Govind Singh Saharan · Prithwi Raj Verma
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White Rust of Crucifers: Biology, Ecology and Management

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White Rust of Crucifers: Biology, Ecology and Management

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Foreword

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सचिव एवं महानिदेशक
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SECRETARY & DIRECTOR GENERAL

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Amongst the cruciferous crops grown world-over, oil yielding species of *Brassica* occupied an area of about 30 m ha producing nearly 53 million t, when averaged over last one decade, and is consistently gaining importance due to its wider adaptability, low water requirement, higher cost-benefit ratio and well defined cultivation and post-harvest management technology. Despite this, wide fluctuations in yield are often observed due to various biotic and abiotic stresses including infestation of white rust disease. White rust, caused by fungus *Albugo candida* is not only a widespread disease of crucifers causing enormous yield losses upto 90 % in Brassica crops during epidemic years, but also infects more than 400 species of plants belonging to 23 families of vegetable crucifers, ornamental plants and numerous weed hosts world over. The publication of present book entitled “White Rust (*Albugo* species) in crucifers: Biology, Ecology and Management” is a very timely and valuable efforts to compile the latest information on white rust.

The subject matter has been distinctly covered in 15 chapters supported with suitable illustrations in form of photographs, graphs, figures and tables. Each chapter comprehensive covers the disease, its distribution, host range, symptomatology, genetics of host-parasite interaction, sources of resistance, biochemistry of host-parasite interaction, identification and mapping of R-genes, disease management and laboratory and field techniques developed.

The authors have put in their knowledge and wisdom to compile white rust research data in the form of book and suggesting priority areas of research which are, thought provoking based on their lif long experience of working with this important disease.

The authors deserve appreciation for their academic task of bringing out an encyclopedia of white rust of crucifers. I am sure this book will be of immense help to the students, teachers, researchers, extension scientists and all those who are interested in growing crucifers for higher returns and improved quality seeds.

10th July, 2013
New Delhi

Preface

White rust caused by the fungus *Albugo* is the most devastating disease of vegetables and oil yielding crops all over the world. The disease is also known as white blisters rust or staghead. In epidemic years the disease may cause losses in the yield upto 90 %. The fungus infects more than 400 species of plants worldwide including important vegetable crucifers, oil yielding Brassicas, ornamental plants and numerous weeds. Extensive yield losses, lack of resistant cultivars, and difficulty encountered in managing the disease under field conditions have prompted researchers for sustainable research on this disease. Since, our first review on "white rusts" (Saharan and Verma 1992) and other numerous publications, very useful information have been published, which encouraged us to compile the data in the form of a present book.

The present book on "white rust" deals with the aspects on "the disease" *viz*., its distribution, host range, symptoms, disease assessment, yield losses, life cycle, survival, epidemiology, and "the pathogen" *viz.*, its taxonomy, morphology, structures and reproduction, species, phylogeny, infection, life cycle, physiological specialization, genetics of host-pathogen interaction, sources of resistance, biochemistry of host-pathogen interaction, identification and mapping of resistant genes, and disease management. In addition, laboratory and field techniques developed on white rust have been included.

The subject matter is vividly illustrated with photographs (macro and microscopic, electron and scanning electron micrograph), drawings, graphs, figures, histograms, and tables etc. for stimulating, effective and easy reading and understanding. Each chapter is arranged in chronolgocal order in the form of headings and sub-headings through numerical series to make the subject contiguous. Inclusion of important references will be helpful in original consultations by the researchers, teachers, and students.

We are sure that this comprehensive treatise on "white rust" will be of immense use to the researchers, teachers, students and all others who are interested in diagnosis and management of white rust diseases of crops worldwide.

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P. R. Verma
P. D. Meena
Arvind Kumar

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E. Websites:

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List of Abbreviations

µm	Micrometer
AC	*Albugo candida*
ACjun	*Albugo candida* derived from *Brassica juncea*
ACnig	*Albugo candida* derived from *Brassica nigra*
ACol	*Albugo candida* derived from *Brassica oleracea*
ACrap-2	*Albugo candida* derived from *Brassica rapa*
AEA	Average-environment coordination abscissa
AFLP	Amplified fragment length polymorphism
At.	Atkinsiellales (informal designation)
AUDPC	Area under disease progression curves
BW	Burpee white
COI	Cross-over interactions
COX mtDNA	Cytochrome c oxidase mitochondrial DNA
CR	Cortex cells
CRW	Chinese Rose Winter
CV	Critical variance
cv	Cultivar
d.a.s.	Days after sowing
d.f.	Degree of freedom
DI	Disease index
DM	Downey mildew
DRMR	Directorate of Rapeseed–Mustard Research
DSSI	Disease stress susceptibility index
DSTI	Disease stress tolerance index
EHM	Extrahaustorial membrane
EP	Epidermis layer
EX	Extrahaustorial matrix
F	Fungus
GEI	Genotypic environment interaction
GMP	Geometric mean productivity
GSH	Glutathione
HA	Haustoria
HP	Host plasma
HP	*Hyaloperonospora parasitica*
HPLC	High-performance liquid chromatography
HR	Hypersensitive response

ICAR	Indian Council of Agricultural Research
IH	Intercellular hypha
IP	Interaction phenotype
ITS rDNA	Internal transcribed spacer ribosomal DNA
ITS	Transcribed spacer region
LAT	Local Apparent Time
Le.	Leptomitales
LM	Light microscopy
LP	Leaf phase
LSU nrDNA	Large subunit nuclear ribosomal DNA
M	Mesophyll cells
MDR	Multiple disease resistance
mm	Millimeter
MP	Mean productivity
MS	Moderately susceptible
mtDNA	Mitochondrial DNA
NMR	Nuclear magnetic resonance
NPQ	Non-photochemical fluorescence quenching
NRCRM	National Research Centre on Rapeseed-Mustard
OAS-TL	O-acetylserine (thiol) lyase
OS	Oospores
P	Pith
PACP	Periodic acid-chromic acid-phosphotungstic
PCR	Polymerase chain reaction
PDS	Per cent disease severity
pFCC	Primary fluorescent catabolite
Ph	Phloem
PM	Plasma membrane
PR	Partial resistant
QTL	Quantitative trait loci
r	Infection rate
R	Resistant
R^2	Multiple regression analysis
RAPD	Random amplified polymorphic DNA
RBS	Round Black Spanish
rDNA	Recombinant deoxyribonucleic acid
RFLP	Restriction fragment length polymorphism
RH	Relative humidity
Rh.	Rhipidiales
RNA	Ribose nucleic acid
ROS	Reactive oxygen species
S	Susceptible
s.str.	Sub-strains
Sa.	Saprolegniales
SAT	Serine acetyltransferase
SDW	Sterilised distilled water
SP	Spraying
SP	Staghead phase

SPI	Single pustule isolation
ST	Seed Treatment
TEM	Transmission electron microscopy
TOL	Disease tolerance
UEP	Upper epidermis layer
UK	United Kingdom
URSS	Unión de Repúblicas Socialistas Soviéticas
USA	United States of America
USDA	United States Department of Agriculture
USDA-ARS	United States Department of Agriculture- Agricultural Research Service
UV	Ultra violet
WA	Western Australia
WMO	World Meteorological Organization
WR	White rust
WRP	White rust pustules
X	Xylary vessels

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1 Introduction

White rust (WR; white coloured pustules), white blister or white blister rust (release white powder or form galls) and stagheads (malformation of inflorescence) are the common names of the disease caused by *Albugo* spp. on more than 400 species of plants worldwide. It is known to cause infection on plants of genera belonging to families of Acanthaceae, Aizoaceae, Allionaceae, Amaranthaceae, Ambrosiaceae, Apiaceae, Asteraceae, Boraginaceae, Brassicaceae, Capparidaceae, Carduaceae, Caryophyllaceae, Chenopodiaceae, Cichoraceae, Cleomaceae, Compositae, Convolvulaceae, Crassulaceae, Cruciferae, Fabaceae, Fumariaceae, Gentianaceae, Nyctaginaceae, Orchidaceae, Papaveraceae, Peperomiaceae, Portulacaceae, Resedaceae, Scrophulariaceae, Solanaceae and Urticaceae (Wilson 1907; Biga 1955; Vasudeva 1960; Kenneth 1968; Jorstad 1964; Saharan and Verma 1992; Dick 2002; Choi and Priest 1995).

