. 1*, a *Tm-22* homolog from *Solanum venturii*, confers resistance to potato late blight. Mol Plant-Microbe Interact 22:589–600

Gevens A, Nicholson RL (2000) In this issue "cutin composition: a subtle role for fungal cutinase?". Physiol Mol Plant Pathol 57:43–45

Horvath H, Rostoks N, Brueggeman R, Steffenson B, von Wettstein D, Kleinhofs A (2003) Genetically engineered stem rust resistance in barley using the *Rpg1* gene. Proc Natl Acad Sci 100:364–369

Ibraheem F, Gaffoor I, Chopra S (2010) Flavonoid phytoalexin dependent resistance to anthracnose leaf blight requires a functional yellow seed1 in Sorghum bicolor. Genetics 184:915–926

Krens FA, Schaart JG, Groenwold R, Walraven AEJ, Hesselink T, Thissen JT (2011) Performance and long-term stability of the barley hordothionin gene in multiple transgenic apple lines. Transgenic Res 20:1113–1123

Osbourn A, Bowyer P, Bryan G, Lunness P, Clarke B, Daniels M (1994) Detoxification of plant saponins by fungi. In: Daniels MJ, Downie JA, Osbourn AE (eds) Advances in molecular genetics of plant-microbe interactions. Springer, Dordrecht

# Part IV
# Sustainable Environment

# Chapter 15
# Root Traits for Improving Nitrogen Acquisition Efficiency

**Joseph G. Chimungu and Jonathan P. Lynch**

**Abstract** Agriculture is the mainstay of most developing countries' economies across the globe and continues to play a vital role in the sustenance of human society. Nitrogen (N) often limits crop production and fertilizer usage is limited in developing nations, while in high-input systems intensive fertilization incurs substantial economic and environmental costs. The development of nitrogen efficient crops would ameliorate these problems. In low-input systems they will increase productivity, permitting farmers to climb out of the poverty trap of low inputs and low yields. In high-input systems they will reduce environmental pollution and crop input costs. The global significance of these issues means that the development of crops with reduced N requirement has become a high priority. There is substantial genetic variation for root traits that have potential utility for enhancing N acquisition. In this chapter, we focus on the potential benefits of root phenes that could enhance N acquisition and therefore could be deployed to improve agricultural sustainability.

**Keywords** Agriculture • Nitrogen • Efficiency • Phenotype • Root architecture

## 15.1 The Need for Nitrogen Efficient Crops

World agriculture is facing a formidable challenge to improve crop production to feed a growing population while enhancing the sustainability and profitability of cropping systems, reducing their environmental impact, and preserving biodiversity. The challenge of food security is further complicated by a changing global climate. In this challenging scenario, better knowledge of the genetic and functional basis of plant phenes that enhance soil resource acquisition will be of paramount importance.

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A. Ricroch et al. (eds.), *Plant Biotechnology: Experience and Future Prospects*, DOI 10.1007/978-3-319-06892-3_15,

Nutrient efficient crops have an important role in modern agriculture. In low-input systems that characterize half of world agriculture, nutrient efficient crops will improve crop productivity. In high-input systems of the developed world, nutrient efficient crops will be valuable by reducing input costs and associated environmental impacts. The key to development of these crops is the utilization of the large genetic variation for root phenes (see Box 15.1) that enhance nutrient acquisition.

One of the most important inputs to agricultural systems is nitrogen. Nitrogen, after carbon, oxygen and hydrogen, is the most abundant mineral element in plants and is one of the primary limiting resources in agricultural systems. The use of inorganic N fertilizers has proven to be indispensable for modern crop production. The almost twofold increase in food production in the past 40 years has been largely attributed to the increased use of N fertilizer. While this is a common solution in high-input systems of the developed world, it is often impossible in low-input systems where farmers are poor and cannot afford to buy fertilizers. Furthermore, it is estimated that less than 50 % of nitrogen fertilizer applied is actually taken up by the crop; the remainder is incorporated into soil organic matter or lost from farmlands through erosion, surface runoff, leaching, and volatilization, consequently causing environmental pollution (Raun and Johnson 1999). Moreover, production of inorganic N fertilizers through the Haber-Bosch process is extremely energy consuming and associated with a large carbon footprint. Another major challenge with the use of inorganic N fertilizer has been the sharp increase in its price, driven mainly by increases in the price of fossil fuel. In rich nations the increasing costs of N fertilizers reduces profit margins, while in developing countries where the majority of the earth's population lives, suboptimal N availability is a primary constraint to food production, and the cost of fertilizer is already considerably higher for poor farmers and access to fertilizer is very limited. The global significance of these issues means that the development of crops with reduced N requirement has become a high priority from a scientific, economic and environmental perspective.

Nitrogen efficiency has two fundamental aspects; acquisition efficiency and utilization efficiency. Ideally, plants should be developed with improvement in both aspects. Efficient acquisition of N is an important factor considering that over half of N applied is lost from cropping system via leaching and other factors. Inherently, root traits have a substantial influence on improving N acquisition from soil. Development of crop genotypes with appropriate root traits might improve crop productivity and sustainable agriculture. In this chapter, we focus on root phenes (where *phene* is to *phenotype* as *gene* is to *genotype*) that have potential to enhance N acquisition and discuss their potential deployment in crop improvement programs.

**Box 15.1: Phene**

A phene is a distinct element of an organism's phenotype. The phenotype is the result from the complex interactions between genes and the environment. The phenotype is the collection of distinct phenes, as genotype is the

(continued)

(continued)

collection of many distinct genes. Phene have variants which are called phene states. As an example, root angle is a phene that is important for N acquisition. This phene that has (at least) two states: “steep” and “shallow”, with the steep state being particularly important for N acquisition in leaching environments. Some phenes are plastic. Plasticity is the ability of an organism to change its phene state in response to changes in the environment. For example plants exhibit plasticity of root growth angles in response to suboptimal nitrogen availability. Root plasticity is potentially heritable and can be an important aspect of a plant’s ability to acquire scarce soil resources.

## 15.2 Root Ideotype for Improved Acquisition of Nitrogen

Improved ability to acquire N from the soil both in high input and low input agriculture could be related to several root phenes. Figure 15.1 shows a root ideotype for efficient N acquisition in maize, as suggested by Lynch (2013). The ‘steep, deep, and cheap’ ideotype consists of several root phenes that may enhance N and water acquisition by maize root systems by improving deep soil exploitation. ‘Steep’ refers to architectural phenes such as growth angles of crown and brace roots that increase the depth of soil exploration. ‘Cheap’ refers to architectural and anatomical phenes that reduce the metabolic cost of soil exploration, thus permitting deeper root growth and development. Significant genetic variation for these phenes exists in maize, and coupled with high throughput phenotyping methods should accelerate the understanding and deployment of these phenes in crop improvement programs. The prospects for screening and understanding the utility of phenes in Fig. 15.1 to enhance N acquisition and crop production in both low and high-input systems as well as the likely interactions affecting their utility are now being considered. In subsequent sections we will briefly discuss some of the phenes in the ideotype.

## 15.3 Root Architecture

Root architecture refers to the spatial configuration of the root system within the soil. Although the root system for monocots and dicots differs, some architectural phenes associated with enhanced N acquisition are common. Root angle is an important component of root architecture in relation to the acquisition of N and appears to be affected by N availability (Trachsel et al. 2013). The angle at which roots penetrate the soil is related to root depth. The angle at which roots emerge from the seed could be used as a proxy for deep rooting characteristics, particularly

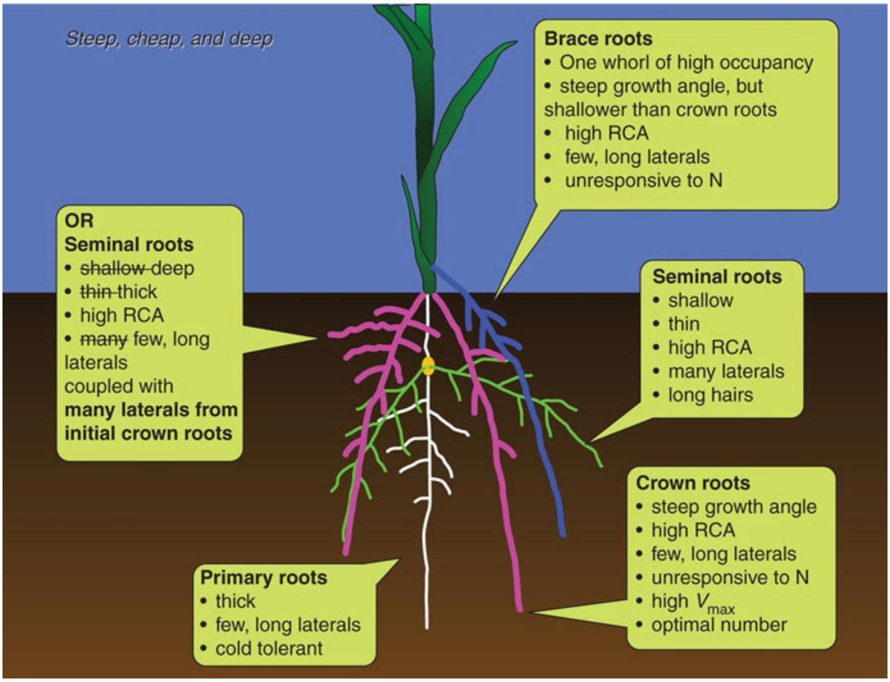

**Fig. 15.1** A hypothetical integrated phenotype for efficient acquisition of nitrogen by maize root systems. The ideotype consists of architectural traits; shallow branched seminal roots, steep, sparsely branched nodal roots, complementary angles of crown and brace roots, non-plastic growth responses, long, dense root hairs, and anatomical traits; abundant root cortical aerenchyma and reduced living cortical area, that reduce the metabolic cost of soil exploration (From Lynch 2013)

if it reflects an underlying gravitropic tendency in the root system. All else being equal, steep root angles are associated with deeper root systems. Deeper roots allow crops to capture the leached N deeper in the soil profile otherwise unavailable for plant growth. This hypothesis has been supported by a recent field study which found that steep angled maize genotypes had better growth under low N than the closely related shallow-angled genotypes (Trachsel et al. 2013). Although axial root growth angle is intrinsically determined by genotype, it also is influenced by the soil environment, including the availability and distribution of nutrients. Trachsel et al. (2013) showed that steeper root angles enabled plastic (see Box 15.1) genotypes to potentially explore similar soil volumes under nitrogen deficient conditions as steep genotypes, thereby not incurring any reductions in grain yield compared to maize genotypes constitutively forming steep root angles. Such evidence demonstrates the underlying importance of plasticity of root traits in improving N acquisition.

Modeling and some experimental evidence suggest that a dimorphic root system, having both shallow and deep roots to enable acquisition of mineralized N in the topsoil as well as leached N at depth, could be useful. Early season topsoil foraging is important for exploitation of immobile resources such as phosphorus, potassium and ammonium as well as mobile resources such as water and nitrate that have not yet been subject to depletion from the topsoil by plant uptake, evaporation (including denitrification and volatilization) and leaching (Lynch 2013). In support of this hypothesis, vigorous wheat lines with faster vertical root growth and early extensive horizontal root development had significantly better N-acquisition than their counterparts in sandy soils (Liao et al. 2006).

Research in both monocots and dicots has shown the potential importance of root system architecture under edaphic stress. Quantitative trait loci (QTL) controlling axial root growth angle have been identified in maize. This phene can be readily measured in seedlings as a proxy for the mature plant and by using high throughput methods such as shovelomics for mature plants in the field (Trachsel et al. 2010).

## 15.4 Phenes that Enhance N-acquisition at Minimal Metabolic Cost

The metabolic costs of soil exploration are quite substantial for plants and can significantly impact the economic yield and plant fitness under edaphic stress. Plant allocation to root growth increases under nutrient stress, and therefore the metabolic cost of root growth becomes a significant component of plant adaptation to nutrient stress and fitness. In general, when N availability decreases, plants allocate relatively more resources to their roots, which is consistent with a resource optimization hypothesis. This strategy comes at a cost in that each unit of leaf area has relatively more non-photosynthetic tissue to sustain. A plant that is able to acquire N at reduced metabolic cost will have superior productivity, because it will have more metabolic resources available for further resource acquisition, growth, and reproduction. Several types of root phenes can alter the cost of root growth and soil resources acquisition. Anatomical phenes may reduce root cost due to alterations in the ratio of respiring to non-respiring tissue. Such phenes include root cortical aerenchyma (Fig. 15.2), low living cortical area, and cortical senescence. Morphological phenes such as root hairs, root diameter and optimal number of nodal roots per plant have potential to enhance N acquisition at minimal root metabolic cost.

### *15.4.1 Anatomical Phenes*

Root cortical aerenchyma (RCA) is usually considered to be an adaptation to hypoxia; however RCA is also induced by suboptimal availability of nitrogen, phosphorus, sulfur, and water. RCA converts living cortical root tissue to large intercellular spaces by programmed cell death (Fig. 15.2), thereby reducing root respiration (Fig. 15.3). RCA may be useful for N acquisition by releasing resources that allow the plant to invest in new root growth for deep soil foraging. Genotypic variation in RCA formation is associated with deeper rooting, better plant water status,

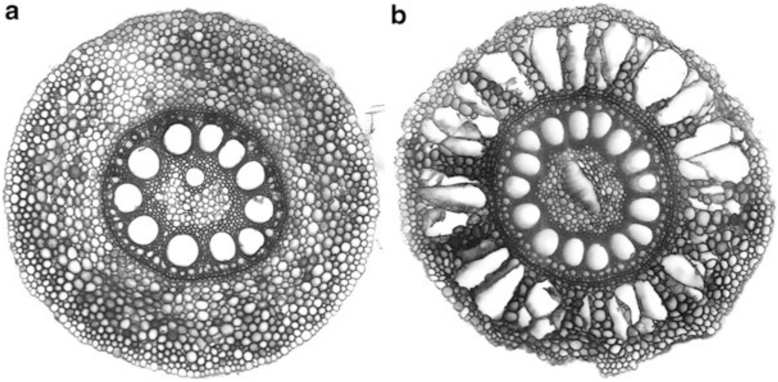

**Fig. 15.2** Cross-sections of second crown root of maize 70 days after planting showing genotypic variation in cortical aerenchyma formation, which replaces living tissue (**a**) with air-filled lacunae (**b**). Genotypes are closely related recombinant inbred lines (*RILs*) of the same parents growing in the field

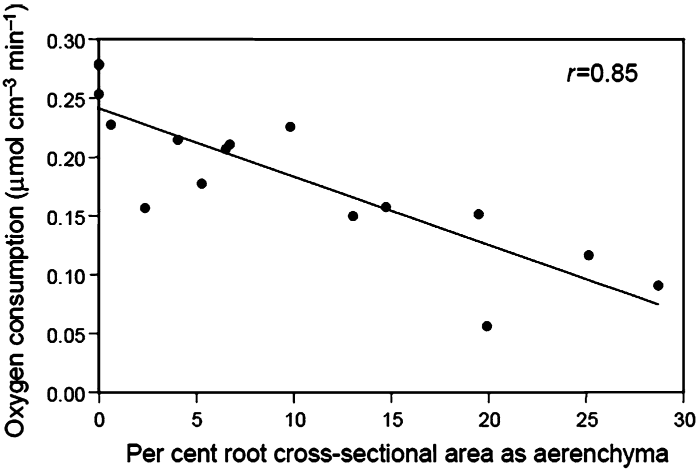

**Fig. 15.3** Root segment respiration is correlated with the proportion of aerenchyma in a root cross section. Cortical aerenchyma formation in maize roots disproportionately reduces root respiration (From Fan et al. 2003)

and 800 % variation in yield under drought stress in the field (Zhu et al. 2010). *SimRoot* modeling indicates that this trait can substantially increase the acquisition of N in maize, especially in low fertility sandy soils with high rates of N leaching (Postma and Lynch 2011). Thus, a deeper root with high RCA is important for efficient capture for mobile soil resources, such as nitrate and water. QTL have been identified controlling RCA formation in maize with potential application in breeding programs to enhance flooding tolerance in maize and other cereals.

Another anatomical modification with the potential to reduce metabolic cost is cortical senescence. Cortical senescence is the eventual loss of the cortex in mature regions of the root. This process is distinct from the formation of aerenchyma discussed above and from the loss of the cortex resulting from secondary thickening in many dicotyledonous plants. Cortical senescence has potential implications in reducing the metabolic cost of root growth and resource acquisition. The loss of living cells may also reduce hydraulic conductivity of the root and thereby affecting radial transport of water and nutrients across the remaining cortex to the stele. This subject remains to be researched to evaluate the utility of this trait under edaphic stresses such as N. Recently it was reported that low living cortical area (LCA) reduces root cortical burden, thereby improving root growth under water-limited conditions (Jaramillo et al. 2013). Low LCA may also be useful for N acquisition by releasing resources that allow the plant to invest in new root growth for deep soil foraging.

### *15.4.2 Morphological Phenes*

The most efficient way of increasing absorbing root surface area is the formation of root hairs. The variation in root hair length and density among plant species is well documented, but the variation among genotypes and its link to N acquisition is poorly understood. Root hair growth has been shown to increase under suboptimal availability of nutrients such as phosphorus. Electrophysiological and molecular evidence supports a role for root hairs in the uptake and transport of both nitrate ($NO_3^-$) and ammonium ($NH_4^+$) (Gilroy and Jones 2000). Unpublished results from field experiments indicate that maize genotypes with long root hairs acquire more N under low N conditions. Root hairs are attractive targets for crop improvement programs because there is large genotypic variation in root hair length and density, relatively simple genetic control, and opportunities for direct phenotypic selection. Genotypic variation in root hair length and density in maize and common bean is controlled by several major QTL, suggesting that this trait could be selected using marker assisted breeding. This raises the possibility that the selection and breeding of crop genotypes with root hairs may contribute to more efficient N-acquisition thereby reducing environmental impact of agriculture and improving crop productivity and sustainability.

Another morphological modification to the root system that can reduce metabolic costs is root diameter or the production of finer roots. If a fixed proportion of assimilates is used for root growth, greater root length can be achieved by reducing root diameter, i.e. ‘specific root length’ (length of root per unit root mass). In general under growth-limiting conditions the construction costs per unit root length should be minimized to enable a large soil volume to be explored at reduced cost. The variation in specific root length (SRL) among plant species is well documented; but variation of SRL among genotypes and its link to N acquisition is poorly understood. Crop genotypes with high SRL may be more efficient in N acquisition than those with low SRL. High SRL genotypes should be able to increase the length of their root system more easily than those with low SRL, thus enabling them to acquire rapidly leaching N in both low and high-input systems. However, the maintenance cost of producing finer roots may be greater, because they may be more vulnerable to adverse edaphic and biotic conditions than thicker roots and have to be replaced more frequently.

## 15.5 Physiological Mechanism of N Uptake

Crop plants acquire N from the soil mainly in the form of $NO_3^-$, through the activity of both high-affinity transport systems (HATS) and low-affinity transport systems (LATS). One adaptation to low N is the upregulation of HATS-N in roots, giving rise to the hypothesis that HATS may enhance N acquisition under low N and low-input agroecosystems. Because LATS is operative only at high N concentrations, the relative contribution of this system to the overall N acquisition of the plant might increase where roots are likely to encounter patches and pulses of high N. In most agroecosystems, however, the concentration of nitrate in bulk soil solution is so low that the relative contribution of the LATS becomes negligible. In most agroecosystems where there is suboptimal N and its availability fluctuates during the season due to mineralization, leaching, and denitrification, the most meaningful component of the root nutrient uptake system is the HATS and its kinetic parameters. The dynamics of N uptake can be quantitatively described using the Michaelis Menten equation, and its two key parameters; Vmax (the maximum velocity of uptake) and Km (the substrate concentration at which half the maximum velocity is attained). They are the measures of maximum uptake rate and the affinity of the uptake sites for the nutrient, respectively. Some studies have suggested that these parameters can be useful to select the genotypes having high nutrient acquisition efficiency.

## 15.6 Genetic Variation for Root Phenes

There is substantial genetic variation for root phenes that have potential utility for enhancing crop N acquisition. For example, there is substantial variation for both root anatomical and architectural phenes among maize RIL populations, maize landraces, and teosintes. The presence of substantial variation for root phenes is pivotal for development of crop cultivars with enhanced N acquisition.

## 15.7 Deployment Strategies of Root Phenes in Crop Improvement Programs

Several phenes have been identified with potential utility in breeding N efficient crops (Fig. 15.1), as discussed above, including deep soil foraging through steep root growth angles, cortical aerenchyma, living cortical area, root hairs, and specific root length. Deployment of these phenes in crop improvement programs will result in the development of crop cultivars with enhanced N acquisition efficiency, benefiting resource-poor farmers in developing countries who were largely left behind by the first green revolution. In developed countries, where there is intensive use of fertilizer, they will reduce environmental pollution and increase profits by enhancing the recovery of applied N. Breeding for enhance N acquisition can be approached with modern breeding tools, based on molecular breeding, transgenic approaches and through conventional breeding that typically rely on some form phenotypic selection in a target environment as described below.

As mentioned in the previous section, genotypic variation for root phenes has been observed in many food crops. This variation can be utilized in development of cultivars that are superior in N acquisition and higher yielding in low-input systems. Conventional breeding involves phenotypic selection for improved root systems in nutrient deficient soils and this has proven to be an inefficient strategy. One potential challenge of conventional breeding for improved nutrient acquisition is the spatiotemporal variability of nutrient availability within screening field plots, which can result in substantial genotype x environmental interaction and, as a result, low heritability of phenes. This may require very extensive and costly field evaluation in multiple environments. These challenges make the prospects of using trait based selection a viable alternative.

Trait based breeding is based on targeting the improvement of specific phenes known to increase N-acquisition, and has merit over breeding for yield *per se* under nutrient stress, because it increases the probability of crosses resulting in additive gene action. Before specific root phenes can be considered for selection, they need to meet certain requirements; namely that (a) genetic variation for the target phenes exist within germplasm being utilized, (b) availability of high throughput evaluation methods of the phene expression, and (c) the utility for genetic variation for target phenes in the agroecosystems of interest. In the case of the phenes discussed here, unfortunately there are few examples that meet all these prerequisites and therefore there is urgent need for focused studies to evaluate them. For example, steep axial root growth angles associated with deep soil foraging is critically important for N acquisition, and genotypic variation for axial root angles is well established in several crops. This strategy has proven to be successful in the deployment of shallow root growth angles, root hair length and density, and basal root whorl number, which are important for phosphorus acquisition in common bean.

Both conventional and trait-based strategies rely on extensive field screening of root phenes. A significant challenge to the selection for root phenes is the difficulty of evaluating phene state (see Box 15.1), since many root phenes are plastic, roots are difficult to extract from the soil, such extraction may change certain architectural phenes, and many root sampling procedures are destructive and impractical for screening large numbers of diverse lines. This potential drawback may be overcome by the use of modern breeding approaches that rely on molecular makers tightly linked to phene expression. Root phenes are controlled by several genes; an appropriate method to dissect multigenic heritance is through the identification of Quantitative Trait Loci (QTL). Identifying and placing QTL on a genetic map is based on the statistically significant association of phene expression with molecular makers that constitute the genetic map. Molecular makers found to be linked to the phene can used for selection in the breeding process. Molecular makers are useful especially for phenes that can only be evaluated on mature plants and with high plasticity resulting in low heritability. The availability of molecular makers in low-input systems is limited. Some architectural phenes can be rapidly phenotyped in the field using a simple method called shovelomics, in which root crowns are excavated and visually scored for several root phenes (Trachsel et al. 2010).

## 15.8 Modeling Approach Towards the Study of Root Phenes

Models that mechanistically simulate processes occurring in biophysical systems provide researchers a powerful investigative tool that complements empirical approaches. Modeling offers several advantages for identifying key plant phenes. Advantages include the ability to assess the utility of a phene under a diverse range of soils, climates, and weather patterns. *SimRoot* is an example of a structural functional plant model that has been useful in elucidating the utility of root anatomical and architectural phenes for soil resource acquisition in maize (*Zea mays* L.) and common beans (*Phaseolus Vulgaris* L.). *SimRoot* is a mechanistic model which uses empirical architectural and anatomical data of plant root systems (Postma and Lynch 2011). *SimRoot* simulations found that RCA increases the efficiency of N-acquisition in coarse textured soils, which are more prone to leaching than fine soils (Fig. 15.4).

The utility of phenes for N acquisition may depend on the expression of other phenes in the plant phenotype. Such interactions may be synergistic, neutral, or antagonistic. For example greater lateral root length and density in deep soil layers would improve nitrogen acquisition. However, increased lateral branching add extra metabolic burden to the plant, and it could influence the growth of other root classes. This tradeoff could be alleviated by reducing metabolic burden. These issues are multifactorial questions for which it is impractical to use empirical approaches. Structural functional plant modeling has been used to test hypotheses regarding phene utility, and offers a convenient and fast way to explore the complexity of phene interactions.

Models also permit the evaluation of the utility of phenes and integrated phenotypes under different climate scenarios, which is especially important in the stressful environments of developing nations, which could be seriously affected by

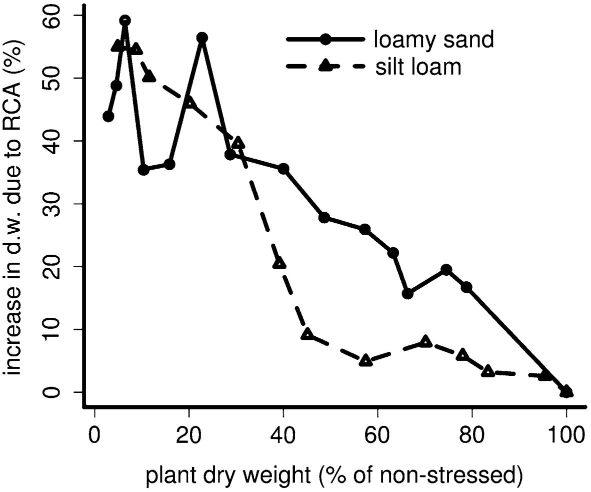

**Fig. 15.4** The predicted utility of root cortical aerenchyma in two different soils. RCA formation was more beneficial in coarse textured soils than in fine textured soils The x axis is relative plant dry weight (ratio of stressed to non-stressed plant dry weight) 40 days after germination while on y axis is the overall benefit of RCA formation (From Postma and Lynch 2011)

global climate change. However modeling has been criticized by some authors in the past for being over-simplistic and failing to take into account the many complex variables of the soil environment and dynamics; nevertheless, recent progress in root biology coupled with advances in understanding the biological, chemical and physical processes affecting root growth in soil means that newer models are now better equipped to integrate complex arrays of variables into one mathematical framework than was previously possible.

## 15.9 Conclusions

In summary, the prospects are bright for the development N efficient crops. Many of the root phenes discussed above can be easily phenotyped using several high-throughput methods and some are well characterized, and QTL have been identified, enabling the development of molecular makers, which will accelerate the process of phene identification and selection. These markers would allow early identification of the desired root phenes that can be advanced to the next stage of selection and enhance the deployment of these phenes in elite advanced breeding lines. In addition, significant genetic variation exists for the targeted phenes. However, there are important knowledge gaps in our understanding of how root phenes interact to affect plant fitness in different agoecologies, tradeoffs associated with other plant functions, and how N efficient cultivars may affect the long-term productivity of agroecosystems, considering factors such as nutrient cycling, intercropping, and the socioeconomic impacts of improved genotype on rural community. However the obvious advantage of N efficient crops in low-input systems is increased crop productivity that might permit farmers to climb out of the poverty trap of low inputs and low yields. In addition N efficient cultivars may represent the leading edge of technical intervention in low input systems, because of the relatively few barriers to their adoption, as well as the large impact they can have on crop yields. In high-input systems N efficient crops will reduce environmental pollution and improve agricultural profitability and sustainability.