The genus *Albugo* is a widely distributed fungus of obligatory plant pathogens, containing more than 50 species infecting more than 400 host plants of 31 families of 12 orders in Dicotyledoneae, and 1 family in Monocotyledoneae crops and common weeds (Biga 1955; Choi and Priest 1995; Walker and Priest 2007). Several species are well-known plant pathogens causing very heavy yield losses on field crops of economic importance, e.g. *Albugo candida* on oil yielding Brassicas and crucifer vegetables, *Albugo tragopogonis* on sunflower, *Albugo ipomoeae-pandurate* on sweet potato, and *Albugo occidentalis* on spinach. Despite its prominent role as a plant pathogen, a detailed modern monographic study of the genus *Albugo* is lacking. Although, compilations of the published data (Biga 1955; Saharan and Verma 1992; Choi and Priest 1995; Dick 2002) gives useful information, but a critical re-examination of the genus *Albugo* using modern techniques like molecular biology, biotechnology, genetic engineering and nanotechnology is urgently required.

The family Albuginaceae J. Schrot was long regarded as comprising of only a single genus *Albugo* (Pers.) Roussel, but recently it was revealed that four distinct lineages exist in this family (Voglmayr and Riethmüller 2006). These lineages represent specific host range, with *Albugo* s.str. being pathogenic to Brassicaceae Burnett, *Albugo* s.l. pathogenic to Convolvulaceae Juss, *Pustula* Thines pathogenic to Asteridae, and *Wilsoniana* Thines pathogenic to Caryophylidae (Thines and Spring 2005).

White rust on cultivated oilseed Brassicas, cruciferous species and others is known to occur in many countries of the world on more than 400 species of plants. Although the genus *Albugo* was discovered in eighteenth century (Persoon 1796, 1797; Gmelin 1792), confusion persisted amongst albugologists regarding the name of *Albugo, Uredo* and *Cystopus*. Now it is finally settled that the *A. candida* is a pathogen of white blister rust all over the world (Biga 1955; Saharan and Verma 1992; Choi and Priest 1995). Initial work on pathogen occurrence, identity, symptoms, morphology, taxonomy, host range and infection was carried out by Wilson (1907), Togashi and Shibasaki (1934), Togashi et al. (1930, 1931),

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Wakefield (1927), Butler and Bisby (1934), Savulescu (1946, 1960), Biga (1955), Baker (1955), Waterhouse (1975) and Burdyukova (1980). Wakefield (1927) and Biga (1955) for the first time constructed a key for the differentiation of *Albugo* species. Later in 1995, Choi and Priest also gave a key to the genus *Albugo.* A revision of genus *Albugo* has recently been published by Thines and Spring (2005).

The disease causes yield losses from 1 to 90 % in oil-yielding Brassica and cruciferous crops depending upon host genotype, planting time, plant population, nutrition and climatic conditions (Saharan and Verma 1992; Saharan 2010). In general, disease symptoms appear on all aerial parts of infected plants in the form of white to creamy-white pustules of pinhead size to various shapes, size and arrangements. Inflorescence malformation of different kinds in the form of staghead is also very common. Symptomatological variations influenced by genotype–pathotype interactions under the influence of environmental conditions can be observed. The pathogen perpetuates in the soil or as a contaminant with the seed through the oospores formed in the hypertrophied plant parts. Zoospores from germinating oospores cause primary infection, whereas the secondary infection/spread is caused by sporangia and zoospores formed in diseased plant parts (Saharan and Verma 1992; Saharan et al. 2005). The disease development after infection is favoured by 12–15 °C temperature, >70 % relative humidity (RH), 2.7–3.4 km/h of wind velocity, and intermittent rains under field conditions (Saharan et al. 2005; Chattopadhyay et al. 2011). The role of host resistance/susceptibility, sowing time, spacing, plant population, nutrition, cultural practices and irrigation, etc. in the development of disease have been investigated (Saharan and Lakra 1988; Saharan and Verma 1992; Saharan and Mehta 2002; Meena et al. 2002a, b; Saharan et al. 2005). However, precise analysis of WR epidemiological parameters needs re-examination to develop effective and reliable disease forecasting system in the climate changing era.

More than 117 races/pathotypes of *A. candida* have been reported, but their status whether they are same or different needs confirmation on a set of internationally accepted host differentials/isogenic lines (Saharan 2010). Although large number of genotypes showing high level of resistance in each *Brassica* and cruciferous species have been identified from various countries, but very few have been exploited to develop resistant cultivars (Saharan and Verma 1992; Saharan 2010).

The studies on genetics of host–pathogen interaction indicate that resistance in host is governed by one, two or more than two dominant genes (*AC*-7-1, *AC*-7-2, *AC*-7-3), additive genes with epistatic effects, and single recessive gene (*WPr*) along with a single gene (*WRR4*) conferring broad spectrum resistance to races, *AC*-2, 4, 7 and 9 (Pound and Williams 1963; Fan et al. 1983; Liu et al. 1996; Saharan and Krishnia 2001; Bansal et al. 2005; Borhan et al. 2008). The inheritance of virulence in *Albugo–Brassica* system suggests that a single dominant gene controls avirulence in race *AC*-2 in *Brassica rapa cv*. Torch (Adhikari et al. 2003). Systemic resistance in *Brassica juncea* to *A. candida* can be induced by pre- or co-inoculation with an incompatible isolates of *A. candida* (Singh et al. 1999). Resistant genes effective against one or more race(s) of *A. candida* have been mapped and identified on the chromosomes of *B. juncea,* viz., *ACr* (Cheung et al. 1998), *AC*-2_1 (Prabhu et al. 1998), *AC*-2 (Varshney et al. 2004), *ACB*1-*A*4.1, *ACB*1-*a*5.1 (Massand et al. 2010), *B. rapa,* viz., *ACA*1 (Kole et al. 1996, 2002), *B. napus,* viz., *ACA*1 (Ferreira et al. 1994), *AC* 2*V*1 (Somers et al. 2002), and *Arabidopsis thaliana, viz., RAC*-1, *RAC*-2, *RAC*-3 and *RAC*-4 (Borhan et al. 2001, 2004, 2008).

Studies on host–pathogen interaction, fine structures of hyphae, mycelium, sporangia, zoospores and oospores have been conducted through histopathology, electron microscopy, scanning electron microscopy and transmission electron microscopy (Berlin and Bowen 1964a, b; Davison 1968; Coffey 1975, 1983; Hughes 1971; Khan 1976, 1977; Tewari and Skoropad 1977; 1980; Kaur et al. 1984 and Baka 2008).

In the infected host tissues, biochemical changes take place in the form of photosynthesis and respiration, chlorophyll pigments, carbohydrate

metabolism, sugars, phenolic compounds, glucosinolate, protein, amino acids, RNA contents, ionic contents, metabolites, growth substances, fatty acids and phytoalexins (Saharan and Verma 1992; Cheng et al. 1999; Aldesuquy and Baka 1992; Chou et al. 2000; Singh 2000; Pruthi et al. 2001; Spring et al. 2005; Pedras and Ahiahonu 2005; Pedras et al. 2007a, b; Pedras et al. 2008; Mishra et al. 2009; Misra and Padhi 1981; Kaur et al. 2011).

Under field conditions no single method or approach is feasible, viable, effective, economical and environmentally safe to manage WR disease. Therefore it has been suggested to integrate control measures like, cultural, chemical, biological and host resistance (Saharan and Verma 1992; Saharan 1992a, b; Mukerji et al. 1999; Saharan and Mehta 2002).

During twenty-first century, molecular studies using amplified fragment length polymorphism (AFLP) and *internal transcribed spacer* 1 (ITS 1) sequencing (Rehmany et al. 2000), 28S rDNA sequencing (Riethmuller et al. 2002), large subunit (LSU) nuclear ribosomal DNA (nrDNA) sequencing (Choi et al. 2006; Voglmayr and Riethmüller 2006), ITS ribosomal DNA (rDNA) and COX mitochondrial DNA (mtDNA) sequencing (Choi et al. 2006, 2007, 2008, 2009, 2011a, b, c; Thines et al. 2008; Thines and Voglmayr 2009; Thines et al. 2009), and phylogeny data for pathogenicity spectrum of the isolates (Kaur et al. 2011a) have resolved pathogenic diversity, evolution, morphological, pathological and phylogenetical relationship of *Albugo* species.

After scanning through more than 1,200 publications from 1792 to 2012, it has been revealed that up to 1955, majority of publications dealt with the pathogens (*Albugo*) identity, taxonomy, morphology, host range, distribution, disease symptoms, infection process and reporting of new species. Detailed study on other aspects of host–pathogen interaction, disease development, host resistance, pathogen virulence, phylogeny, and disease management have been conducted in the latter half of twentieth and twenty-first century. Although very useful information on diseases caused by *Albugo* have been generated by the researchers during the past century, some pitfalls and gaps still exist in the better comprehension of WR disease. In depth research is required through the modern techniques and tools of biotechnology, molecular biology, genetic engineering and nanotechnology on the priority areas of research suggested in Sect. 15.

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2 The Disease

On the basis of symptoms on the host plants, the disease is known as white rust (WR), white blister, stagheads, and white blister rust (Persoon 1801; Kuntze 1891; Biga 1955; Saharan and Verma 1992) all over the world.

2.1 Historical Account

The first species of *Albugo* was described as *Aecidium candidum* (now *A. candida*) by Gmelin in 1792 (Gmelin 1792), which was later placed in genus *Uredo*, subgenus *Albugo* (Persoon 1801). Based on differences in symptom development, Persoon (1801) described two different species of white blister rust, with *Uredo candida* subdivided into three varieties, parasitic to Brassicaceae and Asteraceae. A few years later, *Albugo* was established as an independent genus by De Roussel (1806), although erroneously Gray (1821) is often still given as the author for this genus. De Candolle (1806) added the species *Uredo portulaceae* (now *Wilsoniana portulacae*), and *Uredo candida* beta *tragopogi* to species rank (*Uredo tragopogi,* now *Pustula tragopogonis*) and renamed as *Uredo candida cruciferarum.* In the remainder of the nineteenth century, only a few additional species were described, but a multitude of synonyms were introduced for the already established species, while it was generally perceived that the species had broad host ranges, and were affecting whole host families. Leveilla (1847) described the genus *Cystopus,* and later de Bary (1863) described the sexual state of *Albugo*, adopting the generic name *Cystopus*. Previously, Biga (1955) pointed out that names of sexual form have no precedence over anamorphs in oomycetes, many researchers considered white blister rusts to be members of the superfluous genus *Cystopus* (Wakefield 1927), while the older genus name, *Albugo,* also persisted.

In the early twentieth century, several additional species of *Albugo* were described. Wilson (1907) recorded 13 species, and Biga (1955) accepted 30 species in this genus about 50 years later. Only a few new species were described thereafter; the key to the genus *Albugo* published 40 years later (Choi and Priest 1995) recognised 10 species.

Albugo candida (Pers.) Roussel (Albuginales, Peronosporomycetes), consisting of about 50 species (Biga 1955; Choi and Priest 1995), is an obligate and ubiquitous fungal pathogen responsible for WR in Brassicaceous hosts over widely different geographical areas of the world. *Albugo* has been typified by Kuntze (1891), who gave *Uredo candida* (Pers.) as the type species.

Up to now, the white blister pathogen on oilseed rape has been considered *A. candida* or *Albugo cruciferarum* (Farr and Rossman 2010). *Albugo cruciferarum* is regarded as a synonym of *A. candida* (Choi et al. 2007).

2.2 Host Range

White blister rust is one of the most common diseases in Brassicaceae. Recently, molecular approaches revealed that apart from *A. candida,*

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several other more specialized species of the genus are causing this disease on Brassicaceae (Mirzaee et al. 2012), but the host diversity of this group still remains largely unexplored (details in 2.2.1).

Among the crucifers, a wide variety of life cycles and habitat preferences exist, and it is unknown if the large number of listed hosts of *A. candida* all harbor similar systemic, and asymptomatic infections. According to Fungal data bases at the USDA-ARS Systematic Botany and Mycology Laboratory (Farr et al. 2004), *A. candida* was recorded on more than 300 hosts. The first record seems to be that of *A. candida* from Spain by Colmeiro (1867). It is known as a crop pathogen on various *Brassicaceae* species. Detection of *A. candida* infections across the range of species tested viz., *Lepidium campestre* (L.) R.Br., an annual, *Erysimum menziesii* ssp. *eurekense* R.A. Price, a monocarpic perennial, and *Arabis lyrata* L., a short lived perennial, suggest that asymptomatic infection appears to be quite common throughout the Brassicaceae (Jacobson et al. 1998). However, it remains to be studied if such extended latent infections are universal on other host families attacked by other *Albugo* species. Studies suggested that the disease cycles of *A. candida* should be re-examined, especially in economically important crop species, where this may have implications for management of the WR disease.

Albugo candida on *Raphanus raphanistrum* was first recorded in 1929 from Western Australia (MacNish 1963), on *Brassica napus* var. *Napobrassica* and *Brassica rapa* var. *Rapa* in 1940 (Chambers 1959), on *Raphanus sativus* in 1944 (Chambers 1959), on *Brassica oleracea* var. *Botrytis* in 1965 (MacNish 1967), on *B. napus* in 1970 (Shivas 1989), on *Brassica tournefortii* in 1974 (Shivas 1989), on *B. rapa* var. *Chinensis* in 1984 (Shivas 1989), and on Chinese cabbages (*B. rapa* subsp. *pekinensis*), Japanese mustard (*B. rapa* subsp. *nipposinica*), and tah tsai (*B. rapa* subsp. *narinosa*) in 1996 (Koike 1996), and on *Eruca sativa* (*E. vesicaria*) in 1999 from California, USA.

Although, the Caper bush (*Capparis spinosa* L.) is attacked by relatively few pathogens, the most prevalent being the white blister rust caused by members of the family Albuginaceae. The plant parasites of this family are very distinctive from the second obligate parasitic group of the family Peronosporaceae (Riethmuller et al. 2002; Hudspeth et al. 2003; Thines and Spring 2005; Thines et al. 2008). The Albuginaceae contains four distinct lineages; *Albugo* s.str., parasitic to Brassicales, *Albugo* s.l., parasitic to Convolvulaceae, Pustula Thines, parasitic to Asterales, and Wilsoniana, parasitic to Caryophyllales. Within the Albuginales, about 40 species responsible for white blister rust disease of economically important agricultural crops, and common weeds have been described (Biga 1955; Choi and Priest 1995).

Albugo candida with a broad host spectrum within the Brassicaceae, and existence of a high degree of genetic diversity within the genus (Choi et al. 2006; Voglmayr and Riethmüller 2006), strongly suggest that several of the observed lineages might constitute a distinct species. Based on recent lectotypification of *A. candida*, two specialized *Albugo* species, parasitic to Brassicaceae, have been described within *Albugo* (Choi et al. 2007, 2008). The broad host range of *A. candida* extends from Brassicaceae to Cleomaceae, and Fabales to Capparoceae (Choi et al. 2009, 2011b, c). The natural host of *Albugo* s.str. extends from Brassicales to Fabales via host jumping (Choi et al. 2011b, c).