**Acknowledgements** We thank Hannah Schneider and Jimmy Burridge for their helpful comments.

## References

Fan M, Zhu J, Richards C, Brown KM, Lynch JP (2003) Physiological roles for aerenchyma in phosphorus-stressed roots. Funct Plant Biol 30:493–506

Gilroy S, Jones DL (2000) Through form to function: root hair development and nutrient uptake. Trends Plant Sci 5:56–60

Jaramillo RE, Nord EA, Chimungu J, Brown KM, Lynch JP (2013) Root cortical burden influences drought tolerance in maize. Ann Bot. doi:10.1093/aob/mct069

Liao M, Palta JA, Fillery IRP (2006) Root characteristics of vigorous wheat improve early nitrogen uptake. Aust J Agr Res 57:1097–1107

Lynch JP (2013) Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems. Ann Bot 112:347–357

Postma JA, Lynch JP (2011) Root cortical aerenchyma enhances the growth of maize on soils with suboptimal availability of nitrogen, phosphorus, and potassium1[W][OA]. Plant Physiol 156:1190–1201

Raun W, Johnson G (1999) Improving nitrogen use efficiency for cereal production. Agron J 91:357–363

Trachsel S, Kaeppler SM, Brown KM, Lynch JP (2010) Shovelomics: high throughput phenotyping of maize (Zea mays L.) root architecture in the field. Plant and Soil 341:75–87

Trachsel S, Kaeppler SM, Brown KM, Lynch JP (2013) Maize root growth angles become steeper under low N conditions. Field Crop Res 140:18–31

Zhu J, Brown KM, Lynch JP (2010) Root cortical aerenchyma improves the drought tolerance of maize (Zea mays L.). Plant Cell Environ 33:740–749

# Chapter 16
# Biotech Approaches for Crop Improvement in the Semi-arid Tropics

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**Abstract** The semi-arid tropics (SAT) include parts of developing world that are characterized by unpredictable weather, limited rainfall and nutrient-poor soils. The crops grown in SAT region are exposed to various biotic and abiotic stresses adversely affecting the crop productivity. To meet the rising demand for food production in the context of population growth and climate variability, agricultural crops need to be improved so that they are better adapted to biotic and abiotic stresses, leading to higher crop productivity. The use of genomic approaches for the indirect selection of improved crop varieties can facilitate breeding strategies by alleviating time-consuming traditional approaches. In recent years, developments of significant amount of genomic resources in SAT crops have made them 'genomic resource rich' from the so-called 'orphan' crops. With the advent of next-generation sequencing and high-throughput genotyping methods, large numbers of molecular markers have been developed and mapped in SAT crops. Several quantitative trait loci (QTL) have been identified and are being targeted for introgression into elite lines, using marker-assisted backcrossing (MABC) approach. MABC programs underway in SAT crops include drought tolerance in chickpea, resistance to fusarium wilt and ascochyta blight in chickpea and rust in peanut. The downy mildew resistant pearl millet hybrid 'HHB 67 Improved' has been the first public-bred product of DNA-marker-assisted selection released by the International Crops Research Institute for Semi-Arid Tropics (ICRISAT).

Besides, the use of transgenic technology potentially offers a more targeted gene-based approach for the genetic enhancement of field crops, thereby alleviating some of the major constraints to crop productivity that are difficult to breed due to species barriers or non-availability of traits in the germplasm collections. Transgenic crops

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A. Ricroch et al. (eds.), *Plant Biotechnology: Experience and Future Prospects*, DOI 10.1007/978-3-319-06892-3_16,

are spreading faster than any other agricultural technology in history, and in SAT crops where several research programs are underway to develop transgenic crops.

**Keywords** Biotechnology • Crop improvement • Genomics • Molecular markers • Semi-arid tropics • Transgenics

## 16.1 Introduction

### *16.1.1 Semi-arid Tropics*

The semi-arid tropics (SAT) encompass parts of 48 countries in the developing world including parts of sub-Saharan Africa, Latin America and Southeast Asia, and India. The SAT region is characterized by limited and unpredictable rainfall, nutrient-poor soils and other agricultural constraints. One-sixth of the world's population live in the SAT region, and are the poorest among the poor, where half of them live on less than US $1 per day. Sorghum, pearl millet, cowpea, chickpea, pigeonpea and peanut are among the vital crops that feed the poor people living in the SAT where these crops are exposed to various biotic and abiotic stresses with very low average crop productivity. Besides biotic stresses, drought, high temperatures and other abiotic stresses are the primary causes of crop losses in the SAT region that reduce average yields for most major crop plants by over 50 %. Application of biotechnological approaches has a potential to contribute efficiently to solve or reduce these problems, thereby contributing to a sustainable agriculture.

### *16.1.2 Why Crop Improvement Is Needed?*

Crop improvement programs are required to meet the growing demand for food due to population growth, climate change and malnutrition etc. Agriculture in the twenty-first century faces multiple challenges where it has to produce more food to feed the growing population. According to the United Nations population division estimates, world population is expected to reach nine billion by 2050 and most of this increase will be in Asia and Africa, which, along with the rest of the globe, will face increased strain on already insufficient natural resources. The projections show that feeding the growing world population would require raising overall food production in the developing countries by more than double by 2050.

One of the major factors that affect food production is global climate variability that is expected to affect the four dimensions of food security including availability, accessibility, utilization and stability. Global temperatures are predicted to increase by 2.5–4.38 °C by end of the century, which would severely effect the agriculture production. Estimations have indicated that rising global temperatures between 1981 and 2002 reduced the yields of major cereals by $5 billion per year.

According to the estimation of a FAOs discussion paper, by 2050 developing countries may experience a decline of between 9 and 21 % in overall potential agricultural productivity as a result of global warming (FAOSTAT).

Besides population growth and climate change, malnutrition is also a major concern. Micronutrient malnutrition is a major health burden and widespread among the people in developing and developed countries. Micronutrient deficiencies (MNDs) are of great public health and socioeconomic concern worldwide. According to a report by the World Health Organization (WHO), over two billion people suffer from vitamin and mineral deficiencies mostly involving iron, vitamin A, zinc and iodine with important health consequences worldwide.

Apart from the above factors that are increasing the demand for food supply, globally there are huge losses in food production due to various biotic and abiotic stresses. Only 10 % of the global arable land can be classified under the non-stress category, which implies that crops grown on the other 90 % of arable lands experience one or more environmental stresses. Drought problems are likely to worsen with the projected rapid expansion of water-stressed areas of the world from 28 to 30 countries today, to over 50 countries encompassing three billion people by 2030. Besides drought, soil salinity is one of the main abiotic stresses that plants encounter more frequently and it is expected that by 2050, over 50 % of all arable land will be saline. Furthermore, crops under abiotic stress are usually more susceptible to weeds, insects and diseases, which considerably increase the economic losses. Besides, there is a crucial need to enhance resistance to biotic stresses like fungal, bacterial and viral diseases as well as insect- pests that result in severe economic losses. While, resistance/tolerance to some of the biotic and abiotic constraints to crop productivity is available in the existing germplasm, in many cases biotechnological intervention holds a great potential (Sharma et al. 2002a).

### *16.1.3 Green Revolution and Gene Revolution*

The intensification of agriculture in many parts of the world over the past five decades, supported by appropriate research has led to an increase in global food grain production from approximately 850 million tons in 1960 to 2350 million tons in 2007. The last five decades have seen two waves of agricultural technology development and dissemination to developing countries.

The first wave began with the ‘Green Revolution’ in which an explicit strategy for the development and dissemination of technology aimed at poor farmers in poor countries improved germplasm that was freely made available as a public good. Modern plant breeding, improved agronomy, and the development of inorganic fertilizers and modern pesticides led to dramatic yield increases. Most industrial countries achieved sustained food surpluses by the second half of the twentieth century, and eliminated the threat of starvation.

Thereafter, a second wave generated by the gene revolution in which a global agricultural research system largely by private sector created improved agricultural technologies flowing to developing countries, mainly through market operations. Introduction of biotech approaches like genomic and trans-genomics in crop improvement initiated this gene revolution where on one hand, the recent advances in genomics by the introduction of high throughput sequencing and genotyping technologies have been facilitating the molecular breeding, on the other hand, genetic engineering technologies have been developed and improved for almost all crops facilitating gene transfer from any source. Transgenic crops are spreading faster than any other agricultural technology in history; a few examples include insect resistant varieties of cotton, and maize, and herbicide-tolerant soybeans.

### *16.1.4 Biotech Approaches for Crop Improvement*

Agricultural biotechnology involves application of scientific techniques to modify and improve crops production. Modern agricultural biotechnology has been under development since over past four decades that can contribute to overcoming major bottlenecks of classical plant breeding such as the lack of natural sources of resistance to various biotic (resistance to insect pests, fungal pathogens and viral diseases) and abiotic constraints (tolerance to drought, salinity and temperature) to crop productivity. However, successful application of biotechnology to address these constraints requires a good biological knowledge of the target plant species and the mechanisms underlying tolerance to these stresses. Different approaches, including genomics and transgenenics have been developed to understand the biological mechanisms and ultimately to improve the crop yield and quality.

## 16.2 Genomic Approaches

Since 1990s, genomics has been the most active research field in biological science generating a huge amount of scientific information on crop plants. Genomics involves the development of molecular markers for genetic diversity analysis and to help identify DNA regions tightly linked to agronomic traits called as Quantitative Trait Loci or QTLs in crops. Modern genomic approaches including development of huge sequence data using next-generation sequencing (NGS) technologies and identification of DNA sequence based markers. The use of molecular markers for the indirect selection of improved crop varieties can facilitate plant-breeding strategies by alleviating time-consuming and expensive approaches of direct screening under greenhouse and field conditions over several generations. Following the development of molecular markers, genomics involves construction of genetic maps and QTL mapping with polymorphic markers using segregating population for specific traits. Following these, closely linked marker can be identified to use in marker-assisted selection (MAS) in breeding programs.

## *16.2.1 Genomic Approaches in Legumes of the SAT*

### 16.2.1.1 Sequence Data and Molecular Markers Development in Legumes of the SAT

Molecular diversity analysis has indicated that the major SAT legumes including chickpea, pigeonpea and peanut have a narrow genetic base in cultivated gene pools that poses a big challenge for genotype identification. However, a large number of accessions for each of the three legume species are present in several gene banks of the world. The gene bank at the International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India (ICRISAT) has over 120,000 accessions of these legumes, besides cereals like sorghum and millets. In recent years ICRISAT together with its partners have developed significant amount of genomic resources in SAT crops that have made them ‘genomic resource rich’ crops from so-called ‘orphan crops’. Using different molecular approaches, several mapping populations have been generated towards developing high-density genetic maps (Varshney et al. 2013).

Molecular markers are the most powerful genomic tools to increase the efficiency and precision of plant breeding for crop improvement. For genetic diversity analysis, a range of molecular markers including hybridization-based and PCR-based DNA markers have been used. Amongst these, simple sequence repeats (SSRs), single nucleotide polymorphism (SNP) and diversity array technology (DArT) markers have become popular in the SAT crops. The SSR markers have the advantages of being multi-allelic and co-dominant and during the last few years, a large number of SSR markers in each of three legumes have been developed at ICRISAT. About 2,000 in chickpea, 3,200 in pigeonpea and about 2,500 in peanut, novel SSR markers have been developed by next generation sequence (NGS) and high-throughput (HTP) genotyping methods.

The diversity array technology (DArT) is a high-throughput marker system, which became popular in many other crop species since no sequence information is needed for developing these markers. In collaboration with DArT Pty Ltd., ICRISAT has developed DArT arrays comprising 15,360 clones in each of the three legumes that showed a narrow genetic diversity in the elite genepool as compared to landraces and wild species. DArT markers may prove useful for introgression of segments from alien species to the elite varieties of the legume crops.

Similarly, the SNP markers offer high-throughput and cost-effective genotyping options in legumes and dry land cereals of SAT. With the advent of NGS technologies, large numbers of SNP markers have been developed in recent years in chickpea and pigeonpea at ICRISAT. NGS technologies offer the ability to produce huge sequence data sets at relatively low cost in less time. Although, a number of NGS technologies have become available, 454 (454 Life Sciences), SOLiD (Applied Biosystems) and Illumina (Illumina Inc) have been the most commonly used NGS technologies for the identification of SNPs in pigeonpea and chickpea. The Illumina sequencing was carried out on parental genotypes of mapping populations and 120 million reads for chickpea and 128.9 million reads for pigeonpea was developed.

Alignment of these Illumina reads with respective transcriptome assemblies that were developed using NGS together with Sanger sequencing technology have provided >10,000 SNPs each in chickpea and pigeonpea. Moreover, different platforms including GoldenGate, VeraCode and Competitive Allele Specific PCR (KASPar) assays have also been developed in chickpea and pigeonpea for varying level of throughput of SNP genotyping. When compared to GoldenGate and VeraCode assays, KASPar assays have emerged to be cost effective owing to the requirement of very few markers for genotyping large-scale segregating populations during marker-assisted selection (MAS) programs. Considering this, KASPar assays have been developed at ICRISAT for 2,005 SNPs in chickpea and 1,616 SNPs in pigeonpea.

#### 16.2.1.2 QTL Mapping and Molecular Breeding in SAT Legumes

At ICRISAT, genetic maps in chickpea, pigeonpea and peanut have been developed using different molecular markers. Since, identification of association between the trait of interest and a genetic marker (QTL mapping) is the starting point for molecular breeding, ICRISAT and its partners have made some progress towards QTL mapping for several production constraints in SAT legumes.

**Chickpea** (*Cicer arietinum* L.): Chickpea is the most important food legume of SAT but its yield potential is limited by a series of biotic and abiotic stresses, including Ascochyta blight, Fusarium wilt, drought, cold and salinity. Moreover, terminal drought can reduce chickpea yield from 20 % to more than 50 % and hence, a deep root system capable of extracting additional soil moisture should positively impact yield in drought-prone areas.

For drought related traits, two intra-specific mapping populations of chickpea have been developed at ICRISAT and two SSR based genetic maps have been developed. Using these genetic maps and phenotyping data, QTLs for several root-related traits and other drought-tolerance-related traits have been identified in chickpea. The genomic region contains several QTLs for drought tolerance that were targeted for introgression in elite chickpea lines using marker-assisted backcrossing approach. For drought tolerance, nine leading chickpea varieties have been targeted by ICRISAT and its partners and some back cross lines were developed with varieties JG 11, Chefe and KAK 2. While the screening of these backcross lines for root traits showed encouraging results, their agronomic performance is currently being evaluated. In chickpea, genetics of resistance to Ascochyta blight and Fusarium wilt have been extensively analyzed. Efforts have been initiated to introgress resistance to Fusarium wilt and Ascochyta blight in elite chickpea cultivars from different agro-climatic zones through MABC and some back cross lines have been generated in elite chickpea cultivars.

**Peanut** (*Arachis hypogaea* L.): Peanut which is an important oilseed cash crop containing up to 54 % oil and 16–36 % protein is cultivated in over 100 tropical and subtropical countries of the world, of which 70 % of the world's peanut is produced in the SAT. India has the largest peanut growing area with 6.7 million ha (27.3 %)

and stands second in the production at 6.5 million t (18.2 %). The first genetic map for cultivated peanut, an amphidiploid (4X) species was developed at ICRISAT, that demonstrated its utility for molecular mapping of QTLs controlling drought tolerance-related traits as well as establishing relationships with diploid AA genome of peanut and model legume genome species. The SSR-based genetic linkage maps were constructed with three peanut recombinant inbred line (RIL) mapping populations, segregating for drought-tolerance-related traits. Detailed analysis using the phenotyping and genotyping data in three peanut populations identified 153 main effect and 25 epistatic QTLs for drought-tolerance-related traits. However, a majority of these QTLs contribute relatively low phenotypic variation in peanut and may not be useful for molecular breeding approaches like MABC. The genetic maps based on two mapping populations segregating for fungal foliar disease resistance in peanut were also developed and by using the genotyping and phenotyping data on these populations, a total of 28 QTLs for the late leaf spot disease and 13 QTLs for rust disease were detected. These markers were then used for introgression of this major QTL for rust resistant in three elite cultivars ICGV 91114, JL 24 and TAG 24 using MABC approach. As a result of MABC, several homozygous lines have been generated and initial screening of these lines for rust has identified several promising lines that showed remarkable reduction in the disease spread. The first peanut variety through marker-assisted backcrossing is a root-knot nematode-resistant variety, NemaTAM developed in USA.

**Pigeonpea** (*Cajanus cajan* L.): Pigeonpea is an important food legume crop in the SAT region whose average crop productivity is relatively lower than many other legumes. Narrow genetic diversity in the cultivated germplasm has constrained the effective utilization of conventional breeding as well as genomic approaches, resulting in pigeonpea being often referred to as an ‘orphan crop legume’.

In pigeonpea, availability of genomic resources was limited in the past and this restricted the development of genomic approaches for crop improvement so far. For enhancing the genomic resources of pigeonpea against biotic constraints, ICRISAT has developed large-scale SSR markers from BAC (bacterial artificial chromosome)-end sequences and their subsequent use for genetic mapping. Efforts have been made only recently to develop genetic maps in pigeonpea. Based on an inter-specific mapping population, different markers were used for developing genetic maps. Six QTLs for sterility mosaic disease have been identified using two mapping populations. Furthermore, several QTLs have been identified for fertility restoration (Rf) in pigeonpea. More recently, with the availability of a genome sequence of pigeonpea developed by ICRISAT and its partners, the progress in the crop improvement of this crop is likely to be expedited in near future.

### *16.2.2 Genomic Approaches in Sorghum and Millets*

**Sorghum** (*Sorghum bicolor* L.): It is the fifth most important cereal crop grown on 47 million ha in 104 countries in Africa, Asia, Oceania and the Americas. Sorghum, a heat and drought tolerant C4 crop is a widely consumed cereal staple in subtropical and semi-arid regions of Africa and Asia. Sorghum ranks fifth after maize, wheat, rice, and barley in worldwide grain production, and currently the second important source of grain-based ethanol in the US (after maize).

In the past, several molecular markers have been developed in sorghum, which includes both hybridization and PCR based markers. In recent years, following genome sequencing, significant progress has been made in the development of high throughput marker systems in sorghum. Recently, a German group sequenced five genetically diverse sorghum genotypes, including three sweet sorghums and two-grain sorghums, which were aligned to the sorghum reference genome resulting in the identification of over one million high-quality SNPs.

In sorghum, high-density reference maps of *S. bicolor* and one interspecific *S. bicolor* x *S. propinquum* were developed by mapping different markers. These two maps share one common parent, *S. bicolor* BTx623, which is a leading US sorghum inbred and whose shotgun genome sequence is available. In sorghum, QTLs associated with several traits have been characterized, which include grain quality and productivity, plant height and maturity, characters concerned with plant domestication, disease resistance, and abiotic stresses including post-reproductive stage drought tolerance (stay-green); pre-harvest sprouting, and aluminum tolerance by different groups. Additional morphological characteristics have also been mapped in interspecific and/or intraspecific populations of sorghum. In addition, several sorghum linkage maps have also been generated. Several molecular breeding programs on different trait like drought tolerance, grain yields etc., in sorghum are underway. The small genome of sorghum has long been an attractive model for advancing understanding of the structure, function, and evolution of cereal genomes.

**Pearl millet** (*Pennisetum glaucum* L.): Pearl millet is the world's sixth most important tropical food cereal, grown mostly in semi-arid West Africa and India. Pearl millet is the only major cereal that reliably produces both grain and forage on poor, sandy soils under hot, dry conditions. Several QTLs including drought tolerance, yield etc., have been mapped in pearl millet using different markers. In pearl millet downy mildew can cause devastating yield losses and QTLs were mapped for downy mildew resistance, using different molecular markers by ICRISAT and other institutes. This information has been used for marker-assisted backcrosses and a downy mildew resistant pearl millet hybrid 'HHB 67 Improved' was released in India in the year 2005, which was the first MAS product from a public institute.

Since, drought at the reproductive stage (terminal drought) is a major constraint to pearl millet productivity, several QTLs for grain yield and its components under terminal drought stress conditions have been identified. The major QTL associated with grain yield and drought tolerance has been identified on linkage group 2 (LG 2) and efforts are currently underway to develop closely spaced gene-based markers within this drought tolerance (DT)-QTL. At ICRISAT, cDNAs were prepared from the drought stressed leaf and root tissues of each of the two inbred genotypes of pearl millet and sequenced using NGS technologies. The SNPs and SSR markers were identified from these drought responsive expressed sequence tags (EST) sequences of pearl millet and linkage maps were developed with four recombinant inbred populations. These linkage maps were used to construct a consensus map for pearl millet that contains the largest set of mapped SSRs reported to date in pearl millet.

Notably, the genome sequences of sorghum, chickpea and pigeonpea crops are now available. The genome sequence in combination with the integrated genetic and physical maps will potentially be valuable resources providing powerful and efficient genomic tools to identify and characterize genes or QTLs for agronomic traits of these crops, thereby facilitating marker-assisted breeding and crop improvement options.

## 16.3 Transgenic Approaches

The newly acquired ability to transfer genes between organisms without sexual crossing provides breeders with new opportunities to improve the efficiency of production and to increase the utility of agricultural crops. Genetic transformation technology relies on the conceptual framework and the technical approaches of plant tissue culture and molecular biology to develop commercial processes and products. Despite significant advances over the past decade, development of efficient transformation methods and incorporation of stable engineered resistance that requires selection of numerous independent transformants with the appropriate level of gene expression can take many years of painstaking research. The use of transgenic technology or "trans-genomics" potentially offers a more targeted gene-based approach for the genetic enhancement of field crops, thereby alleviating some of the major constraints to crop productivity that cannot be addressed otherwise. The feasibility of using *Agrobacterium tumefaciens*-mediated gene transfer has been an important breakthrough in transgenic crop research, although the rate of recovery of transgenic lines is still low in many important crops. To date, genetic transformation has been reported in almost all major cereals and legumes crops. Despite being crucial to tropical agriculture, transgenic grain legumes with an exception of soybean have not moved out from laboratories to farm lands. Major crop plants produced by genetic engineering techniques have been so welcomed by farmers. Although genetically engineered crops (GEC) or genetically modified (GM) or biotech crops in agriculture have been available only since the past 15 years, their commercial use and adoption has expanded rapidly. Twelve transgenic crops (corn, tomato, soybean, cotton, potato, rapeseed [canola], squash, sugarbeet, papaya, rice, flax, and chicory) have been approved for commercial production in the USA (James 2013). The most widely grown are "*Bt*" corn and cotton for resistance to insects, and glyphosate-resistant soybeans for tolerance to herbicides.

In traditional plant breeding, DNA from the parents recombines randomly, and undesirable traits will transfer with desirable traits such as pest resistance with lower yield. To separate undesirable traits from desirable is a time-consuming and labor-intensive; plants must be backcrossed again and again. In contrast, with genetic engineering segments of DNA that code genes for a specific characteristics are selected and transferred to the crop of interest. Hence, genetic transformation provides a complementary means to crop breeding, especially for traits that are rare or not readily available in the crop germplasm, thereby, moving crop improvement from resource-based to science-based industry.

### *16.3.1 Peanut Transgenics for Resistance to Fungal Diseases*

Besides viruses, fungal diseases are the most significant limiting factor causing more than 50 % yield losses throughout the world. Leaf spot (early and late), rusts and yellow mold (*Aspergillus flavus* and *A. parasiticus*) causing aflatoxin contamination are the major fungal diseases affecting peanut crop. Chemical control and conventional breeding has yielded only limited success in controlling these fungal diseases. To overcome these bottlenecks, various groups have carried out transgenic options for peanut crop improvement for various diseases. Transgenic peanuts expressing chitinase and glucanase genes have been shown to provide enhanced resistance to these fungal diseases.

Aflatoxin contamination, in peanut besides being a serious health hazard and trade barrier, causes average annual losses of over US$ 26 million in the USA alone. Both biotic and abiotic factors have also been shown to influence *A. flavus* infection and aflatoxin contamination such as drought. As a result, the burden of aflatoxin contamination in semi-arid regions (both pre-harvest and post-harvest) has been exacerbated by the prevailing weather conditions. Since conventional breeding methods for controlling aflatoxin are only partially effective, novel biotechnological methods are needed to develop pre-harvest host-plant resistance to aflatoxin contamination. At ICRISAT transgenic approaches are underway to develop *A. flavus* resistant peanut by using different genes including chitinases, 13S-lipoxygenases, defensisns etc. A few transgenic events have shown reduced levels of fungal infection and aflatoxin contamination in such studies.

### *16.3.2 Abiotic Stress Tolerant Transgenic Peanut*

Drought is the major cause for low and erratic pod yield in peanut that contributes to over 6.7 million t loss in annual world peanut production, resulting in estimated economical losses of over US$ 520 million, annually. Moreover, drought-*Aspergillus* interaction results in occurrence of pre-harvest aflatoxin contamination in peanut. There have been very few efforts on developing peanut transgenics for abiotic stresses. Transgenic peanut plants transformed with AtNHX1 gene were reported to be more resistant to high concentration of salt and water deprivation than their wild type counterparts. Salt and proline level in the leaves of the transgenic plants were also much higher than that of the untransformed plants. At ICRISAT, peanut has been transformed with a single regulatory gene (DREB1A transcription factor), which in turn regulates the expression of downstream genes leading to the activation of many functional genes. Preliminary results of these transgenics showed that several events acquired the capacity to extract more water from the soil profile, or had altered leaf water conductance. Further field level trails are being carried out to evaluate the performance of these transgenic peanut events for field drought tolerance (Bhatnagar-Mathur et al. 2012).

### *16.3.3 Biofortified Transgenic Peanut*

Micronutrient malnutrition is a rapidly growing public health problem affecting over 40 % of the world population. Vitamin A deficiency (VAD) alone causes up to two million deaths annually in children aged between 1 and 4 years. Clearly, VAD is a major food-related primary health problem among populations of the developing world, and fortifying crops with pro-vitamin A or β-carotene to address VAD has high potential in the long-term. There is a considerable interest in the development of food products rich in pro-vitamin A carotenoids for potential and beneficial effects on human health over the alternative dietary supplements. More recently, enhancement of micronutrient density of plant foods through agricultural practices, especially biotechnological tools referred to as “Biofortification” is considered as a potential strategy to alleviate VAD and to improve the nutritional content of staple food crops to benefit global health. The ‘golden rice’ is an example of biofortified biotech crop, which has sufficient β-carotene to meet total vitamin A requirements in developing countries with rice-based diets.

Since, oilseed crops are important for low-income families in the semi-arid tropics as they contribute 40 % of the total calories in their diets. Nevertheless, these crops that otherwise serve as important sources of nutrition in these regions, contain very little carotenoids. To address this, transgenic peanut and pigeonpea transgenic events have been developed by engineering the carotenoid synthesis pathway for enhanced levels β-carotene (pro vitamin A). These legumes, owing to their oil content appear to be suitable matrix for β-carotene aimed at higher bioavailability and bioefficacy. Transgenic peanut developed with single or dual genes of plant origin show up to 20-fold increase in β-carotene levels. To improve the β-carotene levels further, newer technologies are being developed using stacked genes from bacterial origin. This approach will hopefully go a long way in addressing the problem of VAD in the developing countries where the options for food-based vitamin A supplementation are very limited.

### *16.3.4 Insect Resistant Transgenic Pigeonpea and Chickpea*

Amongst the many insect pests, the legume pod borer, *Helicoverpa armigera* is the most devastating pest damaging chickpea and pigeonpea in Asia, Africa, and

Australia. The resistance to *Helicoverpa* in these pulse crops has so far been found to be low to moderate and transgenic technology provides alternate and sustainable levels of resistance to this insect pest. Although, extensive work has been carried out in developing transgenic plants with *Bt* and other insecticidal genes to combat the insect pest *H. armigera*, there has not been a major breakthrough in controlling this devastating pest in these important pulse crops.

The *cry* protein from the soil bacterium *Bacillus thuringiensis* has been used for decades as the active ingredient of some natural insecticides, where transferring gene codes for crystalline (CRY) proteins have developed the transgenic *Bt* crops. In *Bt* crops, the most serious insect pests of those crops were killed after they feed on the plant, while beneficial insects are left unaffected. The *Bt* cotton is the best example for *Bt* crops where it occupied the major share of the cotton crop worldwide and has thereby reduced total world insecticide usage significantly. In chickpea and pigeonpea, conventional breeding methods have not been very successful in developing resistance to this dreaded insect pest, due to the limited genetic variation in cultivated germplasm. This has led to development of transgenic events in these crops carrying the *cry1Ac* and *cry2Aa* genes, that are currently being rigorously evaluated for resistance to *H. armigera*, the legume pod borer.