Albugo candida infects a large number of host plants in Aizoaceae, Cruciferae, Capparidaceae, and Cleomaceae, including leaves of *Reseda alba* (Jorstad 1964; Mukerji 1975a; Walker 1957; Waterhouse 1975). *Albugo candida* is known to infect plants of 241 species in 63 genera of cruciferae family (Biga 1955). Hosts amongst the cultivated crops include *Brassica alba, B. oleracea* var. *Capitata* (cabbage), *B. oleracea* var. *Botrytis* (cauliflower), *Brassica juncea* (Indian mustard), *Brassica nigra* (Black mustard), *Brassica rugosa*, *B. chinensis, Brassica pekinensis, B. rapa* (turnip rape), *B. rapa* var. Toria, *B. rapa* var. Brown Sarson, *B. rapa* var. Yellow Sarson, *B. napus*, *B. tournefortii*, *Raphanus sativus, Armoracia lapathifolia* (horseradish), *Lepidium sativum* (cress), *Rorippa nasturtium* (watercress), *Alys-*

sum montanum, Barbarea vulgaris, *Cheiranthus cheiri* (wallflower), *Matthiola incana* (stocks), *Raphanus raphanistrum*, and many weeds (Butler 1918; Heald 1926; McKenzie 1987; Morris and Knox-Davies 1980; Petrie 1986, 1988; Walker 1957; Whipps and Cooke 1978a, b, c, d; Wiant et al. 1939; Saharan and Verma 1992). *Capsella-bursa-pastoris* (Shepherd's purse), *Lepidium virginicum* (pepper grass), *Sisymbrium officinale,* and *S. irio* are the most common weed hosts (Glaeser 1971; Heald 1926; Kaur et al. 2011b, c; Choi et al. 2011a, b, c). A preliminary check list of Albuginales and Peronosporales (Chromista) prepared by Garcia-Blazquey et al. (2007) after the scrutiny of 188 publications between 1867 to 2005, revealed that 1500 fungus/host combinations of WR and downy mildews (DM) (*Albugo* and *Hyaloperonospora*) have been reported from Iberian Peninsula and Balearic Islands.

Albugo ipomoeae-panduratae infects members of the Convolvulaceae, especially *Ipomoea* and *Convolvulus* spp. including economically important hosts *Ipomoea batatas* (sweet potato), *Lepidium pestigridis* and probably *I. aquatic* (*I. reptans*, water spinach). *I. horsfanae*, *I. putpurea*, and *Calonyction aculeatum* are amongst ornamentals attacked by this fungus (Mukerji and Critchett 1975). Presence of asymptomatic, systemic infections vertically transmitting through seeds suggests that *A. candida* may possibly form endophytic relationships with at least some of its crucifer hosts viz., *L. campestre* (L.) R.Br., *Arabis lyrata* L., and *Erysimum menziesii* ssp. *eurekense* R.A. Price (Jacobson et al. 1998). Capers (*Capparis spinosa*) widely grown in the Mediterranean for their edible flower buds, is also attacked by *A. candida* (Choi et al. 2009).

Legumes are infected by *Albugo mauginii* (Kenneth 1968), while members of the Compositae are infected by *A. tragopogonis* (Jorstad 1964). Ten species of *Albugo* infecting several cultivated and wild hosts have been reported from India (Vasudeva 1960). In addition, Wilson (1907, 1908) listed several wild hosts from 12 different families including Cruciferae, Portulacaceae, Chenopodiaceae, Amaranthaceae, Convolvulaceae, Boraginaceae, Piperaceae, Caryophyllaceae, Ambrosiaceae, Cichoriaceae, Carduaceae, Capparaceae, and Allioniaceae.

It was recently realized that only a high degree of genetic diversity exists among the species parasitic to Brassicaceae, and that a variety of distinct, specialised species are present on Brassicales (Choi et al. 2006, 2007, 2008, 2011a, b, c; Choi and Thines 2011; Ploch et al. 2010; Thines et al. 2009). It was also demonstrated that *A. candida* s.str. has a broad host range, infecting representatives of about 20 genera of the Brassicaceae, and also some Cleomaceae Bercht. & J. Presl and Capparaceae Juss. (Choi et al. 2009). The host range of species with a confirmed placement in *Albugo* s.str. was previously restricted to only four families of the Brassicales, i.e. Brassicaceae, Capparaceae, Cleomaceae, and Resedaceae Martinov, but it now extends to Fabales as well. It is noteworthy that *A. mauginii* and *A. resedae* are found to be sister species, as both do not occur on Brassicaceae s.l. (including Cleomaceae and Capparaceae). Potentially, this group has evolved effectors targeting conserved pathways, and is currently in the process of establishing itself on the new hosts as a consequence of the host jump.

So far, three genera have been described in the Albuginales: *Albugo, Pustula,* and *Wilsoniana*. The members of the later two are parasitic to Asteridae and Caryophyllidae, respectively, while the remaining species in *Albugo* can be further separated into three subgroups: *Albugo* s.str. parasitic to Brassicales (Brassicaceae, Cleomaceae, Capparaceae, Resedaceae), *Albugo* s.l. parasitic to Convolvulaceae, and some white blister rust species parasitic to Caryophyllales, with unresolved affinity to either *Wilsoniana* (Thines and Spring 2005) or *Pustula* (Voglmayr and Riethmüller 2006). Based on the close relationship of *A. mauginii* from Fabales to *Albugo* species from Brassicales, a host-jumping event from Brassicales to Fabales is the most likely explanation; codivergence with the respective host would be a less parsimonious assumption. Host jumping is not rare in obligate parasites, because it has been reported in DM, *Pseudoperonospora cubensis* s.l. (including the possibly nonspecific *P. humuli*), which is parasitic to hosts in three distantly re-

lated families: Cucurbitaceae, Cannabaceae, and Balsaminaceae (Choi et al. 2005; Runge et al. 2011; Voglmayr et al. 2009). Host jumping has also been reported in powdery mildews (Inuma et al. 2007; Matsuda and Takamatsu 2003), and rusts (van der Merwe et al. 2008; Savile 1979). It can, therefore, be assumed that speciation of the white blister rusts can take four different evolutionary paths.

1. Various degrees of coevolution with their plant hosts, eventually resulting in the pathogen's dependence on a specific host genus or even a single species. This is the case in all *Albugo* species investigated so far, with the exception of *A. candida*, leading to clade-limited speciation.
2. Allopatric speciation by geographic isolation as in *A. koreana* (Choi et al. 2007) and possibly *A. voglmayrii* (Choi et al. 2008).
3. Sympatric speciation due to unknown niche adaptation factors, as in *A. laibachii* (Thines et al. 2009).
4. Host jumping, expanding the parasites' host ranges to phylogenetically distant hosts, with possible later speciation, as in *A. candida* and *A. mauginii*

The physiological basis for these host jumps is still largely unknown, but it can be assumed that the rapid evolution of effectors genes (Birch et al. 2006; Morgan and Kamoun 2007) plays a pivotal role in the process of adaptation of a pathogen species to a new host (Thines et al. 2009; Thines and Kamoun 2010; Choi et al. 2011b, c).

2.2.1 Host Diversity

Analysis of host range reported so far reveals an array of host diversity to WR (Thines 2010a, b). Except in Convolvulaceae, Biga (1955) and Choi and Priest (1995) accepted only single or a few species per host family, and they considered them synonymous in many cases when more than one species had been described on a single host family. Thus, it was assumed that the species were host-family-specific, and this stance led to mostly broad species concepts (Wilson 1907; Biga 1955; Kochman and Majewski 1970; Vanev et al. 1993; Choi and Priest 1995). During the last decade, however, molecular phylogenetic studies have revealed some genetic diversity within *Albugo* on Brassicaceae (Rehmany et al. 2000; Choi et al. 2006; Voglmayr and Riethmüller 2006). Detailed molecular phylogenetic and morphological studies have confirmed the validity of *Albugo lepidii* (Choi et al. 2007), and turned up several previously overlooked species (Choi et al. 2007, 2008; Thines et al. 2009; Ploch et al. 2010). Using *Cardamine,* Ploch et al. (2010) recently demonstrated that even within a single host genus there may be multiple specialized species. In Caryophyllales as well, species diversity seems to be higher than previously thought. Voglmayr and Riethmüller (2006) demonstrated that *Wilsoniana amaranthi* and *W. bliti*, both parasitic on species of *Amaranthus*, are indeed independent species. However, we have only scratched the surface of the complex of *Albugo* from Caryophyllales, because, several species may still await discovery. Oospore morphology and ornamentation, previously used mostly for species delimitation on distinct plant families only (with the notable exception of species in Convolvulaceae), have now been shown to be the most important characters for their delimitation (Voglmayr and Riethmüller 2006; Choi et al. 2007). Detailed investigations in the Convolvulaceae are lacking, but judging from the morphological diversity of the species described (Choi and Priest 1995), most of these can be expected to prove phylogenetically distinct. For Asteraceae, which contain *ca* 300 reported host genera (Wilson 1907; Biga 1955; Whipps and Cooke 1978a, b, c; Voglmayr and Riethmuller 2006), about four times the *ca* 70 host genera known for Brassicaceae (Wilson 1907; Biga 1955; Kochman and Majewski 1970), no detailed investigations using both molecular phylogenetic and morphological evidence have yet been published. However, Voglmayr and Riethmuller (2006) have already observed some genetic diversity in nrLSU in this group, its species diversity may be comparable to that of the Brassicaceae-infecting lineages. If species of Asteraceae are as host-specific as in Brassicaceae, dozens of new species await discovery (Thines 2010a, b). Principal crop, ornamental and weed hosts of *A. candida* are listed in Table 2.1.

Table 2.1 Principal crop, ornamental and weed hosts of *A. candida*

Hosts	Scientific name	Reference	Year	Country
Pigweed	*Amaranthus*	Biga (1955)	1955	Italy
Slender amaranth	*A. viridis*	Biga (1955)	1955	Italy
Horse radish	*Armoracia lapathifolia*	Lázaroe e Ibiza (1902)	1902	
Horseradish	*A. rusticana*	Lázaroe e Ibiza (1902)	1902	ESP-M
Garlic mustard	*Alliaria petiolata*			
	Alyssum sp.	Savulescu & Rayss (1930)	1930	Romania
	A. montanum		1896	Poland
	Arabidopsis arenosa		1974	Romania
Thale Cress	*A. thaliana*		1977	Romania, Ilfov
	A. hallerii		1980	Romania, Suceava
Rockcress	*Arabis* spp.	Savulescu & Rayss (1930)	1930	Romania
	A. turrita		1955	Bulgaria
Snow-in-summer	*A. caucasica*			
Hairy rock cress	*A. hirsuta*	Gonzalez Fragoso (1919)	1919	Spain
	A. albida		1977	Tasmania
	A. alpine	Savulescu and Rayss (1930)	1972	Romania
	A. arenosa (L.) Scop	Savulescu and Rayss (1930)	1930	Romania
	A. soyeri	Unamuno (1921b)	1921	Spain
	A. ovirensis	Savulescu and Rayss (1930)	1930	Romania
	Aubrieta deltoidea		1953	Germany, Hessen
Goldkugel	*Aurinia saxatilis*			
	Barbarea intermedia	Unamuno (1921)	1921	Spain
	B. vulgaris		1943	Ireland
	Biscutella laevigata		1903	Switzerland
	B. auriculata	Unamuno (1928)	1928	Spain
	B. intermedia	Durrieu (1966)	1966	Spain
	B. valentine sub sp. *pyrenaica*	Gonzalez Fragoso (1917)	1917	Spain
	Berteroa incana		1987	Austria, Krems
Karashina	*Brassica cernua* Forbes and Hemsl	Togasaki & Shibasaki (1930)	1930	Japan
Indian mustard	*B. juncea*	Togasaki & Shibasaki (1930)	1930	Japan
	B. japonica	Togasaki & Shibasaki (1930)	1930	Japan
Mibua	*B. japonica* Sieb var. *Indivisa* Makino	Togasaki & Shibasaki (1930)	1930	Japan
Gobhi sarson, rape	*B. napus*	Unamuno (1921)	1921	Spain
Black mustard	*B. nigra* (L.) Koch	Gonzalez Fragoso (1916)	1916	Spain
Jersey Kale	*B. oleracea* var. *Palmifolia*			
Kale, Collard	*B. oleracea* var *Acephala* DC			
Kitechen kale	*B. oleracea* var. *Fimbriata* Mill.			
Kohlrabi	*B. oleracea* var. *Gongylodes* L.			
Borecole	*B. oleracea* var. *Sabellica*			
Broccoli, calabrese	*B. oleracea* L. var. *Italica* Plenck			
Brussels sprouts	*B. oleracea* L. var. *Gemmifera* DC			

Table 2.1 (continued)

Hosts	Scientific name	Reference	Year	Country
Burma sarson	*B. oleracea* L. var. *Chinensis* Prain			
Cabbage	*B. oleracea* var. *Capitata*			
Cauliflower	*B. oleracea* var. *Botrytis*			
Candytuft				
Chinese kale	*B. oleracea* var. *Albiflora* Sun [*B.alboglabra*]			
Charlock				
Marrow stem kale	*B. oleracea* var. *Medullosa*			
Nasturtium				
Pakchoi	*B. chinensis* L.	Togasaki & Shibasaki (1930)	1930	Japan
Komatsuna	*B. chinensis* var. *Komatsuna* Matsum and Nakai	Togasaki & Shibasaki (1930)	1930	Japan
	B. chinensis var. *Oleifera* Makino			
Portugese cabbage	*B. oleracea* var. *Costata*			
Chinese cabbage	*B. pekinensis* (Lour.) Rupr.	Togasaki & Shibasaki (1930)	1930	Japan
Collards	*B. oleracea* var. *Acephala*DC/*B. oleracea* var. *Viridis*			
Cole crops	*B. oleracea*			
Rutabaga	*B. rapa* var. *Napobrassica* (L.) DC			
Salsify				
Savoy cabbage	*B. oleracea* var. *Sabauda* L.	Unamuno (1921a)	1921	Spain
Thousand-head kale	*B. oleracea* var. *Fruticosa* Metz. *B. oleracea* var. *Ramose*			
Tronchuda cabbage	*B. oleracea* var. *Tronchuda* L. H. Bailey			
Turnip	*B. rapa* L.	Togasaki & Shibasaki (1930)	1930	Japan
	Cakile maritima	Lagerheim (1890)	1890	Portugal
	Calepina irregularis		1978	Romania
Caper bush	*Capparis spinosa* L.	Winter (1887)	1887	Portugal
	C. rupestris			
Shepherd's-purse	*Capsella bursa-pastoris*	Lagerheim (1890)	1890	Portugal
Nazua	*C. bursa-pastoris* Moench *var. Auriculata*	Togasaki & Shibasaki (1930)	1930	Japan
Bitter cresses	*Cardamine*		1997	
Tanetsukebana	*C. flexuosa* With	Togasaki & Shibasaki (1930)	1930	Japan
	C. halleri subsp. *ovirensis*	Savulescu and Rayss (1930)	1930	Romania
	Cardamine hirsuta	Lagerheim (1890)	1890	Sapin
	Cardaria draba	Hoyos de Onis (1898)	1898	Spain
	Cheiranthus incanus			Northwest Europe
Wallflower, common and western	*C. cheiri/Erysimum cheiri*	Camara (1930)	1930	Portugal
	Cleome anomala			