### 16.3.5 Sorghum Transgenics

Worldwide, sorghum (both grain and sweet stalk) producers face a major threat to their crops from insect pests, and the most destructive of these is the lepidopteran stem borer (*Chilo partellus*) which alone causes over 49 % yield losses in Asia and Sub Saharan Africa estimated over $360 Million. Despite the efforts made over the past four decades to build resistance to stem borer through conventional breeding, this is limited due to a lack of reliable resistance in the primary and secondary gene pools. Although, there is some resistance available in the wild relatives, incorporating this into cultivated germplasm has not been successful due to cross compatibility issues and linkage drags.

Therefore, incorporating host-plant resistance will be the most useful management option for minimizing damage by this dreaded pest. ICRISAT has been working on developing insect resistance in both grain sorghum and sweet stalks cultivars using *Agrobacterium*-mediated transformation using insecticidal crystal proteins, that have proven effective against several other lepidopteran pests. Several promoter gene combinations are being used for developing transgenic varieties of sorghum resistant to stem borer. Several events have been developed for both types and are currently under event selection in contained greenhouse conditions. *Moreover*, *t*o delay evolution of pest resistance, "pyramid" strategy using two or more genes is being used. The promising transgenic events with effective insect resistance would eventually be advanced to field trials. Owing to the extent of losses in sorghum caused by this major pest, transgenic sorghum for stem borer resistance is important for the dry land agriculture in particular and potentially could revolutionize the global sorghum production, which could be key to sustainable economic growth in the drylands. Besides, significant yield losses occur in sorghum due to fungal diseases such as anthracnose caused by *Colletotrichum sublineolum*. Genetic transformation technology has a potential to confer tolerance to anthracnose in sorghum by introducing genes encoding proteins such as chitinases and chitosanases that hydrolyse fungal cell wall.

### *16.3.6 Pearl Millet Transgenics*

Improvement of millets using transgenic technology has largely been un-attempted, even though the transformation system for pearl millet was developed earlier. The first transgenic pearl millet expressing antifungal protein gene conferring resistance to downy mildew has only recently been produced which showed effective disease resistance to an extent of 90 % when compared to non-transformed control plants. Being an abiotic stress tolerant crop, there is not of much relevance to transfer any other stress gene homologue from allied species to millets. However, the abiotic stress tolerant genes of millets, although very little characterized and reported, are of great importance for enhancing stress tolerance in other plant species, indeed, a good example of bio prospecting.

## 16.4 Biosafety and Deployment of Transgenic Crops

Gene transfer techniques to develop transgenic crops can be seen as a logical extension of the crop plants for the future. When compared to the gross genetic alterations using wide-species hybridization or the use of mutagenic irradiation, direct introduction of one or a few genes into crops results in subtle and less disruptive changes that are relatively specific and predictable. The process is also clearly more expeditious, as the development of new cultivars by classical breeding typically takes from 10 to 15 years. However, unlike the development of antibiotics and chemical molecules and materials which are stand-alone commodities, the development of transgenics for even a single trait requires many molecules (sequences) i.e., promoters, DNA vectors, genes and well-adapted varieties and hybrids (Sharma et al. 2002b). It is in the combinations of these elements and the subsequent stacking of gene constructs in adapted varieties bred through recombination breeding that viable products useful to the farmers can be developed.

Keeping in view this, robust regulatory systems need to be established and properly implemented to ensure biosafety to humans, animals and the environment. Although, a decade and a half of extensive testing on non-target insects and predator species that has accompanied the long-term and wide-scale use of *Bt* crops, has till date not detected significant adverse effects. Nevertheless, scientific research aimed at risk analysis, prediction, and prevention, combined with adequate monitoring and stewardship, must continue so that there is no negative ecological impact. Besides, concerted efforts to translate the proof-of-concepts from public sector research activities on crops for subsistence farming needs to be accelerated so that resource-poor farmers can have access to fruits of this modern technology that has so far not benefitted them.

## 16.5 Conclusions

The average crop productivity in the semi-arid tropics is very less due to various biotic, abiotic stresses and climate variability, whereas the demand for food production rising in the context of population growth. Over the years, biotechnology has emerged as a promising tool to overcome constraints in crop productivity and quality. Recently, large number of molecular markers has been developed in SAT crops using high-throughput technologies and several QTLs have been mapped. Genome sequences of some SAT crops are now available and these will be valuable resources to develop powerful and efficient genomic tools for crop improvement. In SAT crops several molecular breeding programs are underway and in near future some improved biotech crops will be released.

The use of transgenic technology potentially offers a more targeted gene-based approach. Although transgenic technologies have been developed recently within the last four decades, commercial use of transgenic crops have been expanding rapidly in developed countries with a rapid adoption rate where the land under biotech crops in both developed and developing countries is almost similar now. There are many transgenic technologies like insect resistance, herbicide tolerance and nutritional quality improvement etc. are underway in crops of the semi-arid tropics and others. However, for further development and deployment of transgenic crops in the developing countries, there is an urgent need to address the issues linked to translational research, biosafety and harmonization of regulatory framework.

## References

Bhatnagar-Mathur P, Palit P, Kumar CS, Reddy DS, Sharma KK (2012) Grain legumes: biotechnological interventions in crop improvement for adverse environments. In: Tuteja N, Gill SS, Tuteja R (eds) Improving crop productivity in sustainable agriculture. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. http://onlinelibrary.wiley.com/doi/10.1002/9783527665334.ch16/summary

FAOSTAT. http://faostat.fao.org/

James C (2013) Highlights global status of commercialized biotech/GM crops: 2012. ISAAA Brief 44-2012. www.isaaa.org

Sharma HC, Crouch JH, Sharma KK, Seetharama N, Hash CT (2002a) Applications of biotechnology for crop improvement: prospects and constraints. Plant Sci 163:381–395

Sharma KK, Sharma HC, Seetharama N, Ortiz R (2002b) Development and deployment of transgenic plants: biosafety considerations. In Vitro Cell Dev Biol Plant 38:93–105

Varshney RK et al (2013) Achievements and prospects of genomics-assisted breeding in three legume crops of the semi-arid tropics. Biotechnol Adv. doi:10.1016/j.biotechadv.2013.01.001

# Chapter 17
# Sustainable Soil Health

**Mary Ann Bruns**

**Abstract** Soil is nature's support medium for plant growth. Soils on only 12 % of Earth's land area possess the physical requirements to function as cropland. However, one-fourth of this land is now moderately to severely degraded. *Soil health* is a measure of how well soil functions in retaining water and nutrients to support robust plant growth. Soil degradation, on the other hand, is any process that lowers the soil's food-producing capacity. Biotechnologies for crop improvement will have little impact if crops must be grown on degraded soils. Even when water and fertilizer are available, agricultural use of degraded soils often results in inefficient resource use and off-site pollution. *Soil quality*, a term sometimes used interchangeably with soil health, applies specifically to observable or measurable soil properties that indicate soil health. Although arable soils around the world differ in inherent properties that determine potential productivity, each soil has alterable properties that can be managed to sustain high productivity into the future. Alterable properties include soil organic matter content, root and microbial density, and macroporosity, all of which are highly dependent on maintaining biological diversity and activity in the soil. Because alterable properties undergo drastic changes when native vegetation is removed and land is disturbed for crop production, sustainable soil health involves restoring biological integrity through proper management. This chapter describes how soils are formed, why soils vary in productivity, and how soil health can be improved through diversified cropping, use of organic amendments, and soil management practices that promote beneficial root-microbe interactions.

**Keywords** Soil quality • Organic matter • Soil aggregation • Rhizosphere • Microbial biomass

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A. Ricroch et al. (eds.), *Plant Biotechnology: Experience and Future Prospects*, DOI 10.1007/978-3-319-06892-3_17,

## 17.1 Introduction

Soil is a dynamic natural body composed of minerals, organic matter, and organisms lying between the Earth's crust and the atmosphere. Soil forms slowly as a function of root and microbial activity, downward water percolation, and mineral weathering. Soil formation rates are estimated at about 0.04–0.08 mm per year, resulting in annual natural accumulations of 0.5–1 t of soil per hectare (Brady and Weil 2007). Soils which can support food production have taken centuries to develop and must not be confused with displaced "dirt" or "dust."

Soils on only 12 % of Earth's land area possess the requirements to function as cropland (i.e., sufficient soil depth, adequate moisture, permissive temperatures, moderate slope), while steeper and shallower soils on 26 % more land permit function as pasture or rangeland (FAO 2013). Our soil resource is threatened, however, by erosion, deforestation, overgrazing and mismanagement, with soil loss rates estimated to be 10–30 times faster than rates of natural soil formation. Global averages of 5–40 t of soil are lost per hectare per year, with wind- and water-borne particles ending up in rivers, reservoirs, and oceans (Pimentel 2006). With a projected world population of nine billion people by 2050, food security depends on sustaining and intensifying agricultural productivity of these soils.

This chapter discusses soil as a critical food-producing resource and explains "soil health" as an integrative management objective for improving agricultural productivity. Despite decades of government-supported soil conservation programs, agricultural soils in developed countries continue to undergo serious degradation. Although developed countries can partly compensate for soil loss by increased use of fertilizer and irrigation, such resources are not available to most farmers in the developing world, where native soils are often less suited for agriculture. Soil conservation practices that focus only on keeping erosion losses to "tolerable levels" will not provide food security. Management practices must instead aim to restore biophysical integrity and biological diversity that characterize soil health.

## 17.2 Definition of Soil Health

*Soil health* is defined as a specific soil's capacity to provide requirements for vigorous plant growth while protecting the environment from off-site losses of soil, water, and nutrients. Healthy soils have biological integrity, making them resilient and capable of providing a self-regulating, low-stress habitat for life. While the terms "soil health" and "soil quality" have sometimes been used interchangeably, soil quality more precisely refers to individual soil properties that can be measured and changed through management (Brady and Weil 2007). Observable or measurable properties include soil color, organic matter content, aggregation, porosity, and biological activity, all of which are used as *soil health indicators.*

Soil health must be assessed on the basis of a "specific soil" because natural soils around the world (and even within a given region) vary greatly in their inherent capabilities to support growth of plants. Once native vegetation is removed and natural soil structure is disrupted, agricultural soils are susceptible to degradation as soil organic matter becomes depleted by exhaustive cropping or overgrazing. Degraded soils cannot function to provide the same range of "ecosystem services" as intact, healthy soils can. Ecosystem services performed by healthy soils include the soil's ability to take up and store water, aerate and facilitate root growth, retain and recycle nutrients, support diverse biota to outcompete pests and pathogens, and prevent water runoff and soil erosion.

## 17.3 The Soil Resource

The suitability of a native soil for agricultural use is determined largely by inherent properties that arise from the integrated effects of five soil-forming factors: climate (temperature, precipitation); plants and other living organisms (above- and belowground); parent material (bedrock or other substrata); relief (topographic or landscape position); and age (length of development). Professional evaluation of a soil to determine appropriate use involves assessment of its surrounding landscape, as well as excavation of the soil to a depth of at least 1 m. This enables observation and measurement of soil layers ("horizons") that differ in color, thickness, permeability, and other properties.

The "soil profile" is a two-dimensional description of the horizons from the surface to the bottom of the excavation, while the "pedon" is the actual three-dimensional assemblage of horizons for a given soil. The pedon represents the smallest volume that can be called a soil and is used as a reference when depicting soil classes as polygons or units in soil mapping. Native topsoil horizons (termed "epipedons") are most directly influenced by plant cover, and because they are enriched with humus from decomposed plant residues, they are typically darker and more cohesive than horizons beneath them. Subsoil horizons are less affected by vegetation, but their thicknesses and physicochemical characteristics reflect local topography, water flow, composition of the parent material, and rates of mineral weathering. Parent materials such as limestone or wind-blown dust provide much more "native" soil fertility than materials like sandstone, which is rich in quartz but lacks minerals needed by plants.

The relative age of a soil and the climate under which it develops strongly influence soil pH and the pool of "base-generating" and "acid-generating" minerals available to plants. Soils subjected for millennia to tropical temperatures may have had practically all essential, base-generating minerals (i.e., calcium, potassium, and magnesium) leached away by heavy rainfall, leaving acid-generating minerals (i.e., aluminum, iron) that have adverse effects on plant growth. Soils exposed to weaker rainfall under moderate climatic regimes, on the other hand, tend to retain more of the basic minerals, especially if nutrients from litter from overlying vegetation are returned to the soil. Climate thus dictates the types and amount of vegetation that contribute to soil development, while topography influences a soil's net accumulations and losses of mineral and organic materials over time.

Differences in these factors and their relative contributions to soil formation account for great spatial variability of soils.

## 17.4 Global Soil Classification

Soils are grouped into classes that reflect wide-ranging differences in the five soil-forming factors. Two frameworks for international soil classification are the World Reference Database (WRB) for Soil Resources of the Food and Agriculture Organization (IUSS 2006) and U.S. Soil Taxonomy (USDA-NRCS 1999a). The WRB system uses two tiers of classification, with the first tier comprising 32 Reference Soil Groups (RSGs) and the second tier consisting of RSGs modified by specific descriptors that can be measured or observed. The U.S. system comprises 12 Soil Orders further divided into more than 20,000 soil series.

Table 17.1 lists the RSGs, their approximately correspondent Soil Orders, and the global percentages of ice-free land classified in each Soil Order. Soils best suited for agriculture (bold font in Table 17.1) include Mollisols, Alfisols, and Inceptisols. These soils possess the greatest native fertilities but account for only 26.4 % of global land area. The most widely distributed soils, Entisols (16.3 %) and Aridisols (12.7 %), have low native fertility and receive inadequate rainfall. Gelisols (8.6 %) are subject to low temperatures, while highly weathered Ultisols (8.5 %) and Oxisols (7.6 %) are high in aluminum and support mainly acid-tolerant plants. Thus, the majority of soils around the world have at least one severe limitation to their use for food production.

World soil maps created at scales of 1:5 million (i.e., 1 cm on the map represents 50 km on the ground) provide a broad understanding of the global distribution of major soil groups (Batjes et al. 2013). However, maps produced at finer-scale resolution are required to depict soil variability at landscape levels sufficient to inform regional decision makers. Maps at scales of 1:10,000 or finer, however, may be required to capture local variation in soil depth, slope, and drainage, all of which influence soil suitability for producing food. Although finer-scale maps accompanied by detailed soil descriptions are available in most countries, understanding the limitations of soil at a given location typically requires direct observation and handling.

## 17.5 Soil Degradation

The Global Assessment of Land Degradation and Improvement (GLADA) conducted by the United Nations Environment Program (UNEP) employed remotely-sensed NDVI data over a 25-year period to assess the vigor of plant cover around the world. The NDVI (Normalized Difference Vegetation Index) is a measure of primary plant productivity based on absorbance of infrared radiation by chlorophyll. It was notable that many *degraded lands* identified by GLADA as undergoing degradation

**Table 17.1** List of World Reference Base (WRB) Reference Soil Groups (RSGs) and approximately equivalent Soil Orders in U.S. Soil Taxonomy

| RSG diagnostic characteristics (horizons, properties, or materials that are observable or measurable in the field) | RSG | Soil order (% of global land area)[a] |
|---|---|---|
| Soils with thick organic layers | Histosols | Histosols (1.2 %) |
| Soils with strong human influence | | |
| Soils with long and intensive agricultural use | **Anthrosols** | |
| Soils containing many artefacts | Technosols | |
| Soils with limited rooting due to shallow permafrost or stoniness | | |
| Ice-affected soils | Cryosols | Gelisols (8.6 %) |
| Shallow or extremely gravelly soils | Leptosols | |
| Soils influenced by water | | |
| Alternating wet-dry conditions, rich in swelling clays | Vertisols | Vertisols (2.4 %) |
| Floodplains, tidal marshes | **Fluvisols** | |
| Alkaline soils | Solonetz | |
| Salt enrichment upon evaporation | Solonchaks | |
| Groundwater affected soils | Gleysols | |
| Soils set by Fe/Al chemistry | | |
| Allophanes or Al-humus complexes | Andosols | Andisols (0.7 %) |
| Cheluviation and chilluviation | Podzols | Spodosols (2.6 %) |
| Accumulation of Fe under hydromorphic conditions | Plinthosols | |
| Low-activity clay, P fixation, strongly structured | Nitisols | Oxisols (7.6 %) |
| Dominance of kaolinite and sesquioxides | Ferralsols | |
| Soils with stagnating water | | |
| Abrupt textural discontinuity | Planosols | |
| Structural or moderate textural discontinuity | Stagnosols | |
| Accumulation of organic matter, high base status | | |
| Typically mollic | **Chernozems** | **Mollisols (6.9 %)** |
| Transition to drier climate | **Kastanozems** | |
| Transition to more humid climate | **Phaeozems** | |
| Accumulation of less soluble salts or non-saline substances | | |
| Gypsum | Gypsisols | |
| Silica | Durisols | |
| Calcium carbonate | Calcisols | |

(continued)

**Table 17.1** (continued)

| RSG diagnostic characteristics (horizons, properties, or materials that are observable or measurable in the field) | RSG | Soil order (% of global land area)[a] |
|---|---|---|
| Soils with a clay-enriched subsoil | | |
| Albeluvic tonguing | Albeluvisols | |
| Low base status, high-activity clay | Alisols | Ultisols (8.5 %) |
| Low base status, low-activity clay | Acrisols | |
| High base status, high-activity clay | **Luvisols** | **Alfisols (9.6 %)** |
| High base status, low-activity clay | **Lixisols** | |
| Relatively young soils or soils with little or no profile development | | |
| With an acidic dark topsoil | Umbrisols | |
| Sandy soils | Arenosols | Aridisols (12.7 %) |
| Moderately developed soils | **Cambisols** | **Inceptisols (9.9 %)** |
| Soils with no significant profile development | Regosols | Entisols (16.3 %) |

*Bold* font indicates groups that are inherently best suited for agriculture. Other groups have some type of limitation (described in column one) which must be addressed prior to agricultural use or which poses management challenges

[a] Percentages of land area not included are either rock- or ice-covered

did not overlap with lands having *degraded soils*, which had been identified in an earlier assessment conducted by UNEP in 1988–1991, the Global Survey of Human-Induced Soil Degradation (GLASOD, Fig. 17.1). The latter assessment defined soil degradation as a "process which lowers the current and/or future capacity of the soils to produce goods or services." The GLASOD study concluded that soils in 38 % of the world's agricultural lands had been degraded either by wind or water erosion or other processes such as salinization and chemical pollution. The lack of spatial overlap between GLADA and GLASOD demonstrates how soil degradation estimates can be influenced by measurement method and reflects the challenges facing decision makers who must interpret available information.

Soils are most resistant to degradation when they serve as biologically intact foundations for "permanent," dense vegetative cover (i.e., native grassland, forest). It can be argued that converting such soils to agricultural use is an inherently degradative process. Plant removal and soil disturbance destroy intact root-microbial networks belowground that may have taken many years to become established but which are not recognized as being important to agricultural productivity. Because native plant-soil systems are co-adapted to resist the destructive forces of local climate, their disruption renders topsoils more vulnerable to drought and displacement by wind or water. The loss of surface soil, which is more nutrient-rich than deeper soil, rapidly reduces the soil's native fertility and water-holding capacity.

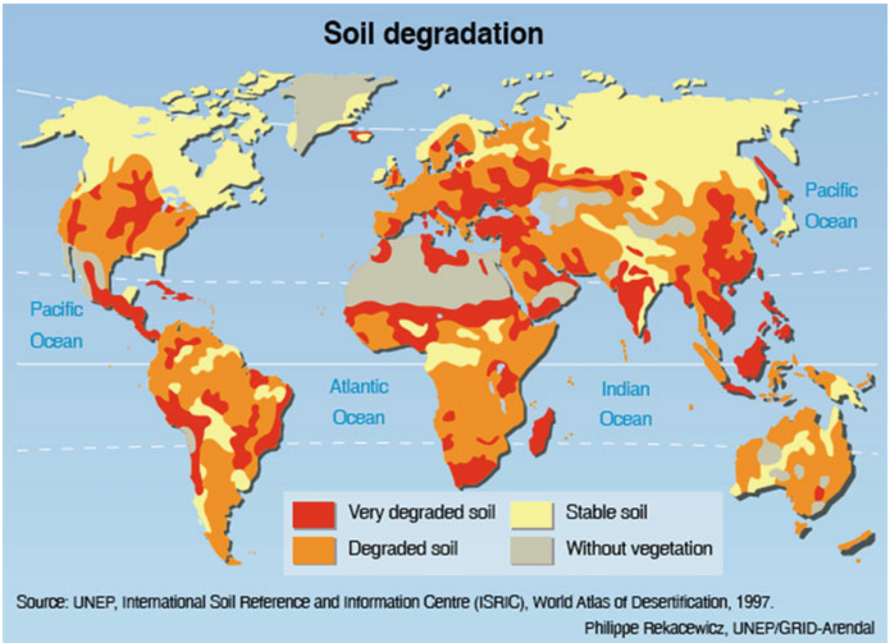

**Fig. 17.1** World map produced with data from the Global Survey of Human-Induced Soil Degradation, Food and Agricultural Organization (Source: Philippe Rekacewicz, UNEP/GRID-Arendal, World Map of Degraded Soils, used with permission)

High NDVI readings in the GLADA study may have been obtained from plant cover growing on degraded soils managed with high inputs of fertilizer and irrigation. Because the negative effects of reduced soil health (i.e., poor soil structure, reduced water-holding capacity, low nutrient-use efficiency) on productivity can be masked by increased expenditures of nonrenewable resources, NDVI data may need to be interpreted in a more comprehensive way to account for all resources used in agricultural production. Similarly, soil conservation programs in North America are shifting from a sole focus on erosion reduction to practices that increase soil carbon (soil organic matter), which helps improve soil structure and increases efficiencies of nutrient and water use (USDA-SARE 2010).

## 17.6 Roles of Inherent and Alterable Soil Properties in Enhancing Soil Health

While a soil's suitability for agricultural use is determined by inherent properties, the soil's relative health and productivity are determined by alterable properties. Two examples of inherent properties are soil texture and depth to bedrock. Both of these are “givens” for the farmer, because it is not physically or economically feasible to modify them. Inherent soil properties can limit the types of crops that can be grown, and they influence the range of alterable properties achievable for that soil. Sustaining the health of a soil requires active management so that its alterable properties remain highly conducive to root proliferation and plant growth. One example of an alterable property is soil structure. The distinction between soil texture and soil structure exemplifies how inherent and alterable soil properties affect soil health.

Texture is an inherent property based on the coarseness or fineness of the soil’s mineral fraction. In the field, the texture of a handful of soil can be estimated manually with some experience. More accurate determination of soil texture is accomplished by completely dispersing the soil and measuring the percentages of sand (diameters between 2 and 0.05 mm), silt (0.05–0.002 mm), and clay (smaller than 0.002 mm) particles. Since only particles smaller than 2 mm are considered soil, rock fragments larger than 2 mm are not considered in soil textural classifications. A specific soil can be grouped into one of 12 textural classes according to the relative distribution of particle sizes. Sands (>90 % sand) and clays (>60 % clay) are the two textural classes least suitable for agricultural use because they lie at the extremes of water and air permeability. Medium-textured soils, classified as loams (sandy loam, silt loam, silt, loam, sandy clay loam, clay loam, and silty clay loam), provide best conditions for air and water exchange in growing agricultural crops. Soils in the remaining textural classes (loamy sand, sandy clay, and silty clay) are intermediate.

Soil structure, on the other hand, is an alterable property based on organic matter as well as mineral fractions. Soil structure is the three-dimensional arrangement of mineral particles and organic matter into soil aggregates and pores of varying sizes. Soil structure reflects the amount and type of vegetation grown in the soil and the degree of physical disturbance to which the soil has been subjected. Imagine yourself standing in a well-managed garden or agricultural field—then envision the cubic meter (1 $m^3$) of soil immediately beneath your feet. Incredibly, about half that volume of soil is void space. The other half consists mainly of weathered minerals (sand, silt, clay, pebbles) and a relatively small, but functionally important, proportion (1–10 %) of soil organic matter derived mainly from decomposing plant litter and root-microbe debris. To a great extent, biological activity controls this spatial arrangement of soil voids and solids.

Soil bacteria use organic carbon from living roots and decaying organic matter to obtain energy for growth and production of “extracellular polymeric substances” (EPS). Bacterial EPS facilitates adherence of cells to soil particles and results in the formation of *microaggregates*. In the presence of degradable organic matter and adequate moisture, bacterial and fungal activity act to bind smaller aggregates into larger ones. Experiments employing microbial inhibitors in soils demonstrate that *macroaggregates* (at least 0.25 mm in diameter) are formed only in the presence of active organisms (Bossuyt et al. 2001). In the field, soil structure is evidenced by its “friability,” or ease with which the soil is broken apart into crumbs, which

**Table 17.2** Relationship between soil texture classes and bulk density values

| Ideal soil bulk densities and root growth limiting bulk densities for soils of different textures | | | |
|---|---|---|---|
| | Ideal bulk densities | Bulk densities that can affect root growth | Bulk densities that can restrict root growth |
| Soil texture | g/cm$^3$ (expressed as Mg m$^{-3}$ in International Scientific Units) | | |
| Sand, loamy sand | $<1.60$ | 1.70 | $>1.80$ |
| Sandy loam, loam, sandy clay loam, clay loam, silt, silt loam, silty clay loam | $<1.40$ | 1.60 | $>1.75$ |
| Sandy clay, silty clay, clay | $<1.10$ | 1.50 | $>1.60$ |

Table from the USDA-NRCS soil quality test kit guide (USDA-NRCS 1999b)

facilitate water infiltration and oxygen availability. Soils which contain more organic matter and which are infrequently disturbed (e.g., soils under long-term perennial vegetation) have more large pores and aggregates than heavily tilled agricultural soils in the same locale. The most effective way to enhance soil structure is to increase the soil's organic matter content (e.g., by tilling in young cover crops or by amending the soil with composts or manures).

Differences in soil structure are reflected in laboratory measurements of soil bulk density, which is determined from the dry mass of soil solids within a known volume. Although bulk density does not quantify soil structure *per se,* it can be used to assess the efficacy of management practices aimed to enhance organic matter content and improve soil health. For a given soil, the higher the bulk density, the less porosity it has for air and water movement. Bulk densities range from 0.8 g cm$^{-3}$ for uncultivated soils under perennial vegetation, to 2.2 g cm$^{-3}$ for heavily compacted soil. Bulk densities of 1.0–1.4 g cm$^{-3}$ provide highly favorable conditions for plant growth in most soils. However, soil texture affects the value of a soil's "ideal" bulk density for agricultural use, as well as the degree to which bulk density can be increased before root growth is restricted (Table 17.2).

Soil organic matter is a heterogeneous component of soil, consisting of freshly added plant material, living and decaying roots and microorganisms, partially decomposed plant, animal, and microbial materials, and fully decomposed humus. Soil organic matter content, like other alterable soil properties, can be measured in the analytical laboratory, although increasing darkness of topsoil color is a good field indicator of higher organic matter levels. Other alterable properties include water-extractable carbon, soil aggregate stability, soil pore size distribution, water-holding capacity, water infiltration rate, carbon and nitrogen availability, microbial biomass content, pH, and salt content. Resources are available for farmers and landowners to learn about soil health indicators, field assessment methods, and management practices that are effective in improving soil health (Cornell University 2009; USDA-SARE 2010).

## 17.7 Why Organic Matter Enhances Soil Health

Food security and sustainable soil health depend on minimizing erosive soil losses. To achieve this, vegetative cover on arable lands must be maintained as continuously as possible to help rebuild root-microbial networks that keep soil in place. Living vegetative cover sustains plant roots that exude and secrete organic compounds. Topsoil under living vegetation has an increased proportion of rhizosphere soil, which is defined as soil immediately adjacent to plant roots (typically within 2 mm of root surfaces). Rhizosphere soil contains greater concentrations of organic carbon and denser populations of active soil microorganisms than non-vegetated soils. Because living roots "pump" carbon into soils, vegetated soils are well-aggregated and more erosion-resistant than non-vegetated soils.