Table 2.1 (continued)

Hosts	Scientific name	Reference	Year	Country
Yellow mesambay	*C. viscose* L.			
	Coincya longirostra	Unamuno (1940)	1940	Spain
	C. monensis subsp. *cheiranthos*	Camara and Luz (1939)	1939	Portugal
	Coronopus squamatus	Camara and Oliveira (1944)	1944	Portigal
	C. procumbens (l.) Gilib.	Savulescu and Rayss (1930)	1930	Romania
Carrot	*Daucus carota*			
	Descurainia sophia		1977	URSS
	Diptychocarpus strictus		1978	URSS
	Diplotaxis virgata	Gonzalez Fragoso (1916)	1916	Spain
	D. erucoides	Gonzalez Fragoso (1917)	1917	Spain
	D. muralis	Gonzalez Fragoso (1916)	1916	Spain
Perennial wall rocket	*D. tenuifolia*		1997	Malta
	Draba nemorosa	Togashi & Shibasaki (1930)	1930	Japan
Inu-nazuna	*D. nemorosa var. Hebecarpa* Ledeb.			
	D. nemorosa var. Macrospora	Togashi & Shibasaki (1930)	1930	Japan
	D. verna			
	Eruca sativa		1968	Pakistan
Purple vein rocket	*E. vesicaria*			
	Erophila verna	Mirzaee et al. (2012)	1979	Romania
Wallflower	*Erysimum cheiri*		1997	
	E. cuspidatum		1979	Romania, Mehedinti
	E. crassicaule Boiss.	Mirzaee et al. (2012)	2009	Iran
Treacle mustard	*E. cheiranthoides*		1980	Romania
	E. crepidifolium		1973	Romania
	E. diffusum		1973	Romania
	Erucastrum gallicum	Sydow and Sydow (1903)	1903	Portugal
	E. nasturtiifolium	Loscos and Pardo (1867)	1867	Spain
Horse-radish	*Eutrema wasambi* Maxim			
	Heliophila meyeri	Vanrhynsdorp (1896)	1896	South Africa (RSA)
	Hirschfeldia incana	Gonzalez Fragoso (1917)	1917	Spain
	Hesperidis matronalis L.	Savulescu and Rayss (1930)	1930	Romania
	Hymenolobus procumbens		1979	Romania
Swamp morning glory	*Ipomoea aquatica*			
Sweet potato	*I. batatas*			
	Iberis amara		1938	USA, California
	Lunaria annua	Savulescu and Rayss (1930)	1930	Romania
Field cress	*Lepidium campestre*	McRitchie (1986)	1986	USA
	L. graminifolium	Unamuno (1941)	1941	Spain
Virginian Peppergrass	*L. virginicum*			
Perennial pepperweed	*L. latifolium*	Winter (1887)	1887	Sapin
Garden cress	*L. sativum*	McRitchie (1986)	1986	USA
	L. perfoliatum L.	Savulescu and Rayss (1930)	1930	Romania
Sweet alyssum	*Lobularia maritima*	Unamuno (1921)	1921	Spain

Table 2.1 (continued)

Hosts	Scientific name	Reference	Year	Country
Waterspinatch	*L. aquatic=I. reptans*			
Honesty	*Lunaria annua*	Savulescu and Rayss (1930)	1930	Romania
Honesties	*L. honesties*		1997	
Stocks	*Matthiola incana*	Lagerheim (1890)	1890	Portugal
	Malcolmia africana	Gonzalez Fragoso (1917)	1917	Spain
	M. littorea	Lagerheim (1890)	1890	Spain
Watercress	*Nasturtium officinale*			
Creeping yellow cress	*Rorippa sylvestris*			
Yellow rocket	*R. nasturtium*			
Yellow marsh cress	*R. islandica*			
Great yellow cress	*R. amphibia*		1997	
Water cress	*R. nasturtium-aquaticum agg.*		1997	
Daikon	*Raphanus sativus* L. *var. Longipinnatus* Bailey			
Daikon	*R. sativus* L. *var. Macropodus* Makino	Togashi and Shibasaki (1930)	1930	Japan
Radish	*R. sativus* L.	Gonzalez Fragoso (1914)	1914	Spain
Wild radish	*R. raphanistrum*	Camara (1916)	1916	Portugal
	Rapistrum rogosum	Unamuno (1929)	1929	Spain
White mustard	*Sinapis alba*			
Wild mustard (Charlock)	*S. arvensis*	Camara (1936)	1936	Portugal
Corn spurry	*Spergula arvensis*			
Hedge mustard	*Sisymbrium officinale*		1997	
Eastern rocket	*S. orientale*	Jorstad (1962)	1962	Spain
London rocket	*S. irio*	Gonzalez Fragoso (1916)	1916	Spain
Tall rocket	*S. altissimum*			
	S. luteum		2002	Korea, Pyongchang
	S. crassifolium	Gonzalez Fragoso (1914)	1914	Spain
African mustard	*Strigosella africana*	Biga (1955)	1955	Russia
	S. brevipes	Biga (1955)	1955	Russia
	S. aegyptica	Melchers (1931)	1931	Egypt
	Syrenia sessiliflora (DC.) Ledeb.	Savulescu and Rayss (1930)	1930	Romania
	Teesdaliopsis conferta	Mayor (1972)	1972	Spain
Common nasturtium	*Tropaeolum majus*			
Field pennycress	*Thlaspi arvense*	Savulescu and Rayss (1930)	1930	Romania

2.3 Geographical Distribution

White rust on cultivated oilseed Brassicas and other cruciferous species is prevalent worldwide where these crops are grown. Although the list is not exhaustive, countries where the disease occurs include the UK (Berkeley 1848), USA (Walker 1957), Brazil (Viegas and Teixeira 1943), Canada (Greelman 1963; Petrie 1973), Germany (Klemm 1938), India (Chowdhary 1944), Japan (Hirata 1954, 1956), Pakistan (Perwaiz et al. 1969), Palestine (Rayss 1938), Romania (Savulescu 1946a, b, 1960), Turkey (Bremer et al. 1947), Fiji (Parham 1942), New Zealand (Hammett 1969), China (Zhang et al. 1981, 1984, 1986), and Korea (Choi et al. 2011a). White rust of sunflower occurs in Russia (Novotel'Nova 1962, 1968; Novotel'Nova and Minasyan 1970; Novotel'Nova and Pystina 1985), Uruguay (Sackston 1957), Argentina (Sarasola 1942), Australia (Middleton 1971; Stovold and Moore 1972), and in many other countries (Kajomchaiyakul and Brown 1976). White rust of salsify occurs in Australia, Canada, USA, South America, Europe, Asia, and Africa (Wilson 1907, 1908). White rust of water spinach is a serious disease in India and Hong Kong (Ho and Edie 1969; Safeefulla 1952a, b; Safeefulla and Thirumalachar 1953), and affects spinach in Texas (Wiant 1937; Williams and Pound 1963; CABI 2001).

2.3.1 Distribution Map

A new distribution map 821 is provided (CABI 2001) for *A. candida* (Pers.) Kuntze (Chromista: Oomycota: Peronosporales) hosts mainly *Brassica* spp. and other Brassicaceae. Information is given on the geographical distribution in EUROPE: Austria, Belgium, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Malta, Netherlands, Poland, Portugal, Romania, Russia, Slovakia, Spain, Canary Islands, Mainland Spain, Sweden, Switzerland, UK, Ukraine, Yugoslavia (Fed. Rep.); ASIA: Bhutan, China, Anhui, Fujian, Gansu, Guangxi, Guizhou, Hebei, Hong Kong, Hubei, Jiangsu, Jiangxi, Jilin, Liaoning, NeiMenggu, Qinghai, Shaanxi, Shandong, Shanxi, Sichuan, Xinjiang, Xizhang, Yunnan, Zhejiang, Cyprus; India: Bihar, Delhi, Haryana, Himachal Pradesh, Jammu and Kashmir, Karnataka, Madhya Pradesh, Maharashtra, Manipur, Punjab, Rajasthan, Sikkim, Tamil Nadu, Uttar Pradesh, West Bengal; Iran, Iraq, Israel, Japan, Korea, Republic of Malaysia, Peninsular Malaysia, Sabah, Sarawak, Nepal, Pakistan, Philippines, Taiwan, Turkey, Yemen; AFRICA: Egypt, Ethiopia, Kenya, Libya, Malawi, Mauritius, Sierra Leone, South Africa, Sudan, Tanzania; Canada: Alberta, British Columbia, Manitoba, New Brunswick, Nova Scotia, Ontario, Prince Edward Island, Quebec, Saskatchewan; Mexico; USA: Alabama, Arizona, Arkansas, California, Colorado, Delaware, Florida, Georgia, Hawaii, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Maine, Massachusetts, Minnesota, Mississippi, Missouri, Montana, Nebraska, Nevada, New Hampshire, New Jersey, New Mexico, New York, North Carolina, North Dakota, Ohio, Oklahoma, Oregon, Pennsylvania, South Carolina, South Dakota, Texas, Utah, Vermont, Virginia, Washington, West Virginia, Wisconsin, Wyoming; CENTRAL AMERICA and CARIBBEAN: Barbados, Bermuda, Cuba, Dominican Republic, El Salvador, Jamaica, Puerto Rico, Trinidad and Tobago, Argentina, Brazil, Parana, Falkland Islands, Guyana, Suriname; OCEANIA: Australia, Western Australia, Cook Islands, Fiji, French, Polynesia, New Caledonia, New Zealand, Papua New Guinea, Samoa, Vanuatu (CABI 2001).

2.4 Symptomatology

Symptoms of the disease can be observed in the form of two categories. Firstly, the general symptoms described in book chapters, reviews, monographs and research papers etc. Secondly, specific symptoms and symptom variability recorded on different genotypes of Brassica crops sown on different dates, with different cultural conditions like, soil type, nutrition, variety, environment and locations. Isolate specific symptoms have also been observed (Jat and Saharan- Personal observation; Gupta and Saharan 2002b).

Fig. 2.1 **a** Circular *creamy* colour pustules on leaf. **b** *White* circular pustules on lower surface of Indian mustard at Solapur, Karnataka, India. **c** *White* rust pustules on lower surface of leaves of Indian mustard at Bharatpur, Rajasthan, India. **d** *White* rust pustules on upper surface of leaves of Indian mustard at Bharatpur, Rajasthan, India. **e** *White* rust pustules on both upper and lower surface on the same plant. **f** Hypertrophies (staghead) on inflorescense.

2.4.1 General Symptoms

All the above ground parts of the plant may show infection. In the initial stage of infection, localized isolated spiny white pustules or blisters up to 1–2 mm in diameter are formed on the aerial parts preferentially leaves and cotyledones. The upper area of the leaf becomes tan yellow. Numerous pustules ultimately coalesce to form larger pustules. Often pustules appear in a circular arrangement around one big central pustule (Fig. 2.1a). Mature pustules rupture to release a mass of white sporangia (Fig. 2.1b, c). In some cases, pustules may be surrounded by a yellow halo. On leaves, initial infection is on the under surface, but under humid conditions, both sides may produce white minute pustules. Pustules (Fig. 2.1d) are also common on inflorescence and floral parts. In the young stem and inflorescence, the pathogen becomes systemic in the infected tissues and stimulates deformities with pronounced hypertrophy. The disease is sometimes called the

Fig. 2.2 *White* to *cream-yellow* raised pustules of variable size on the abaxial surface of the cotyledons and on primary leaves

"cancer" of rapeseed as one phase of this disease shows grotesque malformations of the young shoots and inflorescence. The leaves on the systemically infected plants may become thickened, fleshy, pallid and distorted or rolled. When systemic infection takes place in seedling stage, the entire plant may remain dwarf with small leaves. Stem may show various types of swellings. The pathogen stimulates hypertrophy and hyperplasia consequently resulting in deformation of floral parts (Fig. 2.1e). Later in the season, the infected flowering shoots show very dramatic changes. The axis becomes swollen, curved, twisted giving a zig-zag appearence. The flowers and young fruits may also become malformed. Finally, the flowering shoot may consist only the swollen axis and spiny pedicels of the flowers and fruits. Such structures are reminiscent of the antlers of stags and are therefore referred to as 'stagheads' (Fig. 2.1e). These deformities are attributed to hormonal imbalance induced by the pathogen. There is also evidence of changes in chlorophyll content of diseased leaves. Stimulations of normal buds result into production of lateral shoots. The floral parts show irregular enlargement with distortion of the tissues due to hypertrophy and hyperplasia. The flower stalk may be enormously thickened. The flower organs become wholly or in part swollen, fleshy, green or violet in colour, and persist instead of petals and stamens falling off early. The petals may look like sepals, and stamens become leaf-like or occasionally like carpels; ovules and pollen grains are usually atrophied resulting in the sterile condition of the flowers. The pistil also becomes malformed and larger in size and sometimes the pods transform into leaf like structures. Such malformed pods lack seed development. Persistent, systemic, and asymptomatic infection of *Albugo* has been detected in wild crucifer species (Jacobson et al. 1998).

The mixed infection of WR and DM is conspicuous at floral stage of crop growth. On the malformed floral parts WR produces pustules and DM produces a coating of fine white mass of sporangia. At maturity, under high humidity, malformed inflorescence may be covered with saprophytes turning into dark brown to black colour. Plants may remain stunted due to heavy infection (Saharan 1992a, b; Saharan and Verma 1992; Saharan and Mehta 2002).

2.4.2 Symptoms Variability

Field observations of WR infection reveal greater variations in symptoms on different Brassica crops (Saharan and Jat-personal observations). The first appearance of disease can be seen on cotyledons, right from the beginning of December, under Indian cropping system. The WR disease is initially characterized by the appearance of white to cream-yellow raised zoosporangial pustules of variable shape and size on the abaxial surface of the cotyledons and on primary leaves (Fig. 2.2). Initially, the sori are discrete but later coalesce to cover whole plant organs. The fungus

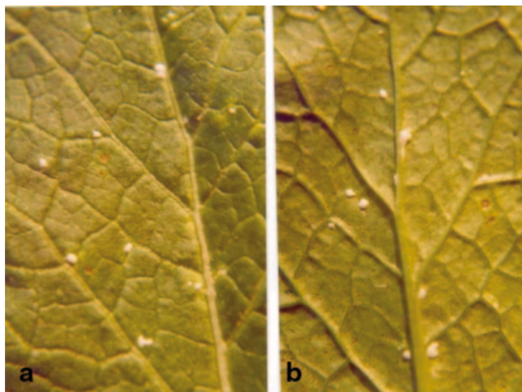

Fig. 2.3 Small pinhead size *creamy-white* pustules on **a** adaxial, and **b** abaxial surfaces of leaf

Fig. 2.5 Pustule may also appear on or near veins of **a** abaxial surface, and **b** bursting of pustules on corresponding adaxial surface

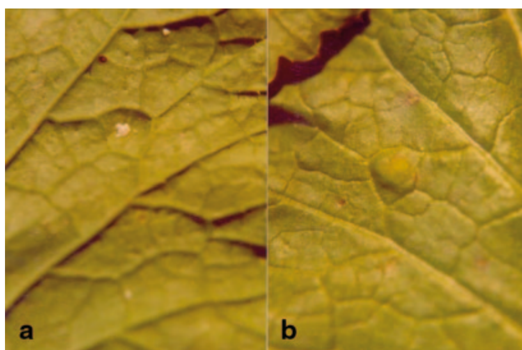

Fig. 2.4 **a** *Dirty white* pustules, with raised appearance on abaxial surface, and **b** *raised green* island on the corresponding adaxial surface of leaf

Fig. 2.6 Pustules of medium size, *creamy white*, with 5 mm diameter regular margin, raised in appearance on abaxial surface of leaf

infects both vegetative (local) and reproductive (systemic) tissues showing symptoms on all plant parts except the roots.