Root-enriched soils harbor diverse communities of bacteria, archaea, and fungi. These three groups comprise the "soil microbial biomass," which is responsible for organic residue decomposition and the release of such inorganic nutrients as ammonium, phosphates, and sulfates. Macro- and micronutrients, when bound within organic compounds in decaying plant tissue, remain unavailable to roots until microbes and their degradative enzymes break these tissues down. Slightly larger organisms, the protozoans and nematodes, prey upon the microbial biomass. Upon consumption of microbial cells, these "grazers" release inorganic nutrients in their wastes that once again become available for plant uptake. Along with other groups of soil organisms, including microarthropods and earthworms, a soil's entire biological assemblage is sometimes referred to as the "edaphon," from the Greek word for "ground" or "soil." Because soil biology is a comparatively young science, much remains to be learned about how the soil influences its edaphon and vice versa. It is widely recognized, however, that the soil edaphon is highly correlated with the amount and quality of soil organic matter, as well as organic matter quality and frequency of addition (SWCS 2000).

Just as plant roots require adequate nutrients, water and air to thrive, so does the soil microbial biomass. Although most of the organic carbon taken up and decomposed by the soil microbial biomass is released by aerobic respiration as carbon dioxide, some of the carbon becomes stabilized when microbial exopolysaccharides bind to clays to facilitate aggregate formation. Soil aggregation acts as positive feedback to improve soil porosity and the microbes' own habitat. As organic compounds become "humified" (i.e., resistant to further microbial degradation), intimate chemical interactions between humus carbon and soil minerals help protect and stabilize the carbon, particularly within microaggregates. Continual additions to topsoil of "fresh" organic matter sources (i.e., legume cover crops, composts, manures) feed this "self-regulating" and stabilizing activity by soil microorganisms. If organic matter is not added continually, either from living plant roots or with soil amendments, net soil loss will occur.

## 17.8 Crop Diversification and Nitrogen Nutrition

Agricultural policies exert pressures on farmers to produce one of a few commodity crops such as maize, wheat, soybean, and rice. Subsidies and disaster payments based on commodity crop acreage are powerful disincentives for farmers to include non-commodity crops in soil-building rotations. Growing the same crop in the same field year after year results in the introduction of a narrow range of organic carbon compounds into the soil, leading to low diversity of soil biota and selection of pathogens or pests that thrive on the predictable food source. More importantly, continuous "monocropping" precludes the use of soil-building crops, the roots and residues of which are especially important sources of organic carbon to enhance soil health. Crop diversification not only helps improve soil diversity, it can improve nitrogen (N) use efficiency, a critical problem for today's agricultural systems which lose on average 50 % of applied N to the environment.

Legumes represent an important group of soil-building crops, because their symbiotic relationship with rhizobia bacteria enables them to incorporate $N_2$ from the atmosphere. This evolutionary relationship, in which $N_2$-fixing rhizobia proliferate in tumor-like root nodules, occurs only within the legume family, which is particularly fortuitous for these plants, because prokaryotes (bacteria and archaea) are the only organisms on Earth that can reduce gaseous $N_2$ to $NH_3$ (ammonia). Biologically fixed N differs from industrial N fertilizer (i.e., $NH_4NO_3$, or ammonium nitrate), because it is fixed directly within the plant.

Another distinction is that biologically fixed N is less likely to be lost from soils than industrially fixed N, because it is coupled immediately to carbon in such intracellular organic molecules as proteins and amino acids. Organic N is recycled in the soil more slowly than inorganic N, because it first must undergo microbial decomposition before $NH_4^+$ is released into the soil. Moreover, the majority of ion exchange sites on soil particles are negatively charged, so that the $NH_4^+$ tends to be held in soils by ionic forces. As a tightly held cationic nutrient, $NH_4^+$ is less mobile and therefore less subject to leaching losses after heavy rainfall than the $NO_3^-$anion.

The carbon added to soils in decaying legume residues can also stimulate new growth of microbial biomass, leading to cellular re-incorporation of inorganic N. The shuttling of inorganic N in and out of microbial biomass helps to prolong "N soil residence time." The majority of soil microorganisms are heterotrophs (dependent on organic carbon for energy and cell material.) When heterotrophic competition for $NH_4^+$ is sustained with new supplies of organic carbon, soil $NH_4^+$ is less available for oxidation to $NO_3^-$by the specialist microbes known as "nitrifiers."

Nitrifying bacteria and archaea do not need organic carbon because they are "lithoautotrophs", i.e., they depend on $NH_4^+$ or $NO_2^-$oxidation for energy generation and $CO_2$ fixation for cell material. By enhancing organic carbon inputs, heterotrophic uptake of $NH_4^+$ makes it less available to nitrifiers, preventing or delaying autotrophic conversion to the more mobile form of $NO_3^-$. In addition to being more leachable, $NO_3^-$also can be denitrified to $N_2O$ or $N_2$ in wetter soils and lost to the atmosphere. Thus, lower N losses which have been observed for legume-based cropping systems (Drinkwater et al. 1996) may be explained in part by heterotrophic suppression of nitrifier activity and the predominance of reduced over oxidized forms of soil N.

## 17.9 Reduced Tillage

When permanent vegetation is removed and soils are disturbed, soil organic matter levels begin to decline unless organic carbon is added back in the form of living roots, cover crop residues, composts, or animal manures. If organic matter is not returned to the soil, reductions in soil organic matter occur rapidly, particularly in systems that employ conventional (inversion) tillage. In this type of tillage, a moldboard plow is used to cut into and turn over topsoil. Additional equipment such as cultivators and diskers are used to break up the soil further so that the seedbed can be smoothed with a harrow. Repeated physical disruption promotes soil aeration and mixing of soil microbial biomass with crop residues, resulting in rapid oxidation of organic matter, reducing the amount of carbon that will be processed slowly into stable humus.

As soil organic matter declines, the soil becomes more susceptible to erosion, reinforcing a feedback cycle that leads to even more soil carbon being lost. Because moldboard plowing of extensive areas of prairie sod in the Midwestern United States led to the Dust Bowl in the 1920s and 1930s, the United States Soil Conservation Service and cooperating farmers began to investigate tillage methods that entailed less physical disturbance and left more crop residue on the soil (Montgomery 2007). These first forms of "conservation tillage" employed chisel plows with narrow points spaced to create furrows for seed introduction without the need to invert the entire soil or bury all crop residues. Subsequent types of conservation tillage (i.e., no-till planting, mulch tillage, ridge tillage) all result in less soil disturbance than conventional tillage.

Although conservation tillage has focused on the amount of crop residues left on the soil surface (must be at least 30 %), an additional benefit of reduced tillage is the slowed rate of organic matter oxidation within the soil. In addition, reduced tillage alters the composition of the soil microbial biomass, resulting in fungi making up a greater proportion of total biomass. No-till methods reduce physical breakage of fungal hyphae, thus favoring fungal proliferation and enhancing formation of macroaggregates, which improves soil porosity. Reduced tillage also is less damaging to endomycorrhizal fungi (i.e., root "endophytes"), which produce fine networks of nutrient-scavenging hyphae extending beyond roots to distances of several cm. Tips of these hyphae can tap into water and nutrient supplies otherwise unavailable to plant roots and bring nutrient-laden water back to the plant. Reduced tillage methods thus improve soil biological integrity by slowing organic matter loss and preserving root-microbe symbioses.

## 17.10 Microbial Augmentation

In addition to maintaining the biophysical integrity of soils, many microorganisms interact with plant roots in positive ways. “Plant growth-promoting rhizobacteria” (PGPR) comprise a diverse array of beneficial bacteria that have been recovered from plant roots and studied for several decades. Various functions of PGPR have been observed under laboratory conditions, but the extent to which these functions can be replicated in the field is uncertain. Beneficial functions provided by PGPR include production of phytohormones (e.g., indoleacetic acid), which influence root growth, and release of phosphate-solubilizing enzymes that make the phosphorus in soil minerals more available to the plant. Recognition of PGPR has spurred interest in increasing their presence in the rhizosphere by developing their use as inoculants.

Inoculation as a plant-growth-promoting technology has been used for decades, mainly with rhizobia and legume seeds to promote the establishment of the $N_2$-fixing symbiosis. Most inoculant products contain a mixture of rhizobial strains that have been proven to be “rhizosphere-competent,” or capable of surviving in rhizosphere soil, entering host plant roots, and inducing the host to develop “effective” nodules. Many steps involving chemical signaling and recognition between the legume and the symbiont are required for an effective symbiosis to be established. Effective nodulation, which results in $N_2$ fixation, can be determined in the field by observing the red interiors of nodules when cut open. The red coloration is due to the presence of leghemoglobin, a protein produced by the plant to prevent $O_2$ from interfering with rhizobial enzymes responsible for $N_2$ fixation.

The problems and pitfalls that have been encountered with rhizobial inoculants should be taken into account when developing novel inoculants. Failure to use appropriate rhizobial strains which are compatible with a specific legume variety can make inoculation futile. Since specificity is clearly observed between legume varieties and their compatible rhizobial strains (i.e., “cross-inoculation groups”), similar relationships may exist between other plant hosts and microbial associates. Other factors can interfere with effective symbiosis, even when the correct rhizobial strain is present. For example, high levels in the soil of ammonium and nitrate render it unnecessary for plants to invest in the energy and carbohydrate delivery needed to support effective nodules. Soil conditions such as phosphorus or trace element deficiencies can also result in ineffective nodulation.

It is reasonable to expect that any beneficial microorganisms introduced through inoculation or soil augmentation will interact with and encounter competition from the indigenous microbial community (i.e., the edaphon). Inoculant technologies must be based on a recognition that introduced organisms will face competition from resident microorganisms which are already adapted to soil conditions. Resident soil rhizobia from previous legume crops, for example, can also persist in soil and compete with inoculated strains.

Considering the investment required to develop new inoculants, a more feasible alternative to promoting beneficial root-microbe relationships is to enhance generalized activity of the entire edaphon by adding organic matter and using management practices that promote biophysical integrity. The concept of the "plant microbiome"—the array of microorganisms living in and on plant tissues—is analogous to the "human microbiome" but is likely to be strongly influenced by the "soil microbiome" (Chapparo et al. 2012). The same biotechnological tools that inform us about how our own health is affected by microorganisms residing on and in the human body are therefore applicable to elucidating how soil- and plant-associated microorganisms contribute to crop health.

## 17.11 Conclusions

Agricultural management goals often focus on attaining maximal yields of the most profitable crops in the short-term, rather than on sustaining long-term soil productivity. As much as 25 % of the world's agricultural lands are considered moderately to severely degraded (UNEP 1997), but soil damage can be masked with added fertilizer and irrigation. Such inputs are often unavailable in developing countries, but even where water and fertilizer are abundant, agricultural use of degraded soils leads to increasing resource inefficiencies. Improving soil health depends on maintaining or increasing organic matter content and minimizing disturbance to promote beneficial biological processes known to occur in plant-soil systems. A key challenge for farmers, researchers, and policy makers is to determine how crop production practices can complement or accommodate these processes in soils. Policy innovations for sustainable soil health will be based on explicit recognition that soil organic matter and biota are critical to erosion control and keeping soils in place.

**Acknowledgements** I thank Dr. Claudia Rojas-Alvarado for her helpful review and comments during the preparation of this chapter.

## References

Batjes NH, Bindraban PS, Reuter HI (2013) Towards improved soil information for quantification of environmental, societal, and economic sustainability. Report 2013/05. ISRIC-World Soil Information, Wageningen, 44 pp

Bossuyt, H, Denef K, Six J, Frey SD, Merckx R, Paustian, K (2001) Influence of microbial populations and residue quality on aggregate stability. Appl Soil Ecol 16:195–208

Brady NC, Weil RR (2007) The nature and properties of soils, 14th edn. Prentice-Hall, Upper Saddle River, 980 pp

Chapparo JM, Sheflin A, Manter DK, Vivanco JM (2012) Manipulating the soil microbiome to increase soil health and plant fertility. Biol Fertil Soils 48:489–499

Cornell University (2009) Cornell soil health assessment training manual, 2nd edn. Cornell University, Ithaca NY. Available online http://soilhealth.cals.cornell.edu/extension/manual.htm

Drinkwater LE, Wagoner P, Sarrantonio M (1996) Legume-based cropping systems have reduced carbon and nitrogen losses. Nature 396:262–265

Food and Agriculture Organization of the United Nations (2013) FAO statistical yearbook 2013. World Food and Agriculture, Rome, 289 pp

IUSS (International Union of Soil Sciences), Working Group WRB (2006) World reference base for soil resources 2006. World Soil Resources Reports No. 103. FAO, Rome, 128 pp

Montgomery DR (2007) Dirt—the erosion of civilizations. University of California Press, Berkeley, 285 pp

Pimentel D (2006) Soil erosion: a food and environmental threat. Environ Dev Sustain 8:119–137

Soil and Water Conservation Society (SWCS) (2000) Soil biology primer. Rev. edn. Soil and Water Conservation Society, Ankeny, Iowa. Soil biology primer. Online resource available at soils.usda.gov/sqi/concepts/soil_biology/biology.html

United Nations Environment Programme (UNEP), International Soil Reference and Information Centre (ISRIC), World Atlas of Desertification (1997)

United States Department of Agriculture, Natural Resources Conservation Service (1999a) Soil taxonomy: a basic system of soil classification for making and interpreting soil surveys, NRCS, Washington, DC, 871 pp. Available online at ftp://ftp-fc.sc.egov.usda.gov/NSSC/Soil_Taxonomy/tax.pdf

United States Department of Agriculture, Natural Resources Conservation Service (1999b) Soil quality test kit guide. USDA, Washington, DC, 82 pp

United States Department of Agriculture, Sustainable Agriculture Research and Education, Sustainable Agriculture Research and Education (2010) Building soils for better crops, 3rd edn. Available online http://www.sare.org/Learning-Center/Books/Building-Soils-for-Better-Crops-3rd-Edition

# Part V
# Contributions to Food, Feed, and Health

# Chapter 18
# Approaches for Vegetable and Fruit Quality Trait Improvement

**Li Li, Yaakov Tadmor, and Qiang Xu**

**Abstract** Improving food quality traits has become a major goal of fruit and vegetable breeding due to the increasing public awareness of nutraceutical compounds to human nutrition and health. During domestication and breeding of modern varieties, many traits were left behind in the wild and in the primitive and old varieties. Thus, natural variation in such exotic germplasm of fruits and vegetables provides rich sources for discovery of novel genetic elements to improve nutritional quality and health-promoting properties of food crops. Advanced 'omics' tools including genomics, transcriptomics, proteomics, and epigenomics facilitate the hunting of important genes that control quality traits of fruits and vegetables. Plant biotechnology enables the utilization of those novel discovered genetic elements to breed new varieties of agricultural crops with value-added traits.

**Keywords** Biotech vegetables and fruits • Carotenoids • Natural variation • Omics • Quality traits

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A. Ricroch et al. (eds.), *Plant Biotechnology: Experience and Future Prospects*, DOI 10.1007/978-3-319-06892-3_18,

## 18.1 Introduction

Edible plant products, such as fruits, leaves, and roots, are not only dietary sources for proteins, fats, and carbohydrates, but also invaluable and in many case the only sources for essential nutrients and phytonutrients in human diet. Vegetables and fruits are particular rich in vitamins and minerals as well as many phytonutrients such as carotenoids, flavonoids, glucosinolates, and phytoestrogens, which are collectively called nutraceuticals. Various varieties within a species are developed, as exemplified in cauliflower, melon and citrus (Fig. 18.1).

From ancient periods, human kind knew how to channel agronomic desired traits through selection and cross breeding. This is how domesticated crops have evolved and how all of past and most of today's leading cultivated varieties were developed. Yield was, and still is, the major concern of crop breeders. During domestication and selection for high yield crops, many quality traits including health benefiting traits were left behind, either by chance ('founder effect') or due to their association with decreased yield or other undesirable traits such as infertility, susceptible to diseases, or bad taste. Such association could result from 'pleiotropic effect' of genes, i.e. the gene that increases a quality trait decreases yield or increases undesired trait. It could also result from tight genetic linkage, i.e. the gene that affects a quality trait is physically located on the chromosome region close to a gene that increases undesired trait and thus they are inherited together ('linkage drag'). As a result, only a fraction of the variation for a certain species is present in

**Fig. 18.1** Photo images of cauliflower, melon and citrus varieties

its modern variety gene pool. The ‘omics’ era creates tools for novel gene discovery and understanding of the genetic elements that are used to synthesize and regulate the formation of quality associated compounds from whole genome level. This leads to the development of new breeding tools including precise cross breeding and genetic engineering. With these tools in hands, we can now search the genetic elements that control quality traits in old varieties and wild relatives, termed exotic germplasm, and introduce them into modern varieties by advanced cross breeding. We can even search the important genes underlying the desired quality traits and transferred them into cultivated varieties utilizing genetic engineering.

This chapter will describe plant products as sources for nutraceuticals; natural variation that is available for improving nutritional and health benefiting quality of plant products with emphasis on carotenoids; ‘omics’ tools that are available today to identify novel genes and to understand genetic mechanisms underlying nutraceutical accumulation in vegetable and fruit products; and biotechnological approaches to utilize genes involved in quality traits for agricultural product improvement, particularly in vegetables and fruits.

## 18.2 Fruits and Vegetables as Source for Nutraceuticals

Fresh fruits and vegetables have long been regarded as healthy foods due to their general and unique content of health beneficial compounds. These include compounds with general and specific antioxidant activities that decrease the risk for cardiovascular diseases, cancer and other age-related degenerative diseases. In recent years public awareness of preventive medicine has increased, bringing new focus on diverse fruit and vegetable consumption as major contributors to general health and specific nutraceuticals. This trend is reflected in major breeding efforts to increase health benefiting compounds in edible fruits and vegetables. Domestication of selected individuals carrying rare mutations and extensive breeding efforts for high yield have led to narrow genetic background in many leading crops, leaving many gene alleles with increasing nutraceutical ability in exotic germplasm of old varieties, landraces and crossable wild species. This makes such exotic germplasm critical and major sources for novel genetic element discovery useful for plant food quality improvement (Fernie et al. 2006). The following section will describe how domestication ‘left behind’ important nutraceuticals, how breeding contributes to their reduction, and how exotic germplasm serves as source for bringing back important nutraceuticals to improve agriculture product quality.

### *18.2.1 The Bottleneck of Domestication*

Humans began domesticating crops around 10,000 years ago by selecting plants that differ from their ancestors with desired traits, such as inhibition of seed shattering,

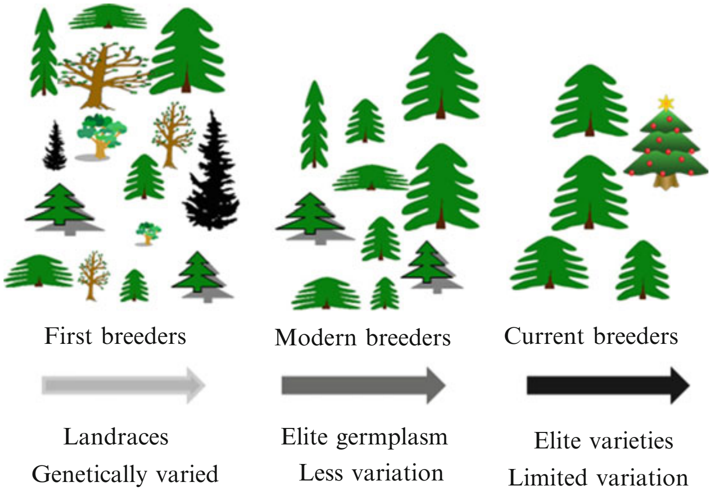

**Fig. 18.2** Schematic outline of domestication process accompanied by breeding efforts. First breeders selected plants from local variation and maintained high genetic variation within landraces varieties that they developed. Modern breeders put emphasis on uniformity and on yield. This and the development of international seed markets decreased the number of variety types and varieties that resulted in significant decrease of genetic variation within each crop species. Current breeders are even more precise with their breeding tools that produce even more uniform high yielding varieties leading to additional significant decrease of genetic variation within each crop species. However, genetic engineering and advanced utilization of exotic germplasm enable current breeders to develop new varieties with new traits

larger fruits, larger seeds, good taste, and intense color. Domestication is considered as a bottleneck for variation as it severely reduces diversity for genes targeted by domestication. Because relatively few ancestral plants, which can't represent the whole genetic variation within a species, wind up being expanded into a new crop, the new crop has less genetic diversity than its wild species, a phenomenon termed as the ‘founder effect’. Moreover, agriculture supports crops in a more uniform environment compared to the environments in which wild species grow. This contributes to an additional narrow down of the genetic variation of domesticated crops. Nutraceutical content and composition were not selection criteria in those days and thus many favorable alleles were left behind in the wild. Additionally, in some cases high content of certain nutraceuticals brings bitterness and off flavors, and thus they were selected against during domestication.

### *18.2.2 The Bottleneck of Breeding*

The process of plant domestication is accompanied by breeding efforts (Fig. 18.2). First breeders were looking for uniform performance and stable yield to enable efficient plant cultivation and processing of the plant products. The first breeders were local and developed varieties that were adapted to their local environments. These breeding efforts gave rise to the first landraces. Early landraces were highly heterozygous and contained broad genetic variation that was necessary to confront natural environmental fluctuation in minimal managed condition. The first target of modern breeders is to increase yield. Genetic methods for increasing yield have been discovered and developed. Elite germplasm that is capable of bringing higher yield has been selected. This process leaves behind large portion of genetic variation. Moreover, current modern breeding is governed mostly by large global seed companies that utilize limited selected portion of the genetic variation to develop elite high yielding competitive varieties for optimal performance within a narrow range of highly managed environmental conditions. These modern breeding efforts yield crop varieties that manage to feed the growing population of our world. However, the modern breeding selects only fraction of the natural variation and thus creates additional bottleneck for natural variation. Part of what is left behind has the potential for quality trait improvement and even for additional increase in yield. Thus, exotic germplasm of old varieties, landraces and wild species, which still contain larger amount of genetic variation compared to modern varieties, is invaluable source for discovery of genetic elements governing quality traits. These genetic elements can be transferred to elite cultivars by either crossing breeding or genetic engineering.

### *18.2.3 Exotic Germplasm and Wild Species*

The bottlenecks of domestication and breeding leave modern varieties with a limited gene pool that can't supply all the needs for quality attributes. Many of such quality traits are left behind in primitive landraces and in wild species. Bringing back these traits from exotic germplasm is critical to improve the quality of food crops including fruit and vegetables. Using molecular and metabolic markers, tools are developed to efficiently detect and transfer desired traits from crossable exotic germplasm to modern varieties.

An approved efficient example for an approach to dissect fruit quality traits in exotic germplasm and in wild species has been shown in tomato. An accession of the green and small fruited wild species *Solanum pennellii* was crossed with M82, a *S. esculentum* processing tomato variety. The interspecific hybrid was crossed back with M82 (a 'backcross') for six generations ('recurrent backcross'). At this time, individuals that were heterozygous to a defined chromosomal segment were identified and self-pollinated to create introgression lines (ILs). Each IL is homozygous in a defined chromosomal segment from *S. pennellii*, while the rest of its genome is homozygous M82. With each IL containing a unique single chromosomal segment of *S. pennellii* in the *S. esculentum* background, the ILs together cover all of the *S. pennellii* genome. The resulting exotic library can be screened for any trait of interest (Zamir 2001). Many *S. pennellii* genes and chromosomal regions that improve tomato fruit quality traits have been found, including those that affect carotenoid content and composition, vitamin C content, volatile compound content and composition, antioxidant content, sugars, and acids. Similar IL populations have been developed with other wild *Solanum* species, as well as with wild species of other crop species, including rice, barley, sorghum, soybean, and pepper.

## 18.3 Natural Variation for Nutritional and Health Benefiting Compounds with Emphasis on Carotenoids

Carotenoids are fat soluble plant pigments that are part of the photosynthesis apparatus. Carotenoids serve as fruit and flower colorants and as precursors for important plant hormones and aroma volatile compounds. Carotenoids that contain a beta ring, such as β-carotene, α-carotene, and β-cryptoxanthin, are pro-vitamin A molecules and the major source for vitamin A in human diet. Additionally, carotenoids have been demonstrated to be potent phytonutrients that possess antioxidant activity and prevent chronic disease including coronary disease and certain types of cancer. Biosynthesis of carotenoids in plants occurs in special organelles (plastids) within the cells, mainly in chloroplasts, the green photosynthesis plastids, and chromoplasts, the special colored plastids devoted to carotenoids biosynthesis and accumulation in fruit, roots, and flowers. Utilizing natural variation to increase the accumulation of nutraceuticals requires studying genetic elements that participate and regulate the production of the metabolites of interest, the degradation, and the storage of these metabolites. These will be demonstrated and discussed in the following section for carotenoids. We will show how variation in key genes involved in the production, degradation, and storage of carotenoids affects the net outcome of carotenoid level in different plant products and bring a few examples to make such natural variation an efficient tool for crop improvement.

### *18.3.1 Biosynthesis of Carotenoids*

Carotenoids are 40 carbon terpenoids derived from five carbon plastidial isopentenyl pyrophosphate and dimethylallyl pyrophosphate. The first committed step in carotenoid biosynthesis is the condensation of two geranylgeranyl diphosphate molecules by phytoene synthase (PSY) to yield phytoene, a non-colored carotenoid (Fig. 18.3). A set of desaturations accompanied by trans-isomerization leads to the production of lycopene, the red carotenoid pigment of tomato and watermelon. The subsequent cyclization of lycopene molecule ends to form either epsilon or beta ring results in the production of β-carotene with two beta rings and α-carotene with one beta and one epsilon ring found abundant in carrot and melon. The rings can be further hydroxylized, epoxidized, and modified to yield more than 700 types of carotenoids.

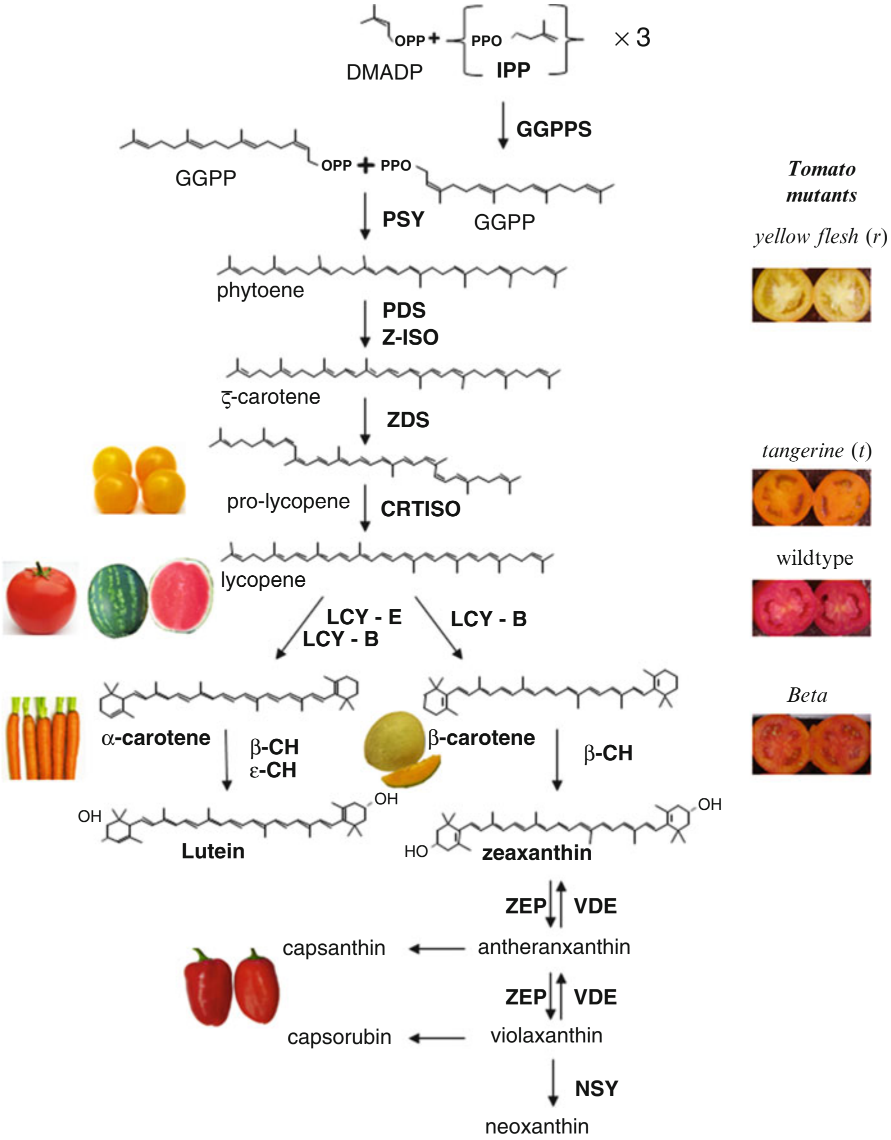

**Fig. 18.3** Schematic biosynthetic pathway of some major carotenoids. *DMAPP* dimethylallyl pyrophosphate, *IPP* isopentenyl diphosphate, *GGPP* geranylgeranyl pyrophosphate, *GGPPS* GGPP synthase, *PSY* phytoene synthase, *PDS* phytoene desaturase, *ZDS* ζ-carotene desaturase, *Z-ISO* ζ-carotene isomerase, *CRTISO* carotenoid isomerase, *LCY-B* lycopene β-cyclase, *LCY-E* lycopene ε-cyclase. Fruits and vegetables that contain high levels of particular carotenoids are aligned in the pathway. Tomato mutant images are also aligned with the carotenoids in the pathway

Tomato serves as a model plant for understanding fruit carotenoid accumulation. Breeding of different colored tomato fruit directly benefits from studying natural genetic variation that governs tomato fruit colors and intensities. The genetic basis of fruit color variation in tomato and its association to carotenoid composition are well established. The characteristic red color of tomato fruit is determined by the accumulation of lycopene. Mutant fruit colors are assigned to specific carotenoid biosynthetic genes. The yellow fruit *r* results from a mutated PSY-1. The yellow-orange fruit *tangerine* that accumulates pro-lycopene is due to a mutated carotenoid isomerase (CRTISO). The orange-red fruit *Delta* with high δ-carotene level is due to a mutation in lycopene ε-cyclase (LCY-E). The orange *Beta* fruit with high β-carotene content is determined by a dominant allele of LCY-B (Fig. 18.3). The crimson fruit (*og*, *ogc*) that accumulate only lycopene and no β-carotene is a null allele of LCY-B. In cassava, a single nucleotide polymorphism in a highly conserved region of *PSY* is associated with increased PSY catalytic activity that results with a colored root and accumulates pro-vitamin A (β-carotene). These examples show that genetic variation that alters specific gene expression and functions could effectively modify the content and composition of carotenoids in plants.

### *18.3.2 Degradation of Carotenoids*

Carotenoid degradation occurs spontaneously or enzymatically by a group of enzymes known as carotenoid cleavage dioxygenases (CCDs). Degradation of carotenoids by CCDs yields important plant hormones that signal plant development programs, respond to abiotic and biotic stress, and mediate responses to the presence of beneficial and non-beneficial organisms. CCD activities yield potent volatile compounds that could mediate plant–animal interactions, for example attracting or repelling insects, and enhance the flavor characteristics of food crops. CCD activities also directly affect carotenoid accumulation as recently demonstrated in peach, where a CCD gene variation governs dominantly the white flesh color. Thus genetic elements that regulate the degradation of carotenoids should be taken in account when one seeks increasing carotenoid content.

### *18.3.3 Storage of Carotenoids*

Carotenoids in plants are synthesized and stored in nearly all types of plastids, but accumulate in high levels in chromoplasts. Thus, regulation of chromoplast formation represents a critical mechanism underlying carotenoid accumulation in plants. Genetic variation in genes that affect chromoplast compartment size and number has been shown to impact carotenoid level, making these genes important tools for increasing carotenoids content. High carotenoid contents in tomato *high pigment* (HP) mutants are linked to an increased plastid number and size, which enables greater biosynthesis and a higher storage capacity. A cauliflower orange curd mutant contains high level of β-carotene. Recent findings reveal that orange phenotype is due to a variation in the *Orange* (OR) gene that governs the biogenesis of chromoplasts from non-colored plastids. Introduction of the *Orange* allele into different plant species leads to high level of carotenoid accumulation. Enhancement of storage sink strength represents another strategy for carotenoids enhancement in agricultural products.

## 18.4 Omics Tools for Genes ‘Hunting’: Focusing on Fruit and Vegetable Crops

The term ‘Omics’ is a suffix derived from genomics, transcriptomics, proteomics and epigenomics. Omics-based techniques are new generation of approaches for gene ‘hunting’ when compared with the traditional genetic tools. Omics tools hold much promise for both basic researches such as gene discovery and applied researches such as breeding programs (Langridge and Fleury 2011). The most important features of omics-tools are fast, comprehensive, and cost-effective. In the past 10 years, the omics tools have rapidly penetrated in different fields of biology. Here, we will focus on the application of omics tools in fruit and vegetable crops.

### *18.4.1 Genomics*

The advance in genomics technologies of DNA sequencing and bioinformatics enables the study of many organisms at whole genome level. So far more than 35 crop genomes have been published. The genome platform provides an important approach to identify potential key genes, like those that control interesting or specific traits in exotic germplasm or in non-model plants. The genome platform also revolutionizes the scale and density of molecular marker identification. The next-generation sequencing (NGS) technologies, including Illumina, SOLiD, and 454 platforms, make the generation of tens of thousands or millions of high throughput markers, such as single nucleotide polymorphism (SNP) markers, across the genome readily achievable by a single step. This NGS-based approach will be even widespread when the cost plummets to a standard that could be broadly accepted by an individual research group.

Genomics technologies dramatically accelerate rapid identification of genes or mutant alleles responsible for interesting traits in various fruit and vegetable crops. One example is the use of the NGS-based mapping approach to quickly identify two gene loci that are responsible for the ovate fruit shape in tomato.

The genome also provides basis toward a mechanistic understanding of important traits. For example, sweet orange contains high level of vitamin C. Comparative analysis of sweet orange genome with those of closely related varieties indicates that the improved vitamin C metabolism in sweet orange is likely due to the gene expansion and transcriptional regulation of key steps in the galacturonate pathway. Citrulline, a α-amino acid enriched in watermelon, is associated with health benefits. Genome-wide analysis suggests a potential mechanism underlying the accumulation of citrulline in watermelon, which is by down-regulation of arginosuccinase and arginosuccinate synthase genes.

### *18.4.2 Transcriptomics*

Transcriptomics studies transcriptome that represents a whole set of gene transcripts expressed by an organism. NGS-based RNA-sequencing (RNA-seq) emerges as the method of choice to determine transcriptome, although the older hybridization-based approaches typically called microarrays are still in use. RNA-seq has been approved to be powerful in discovering, profiling, and quantifying RNA transcripts across the whole transcriptome. It has been widely employed to examine transcriptome of various organisms and even on crops with complex genetic backgrounds and highly heterozygous nature. This approach is particularly useful in non-model plants such as fruit and vegetable crops as it does not require the existing whole genome information.

Transcriptome analysis of fruits and vegetables by RNA-seq has been successful in identifying potential factors controlling quality traits. The red-flesh mutant in citrus accumulates more than 1,000-fold of higher levels of lycopene than the regular yellow fruit. RNA-seq analysis (Fig. 18.4) reveals that 582 genes show differential expression between the mutant and the regular fruit, and the genes that partially impair lycopene downstream flux are critical for the formation of lycopene accumulation trait in the mutant. Green curd cauliflower mutant contains high level of some health beneficial compounds due to the formation of chloroplasts. The genome-wide profiling of gene expression identifies a large number of genes associated with chloroplast development and indicates the key role of regulatory genes in the high hierarch of light signaling pathways in mediating green curd phenotype.

### *18.4.3 Proteomics*

Proteomics provides tools to examine proteins at a global level in an organism. Proteomics incorporates technologies of gel electrophoresis or multidimensional high-pressure liquid chromatography, tandem mass spectrometry (MS/MS), and database search. The tools have been widely used in fruit and vegetable crops. Fruit development and ripening is a hot topic as these processes are directly associated with the production of nutraceuticals in addition to many other compounds. Comprehensive proteomic analysis is applied to explore the potential key processes

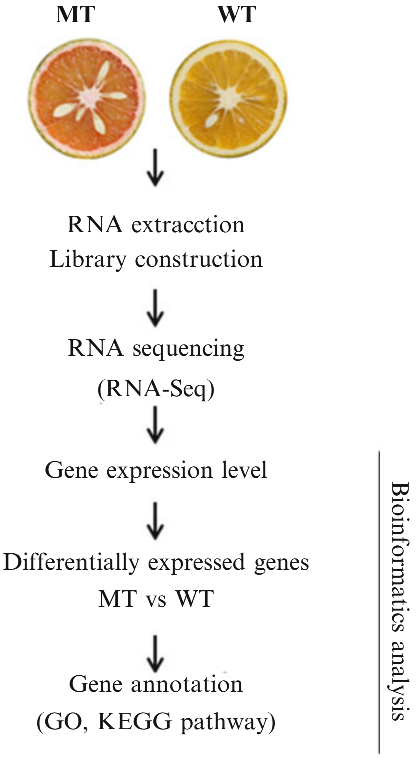

**Fig. 18.4** Outline of transcriptomics analysis using RNA-seq

involved in fruit development in citrus, tomato, apple, apricot, grape, strawberry, peach, etc. Fruit ripening in many cases is characterized with massive synthesis and accumulation of carotenoids. In orange fruits, comparative proteomics in a red-flesh mutant and its regular orange-flesh fruits suggests a regulatory role of oxidative stress on carotenoid metabolisms during fruit ripening, which is confirmed using oxidative stress treatment.

### *18.4.4 Epigenomics*

Epigenomics studies a complete set of epigenetic modifications on epigenome, which represents the set of DNA or histone modifications that do not change the DNA sequence across the genome. Epigenomics is a hot topic in plant biology. The most published studies in fruit and vegetable crops are epigenetic studies that demonstrate DNA methylation during plant development, secondary metabolisms, micropropagation and long-time preservation. DNA methylation of MYB transcription factors is found to play important roles in genetic regulation of apple fruit anthocyanin metabolism. Study of the epigenome of tomato by using the NGS-based approach reveals that the promoter regions of ripening genes are frequently de-methylated, which suggests a critical role of changes in epigenome in controlling tomato fruit ripening.

The omics tools provide unprecedented opportunities to study genome, transcriptome, proteome and epigenome along with metabolome at a global scale for better understanding of metabolisms and gene hunting. The omics studies will form a huge wave in fruit and vegetable research, particularly when the cost can meet requirement of an individual research group. We would anticipate that many agriculturally important traits will be dissected via omics and functional studies. The results from omics studies are valuable for genome-assisted breeding when the genomics data are integrated with agronomic phenotypes or trait data. The genes or variants identified to be linked with specific trait can be used to design efficient and precise breeding strategies. Moreover, genes governing important agronomic or quality phenotypes can be verified and transferred by modern biotechnology tools into elite cultivars to ultimately achieve genetic improvement in fruit and vegetable crops.

## 18.5 Biotechnological Approaches for Vegetable and Fruit Quality Trait Improvement

For centuries, mankind has been improving crops through selective breeding. This process underwent a paradigm shift in the early 1970s, when scientists develop the ability to isolate genes that control specific traits. In 1980s, techniques were developed to allow genes from any sources to be introduced into the recipient genomes. Using the techniques of recombinant DNA technology and transformation, genetic engineering was established to develop new varieties, which paves the way for great improvement in plant breeding.

Plant biotechnology has been established as an import adjunct to traditional plant breeding for crop improvement because of its advantages over other technologies. This technology permits the use of diverse genetic resources from all the living kingdoms and the combination of different species. By alteration one or a few genes of interest either through overexpression or suppression of their expression under specific gene promoters, the technology provides a more precise breeding and allows controllable expression of those introduced genes in specific targeted organs and tissues. The safety of genes and gene products can be tested before introduction into crops. A number of techniques are developed to produce marker-free biotech crops. Because of its precise introduction of a few genes into crop genomes, plant biotechnology provides a fast and efficient approach for crop improvement. Moreover, as some crops can not be easily bred or lack adequate traits, plant biotechnology offers an alternative to traditional breeding. The main disadvantages at present are costs of regulatory approval and lack of consumer acceptance in some areas. Noticeably, since the first biotech crop was grown commercially in 1994,

**Table 18.1** Biotech vegetables and fruits that are/were grown commercially or obtained regulatory approval

| Species | Trade name | Traits | Commercialization |
|---|---|---|---|
| Tomato | FlavrSavr | Delayed fruit ripening | Not anymore |
| Papaya | Rainbow | Resistance to ringspot virus | Yes |
| Plum | HoneySweet | Resistance to plum pox virus | Not yet |
| Potato | | Resistance to Colorado beetle | Not anymore |
| | | Resistance to leafroll/Y virus | Not anymore |
| Sugar beets | | Herbicide resistance | Yes |

global adoption of biotech crops steadily increased to a record 170.3 million hectares in 28 countries in 2012 (James 2012, http://www.isaaa.org/). While the biotech crops in fields are predominantly the major commodity crops such as soybean, maize, cotton and canola with herbicide tolerance and insect resistance traits, a few biotech vegetables and fruits with agronomic traits are/were grown commercially (Table 18.1).

### 18.5.1 *Biotech Vegetables and Fruits Grown Commercially or Obtained Regulatory Approval*

The “Flavr Savr” tomato developed by Calgene was the first genetically engineered crop grown commercially in 1994 for delaying fruit ripening and increasing shelf life. This novel trait was produced by *Agrobacterium*-mediated transformation with a DNA construct containing a polygalacturonase gene in an anti-sense orientation. Expression of this gene in the genetically engineered tomato suppresses the endogenous polygalacturonase enzyme activity, resulting in decreased breakdown of pectin and causing fruits with reduced cell wall degradation, improved viscosity, and delayed softening. The Flavr Savr tomato is out of market now although the transgenic tomato product “tomato puree” was a big success in late 1990s in Great Britain. In addition, some other genetically engineered tomato varieties with delayed ripening trait are generated by reducing the production of ethylene, a phytohormone required for tomato fruit ripening (GM Crop Database: http://www.cera-gmc.org/), but they are not in commercial production.

The Rainbow papaya is the first genetically engineered fruit tree grown commercially for resisting infection by papaya ringspot virus (PRSV), a major disease limiting papaya production. The Rainbow papaya is a hybrid variety of cross between Hawaii’s primary variety Kapoho with the genetically engineered papaya named SunUp. SunUp was developed via microparticle bombardment of the PRSV coat protein into papaya, which makes the papaya plants immune to the ringspot virus infection and subsequently the disease caused by it. The Rainbow papaya was commercialized in 1998 and produced immediate results to stop the rapid decline of papaya production in Hawaii. It remains in use today. Additional experiments are underway to generate papaya varieties that are resistant to other PRSV strains to be used in other areas of the world.

A few other genetically modified vegetables and fruits with delayed fruit ripening, resistant to disease and insect, and herbicide tolerance are available and in various stages for commercial production (GM Crop Database: http://www.cera-gmc.org/). Genetically engineered HoneySweet plum was produced to resist the plum pox virus (PPV) through RNA silencing of the viral coat protein gene. It was deregulated by USDA-APHIS and is available for commercial production. As PPV disease is currently under control through agronomic practices, no plans have been made to release the transgenic HoneySweet varieties for commercial use in US. Transgenic squash resistant to infection of a number of viruses was created and regulatory approval was obtained. Transgenic potato resistant to Colorado potato beetle and potato leafroll virus/the Y virus were generated by introducing cry3A Bt gene, viral coat protein or helicase gene and were commercialized. Transgenic glyphosate and glufosinate tolerant sugar beets were produced to allow the use of herbicides for weed control in sugarbeet fields. This trait was introduced into sugarbeet either by expressing an herbicide tolerant form of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) involved in the synthesis of the aromatic amino acids or by expressing phosphinothricin-N-acetyltransferase (PAT) to inactivate glufosinate. Transgenic sugarbeets are under commercially production.

### *18.5.2 Genetically Engineered Vegetables and Fruits with Quality Traits*

Plant biotechnology not only produces vegetables and fruits with traits that improve their performance in the field, but also offers effective means in generating novel or value-added products with the potential to impact human nutrition and health. A number of biotech crops with value-added traits such as Golden Rice and Plenish$^{\text{TM}}$ high oleic soybeans are on the way for farmer or commercial production. Many biotech vegetables and fruits with enhanced nutritional quality and health-promoting properties are in research and/or development (McGloughlin 2010) (Table 18.2).

*Tomato*: Tomato is a nutrient-rich vegetable fruit that contains high level of carotenoids, vitamin C, and other phytonutrients. Tomato has been engineered to enhance or produce a vast range of health beneficial compounds. Tomato contains high level of lycopene with low levels of other carotenoids. Lycopene is strong antioxidant and high β-carotene level provides more provitamin A precursor for vitamin A synthesis. Thus, they are targets for engineering. Overexpression of *PSY-1* gene leads to twofold increase in total carotenoid levels. Up-regulation of endogenous *LYC-B* or down-regulation of β-carotene hydroxylase diverts flux and

**Table 18.2** Examples of genetic engineered vegetables and fruits with improved quality traits intended to benefit human nutrition and health

| Species | Quality traits |
|---|---|
| Tomato | Carotenoids (β-carotene; lycopene) |
| | Folate (vitamin B9) |
| | Flavonoids (anthocyanins) |
| | Phytosterols |
| | Phenolics (resveratrol; stilbene; chlorogenic acid) |
| Potato | Carotenids (β-carotene; lutein) |
| | Essential amino acids (methionine) and total proteins |
| | Carbohydrates (fructan; inulin) |
| | Phenolics |
| Carrot | Carotenoids (astraxanthin) |
| | Calcium |
| Lettuce | Iron |
| Strawberry | Vitamin C |
| Citrus | Carotenoids (β-carotene) |
| Apple | Phenolics (Stilbenes) |
| Kiwi | Phenolics (resveratrol) |

leads up to 12-fold increase of β-carotene. In one case, strong overexpression of tomato *LYC-B* produces orange fruits with compete conversion of lycopene into β-carotene. Alteration of genes outside of carotenoid biosynthetic pathway, i.e. photomorphorgenesis regulator *DE-ETIOLATED1* and carotenoid storage structure protein fibrillin, also increases lycopene and β-carotene contents.

Tomato as one of the healthiest foods contains very low level of folate (vitamin B9) content. By combining fruit-specific overexpression of GTP cyclohyrolase I and aminodeoxychorismate synthase genes that encode the first enzyme for pteridin and *p*-aminobenzoate synthesis of the folate molecules, respectively, genetically engineered tomato produces an over 25-fold increase of folate, a level in 100 g fruit that can completely satisfy daily folate requirement.

Anthocyanins are a group of flavonoid compounds with potent health beneficial roles. Purple tomato fruit that contains substantially high level of anthocyanins is produced via fruit-specific expression of two snapdragon transcription factors *Delila* and *Rosea1* that control anthocyanin biosynthesis. The engineered purple tomato fruit exhibits over threefold of enhanced hydrophilic antioxidant capacity and has a significant extension of life span in the cancer-prone mice by as much as 30 %. A recent study shows that the purple tomato not only is more healthy food, but also has longer shelf life and less disease. In another case by introducing a petunia *chi-a* gene encoding chalcone isomerase, high flavonol tomato with up to 78-fold increase is produced, which has potential to help reduce risk of heart diseases.

Tomato is also used as plant factory to produce new health benefit compounds not present in the regular tomato fruit. Tomato transformed with a grape stilbene synthase gene produces antioxidant metabolite resveratrol, which is rich in red wine and known to play an important role in reducing the incidence of some chronic diseases, such as heart diseases, cancer and diabetes.

*Potato*: Potato is a major staple food and also the number one vegetable crop in the world. Potato has been engineered to enrich a number of nutrients or phytonutrients. While there are some yellow potato varieties that contain good quantity of carotenoids, the most common grown potatoes are low in carotenoid content. Genetically engineered potato with altered levels of carotenoids is generated by altering either the biosynthetic activity or storage capacity. "Golden potato" is produced by overexpression of a mini-set of the carotenoid biosynthesis pathway genes under the control of tuber-specific promoters, producing up to 114 μg/g dry weights of carotenoids with β-carotene level to 47 μg/g, a level higher than that found in Golden Rice. Orange-yellow flesh potato is also generated by tuber-specific expression of the cauliflower *Orange* allele to enhance the storage sink capacity. The genetically engineered *Or* tubers not only contain high level of carotenoids, but continuously synthesize carotenoids during long term of storage.

Genetically engineered potato with enhanced content of essential amino acids, such as methionine, is produced. Insulins are long chain fructans and positively influences gut microfloral composition to benefit micronutrient absorption, blood lipid composition, and colon cancer prevention. By overexpression of two inulin biosynthetic genes from artichoke, transgenic tubers produce inulin that makes up 5 % of dry weight.

*Carrot*: Carrot is among the most popular vegetable. The orange varieties contain high levels of α- and β-carotene. Ketocarotenoids such as astaxanthin, the carotenoid responsible to the red color in cooked shrimp and lobsters, are produced mainly by algae and cyanobacteria, and rare in plants. By introducing a β-carotene ketolase gene from algae into carrot root, the transgenic carrot accumulates ketocarotenoids to 2,400 μg/g root dry weight with up to 70 % of total carotenoids converted into novel ketocarotenoids, which makes it suitable for biopharming ketocarotenoid production.

Dietary calcium intake is known to be directly correlated with the risk of osteoporosis. Carrot like many vegetables is a poor source of dietary calcium. Carrot was engineered through expression of an Arabidopsis calcium transporter CAX1. The transgenic carrot contains approximately twofold increase of calcium and shows an enhanced calcium absorption in both mice and human feeding trails.

In addition to vegetables, a number of fruits have been engineered to further enhance nutraceutical content or contain novel compounds to improve their quality traits. Citrus fruit is an important source for health-promoting bioactive compounds. Through suppression of endogenous β-carotene hydroxylase gene to block the conversion of β-carotene to downstream xanthophylls, transgenic orange with a deep yellow color was generated that contains up to 36-fold increase in β-carotene content in the pulp and shows protection against oxidative stress in experimental animal model. Like the case in tomato, apple was engineered through expression of the stilbene synthase gene from grapevine. The transgenic apple contains novel compounds of resveratrol derivate (Table 18.2).

While significant progress has been made in generating vegetables and fruits with value-added traits, much more are coming with the discovery of novel genes and better understanding of genetic and molecular mechanisms underlying quality traits.

## 18.6 Conclusions

Fruits and vegetables are important components of human and animal diets in providing various nutraceuticals. Improving quality traits becomes a major goal of fruit and vegetable breeding programs due to the increased public awareness of different plant nutraceuticals. During domestication and development of very first varieties, most attention of breeders was concentrated on increasing yield. Many quality traits, especially those that are associated with nutraceutical content and composition, did not serve as selective criteria. Thus, in many cases biosynthetic and regulative genes that have the potential to improve vegetable and fruit qualities were ‘left behind’ in the ‘hidden’ natural variation, which resides within wild relatives as well as primitive and landrace varieties of our crops. Advanced ‘omics’ tools including genomics, transcriptomics, proteomics, and epigenomics facilitate the discovery of important genes that control quality traits. Plant biotechnology enables the utilization of such novel genes to improve health and nutritional qualities of fruits and vegetables. Moreover, the novel genetic elements discovered provide a golden opportunity to breed new varieties of other major agricultural crops with much needed value-added traits.

**Acknowledgement** We are grateful to colleagues for their contribution to this work. We thank Xiangjun Zhou for photographs. We acknowledge the support of the United States-Israel Binational Agricultural Research and Development Fund to LL and YT (US-4423-11) and the National Natural Science Foundation of China to QX (31272148).

## References

Fernie AR, Tadmor Y, Zamir D (2006) Natural genetic variation for improving crop quality. Curr Opin Plant Biol 9:196–202

James C (2012) Global status of commercialization biotech/GM crops: 2012. ISAAA Brief No. 44. ISAAA, Ithaca

Langridge P, Fleury D (2011) Making the most of ‘omics’ for crop breeding. Trends Biotechnol 29:33–40

McGloughlin MN (2010) Modifying agricultural crops for improved nutrition. New Biotechnol 27:494–504

Zamir D (2001) Improving plant breeding with exotic genetic libraries. Nat Rev Genet 2:983–989

# Chapter 19
# Biofortification: Vitamin A Deficiency and the Case for Golden Rice

**Robert S. Zeigler**

**Abstract** Worldwide, the most seriously limiting nutrient deficiencies in the human diet are iron, zinc, iodine—and vitamin A. Vitamin A is an essential nutrient needed for the visual system, growth, development, and a healthy immune system. Vitamin A deficiency (VAD) is most prevalent among young children and pregnant and nursing women. Despite significant efforts, including capsule supplementation, dietary diversification, fortifying commonly used foods, such as cooking oil with vitamin A, and optimal breastfeeding practices, VAD continues to adversely affect an estimated 190 million preschool children and 19 million pregnant women in the developing world. Since rice is widely produced and consumed in poor developing countries, it seems logical that, if this staple could be made to provide a source of vitamin A, it has the potential to reach millions of people who do not have reliable access to or cannot afford other sources of the vitamin. The nearly 30-year history of the development of Golden Rice, a genetically modified (GM) variety of the cereal that contains beta carotene in the grain, a source of vitamin A, is an enlightening story of vision, imagination, technological creativity, and persistence. Many organizations and individuals in the public and private sector have been involved in this effort that has attracted more than its share of controversy. But, hopefully, Golden Rice's delivery to farmers and consumers will not be delayed much longer by those who oppose the use of this new, promising technology.

**Keywords** Human nutrition • Beta carotene • Vitamin A • Golden Rice • Biotechnology

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A. Ricroch et al. (eds.), *Plant Biotechnology: Experience and Future Prospects*, DOI 10.1007/978-3-319-06892-3_19,

## 19.1 The Beginning of the Golden Rice Saga

“Yellow endosperm” was the answer that silenced the group.

It was April 1984 and Gary Toenniessen, head of the Rockefeller Foundation’s (RF) new International Program on Rice Biotechnology, was having an informal evening beer with some of the world’s leading rice breeders on the verdant guest house patio at the International Rice Research Institute (IRRI) in Los Baños, Philippines. The breeders had gathered for the inaugural meeting of what was to be a nearly 20-year effort to change the face of the application of biotechnology in developing countries (IRRI 1985). RF had not yet decided how to structure the program, where to focus its efforts, or even who should do the work. But, they knew that the powerful scientific tools beginning to emerge would leave the developing world behind unless someone took action. And rice, by far the most important food crop in developing countries and still an orphan crop scientifically, was clearly the best place to start.

So, Gary asked the breeders a simple question: “What is your favorite trait” (Hettel 2009a)? The answers were much what everyone expected, engendering the usual good-natured arguments: high yield, resistance to blast disease, tolerance of drought, etc. That is, until Peter Jennings uttered, in his inimitable style (spoken a bit between a grimace and a self-satisfied sneer, probably wrapped in cigarette smoke), “yellow endosperm” (Fig. 19.1). In the ensuing silence, he explained to his dumbfounded colleagues that, in his view, vitamin A deficiency (VAD) was far and away the greatest health issue among the world’s rice eaters. If yellow endosperm (endosperm being the part of the rice grain that people eat) could be found in rice,

**Fig. 19.1** Rice with yellow endosperm, later to be called Golden Rice, here compared with regular white rice, was only an idea in rice breeder Pete Jennings’ head back in April 1984

it might be caused by a high concentration of beta carotene, a plant molecule like that found in carrots and other fruits and vegetables. Chemically, beta carotene can be converted by a specific enzyme into two molecules of vitamin A. However, for many reasons related to vitamin A status, the nature of the food source, and genetic variation among humans, this conversion can sometimes deliver only as little as 5 % of the beta carotene as vitamin A in the body (Goswami et al. 2003). Jennings admitted that he had been quietly looking in his breeding plots for yellow rice since his earliest days as a rice breeder with the RF program in Colombia during the 1950s. He had never found it and his colleagues had never even considered looking for it.

Rice breeders pay attention to what Peter Jennings says. In the early 1960s, at IRRI, he helped develop the first semidwarf rice varieties that led to the Green Revolution in Asia. He made the cross that led to IR8, the rice variety that changed everything, and he was a significant driver behind the amazing transformation of rice production in Latin America (Hettel 2008).

Gary and the RF subsequently challenged the breeders to find or create rice with endosperm that is yellow and contains beta carotene by officially adding it to the RF's top-20 list of desirable traits in rice that could be aided by biotechnology applications (Khush and Toenniessen 1991). They had done their homework and agreed with Jennings that improving the nutritional value of rice, particularly for vitamin A, could have massive impact on the lives of millions of the world's poorest people.

Renowned geneticist Neil Rutger, then with USDA, who had created a renaissance in the application of induced mutation as a breeding tool for the rapid development of new rice cultivars (Rutger 2009), thought that mutation breeding might also be the way to go to develop rice with yellow endosperm. He proceeded to screen hundreds of thousands of rice mutants for the spontaneous emergence of the trait.

In the meantime, scientists at IRRI felt that the best place to start was to screen the tens of thousands of different samples of native rice varieties that they had collected over the previous 25 years and stored in IRRI's International Rice Genebank. When both of these efforts came up short in just a couple of years, Gary and the international panel of experts that advised him were forced to conclude that beta carotene was not going to be produced in rice endosperm using conventional approaches.

By that time (the early 1990s), Ingo Potrykus, prodded by a talented young husband-and-wife team of postdoctoral fellows, Swapan and Karabi Datta, became interested in creating rice that carried beta carotene in its endosperm (Potrykus 2000) to fight VAD. The Datta couple were supported by the RF program and hailed from eastern India, where the scourges of VAD were particularly severe. Ingo was a professor of plant sciences, specifically the biotechnology of plants, at Albert Einstein's alma mater, the Swiss Technical University, ETH, in Zurich. He was already an accomplished and well-regarded scientist with an impressive track record in plant biotechnology. But, he wanted to make a real difference in the world. So, he took up the Dattas' challenge and approached RF's International

Rice Biotechnology Program for funding (Potrykus 2001). With RF's agreement to get involved, Ingo enlisted the help of the brilliant biochemist Peter Beyer from the University of Freiburg. Between them, they harnessed the intellectual power of many of their students and postdoctoral fellows to translate a dream into a reality.

So, what is rice with yellow endosperm? How was it developed? How do we know it will make a difference? Why are some so fanatically opposed to GM crops such as Golden Rice? And, why do we call it *Golden Rice* anyway?

To put this discussion in context, I will first make a slight diversion to consider how our domesticated crops came to be and why they are not perfect.

## 19.2 Our Crops Are Not Perfect: Blame Our Ancestors

Although the domestication of our staple agricultural crops (rice, wheat, maize, and others) and the development of agriculture are prehistoric, an impressive amount of detective work has been carried out on the origins of our crops. It is fairly safe to say that rice was being cultivated at least 10,000 years ago and that it was domesticated from *Oryza rufipogon* (Khush 1997). As early farmers and traders took rice from outside its center of origin, it could continue to intercross with *O. rufipogon*, with farmers choosing to keep those new traits that were of interest to them (Kovach et al. 2007). This "outcrossing" with its wild ancestor continues today, with "weedy rice" being one of the present-day results that trouble most farmers at one time or another.

What is most telling is the relationship between the wild relatives from which the crops were domesticated and the crops themselves. Our ancestors had to eliminate a large number of traits which, while useful to a plant for survival in the wild, did not fit particularly well with life in a cultivated field or as a main food source for farmers. It is certain that many potentially useful traits were left behind as farmers selected against obviously bad traits, such as a cereal's propensity to shed its seed early on the ground—a great survival trick for a plant spreading its seed, but not so great for farmers wishing to harvest a grain crop. Farmers had no way to tell whether they were leaving behind invisible, but valuable, traits such as vitamins or minerals that were linked to undesirable traits.

Domestication was a hit-or-miss kind of affair. Early farmers had no concept of genetics and certainly no idea that the plants they selected might be leaving behind characteristics that could prove useful in environments that had not yet been created. We need not succumb to a romantic version of early agriculture to conclude that modern economies have produced a much narrower range of crops produced at the scale needed to support increasingly urban societies. Commoditization of a few crops, combined with government subsidy policies, can make basic staples affordable for most people, while leaving sources of broader nutrition essentially unaffordable.

Denison (2012) discusses how breeders today might combine biotechnology and traditional plant breeding by using Darwinian insights to identify promising routes for crop genetic improvement and avoid costly dead ends. This is indeed intriguing. And, we can perhaps benefit from more sophisticated comparisons among natural communities and from the study of wild species in the landscapes where they evolved. But, unfortunately, if a trait was left behind during the process of domestication or even earlier in nature's equivalent process of natural selection and speciation, as was the case for rice and its relatives, we will never find it in our crops, no matter how hard we try.

## 19.3 Malnutrition Due to Nutrient Deficiency: A Consequence of Poverty

As populations have exploded over the last century in various parts of the world, the sources of balanced nutrition have become exotic luxuries, which are out of reach of millions upon millions of poor in Asia, Africa, and Latin America. As our societies and economies have become more stratified and as urbanization has become the norm, people, especially the poor, are relying more and more on just a few of the crops that were domesticated some 10,000 years ago. The cereals, while excellent sources of food energy—rice, maize, and wheat provide 42 % of global caloric intake for the entire human population (GRiSP 2013)—are deficient in a range of essential minerals, vitamins, and nutrients. Advances in agricultural productivity have meant that the threat of starvation has been removed for many populations, and although this has been due largely to such advances in the starchy staples, such as rice, wheat, cassava, etc., the productivity of more nutritious vegetables and legumes has lagged. Hence, the dilemma we are in today.

The most seriously limiting nutrient deficiencies worldwide are iron, zinc, iodine, and vitamin A (UNICEF 2000; WHO 2001). Deficiencies can be caused not only by shortages in the diet but also to some extent by other dietary components that impede absorption of the small amounts of essential nutrients that are consumed. Approximately half of the global population, primarily in developing countries, depends on rice as a staple food (GRiSP 2013); however, this cereal is deficient in both vitamin A and iron. Although food additives (fortificants) have been promoted for many years as a solution to vitamin and mineral deficiencies, and some programs have been successful over the medium term, it is clear that this is not a fully sustainable strategy (Zeigler 2001).

There is nothing new about these deficiencies, and, considering how short life expectancies were in the past, it is likely that dietary deficiencies contributed a lot to truncated life spans. Given the size of our populations, what is new is the staggering number of people affected by nutrient deficiencies. And now, knowing what causes these deficiencies, it is increasingly seen as unacceptable for societies to allow such deficiencies to continue.

## 19.4 Vitamin A: Why It Is Important

Vitamins are nutritionally essential, relatively complex, organic[1] molecules that humans cannot synthesize from simple precursors in our food. Vitamin A is needed for overall growth and development of the visual system and a healthy immune system (IRRI 2013a) and is found in animal products and breast milk. We all need vitamin A to grow and thrive, and the need is especially acute in mothers and young children. If vitamin A is not available directly in the food we eat, there are plant pigments called carotenoids (such as beta carotene) that our bodies can convert into vitamin A. These substances are found in orange-colored fruits and vegetables and in dark-green leafy vegetables.

VAD is most prevalent among young children and pregnant and nursing women as they have increased nutrient requirements. The World Health Organization estimates that 190 million preschool children and 19 million pregnant women are vitamin A-deficient globally (WHO 2009). Children with vitamin A deficiency are more likely to suffer from poor health and die prematurely. Each year, it is estimated that 670,000 children under the age of 5 die from VAD. VAD is the number-one cause of preventable blindness among children in developing countries; as many as 350,000 go blind every year (Whitcher et al. 2001). It is important to keep in mind that these horrific statistics are after society has implemented decades of vitamin A supplement programs. As Peter Jennings was already pointing out to the breeders on that April night back in 1984 at the IRRI guest house, the poor in the developing world, who live primarily on a diet of starchy staples (such as rice) that lack vital micronutrients such as vitamin A, are particularly vulnerable to VAD.

Asia has the highest prevalence of VAD in the world, with the most clinical cases found there (see Fig. 19.2). VAD is still considered a public-health problem in many countries of Asia and 33.5 % of preschool children are affected by it (WHO 2009). WHO (2009) also reported that, in the Philippines, VAD affects approximately 1.7 million children (15.2 %) aged 6 months to 5 years. Subclinical VAD affects one out of every ten pregnant women there. In Bangladesh, one in every five children aged 6 months to 5 years is estimated to be vitamin A-deficient. Among pregnant Bangladeshi women, 23.7 % are affected by VAD.

## 19.5 What Is Being Done About VAD?

There are a number of ongoing efforts to fight VAD in the world. Particularly, Helen Keller International (HKI), a cutting-edge global health organization, has been leading the charge to reduce VAD and the resultant child mortality. These programs include (HKI 2013):

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[1] "Organic" in this sense refers to chemicals that have a carbon-based structure, not a philosophical approach to agriculture.

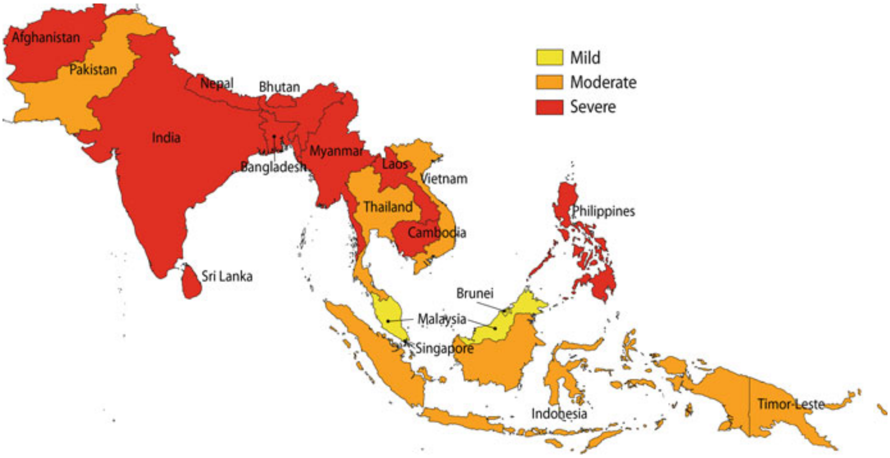

**Fig. 19.2** Severity of VAD in South and Southeast Asia

- Vitamin A capsule supplementation: In 2011, HKI enabled the distribution of 85 million capsules to children ages 6–59 months and postpartum women in the developing world. It takes two capsules a year to prevent VAD in children at a cost of $1 per child per year.
- Dietary diversification: HKI's Homestead Food Production programs enable households to produce their own micronutrient-rich foods and earn additional income. HKI helps communities establish homestead gardens that produce fruits and vegetables and develop animal husbandry programs to provide food rich in vitamin A and other micronutrients year round.
- Large-scale fortification: HKI leads initiatives to fortify commonly used foods, such as cooking oil with vitamin A, and engages in social marketing to encourage their consumption. Enriching food products with vitamin A is a cost-effective and long-term means to address VAD with limited behavior change on the part of consumers.
- Crop biofortification: HKI promotes the production and consumption of orange-fleshed sweet potatoes (Coghlan 2013) that have been bred to produce higher beta carotene, especially when compared with the traditionally grown white-fleshed varieties, which don't have any beta carotene.

Regarding sweet potatoes, they naturally contain beta carotene, so breeders can use traditional methods to improve its content (Coghlan 2013). Since neither cultivated nor wild rice contains any beta carotene, it's impossible to introduce it without genetic engineering. Because the sweet potato breeding does not involve genetic modification, anti-GM activists don't complain about it despite the aim being identical to Golden Rice. But, as Potrykus points out (Coghlan 2013), the experience with sweet potatoes shows that what we're trying to achieve with rice is realistic. As soon as people get the sweet potato, it improves their vitamin A status.

Unfortunately, despite the very best and diversified efforts, VAD continues to adversely affect millions of people, especially the last 10–20 % in the hardest-to-reach areas of the developing world. In the Philippines, as in many developing countries, effective distribution systems for vitamin A supplementation are not in place to reach all people in need adequately and consistently so that the most vulnerable children and women in remote areas are often missed. What is worse, a supplement-only approach must be continued essentially forever, requiring open-ended investments and a complex enabling and supporting infrastructure.

Rice is widely produced and consumed in areas of the developing world where VAD continues to be a persistent problem. If this staple could be fortified with beta carotene precursor of vitamin A, it would have the potential to reach millions of people, including those who do not have reliable access to or cannot afford other sources of vitamin A (IRRI 2013a).

## 19.6 Enter Golden Rice

Why doesn't the rice that people eat have vitamin A or any of its precursors in the first place? Quite simply, the rice plant does not need to store vitamin A or carotenoids in its seed. The growing seedling can produce as much as it needs as it grows. The rice pericarp, or bran, is rich in B vitamins, oils, and some minerals, but not vitamin A (Zeigler 2001). So, even though Peter Jennings laid out the argument for developing rice with yellow endosperm way back in 1984, which spurred RF to add it to its biotechnology program's list of desirable traits, the challenge was enormous.

One cannot overstate the magnitude of the challenge to engineer rice to accumulate vitamin A precursors in the endosperm (Ye et al. 2000). Please excuse me if I get a bit technical here, but I think it is important to show the complexity involved. Biosynthesis of 20-carbon beta carotene entails multiple steps, and when the project was initiated by Potrykus and his team in the late 1980s, plant transformation was in its infancy. Adding new processes that typically involved only a single novel reaction was a massive undertaking. Second, the precursor to carotenoids, the 20-carbon geranyl geranyl diphosphate (GGPP), is present in endosperm of immature seeds. However, it is also the precursor, via the isoprenoid pathway, of other compounds, such as chlorophyll and the gibberellins, which are essential for seed germination and early seedling growth. It was unknown how diverting a portion of GGPP to beta carotene synthesis would affect germination and early seedling vigor (Zeigler 2001).

In plants, four enzymatic steps are required to synthesize 40-carbon beta carotene from GGPP. The carotene phytoene is synthesized from two GGPP in a reaction catalyzed by phytoene synthase. Then, two successive reactions transform the phytoene molecule into lycopene. Lycopene is then cyclized in a subsequent step to produce beta carotene and other carotenoids. The first step, phytoene synthesis in rice endosperm, was achieved in 1997 (Burkhardt et al. 1997). The gene for phytoene synthase from daffodil (*Narcissus pseudonarcissus*), along with either constitutive or endosperm-specific promoters, was incorporated into a rice variety, and seeds were found to accumulate phytoene in the endosperm.

Potrykus and his team had to overcome the problem of multiple transformation events required to complete the reaction from phytoene to lycopene. First, they took advantage of a bacterial phytoene desaturase from the common soil bacterium *Erwinia uredovora* (now renamed *Pantoea ananatis*), which carries out all the reactions for all four required changes (Ye et al. 2000). Then, using *Agrobacterium*-mediated transformation, they introduced the entire beta carotene pathway in one transformation event involving two constructs. One plasmid carried phytoene synthase and phytoene desaturase, in the absence of a selectable antibiotic marker. The phytoene synthase is driven by an endosperm-specific promoter, while the phytoene desaturase is controlled by a constitutive promoter, and the coding sequences for both included transit peptides for plastid import that were intended to localize synthesis of lycopene to endosperm plastids where GGPP is found. The second plasmid carried lycopene beta-cyclase, also targeted to the plastid, which catalyzes the final step of beta carotene synthesis, controlled by endosperm-specific rice glutelin promoter along with a selectable antibiotic (Hygromycin) resistance marker in the same transformation vector.

Endosperm of rice seed from transformants for the full complement of enzymes exhibited the expected yellow color [see Box 19.1 on how rice with this yellow endosperm started to be called Golden Rice in 1999] and was shown to contain varying amounts of beta carotene. As a proof of concept, this was a resounding success. Lost in both the excitement and the criticism by those opposed to any form of transgenic crops was that the level of beta carotene as related to the vitamin A needs of VAD populations was irrelevant at this stage. “Would it be enough to help provide children with their daily vitamin A requirement?” was a question better to be asked much later. In any event, a significant finding was that rice transformants for only the first construct did not just accumulate lycopene in the endosperm as expected. Rather, they showed beta carotene as well as two other carotenoids: lutein and zeaxanthin. Apparently, enzymes for the synthesis of these carotenoids from lycopene are either always expressed in the endosperm or induced by the presence of lycopene (Ye et al. 2000). The accumulation of lutein and zeaxanthin are produced at only very low concentrations, but may be of additional interest because they have different benefits, such as the reduction of macular degeneration in elderly patients (UNICEF 1997).

This work culminated in the late 1990s and early 2000s and was given high-profile coverage in the media, ultimately landing Potrykus on the cover of *Time* magazine (Nash 2000). For the next few years, research continued. And then, as Potrykus recalls (Coghlan 2013), the next big step was in 2005 when scientists at the biotech company Syngenta (Paine et al. 2005) replaced the original gene from daffodil, which makes phytoene synthase, with a more efficient gene from maize (*Zea mays*) and used a nonantibiotic selectable marker system. By then, *Erwinia*

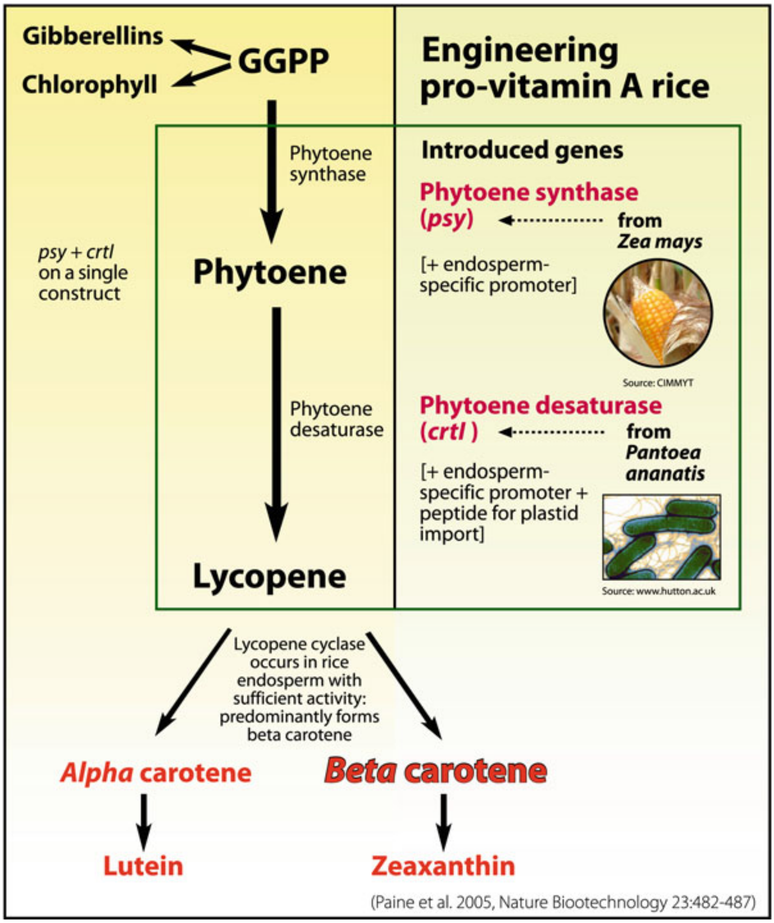

**Fig. 19.3** Steps, gene sources, and components for the development of Golden Rice

*uredovora*, the source of the bacterial phytoene desaturase, had been renamed *Pantoea ananatis* (see Fig. 19.3). This new strain, with the bottleneck gene from daffodil removed and replaced with the maize counterpart, produced a dramatic 20 times more beta carotene (Coghlan 2005). It was a major achievement, according to Potrykus and everyone interested in the success of Golden Rice. At this point, it was clear that adequate levels of beta carotene production in rice endosperm were possible.

Critics of the original Golden Rice were fond of saying that its amounts of pro-vitamin A—1.6 micrograms per gram of rice—were too low to make the rice a practical proposition (Coghlan 2005). But, each gram of the new strain contains up to 37 micrograms of the pro-vitamin A. Tang et al. (2009) showed that daily consumption of a very modest amount of Golden Rice—about a cup (or around 150 g uncooked weight)—could supply 50 % of the Recommended Daily Allowance of vitamin A for an adult.

Then, most recently, this same group from Tufts University with colleagues from China (Tang et al. 2012) found that the beta carotene molecules in Golden Rice are converted very efficiently in the body to one molecule of vitamin A, which approaches the levels obtained with beta carotene in oil, the practical maximum! The conversion is four to eight times better than the conversion from spinach and other green leafy vegetables, which take between 7 and 16 molecules of beta carotene to result in one molecule of vitamin A in the body. This means that a single serving of Golden Rice could provide up to 60 % of the Chinese recommended daily intake for children.

### Box 19.1: How Golden Rice Got Its Name

In an IRRI Pioneer Interview (Hettel 2009b), Gary Toenniessen recalls how Golden Rice got its name.

"Ingo Potrykus first presented his [yellow endosperm] results during a meeting of the Rockefeller Foundation's Rice Biotechnology Network held in Phuket, Thailand, in September 1999. It was one of several important scientific breakthroughs that were presented at that meeting. Following the meeting, some colleagues and I stayed on in Bangkok . . . . We had dinner one evening with Mr. Mechai Viravaidya, Thailand's 'Condom King'—so-called due to his extensive work promoting accessible contraceptives over 30 years in Thailand—and a former member of IRRI's Board of Trustees (1995–2000). He was a true marketer, having distributed those condoms in very innovative ways. He had colored condoms. He gave away condoms if you bought 10 l of gasoline at the local gas station and the like. He made it acceptable to be seen buying condoms and almost made it a treat.

So, we were telling him of some of the exciting results that had occurred at the meeting in Phuket . . . including the recent development of yellow endosperm rice, which produces beta carotene. He immediately recognized the importance of this because he'd been heading an NGO [Population and Community Development Association] that had been dealing with VAD problems in Thailand.

I can remember him saying as we listened intently, 'You Aggies do not understand marketing. Don't call it yellow endosperm rice . . . call it Goooolden Rice. You got to have a marketing campaign behind this. You got to make it a treat to eat Golden Rice. It's got to be better than white rice.' I remember going back and telling Ingo that we had to start calling his invention Golden Rice. Ingo caught on and the rest is history."

## 19.7 The Golden Rice Project

All along, the proponents of Golden Rice have intended for it to be used in combination with existing approaches to overcome VAD, including eating foods that are naturally high in vitamin A or beta carotene, eating foods fortified with vitamin A, taking vitamin A supplements, and optimal breastfeeding practices (IRRI 2013a).

In what is being called the Golden Rice Project (IRRI 2013b), IRRI is working with leading nutrition and agricultural research organizations to further develop and evaluate Golden Rice to reduce VAD, particularly in the Philippines and Bangladesh—two countries where millions of children and women are vitamin A-deficient. Each of the participating organizations is playing a different and important role to contribute to the project.

### *19.7.1 Developing Varieties*

Breeders at the Philippine Rice Research Institute (PhilRice) and the Bangladesh Rice Research Institute (BRRI) are developing Golden Rice versions of existing rice varieties that are popular with local farmers. The new varieties would produce beta carotene while still having the same yield, pest resistance, and grain qualities as before. Cooking and taste tests are being done to help make sure that Golden Rice meets consumers' needs and preferences. Golden Rice seeds are expected to cost farmers the same as other rice varieties. Golden Rice grain should be available to consumers at the same prices of other good-quality rice.

### *19.7.2 Assessing Safety*

To help assess the safety of Golden Rice in the environment, IRRI, PhilRice, BRRI, and others are working together on field tests and other evaluations in both the Philippines and Bangladesh. Golden Rice is analyzed according to internationally accepted guidelines for food safety (FAO 2003). It has always been a condition within the project and the position of all parties concerned that, if Golden Rice were found to be unsafe, or unfit, for human consumption, the project would be terminated. There has been no evidence for any safety or quality concerns identified during the life of the project, despite explicit and concerted efforts by scientists to uncover them should they exist.

PhilRice and BRRI plan to submit all safety information to their national government regulators as soon as all required tests are completed. Regulators will review these data as part of the approval process for Golden Rice before it can be released to farmers or made available to consumers. In late October 2013, PhilRice predicted that Golden Rice would be available in the Philippine market by 2015 (PNA 2013); however, such timelines in the development of any transgenic crop are notoriously difficult to predict.

### 19.7.3 Demonstrating Efficacy

Once Golden Rice is approved by national regulators as safe, HKI will evaluate whether Golden Rice improves vitamin A status (meaning it is efficacious). Working with other international experts and local partners, a study under controlled community conditions will be conducted to determine whether eating Golden Rice every day improves vitamin A status and could therefore contribute to reducing VAD.

### 19.7.4 Reaching Those in Need

Once Golden Rice is shown to be safe and to improve vitamin A status, a comprehensive delivery program will ensure that it is acceptable and accessible to any farmer and consumer, with an added emphasis and efforts for communities suffering from VAD. The aim is to make it sustainable or self-supporting, meaning that, eventually, communities could have ongoing access to Golden Rice without any additional aid or intervention—it would be theirs to grow and consume as they do other rice.

### 19.7.5 Looking Ahead

As the Golden Rice effort has progressed, a number of donors in addition to the RF have come forward to lend their support. Notably, USAID has provided critical support since 2001 and the Bill & Melinda Gates Foundation joined the effort with important backing in 2010. IRRI and others will continue to work together to introduce Golden Rice in the Philippines and Bangladesh as another approach to reducing VAD, if Golden Rice is found to be safe and to improve vitamin A status. Additional research in this project is being conducted by the University of Freiburg and IRRI to develop Golden Rice varieties with even higher beta carotene. Figure 19.4 shows the Golden Rice timeline from its invention in 1999 to future plans for 2014 and beyond.

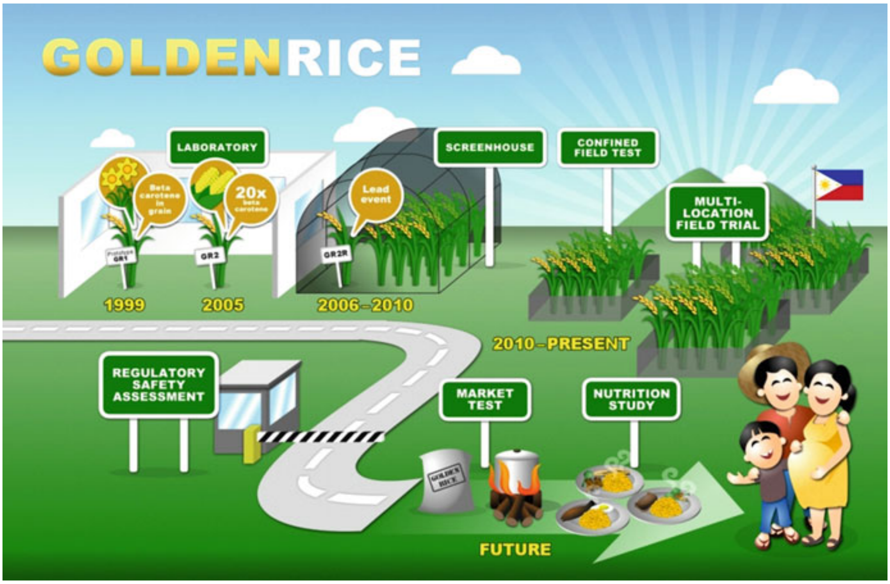

**Fig. 19.4** Timeline below showing the winding path for Golden Rice research

**Laboratory: 1999:** Golden Rice is invented by Professor Ingo Potrykus, then of the Institute for Plant Sciences, Swiss Federal Institute of Technology, and Professor Peter Beyer of the University of Freiburg, Germany. They use genes from daffodil and a bacterium to develop a prototype of Golden Rice that can produce beta carotene in its grain

**2005:** Scientists develop the current version of Golden Rice, now using genes from maize and a common soil microorganism that together produce about 20 times more beta carotene than the 1999 prototype

**2006–2010:** The "lead event" of the current version of Golden Rice is selected and moved to the next phase of research and development. It is transferred to rice research institutes in the Philippines and elsewhere to begin breeding Golden Rice versions of important Asian rice varieties

**Field: 2010 to present:** Crops grown in screen houses and in confined and multilocation field trials help breeders develop new Golden Rice varieties that retain the same yield, pest resistance, and grain qualities. Field tests and other evaluations also generate environmental and other data that will be used to help assess the safety of Golden Rice

**Future:** Regulators in countries where Golden Rice is to be grown will review the data and assess the food, feed, and environmental safety of Golden Rice as part of the approval process before it can be made available to farmers and consumers

If Golden Rice is approved by national regulators as safe for human consumption, then an independent nutrition study will evaluate whether eating Golden Rice every day will improve vitamin A levels in real-world conditions.

Information will be collected on the best practices for storing and cooking Golden Rice, and to develop strategies to ensure that Golden Rice can reach farmers and consumers

### *19.7.6 Other Work on Golden Rice*

Our experiences in developing, evaluating, and planning the delivery of Golden Rice in the Philippines and Bangladesh will be important in designing plans for

Golden Rice in other countries. In addition to the IRRI-led Golden Rice Project in Bangladesh and the Philippines, work to develop Golden Rice is underway at research institutes in India, Indonesia, and other countries. More information about their work, and current activities of the inventors and the Golden Rice Humanitarian Board, can be found in Golden Rice Humanitarian Board (2013).

## 19.8 Opposition to GMOs in General and Golden Rice Specifically

The general public largely became aware of Golden Rice in that *Time* cover story at the turn of the century (Nash 2000). Readers of that article were asked to decide whether genetically engineered crops or organisms (GMOs) would revolutionize farming on the one hand or destroy the ecosystem on the other. Over the next 13 years, the invention has been in the eye of a raging storm between the proponents and opponents of GMOs. Actually, depending on how broad one's definition is for a GMO, GMOs have been a topic (although not with the specific GMO moniker) since the days of Gregor Mendel in the 1860s. In a recent article in the *Seattle Times*, Shea (2013) presents an excellent graphic on the timeline on GMO research and a primer on how the technology works, how it differs from conventional breeding, and the prevalence of genetically engineered foods in American fields and markets.

In August 2013, Golden Rice received major coverage in the *New York Times* when 400 protesters, in an act of vandalism, smashed down the fences surrounding an experimental field of Golden Rice right here in the Bicol region of the Philippines and uprooted the plants growing inside (Harmon 2013). The protestors claimed that Golden Rice could pose unforeseen risks to human health and the environment and that it would ultimately profit large multinational corporations. One purpose of the experiment was to determine whether there were any negative impacts on the environment from Golden Rice cultivation. So, activists destroyed experiments designed specifically to address their concerns. The destruction of the field trial in Bicol, and the reasons given for it, unleashed an unexpected backlash, touching a nerve among scientists around the world, prompting them to counter assertions of Golden Rice's health and environmental risks. At this writing, the controversy rages on.

As the father of Golden Rice, Ingo Potrykus, points out, since the early 1990s, the majority of the media has repeated the mantra that GMOs are highly dangerous for the environment and consumers (Coghlan 2013). This is fueled continuously by a well-financed and well-organized anti-GMO lobby. One of the cleverest tricks of the anti-GMO movement is to link GMOs so closely to the multinational corporations because these companies are perceived to have no friends. That strategy guarantees millions of supporters because people are usually against multinationals and in favor of "organic" farming because of the perception that organic farming is run by idealists who protect nature and don't make money from it.

The controversy over Golden Rice has been very frustrating to many of us. As Potrykus says, it is unfortunate that offering a technology for free that could save so many children and pregnant mothers has been delayed for so long. Since the invention of Golden Rice, 2.5 million children are estimated to have died each year from VAD. Around 500,000 go blind each year, of whom 70 % die. They wouldn't all have been saved by Golden Rice, of course, but every delay means many unnecessary dead or blind children (Coghlan 2013).

As IRRI's director general, I believe that it is high time that activists stop using their cynical and destructive ways to oppose Golden Rice and instead support scientific research, development, and assessment of the product because of its strong potential benefit to humanity. Although the activists claim that Golden Rice is inadequately tested, may discourage food diversification, and may ultimately lead to the monopoly of some seed manufacturing companies, I believe these claims are baseless. Golden Rice was developed by government scientists using public funds. So, why should these activists block a technology developed by the public sector and hold the world's poor hostage over a fight about private control of agriculture in rich countries? The activists claim that there is not enough information to decide whether Golden Rice and these crops are safe, yet they destroy the very trials, like one in Bicol, designed to provide the answers they are demanding. Could it be that they do not want to see the answers if the trials show that Golden Rice is safe, productive, and effective?

The UK Secretary of State for Environment, Food and Rural Affairs, Owen Paterson, feels strongly about this issue as well. He has referred to groups opposing Golden Rice as "wicked" (BBC 2013). Whether GMO opponents are wicked or not, I leave to others to decide, but, as a scientist, I am "genetically" hardwired to be objective, factual, and as truthful as I can be (often to a fault). Therefore, I find the internal inconsistency and self-contradiction of many arguments against GMOs simply astounding.

The development of Golden Rice is an enlightening story of vision, imagination, technological creativity, and persistence. Its dissemination to poor rice consumers will not only fulfill the dream that Peter Jennings articulated back in 1984 and reward the enormous personal investments of professors Potrykus and Beyer but also justify Gary Toenniessen's recommendation to the RF that yellow endosperm be added to its list of desirable traits in rice that might be attained through applications of biotechnology. It would be a pity to see its delivery further delayed by those opposing the adoption of a new technology at any cost—especially when it is millions of very poor people who must shoulder those costs, not the opponents of Golden Rice.

**Acknowledgment** The author gratefully acknowledges the extensive contributions of Gene Hettel of IRRI in the drafting and background research for this chapter.

## References

BBC (2013) GM ‘Golden Rice’ opponents wicked, says Minister Owen Paterson. Viewed online 1 November: www.bbc.co.uk/news/uk-politics-24515938

Burkhardt PK, Beyer P, Wunn J, Kloti A, Armstrong GA, Schledz M, von Lintig J, Potrykus I (1997) Transgenic rice (*Oryza sativa*) endosperm expressing daffodil (*Narcissus pseudonarcissus*) phytoene synthase accumulates phytoene, a key intermediate of provitamin A biosynthesis. Plant J 11(5):1071–1078

Coghlan A (2005) New ‘Golden Rice’ carries far more vitamin. New Scientist, 27 March: www.newscientist.com/article/dn7196-new-golden-rice-carries-far-more-vitamin.html#.Um4WbXAbBuI

Coghlan A (2013) Golden Rice creator wants to live to see it save lives. New Scientist, 16 October: www.newscientist.com/article/dn24417-golden-rice-creator-wants-to-live-to-see-it-save-lives.html?full=true#.Um4Ye3AbBuK

Denison RF (2012) Darwinian agriculture: how understanding evolution can improve agriculture. Princeton University Press, Princeton, 272 p. View a series of lectures based on the book: www.youtube.com/playlist?list=PLNowyW0S1hI8GsRZ_RZNcECgH-TPJhrjt

FAO (Food and Agriculture Organization) (2003) Guidelines for the conduct of food safety assessment of foods derived from recombinant-DNA plants. Food and Agriculture Organization (Rome). Online at www.fao.org/fileadmin/user_upload/gmfp/docs/CAC.GL_45_2003.pdf

Golden Rice Humanitarian Board (2013) The Golden Rice Project. Viewed online, 31 October: www.goldenrice.org

Goswami BC, Ivanoff KD, Barua AB (2003) Absorption and conversion of 11,12-$^3$H-β-carotene to vitamin A in Sprague-Dawley rats of different vitamin A status. J Nutr 133(1):148–153

GRiSP (Global Rice Science Partnership) (2013) Rice almanac, 4th edn. International Rice Research Institute, Los Baños, p 283

Harmon A (2013) Golden Rice: lifesaver? New York Times Sunday Review: 24 August. www.nytimes.com/2013/08/25/sunday-review/golden-rice-lifesaver.html

Hettel GP (2008) Luck is the residue of design. Rice Today 7(1):10–11, YouTube video: http://youtu.be/OxdJ_wiimc0

Hettel GP (2009a) Yellow endosperm as a trait in rice: prelude to Golden Rice. YouTube video: http://youtu.be/a7bGykLVm2E

Hettel GP (2009b) How Golden Rice got its name. YouTube video: http://youtu.be/QzuV4HM-gJI

HKI (Helen Keller International) (2013) Vitamin A supplementation. Online at www.hki.org/preventing-blindness/vitamin-a-supplementation. Viewed 31 October

IRRI (International Rice Research Institute) (1985) Biotechnology in international agricultural research. In: Proceedings of the Inter-center seminar on international agricultural research centers (IARCs) and biotechnology. International Rice Research Institute, Los Baños, 23–27 Apr 1984, 435 p

IRRI (International Rice Research Institute) (2013a) Vitamin A deficiency. IRRI Web site (viewed 22 October): http://irri.org/golden-rice/vitamin-a-deficiency

IRRI (International Rice Research Institute) (2013b) The Golden Rice Project. IRRI Web site (viewed 25 October): http://irri.org/golden-rice/the-project

Khush GS (1997) Origin, dispersal, cultivation and variation of rice. Plant Mol Biol 35:25–34

Khush GS, Toenniessen GH (1991) Rice biotechnology. Biotechnology in Agriculture No. 6. CAB International (UK) and International Rice Research Institute (Philippines), 320 p

Kovach MJ, Sweeney MT, McCouch SR (2007) New insights into the history of rice domestication. Trends Genet 23:578–587

Nash JM (2000) This rice could save a million kids a year. Time, 31 July. http://content.time.com/time/magazine/article/0,9171,997586,00.html

Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G, Wright SY, Hinchliffe E, Adams JL, Silverstone AL, Drake R (2005) Improving the nutritional value of Golden Rice through increased pro-vitamin A content. Nat Biotechnol 23(4):482–487

PNA (Philippine News Agency) (2013) Golden Rice to be available by 2015. PNA via Manila Standard Today, 31 October: http://manilastandardtoday.com/2013/11/01/-golden-rice-to-be-available-by-2015/

Potrykus I (2000) The "Golden Rice" tale. AgBioWorld: Home Page: Archive. Message #906. Published on-line: www.agbioworld.org/biotech-info/topics/goldenrice/tale.html

Potrykus (2001) Golden Rice and beyond. Plant Physiol 125:1157–1161

Rutger JN (2009) The induced *sd1* mutant and other useful mutant genes in modern rice varieties. In: Shu QY (ed) Induced plant mutations in the genomics era. Food and Agriculture Organization, Rome, pp 44–47

Shea K (2013) The ABC's of GMOs. In: Allison M (ed) On voters' plates: genetically engineered crops. Seattle Times, 10 August. http://seattletimes.com/flatpages/local/abcsofgmos.html

Tang G, Qin J, Dolnikowski GG, Russell RM, Grusak MA (2009) Golden Rice is an effective source of vitamin A. Am J Clin Nutr 89:1776–1783

Tang G, Hu Y, Yin S, Wang Y, Dallal GE, Grusak MA, Russell RM (2012) β-carotene in Golden Rice is as good as β-carotene in oil at providing vitamin A to children. Am J Clin Nutr 96(3):658–664, http://ajcn.nutrition.org/content/96/3/658

UNICEF (1997) Vitamin A global initiative: a strategy for acceleration of progress in combating vitamin A deficiency. United Nations, New York

UNICEF (2000) Vitamin A deficiency—a cause of child death that can be easily prevented. Vitamin A-global initiative. United Nations, New York

Whitcher JP, Srinivasan M, Upadhyay MP (2001) Corneal blindness: a global perspective. Bull World Health Org 79(3):214–221

WHO (World Health Organization) (2001) Iron deficiency anaemia: assessment, prevention, and control. United Nations, New York, Published on-line: www.who.int/nutrition/publications/en/ida_assessment_prevention_control.pdf

WHO (World Health Organization) (2009) Global database on vitamin A deficiency: global prevalence of vitamin A deficiency in populations at risk, 1995–2005. WHO, Geneva, Online at http://whqlibdoc.who.int/publications/2009/9789241598019_eng.pdf

Ye X, Al-Babili S, Kloti A, Zhang J, Lucca P, Beyer P, Potrykus I (2000) Engineering the provitamin A (B-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science 287:303–305

Zeigler RS (2001) Agricultural biotechnology: reducing poverty in developing countries. Plant Dis 85(6):568–579

# Chapter 20
# Production of Medicines from Engineered Proteins in Plants: Proteins for a New Century

**Mary E. Mangan**

**Abstract** Recombinant proteins have already delivered major benefits to human health in the relatively short time they have been available. Plant-based production strategies for these proteins—sometimes called molecular pharming—are becoming widespread and offer major utility, as well as overcoming some of the drawbacks of microbial and mammalian production systems. Flexible and rapid engineering methods, combined with benefits of high volume expression for protein isolation, or seed-based long-term storage, offer many options for medically-relevant protein production with direct benefits for people who need to use them. Metabolic and infectious disease treatments are among the early targets, but cancer treatment, circulatory aliments, allergy reduction, and wound repair and tissue regeneration support may result from proteins produced in plant systems. Selected samples of projects are provided to illustrate the current directions, including the first FDA approved recombinant plant drug to treat a disease. Other examples of projects aimed at communicable diseases, cancer, heart disease, and wound repair are included. When safety and efficacy are demonstrated, and with adherence to appropriate regulatory and biosafety frameworks, plant-derived recombinant proteins may offer high-volume and cost-effective delivery systems for many medical applications in this century.

**Keywords** Molecular pharming • Enzymes • GMO • Vaccines • Bioreactors

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A. Ricroch et al. (eds.), *Plant Biotechnology: Experience and Future Prospects*, DOI 10.1007/978-3-319-06892-3_20,

## 20.1 Recombinant Protein Historical Milestones

In the early twentieth century, remarkable technical and societal changes were impacting the planet. Medical advances were occurring, but their translation to the clinic to fight the ongoing scourges of infectious and chronic diseases was in its infancy. In the first decade of the early 1900s, the top three causes of death in the United States were influenza and pneumonia, tuberculosis, and gastrointestinal infections (Jones et al. 2012). Human infectious diseases persist, of course, but are reduced in prevalence and more manageable today in the developed world due in part to tremendous biomedical advances, including antibiotics, vaccines, and supportive treatments, developed in that century. Our battle with illness dramatically changed due to scientific advances of the twentieth century.

Another condition whose treatment transformed over that period is diabetes. Before the 1920s, diagnosis of type 1 diabetes was a brutal blow. Previously healthy youngsters would have less than a year to live following the onset. The body's breakdown proceeded quickly, with an excruciating course for the patient and family, inevitably ending with this typical scenario:

> The wasting away of the flesh from lack of nourishment could be dreadful in itself: "When he came to the hospital he was emaciated, weak and dejected; his thirst was unquenchable; and his skin dry, hard and harsh to the touch, like rough parchment." . . . . The diabetic suffering from acid-intoxication or acidosis (often used synonymously with ketosis) was losing the battle. Food and drink no longer mattered, often could not be taken. A restless drowsiness shaded into semi-consciousness. As the lungs heaved desperately to expel carbonic acid (as carbon dioxide), the dying diabetic took huge gasps of air to try to increase his capacity. "Air-hunger" the doctors called it, and the whole process was sometimes described as "internal suffocation." The gasping and sighing and sweet smell lingered on as the unconsciousness became a deep diabetic coma. At that point the family could make its arrangements with the undertaker, for within a few hours death would end the suffering. (Bliss 2013, p 22)

This changed dramatically with the discovery that insulin—a protein that could be obtained outside the body—could be extracted, isolated, and provided by injection to supplement the failure of the patient's pancreas to supply it. This discovery was awarded the Nobel Prize in Medicine in 1923 to Banting and Macleod (www.nobelprize.org/nobel_prizes/medicine/laureates/1923/). Initially, purified insulin was extracted from tons of pancreas tissue of cows and pigs obtained from slaughterhouses. By mid-century the specific amino acid sequence of the insulin protein had been deciphered by Frederick Sanger, for which he was awarded a Nobel Prize in Chemistry (http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1958/). These crucial steps, combined with other technological developments of the period that were taking place in parallel, provided the opportunity for the next major advance—the production of this protein-based medicine from an engineered bacterial cell, known as recombinant protein production (Goeddel et al. 1979). A protein researcher described insulin as the "Protein of the 20th Century" because of this important trajectory (Fig. 20.1, Structure of Insulin, PDB ID: 1AIY; Gerritsen 2001).

Symmetry: D3 view
Stoichiometry: Hetero 12-mer - A6B6

**R6 HUMAN INSULIN HEXAMER (SYMMETRIC), NMR, 10 STRUCTURES**
**PDB ID: 1AIY**
**DOI:10.2210/pdb1aiy/pdb**

**Fig. 20.1** A milestone of biotechnology was the production of human insulin by bacteria, the first such life-saving drug approved by the FDA. NMR spectroscopy reveals the structure of insulin. DOI:10.2210/pdb1aiy/ pdb (Chang et al. 1997; Berman et al. 2000)

In the 1980s, insulin became the first genetically engineered human protein generated from recombinant biotechnology processes in *E. coli* bacteria approved by the US Food and Drug Administration (FDA), developed by Genetech and made available for use by Eli Lilly and Company in 1983 as Humulin®. It made headlines as a major advance, and recombinant human insulin expressed in bacteria has gone on to become the mainstay of treatment for insufficient production of insulin in diabetic patients. Yet research continues, and novel ways to create high quality and effective insulin at lower costs has been explored using recombinant protein expression plant systems to produce proteins (Boothe et al. 2010).

### *20.1.1 Recombinant Proteins Turning to Plant Production*

From our vantage point this process of the initial discovery of the effectiveness of insulin replacement to the access to recombinant insulin today as standard for care may seem lengthy. Some people remain unaware of the fact that genetically-modified bacteria have been providing insulin for us for three decades, taking its availability for granted. But these foundational steps were necessary, and provided millions of families with a life-saving strategy. Today we stand on the shoulders of giants to move forward with similar strategies and technologies to produce recombinant proteins in other ways. We can now rapidly produce proteins in yeast and other microbial systems in addition to bacteria, in animals and plants, and in cell cultures and whole organisms, eventually in artificial cells and cell-free systems via synthetic biology. We can develop treatments for conditions that benefit not only the developed world but also those in the developing world. This technology can be accessible for researchers and recipients of treatments worldwide.

The choice of expression system depends on many factors. In some cases, bacterial production is most appropriate. In others, eukaryotes—cells with a nucleus and additional structures—like yeast are more suited. For certain outcomes insect or mammalian cell culture is more effective. In other scenarios, plants in either field-scale systems or cell cultures may be most scalable, stable, and cost-effective. Expression in leaf tissue, seeds, or root structures may be preferable depending on the application. These decisions rely on evaluation of the type of molecule to be produced, the scale required, the preferred storage or delivery strategy, the benefits, and the risks.

In this chapter we turn our focus to plant-based strategies for recombinant molecule production. For more detailed scientific and technical issues associated with the choices that are weighted in determining a plant based production system, Goldstein and Thomas (2004) provide a helpful overview and analysis. For "molecular pharming" in general, reviews are available (Paul et al. 2013). For a peek at the state-of-the-art at this time, a conference report on "molecular pharming" provides information on the strategies and challenges being tackled (Lössl and Clarke 2013).

In the rest of this chapter we explore examples of specific projects underway at this time, using plants as the vehicle for expression of recombinant molecules. Individual examples will illustrate the methods being developed, as well as the range of medical issues addressed. This is not designed to be a comprehensive look at the entire field and associated technical details, rather as an introduction for readers new to the topic. In these early days it isn't possible to predict which ones will succeed, as there are many biological, regulatory, corporate decisions, and even social barriers affecting the outcomes. New strategies such as editing methods may also emerge (Belhaj et al. 2013). The goal is to provide an understanding of our current experiences and to point out future prospects. I hope to convey the excitement and potential of these proteins for treating medical challenges we face in a new century.

### 20.1.2 The First Approved Recombinant Plant Protein Drug

When I first read this story of a young Costa Rican girl, I was struck by the similarity to that of diabetic patients in the beginning of the previous century:

> As a toddler, Tania had been a bright girl with a vivid smile and a penchant for dancing. But by age 8 she was in a strange and frightening decline. She struggled on frail limbs to carry her swollen abdomen. As other children rode their bikes on the tiny fishing village's dirt roads, Tania lay on the sofa in her orange cinderblock house, inert. (Heuser 2009)

Tania was eventually diagnosed with a rare disease that is different from diabetes, but shares features. Her body is unable to make a functional protein, acid beta-glucosidase (GBA), leading to the condition known as Gaucher disease. The role of GBA in cells is to help the body break down molecules for the body to recycle. In patients with damage to GBA, the breakdown fails and toxic products accumulate. Depending on the type of mutation, these toxic products can build up and affect several body systems and lead to death if untreated.

After the identification of this protein's role and the pathway in which it acts, researchers tried supplementing the protein in a similar way to the insulin story. However, simply providing purified protein was ineffective, but placenta-extracted human proteins were functional if the protein surfaces were treated and tweaked. This enabled the Genzyme Corporation to develop an effective treatment, Ceredase®, relying on this modified protein preparation of placenta extracts (Cox 2010). But reliance on these human placenta sources has additional risks including availability and contamination, and as in the case of insulin, a more reliable and less costly recombinant production system would be very useful.

It also became clear that this protein requires modifications that happen beyond the basic assembly of the amino acid sequence: glycosylation steps are also required. This process attaches sugar-based molecule residues to proteins to impact their functions. This requirement for modification of the protein after initial protein assembly (known as post-translational modification) means that bacteria can't help us in this case. Some proteins need additional folding assistance or machinery that's not available to bacteria. Numerous types of post-translations modifications of protein chains occur for full functionality of some proteins. Some assemble into multi-subunit complexes consisting of several protein chains that are beyond the capacity of bacterial protein synthesis capacity. In this case, the GBA protein needs to be processed in the eukaryotic organelles called the endoplasmic reticulum and Golgi apparatus, where the necessary modifications can occur. Genzyme did eventually create a production system in a mammalian cell culture model in Chinese Hamster Ovary cells or treating Gaucher patients, and another provider has developed a system in a human cell line (VPRIV® from Shire Human Genetic Therapies).

Another research team took a different approach. Because production of recombinant proteins in mammalian cell culture systems has additional challenges—including expense of cell culture reagents and the possibility of contamination with infectious agents like viruses that could affect patients and has affected production—a plant-cell culture based system was developed to generate the effective modified protein (Fig. 20.2, Structure of Taliglucerase alfa, PDB ID: 2V3F Shaaltiel et al. 2007; Zimran et al. 2011).

Shown to be safe and effective in clinical trials, the taliglucease alfa (Elelyso®) recombinant protein generated in carrot cell cultures from Protalix Biotherapeutics

No symmetry
Stoichiometry: **Monomer**
Biological assembly 1 assigned by authors and generated by PQS (software)

**ACID-BETA-GLUCOSIDASE
PRODUCED IN CARROT
PDB ID: 2V3F
DOI:10.2210/pdb2v3f/pdb**

**Fig. 20.2** Another milestone of biotechnology was the production of human acid-beta-glucosidase protein in carrot cell cultures. It was the first such case of a plant-generated human protein drug approved by the FDA. DOI:10.2210/ pdb2v3f/ pdb (Shaaltiel et al. 2007; Berman et al. 2000)

made history in 2012 as the first plant-derived recombinant enzyme drug treatment approved by the FDA. It has been estimated that this novel production method could reduce the cost of this treatment by 75 % (Maxmen 2012). For children around the world with a rare disease like Gaucher who need access to this crucial recombinant enzyme, a cost-effective solution may be the difference between life and death as insulin was a century ago.

## 20.2 Recombinant Proteins from Plants Can Help Battle Various Diseases

As noted above, the disease burden in the developed world has shifted from infectious diseases because biotechnology has helped us to battle communicable diseases. Overall improvements in prevention, combined with supportive medical

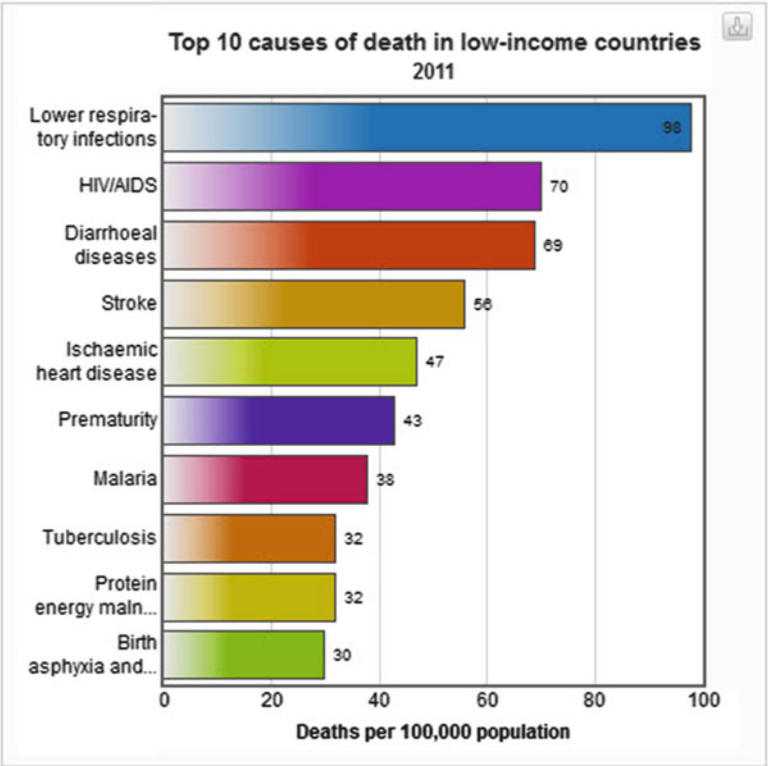

**Fig. 20.3** The list of the top 10 causes of death in low income countries includes diseases that require inexpensive production of effective vaccines and treatments. Manufacture in from plant-based systems may offer many benefits for rapid, local production and stability. From The World Health Organization (WHO) website, http://www.who.int/mediacentre/factsheets/fs310/en/index1.html, accessed April 17, 2014

treatments when prevention isn't possible, have dramatically reduced deaths from infection in the developed world (WHO 2012). However, guard must be maintained against new threats, and new strategies may be needed against evolving pathogens. In the developing world, however, many basic prevention strategies must still be deployed. The top causes of death in developing countries continue to include large numbers of infectious disease triggers (Fig. 20.3, WHO Top 10 causes of death 2011). There is extensive room for additional strategies to be used to tame communicable diseases.

Diarrheal diseases, including cholera, are foes that have had dramatic impact on humans over the centuries. Millions have suffered and died from the vomiting and diarrhea that result from infections with these pathogens. Outbreaks continue to emerge periodically. Even places that had largely vanquished diseases like cholera, such as Haiti, may find that in times of disaster conditions can re-emerge that enable the bacteria to take a toll.

Although traditional injection-based vaccines using components of the viral proteins are available for cholera, efficacy is not 100 %, and immunity may not persist for extended periods (Sinclair et al. 2011). Additionally, in times of natural disasters, deployment of standard vaccines that may require cold-chain refrigerated maintenance to preserve them properly is challenging. Researchers have sought other ways to assist in the battle against debilitating diarrheal diseases, and one promising strategy is rice-based vaccines.

Rice-based protein expression has many advantages (Greenham and Altosaar 2013). Knowledge and methods for rice-growing exist in many of the locations where a plant-based vaccine could be most effective. Self-pollination strategies of rice with its own pollen reduce the chance of cross-pollination with other varieties. Well-studied genetics and numerous tools for manipulation of rice genomes with biotechnology are in place, and appropriate protein expression can be driven to the rice seed grains. Single gene or multi-gene strategies can be employed. Seed proteins are known to be stable for long periods, longer than that of leafy tissues. Rice transports and stores well, meaning that supplies could be on hand in areas with potential outbreaks quickly in times of need. Further, if vaccines can be delivered without needles and the medical personnel to administer them, the reach of this intervention could potentially be much quicker and more widespread (Nochi et al. 2007).

A cholera vaccine based in rice protein expression is under development. Japanese researchers have explored a rice-based delivery system for the cholera toxin B subunit called MucoRice-CTB (Nochi et al. 2009). In model systems this oral treatment with rice powder has delivered immune responses that could be protective against infections. Human testing and further studies of the effectiveness of this method are still needed, and modifications of the specific proteins involved may still be required, but this promising direction could have many benefits for vulnerable populations in the near future.

Cholera is not the only diarrheal pathogen that researchers want to combat. Enterotoxigenic *E. coli* (ETEC) causes illness in developing countries and for travelers to those locations, and the MucoRice-CTB may offer cross-protection (Tokuhara et al. 2010). Encouragingly, this study employed rice stored for over 3 years at room temperature, demonstrating the stability of this recombinant protein method. These researchers have also tackled rotavirus, finding prevention and treatment opportunities for this scourge of low-income countries (Tokuhara et al. 2013). Edible vaccine development has also been discussed for combatting malaria, which has proven a difficult target of traditional vaccine development (Kumar et al. 2012).

### *20.2.1 Tobacco-Based Production of Proteins with Health Benefits*

A new killer emerged in the last century with devastating consequence for many low-income countries, also reaching into the developed world. HIV/AIDS is caused by a virus that spread quickly and killed millions worldwide. Anti-retroviral pharmaceuticals that combat the virus have provided effective treatment and some prophylaxis, yet remain costly and may not be accessible for every patient. Preventing infection is a preferred goal, and biomedical and social intervention strategies are underway. Biomedical prevention methods, including vaccination strategies and topical treatments, have been attempted with varying success. Other candidate drugs have been devised—one using a tobacco plant expression system—that could be widely grown and prove a cost-effective method with broad reach, if effective in clinical trials.

In one case, an antibody—nature's own recombinant proteins in mammalian bodies that target invading and unrecognized proteins—may be used to recognize viruses. Antibodies adapted for use as medical interventions have a long history, and the mechanism of producing antibodies in plants has over two decades of history already. For an excellent overview of the history and technology, advantages and disadvantages, consult the review by De Muynck et al. (2010). A recombinant antibody made in a plant and provided to a patient would assist the body by targeting the HIV/AIDS virus, flagging it for treatment by the patient's own immunity systems. This method has begun the process of clinical evaluation (Boseley 2011).

Another type of recombinant protein that could offer benefits for battling HIV and many other viruses are lectins. These are not antibodies, but a different type of protein that can act as a microbicide to block a virus' ability to infect cells (Huskens and Schols 2012). Griffithsin, a protein from algae, is being expressed in tobacco, but other lectins are being investigated as well. Some of these lectins may have broad-spectrum antiviral activity which could mean even wider benefits on other infection fronts.

Vaccines via plant-based systems for other conditions are considered for other infectious disease situations. Tropical diseases could benefit from cost-effective local focus and needle-free deployment, and several deserving research threads are underway (Rosales-Mendoza et al. 2012). Also progressing at this time is a similar treatment for rabies virus, which still kills many children in the developing world each year (Both et al. 2013).

More widespread viral pathogens may also become the target of plant-based antiviral strategies. Influenza still takes a toll each year around the world, and for medically fragile patients can still be deadly. We also need to be prepared in case another flu with increased virulence strikes, as the world saw in 1918. As we saw before, tobacco plants may be the bioreactor system that can make faster and cheaper recombinant proteins to battle influenza (Danigelis 2012). One organization has performed a clinical trial of a transiently expressed virus-like particle in tobacco which may serve as a vaccine, and can be done within 3 weeks of the access to sequence information for a novel flu strain (Landry et al. 2010). Paired with new strategies such as access to "synthetic vaccines seeds" to rapidly generate DNA constructs, the battle against newly emerged or recently altered virus threats will be transformed (Dormitzer et al. 2013).

Of course, safety and efficacy of any of these methods and recombinant protein choices for the treatment of human illness must be demonstrated in appropriate clinical trial settings. Like any other drugs, all of the regulations for development and testing must be complied with, and biosafety will also need to be explored and ensured (Paul et al. 2011; Guan et al. 2013; Mehrotra and Goyal 2013). If efficacy is shown, expanded production of these proteins in plant tissues may have consequences for plant growth that need to be explored for the yields to be fully maximized. For some cases, transient expression of proteins might be more rapid and effective (Rosenberg et al. 2013).

### *20.2.2 Plant Proteins for Other Medical Conditions*

Soon it will be possible to treat many major health issues with recombinant plant-derived proteins and compounds. I've highlighted some conditions that readers may already be familiarity with—diabetes, HIV, cholera, cancer, flu, and such. But other health situations may also be aided by the same strategies. Now that cancer and heart disease figure more prominently as health issues in the developed world, new plant-produced treatments for related conditions are being explored.

Besides infection, antibody treatments for other conditions are being. Cancer treatment, using antibodies to target tumors, is under investigation and several antibody treatments are currently in use with existing expression methods for antibodies. Research has already been underway for expression of antibody-based tumor targeting recombinant proteins in plant systems. One researcher noted the "irony" of treating cancer with a tobacco-produced recombinant protein, noting that the solutions are not fully in hand yet, but that there is great promise (McCormick 2011).

Numerous strategies for treating heart disease associated conditions are being considered. Certain proteins associated with atherosclerosis and hypertension are targets for vaccination (Salazar-González and Rosales-Mendoza 2013; Rosales-Mendoza 2012).

Researchers are pursuing a rice-based vaccine to reduce impact of a public health issue in Japan—cedar pollen allergy (Domon et al. 2009). The adaptability of these plant expression systems and the speed with which they can generate useful proteins can help target the less widespread or regional medical scenarios underserved by traditional drug development projects.

Another important medical situation is the repair of damaged tissues. The human protein collagen forms an important framework for structural features of skin, tendons, and bone. It also been long sought for engineering because it has biomedical uses for tissue repair and wound healing. The broad utility of collagen has led to attempts to obtain it from numerous sources, including human cadavers, animals, and recombinant sources in various culture systems. Collagen molecules have now been successfully expressed in both tobacco and maize, and could offer high levels of quality proteins for many purposes (Xu et al. 2011; Shoseyov et al. 2013).

Investigation of the effectiveness of tobacco-based collagen for chronic wound healing is underway, and products are in clinical testing and use (Shilo et al. 2013).

There are other public health situations that may be impacted by altering plant characteristics via genetic engineering, but are not directly injected or applied types of products. Improved nutritional properties such as the increased availability of vitamin precursors, or micronutrients, cholesterol-reducers, or healthier oils or compounds could have benefits for consumers and public health. Increased production of plant-based insect repellents or other biocides could have consequences for public health (Hood 2013). Recombinant plant proteins for battling diseases of plants will have major consequences for food security in future years (Bruce 2012). Using plants as factories for the production of plastics, fibers like spider silk, and other materials could have benefits for sustainability (Hauptmann et al. 2013; Stöger 2013). These additional directions associated with genetic modification of plants to make novel proteins are beyond the scope of this chapter, but are key projects to consider.

## 20.3 Proteins for a New Century

The selected examples in the chapter illustrate some of the medical and public health situations being investigated and targeted for plant-based recombinant protein interventions. They represent fraction of the possibilities. And methods are continually being tested, assessed, and improved. Entirely new technologies will emerge to generate needed molecules, and the speed with which we can generate them will increase. The availability of new proteins from genome mining efforts will broaden our toolkits. Multiple strategies are being used now, and will certainly continue to be developed, that will likely offer efficient and effective drug development (Paul et al. 2013). Like all medical interventions, safety and effectiveness studies need to be rigorous and conform to ethical and legal requirements appropriate for their production and usage. It will also be important to be aware of biosafety issues related to plant productions systems that may be different from other factory-style protein production (Cartagena Protocol; Mehrotra and Goyal 2013). Awareness that the implications of policy choices around the use of recombinant proteins generated in plants, and the resulting genetically-modified organisms, could affect the production of these useful tools is also important, and policy makers should be sure to carefully assess the benefits and the risks with appropriate metrics when crafting legislation on this topic (Ammann 2013).

My focus in this chapter was direct benefits to human health from medically-relevant recombinant proteins expressed in plants. The beneficiaries may not only be humans—important agricultural species and companion animals may access plant-based vaccines, proteins, and compounds in the future. Plant-based production of recombinant proteins is certainly not the only path to products with public health benefits, but is among several tools in the global toolbox that should be considered. Synthetic biology production systems will likely become important.

Recombinant proteins generated in plant expression systems stand to offer many benefits for the public health challenges we face, benefits for the sustainability of the planet, and positive outcomes for the world's inhabitants in the coming century in times of rapid change.

## References

Ammann K (2013) Genomic misconception: a fresh look at the biosafety of transgenic and conventional crops. A plea for a process agnostic regulation. N Biotechnol 31:1

Belhaj K, Chaparro-Garcia A, Kamoun S, Nekrasov V (2013) Plant genome editing made easy: targeted mutagenesis in model and crop plants using the CRISPR/Cas system. Plant Methods 9(1):39

Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TH, Weissig H, Shindyalov IN, Bourne PE (2000) The protein data bank. Nucleic Acids Res 28:235–242, www.pdb.org

Bliss M (2013) The Discovery of Insulin. University of Chicago Press, Chicago. ISBN 022607563X

Boothe J, Nykiforuk C, Shen Y, Zaplachinski S, Szarka S, Kuhlman P, Murray E, Morck D, Moloney MM (2010) Seed-based expression systems for plant molecular farming. Plant Biotechnol J 8(5):588–606

Boseley S Anti-HIV drug made by GM plants begins trials in humans. The Guardian. July 19, 2011. Accessed on the web: http://www.theguardian.com/science/2011/jul/19/anti-hiv-drug-gm-plants

Both L, van Dolleweerd C, Wright E, Banyard AC, Bulmer-Thomas B, Selden D, Altmann F, Fooks AR, Ma JK (2013) Production, characterization, and antigen specificity of recombinant 62-71-3, a candidate monoclonal antibody for rabies prophylaxis in humans. FASEB J 27(5):2055–2065

Bruce TJ (2012) GM as a route for delivery of sustainable crop protection. J Exp Bot 63(2):537–541

Chang X, Jorgensen AM, Bardrum P, Led JJ (1997) Solution structures of the R6 human insulin hexamer. Biochemistry 36(31):9409–9422

Cox TM (2010) Gaucher disease: clinical profile and therapeutic developments. Biologics 4:299–313

Danigelis A (2012) Tobacco: more efficient flu vaccine-maker? Discovery.com. Sept 25. Accessed on the web: http://news.discovery.com/earth/weather-extreme-events/flu-vaccine-tobacco-120925.htm

De Muynck B, Navarre C, Boutry M (2010) Production of antibodies in plants: status after twenty years. Plant Biotechnol J 8(5):529–563

Domon E, Takagi H, Hirose S, Sugita K, Kasahara S, Ebinuma H, Takaiwa F (2009) 26-Week oral safety study in macaques for transgenic rice containing major human T-cell epitope peptides from Japanese cedar pollen allergens. J Agric Food Chem 57(12):5633–5638

Dormitzer PR, Suphaphiphat P, Gibson DG, Wentworth DE, Stockwell TB, Algire MA, Alperovich N, Barro M, Brown DM, Craig S, Dattilo BM, Denisova EA, De Souza I, Eickmann M, Dugan VG, Ferrari A, Gomila RC, Han L, Judge C, Mane S, Matrosovich M, Merryman C, Palladino G, Palmer GA, Spencer T, Strecker T, Trusheim H, Uhlendorff J, Wen Y, Yee AC, Zaveri J, Zhou B, Becker S, Donabedian A, Mason PW, Glass JI, Rappuoli R, Venter JC (2013) Synthetic generation of influenza vaccine viruses for rapid response to pandemics. Sci Transl Med 5(185):185ra68

Gerritsen VB (2001) Protein of the 20th century. Protein spotlight, vol 9, April. Accessed on the web: http://web.expasy.org/spotlight/back_issues/009

Goeddel DV, Kleid DG, Bolivar F, Heyneker HL, Yansura DG, Crea R, Hirose T, Kraszewski A, Itakura K, Riggs AD (1979) Expression in Escherichia coli of chemically synthesized genes for human insulin. Proc Natl Acad Sci U S A 76(1):106–110

Goldstein DA, Thomas JA (2004) Biopharmaceuticals derived from genetically modified plants. QJM 97(11):705–716

Greenham T, Altosaar I (2013) Molecular strategies to engineer transgenic rice seed compartments for large-scale production of plant-made pharmaceuticals. Methods Mol Biol 956:311–326

Guan ZJ, Guo B, Huo YL, Guan ZP, Dai JK, Wei YH (2013) Recent advances and safety issues of transgenic plant-derived vaccines. Appl Microbiol Biotechnol 97(7):2817–2840

Hauptmann V, Weichert N, Rakhimova M, Conrad U (2013) Spider silks from plants—a challenge to create native-sized spidroins. Biotechnol J 8(10):1183–1192

Heuser S (2009) One girl's hope, a nation's dilemma. Boston Globe, Boston

Hood G (2013) Finding an all-natural alternative to DEET. KUNC Radio 91.5. Accessed on the web 19 Oct 2013. http://kunc.org/post/finding-all-natural-alternative-deet

Huskens D, Schols D (2012) Algal lectins as potential HIV microbicide candidates. Mar Drugs 10(7):1476–1497

Jones DS, Podolsky SH, Greene JA (2012) The burden of disease and the changing task of medicine. N Engl J Med 366(25):2333–2338

Kumar CS, Deepesh G, Mahavir Y, Archana T (2012) Edible vaccine: a new platform for the development of malaria vaccine. Crit Rev Eukaryot Gene Expr 22(3):243–248

Landry N, Ward BJ, Trépanier S, Montomoli E, Dargis M, Lapini G, Vézina LP (2010) Preclinical and clinical development of plant-made virus-like particle vaccine against avian H5N1 influenza. PLoS One 5(12):e15559

Lössl AG, Clarke JL (2013) Conference scene: molecular pharming: manufacturing medicines in plants. Immunotherapy 5(1):9–12

Maxmen A (2012) Drug-making plant blooms. Nature 485(7397):160

McCormick AA (2011) Tobacco derived cancer vaccines for non-Hodgkin's lymphoma: perspectives and progress. Hum Vaccin 7(3):305–312, Epub 2011 Mar 1

Mehrotra S, Goyal V (2013) Evaluation of designer crops for biosafety—a scientist's perspective. Gene 515(2):241–248

Nochi T, Takagi H, Yuki Y, Yang L, Masumura T, Mejima M, Nakanishi U, Matsumura A, Uozumi A, Hiroi T, Morita S, Tanaka K, Takaiwa F, Kiyono H (2007) Rice-based mucosal vaccine as a global strategy for cold-chain- and needle-free vaccination. Proc Natl Acad Sci U S A 104(26):10986–10991

Nochi T, Yuki Y, Katakai Y, Shibata H, Tokuhara D, Mejima M, Kurokawa S, Takahashi Y, Nakanishi U, Ono F, Mimuro H, Sasakawa C, Takaiwa F, Terao K, Kiyono H (2009) A rice-based oral cholera vaccine induces macaque-specific systemic neutralizing antibodies but does not influence pre-existing intestinal immunity. J Immunol 183(10):6538–6544

Paul M, van Dolleweerd C, Drake PM, Reljic R, Thangaraj H, Barbi T, Stylianou E, Pepponi I, Both L, Hehle V, Madeira L, Inchakalody V, Ho S, Guerra T, Ma JK (2011) Molecular pharming: future targets and aspirations. Hum Vaccin 7(3):375–382

Paul MJ, Teh AY, Twyman RM, Ma JK (2013) Target product selection—where can molecular pharming make the difference? Curr Pharm Des 19(31):5478–5485

Rosales-Mendoza S (2012) Can a plant-based vaccine treat hypertension? Med Hypotheses 79(5):555–559

Rosales-Mendoza S, Govea-Alonso DO, Monreal-Escalante E, Fragoso G, Sciutto E (2012) Developing plant-based vaccines against neglected tropical diseases: where are we? Vaccine 31(1):40–48

Rosenberg Y, Sack M, Montefiori D, Forthal D, Mao L, Hernandez-Abanto S, Urban L, Landucci G, Fischer R, Jiang X (2013) Rapid high-level production of functional HIV broadly neutralizing monoclonal antibodies in transient plant expression systems. PLoS One 8(3):e58724

Salazar-González JA, Rosales-Mendoza S (2013) A perspective for atherosclerosis vaccination: is there a place for plant-based vaccines? Vaccine 31(10):1364–1369

Shaaltiel Y, Bartfeld D, Hashmueli S, Baum G, Brill-Almon E, Galili G, Dym O, Boldin-Adamsky SA, Silman I, Sussman JL, Futerman AH, Aviezer D (2007) Production of glucocerebrosidase with terminal mannose glycans for enzyme replacement therapy of Gaucher's disease using a plant cell system. Plant Biotechnol J 5(5):579–590

Shilo S, Roth S, Amzel T, Harel-Adar T, Tamir E, Grynspan F, Shoseyov O (2013) Cutaneous wound healing after treatment with plant-derived human recombinant collagen flowable gel. Tissue Eng Part A 19(13–14):1519–1526

Shoseyov O, Posen Y, Grynspan F (2013) Human collagen produced in plants: more than just another molecule. Bioengineered 5(1):2165

Sinclair D, Abba K, Zaman K, Qadri F, Graves PM (2011) Oral vaccines for preventing cholera. Cochrane Database Syst Rev 16(3):CD008603

Stöger E (2013) Editorial: from plant biotechnology to bio-based products. Biotechnol J 8(10):1122–1123

Tokuhara D, Yuki Y, Nochi T, Kodama T, Mejima M, Kurokawa S, Takahashi Y, Nanno M, Nakanishi U, Takaiwa F, Honda T, Kiyono H (2010) Secretory IgA-mediated protection against V. cholerae and heat-labile enterotoxin-producing enterotoxigenic Escherichia coli by rice-based vaccine. Proc Natl Acad Sci U S A 107(19):8794–8799

Tokuhara D, Álvarez B, Mejima M, Hiroiwa T, Takahashi Y, Kurokawa S, Kuroda M, Oyama M, Kozuka-Hata H, Nochi T, Sagara H, Aladin F, Marcotte H, Frenken LG, Iturriza-Gómara M, Kiyono H, Hammarström L, Yuki Y (2013) Rice-based oral antibody fragment prophylaxis and therapy against rotavirus infection. J Clin Invest 123(9):3829–3838

WHO (2012) The top 10 causes of death. World Health Organization, Geneva. Fact sheet no. 318. http://www.who.int/mediacentre/factsheets/fs310/en/index.html. Accessed 15 Oct 2013

Xu X, Gan Q, Clough RC, Pappu KM, Howard JA, Baez JA, Wang K (2011) Hydroxylation of recombinant human collagen type I alpha 1 in transgenic maize co-expressed with a recombinant human prolyl 4-hydroxylase. BMC Biotechnol 11:69

Zimran A, Brill-Almon E, Chertkoff R, Petakov M, Blanco-Favela F, Muñoz ET, Solorio-Meza SE, Amato D, Duran G, Giona F, Heitner R, Rosenbaum H, Giraldo P, Mehta A, Park G, Phillips M, Elstein D, Altarescu G, Szleifer M, Hashmueli S, Aviezer D (2011) Pivotal trial with plant cell-expressed recombinant glucocerebrosidase, taliglucerase alfa, a novel enzyme replacement therapy for Gaucher disease. Blood 118(22):5767–5773

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**Pooja Bhatnagar-Mathur** is a Cell and Molecular Biologist associated with ICRISAT, India with over a decade of expertise in genetic engineering of various legumes and sorghum. She has been involved with research aimed at reconciling the best of biotechnological approaches to integrate useful traits including enhanced diseases and pest resistance, abiotic stress tolerance and nutritional enhancement. Pooja has to her credit, several advanced certifications from WIPO on components

A. Ricroch et al. (eds.), *Plant Biotechnology: Experience and Future Prospects*, DOI 10.1007/978-3-319-06892-3,

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**Joseph G. Chimungu** is a graduate student pursuing a PhD in the Department of Plant Science at The Pennsylvania State University. His research focuses on identifying and evaluating the utility of root anatomical traits that affect the root metabolic cost of soil exploration under various edaphic stresses, and the interaction and the possible tradeoffs for the traits.

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**William Dollente Dar** Ph.D., is the Director General of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). He served as Presidential Adviser for Rural Development; Secretary of Agriculture in the Philippines; Executive Director, Philippine Council for Agriculture, Forestry, and Natural Resources Research and Development (PCARRD); and Director, Bureau of Agricultural Research (BAR). He holds a PhD in Horticulture from the University of the Philippines Los Baños, an MS (Agronomy) and BS in Agricultural Education from Benguet State University (BSU) and several honorary doctorates. The latest international recognition of his work includes the “Excellence in Agricultural Leadership Award” by the Association of Agricultural Technology in Southeast Asia (AATSEA) in November 2013, and the prestigious MS Swaminathan Award for Leadership in Agriculture in June 2013.

**J. Franklin Egan** is a research ecologist with the U.S. Department of Agriculture’s Agricultural Research Service (USDA-ARS). His research explores the agronomic and environmental implications of alternative agricultural systems assessed at landscape and regional scales. For instance, a current project compares the land resource requirements of confinement versus pasture-based dairy farming across the northeastern U.S. region. Before joining the ARS, he completed his Ph.D. studies in Ecology at the Pennsylvania State University. His dissertation research examined some of the environmental risks and implications from second-generation herbicide-resistant that are genetically modified to be resistant to the herbicides dicamba and 2,4-D. The potential for new technologies to both open and close opportunities for sustainable agriculture is a running theme in his research and thinking.

**Bryce Falk** received his Ph.D. from the University of California, Berkeley in 1978. He was an Assistant Professor at the University of Florida from 1980 to 1985 and has been member of the faculty of the Plant Pathology Department at UC Davis since 1985. His research program is focused primarily on plant viruses and virus:plant:vector interactions. His laboratory also works with RNA interference targeting insect vectors of plant pathogens. He teaches General Plant Pathology, General Virology and Advanced Plant Virology.

**Shelby J. Fleischer** is a Professor of Entomology at Penn State. His research deals with the structure, dynamics, and management of insect populations and communities, and he strives to advance Integrated Pest Management based on improved understanding of biological and ecological processes. Priority is placed on advancing economically feasible management that improves worker and environmental safety. He has authored or co-authored over 75 publications, presented over 140 papers at professional meetings, been an invited speaker at over 30 venues, and served as a subject editor for *Environmental Entomology*. Dr. Fleischer conducts educational programs with farmers and agricultural industry personnel, where he integrates scientific principles with problem-solving and natural history of arthropods.

**Iffa Gaffoor** is a Postdoctoral Scholar in the laboratory of Dr. Surinder Chopra in the Department of Plant Science at The Pennsylvania State University – University Park, PA, U.S.A. She holds a PhD degree in Plant Science from Michigan State University – East Lansing, MI, U.S.A. She is working toward a better understanding of plant-pathogen interactions, specifically with sorghum and *Colletotrichum sublineolum*. Her research focuses on identifying plant compounds that play a role in making the plant more resistant to disease that will aid in the breeding of sorghum lines that are more disease resistant.

**Leland Glenna** is Associate Professor of Rural Sociology and Science, Technology, and Society at The Pennsylvania State University. His research interests are in agricultural, food, and environmental science and technology. He received his Ph.D. from the University of Missouri in 1997.

**William D. Hutchison** is Professor and Head in the Department of Entomology at the University of Minnesota. He led studies documenting areawide suppression of Bt-corn on European corn borer and resulting economic benefits in the U.S. Corn Belt. Current work includes assessment of transgenic crops on insect populations and insect resistance management for Bt corn. He has published over 125 papers, several book chapters and extension publications on sampling; integrating reduced-risk insecticides with biocontrol; invasive species; and risk-based frameworks for IPM. He is co-editor for Radcliffe's World IPM textbook, an interactive teaching resource used worldwide, and *Integrated Pest Management: Concepts, Tactics, Strategies and Case Studies*. He served as an Editor for the *Journal of Economic Entomology* and *Crop Protection,* and is currently on the Editorial Boards of *Crop Protection* and *GM Crops and Food*. His international activities include the Harvest Choice program at the University of Minnesota.

**Kristal Jones** holds a Ph.D. in Rural Sociology and International Agriculture and Development at the Pennsylvania State University. Her current research examines the impacts of market-oriented seed system development on contemporary seed systems in West Africa.

**Calestous Juma** is an internationally recognized authority on the role of science, technology, engineering and innovation in sustainable development. He is Professor of the Practice of International Development and Director of the Science, Technology, and Globalization Project at Harvard Kennedy School (HKS). He is Faculty Chair of the School's Innovation for Economic Development Executive Program and the Mason Fellows Program. Juma also directs HKS's Agricultural Innovation Policy in Africa Project funded by the Bill and Melinda Gates Foundation. He has been elected to several scientific and engineering academies including the Royal Society of London, the US National Academy of Sciences, the World Academy of Sciences (TWAS), the UK Royal Academy of Engineering and the African Academy of Sciences. He co-chaired the African Union's High-Level Panel on Science, Technology and Innovation and is on the board of the Aga Khan University. His previous positions include: founding Executive Director of the African Centre for Technology Studies in Nairobi; Executive Secretary of the UN Convention on Biological Diversity; and Chancellor of the University of Guyana. He is on the judging panel of the Queen Elizabeth Prize for Engineering and the Africa Prize for Engineering Innovation. Juma holds a D.Phil. in science and technology policy studies from the University of Sussex (UK) and has received numerous international awards and honorary degrees for his work on sustainable development. His latest book, *The New Harvest: Agricultural Innovation in Africa*, was published in 2011 by Oxford University Press. He is currently completing two books: *Innovation and Its Enemies: Resistance to New Technologies* and *Schumpeter's Revenge: Innovation, Entrepreneurship and Development.* Twitter @calestous

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**Marcel Kuntz** is Director of research at Centre National de la Recherche Scientifique (CNRS) and also teaching at University Joseph Fourier (Grenoble, France). His research as a plant biologist are conducted in the Laboratory of Plant Cellular Physiology, a joint laboratory of CNRS, University Joseph Fourier, Institut National de la Recherche Agronomique (INRA) and Commissariat à l'Energie Atomique et aux Energies Alternatives (CEA). Marcel Kuntz is also publishing articles in scientific journals on the political controversy over GMOs and on aspects relevant to the philosophy of science (foundations, methods, implications of science) with examples taken from this controversy.

**Bernard Le Buanec** is member of the Academy of Agriculture of France and founding member of the National Academy of Technologies of France. From 1965 to 1975 he was agronomist at CIRAD, from 1984 to 1993 senior vice-president research and development at Groupe Limagrain and from 1994 to 2008 General Secretary of the International Seed Federation. He has been President of the International Association of Plant Breeders (ASSINSEL) and members of several committees in France, of the working group of the World Bank on biotechnology and intellectual property and of the plant genetic resources committee of the CGIAR. In 2007 Bernard Le Buanec was awarded the Gold Medal from UPOV in recognition of his work for the plant breeding industry. In 2008 he was awarded a Medal of the Livestock and Seed Program of the Agricultural Marketing Service of the US Department of Agriculture in recognition of his work for the US and international seed industry.

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**David B. Sawaya** is a consultant in science and technology policy. He worked on biotechnology policy and conducted original quantitative research on the future of the bioeconomy while working within the Strategic Foresight office of the OECD Secretary General. He is the co-author of a book on the future of biotechnology entitled, "The Bioeconomy to 2030: Designing a Policy Agenda", and has published detailed projections on agricultural and health biotechnologies expected to be commercialized by 2015.

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# Index

A. Ricroch et al. (eds.), *Plant Biotechnology: Experience and Future Prospects*, DOI 10.1007/978-3-319-06892-3,