The critical examination of different oilseed Brassicas show variation in symptoms on different species and genotypes under field conditions. The symptom variability exhibited on the leaves (vegetative phase) of *Brassica juncea, B. rapa* var. Brown Sarson, *B. nigra* and *B. tournifortii* is in the form of small pinhead size, creamy-white pustule of 1–2 mm in diameter, raised in appearance on abaxial surface, but no island on the corresponding adaxial surface; similar, small pinhead size creamy-white pustules are also observed on both abaxial and adaxial surfaces of the leaves of these species (Fig. 2.3). In some cases, *B. rapa* var. Brown Sarson exhibited depressed groove shaped dirty white pustules, with raised appearance on abaxial surface, and raised green island on the corresponding adaxial surface of leaf (Fig. 2.4). In another case, however, small pinhead pustule may also appear on or near veins of abaxial surface and bursting of pustules on corresponding adaxial surface (Fig. 2.5). *Brassica nigra,* produces pustules of medium size, creamy white, with 5 mm diameter regular margin, raised in appearance on abaxial surface of leaves (Fig. 2.6). The genotypes of *B. juncea* exhibit an array of symptoms variability on leaves as described below:

Fig. 2.7 **a** Fainted *Green* Island on adaxial surface and **b** slightly bigger, *creamy white* pustules on abaxial surface

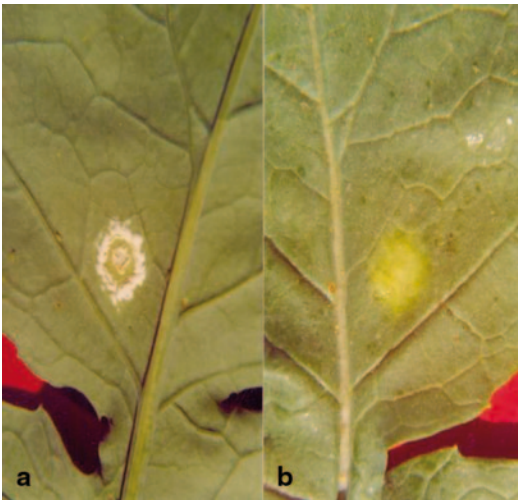

Fig. 2.8 **a** Single raised, *creamy white* pustule surrounded by a concentric ring on the abaxial surface and **b** *light yellowish green* island on adaxial surface

A. Symptoms Variability on Leaves

1. Small pinhead size, creamy white pustule without halo zone on the abaxial surface (Fig. 2.2).
2. Crust like creamy white pustules with irregular margins and yellow halo zone on the abaxial surface (Fig. 2.2).
3. Small pinhead size, creamy white pustules on or near the veins on abaxial surface (Fig. 2.5).
4. Small pinhead size, creamy white pustules on or near the veins on both abaxial and adaxial surfaces (Fig. 2.5).
5. Small pinhead size, creamy white pustules on the abaxial surface and small pinhead size green island on the adaxial surface (Fig. 2.4).

Fig. 2.9 Twin, *creamy white* pustules with *green* centers surrounded by a concentric ring on the both surface **a, b**

6. Slightly bigger, creamy white pustules on abaxial surface and fainted Green Island on the corresponding adaxial surface (Fig. 2.7).
7. Slightly bigger, single, raised, creamy white pustules surrounded by a concentric ring on the abaxial surface, and light yellowish Green Island on the corresponding adaxial surface (Fig. 2.8).
8. Slightly bigger, twin, creamy white pustules with green centers surrounded by a concentric ring on the adaxial surface (Fig. 2.9).
9. Slightly bigger, creamy white pustules surrounded by two diffused concentric rings on the abaxial surface, and violet colour island on the corresponding adaxial surface (Fig. 2.10).
10. Slightly bigger, twin, creamy white depressed pustules surrounded by a single concentric ring on the abaxial surface, and light yellowish green island on the corresponding adaxial surface (Fig. 2.11).
11. Three to four times bigger, creamy white pustules surrounded by a single concentric ring on the abaxial surface (Fig. 2.12).
12. Five to seven raised pustules coalesced to form large pustule without concentric ring on or near the veins on the abaxial surface (Fig. 2.13).
13. Large size, creamy white pustules on the veins with blighted appearance on abaxial surface (Fig. 2.14).

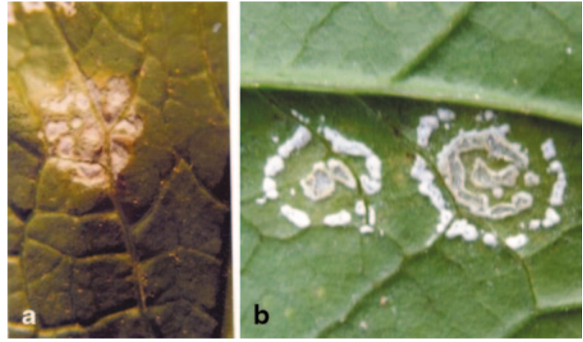

Fig. 2.10 **a** *Violet colour* island on adaxial surface and **b** *creamy white* pustules surrounded by two diffused concentric rings on abaxial surface.

Fig. 2.11 **a** Twin, *creamy white* depressed pustules surrounded by a single concentric ring on the abaxial surface and **b** *light yellowish green* island on adaxial surface

Fig. 2.13 **a, b** Five to seven raised pustules coalesced to form large pustule without concentric ring on or near the veins on the abaxial surface

Fig. 2.12 Three or more bigger, *creamy white* pustules surrounded by a single concentric ring on the abaxial surface

Fig. 2.14 **a, b** *White* rust pustules on the leaf vein showing carpet type growth

Fig. 2.15 **a** Pustule with necrosis on the abaxial surface and **b** complete necrotic island on the adaxial surface

14. Pustule with necrosis on the abaxial surface, and complete necrotic island on the corresponding adaxial surface (Fig. 2.15).
15. Pustule on or near the leaf veins showing scattered powdery mass of sporangia on the abaxial surface, and centre of island having necrosis and necrotic halo zone on the corresponding adaxial surface (Fig. 2.16).
16. Large size, depressed dirty white pustule with irregular margin on the abaxial surface (Fig. 2.17).

B. Staghead Phase (SP)

The staghead infection in different oilseed *Brassicas* and their genotypes also exhibit an array of symptoms variability as under:

1. The tips of main raceme converted into long hypertrophied malformed structure with white pustules in linear longitudinal fashion (Fig. 2.18). Such symptoms often produce in susceptible *B. juncea* cv. RH 30, *B. rapa* cv. Torch, and *B. nigra* cv. Local (Fig. 2.19); the thickness of malformed staghead is almost two to three times or more than the width of normal main raceme.
2. The tips of systemically-infected main raceme appear twisted and swollen with white crust like pustules on their surface (Fig. 2.20).
3. The whole main raceme converted into malformed structures, except a portion of it emerged as healthy branch bearing healthy siliquae in *B. juncea* cv. RH 30 (Fig. 2.20).
4. Hypertrophied primary branches bearing healthy small siliquae (Fig. 2.21).

Fig. 2.16 Pustule showing scattered powdery mass of sporangia on the abaxial surface **a**, and *centre* of island having necrosis and **b** necrotic halo zone on the adaxial surface

Fig. 2.17 **a, b** Large, depressed dirty *white* pustule with irregular margin on the abaxial surface

5. Hypertrophied primary branches with abortive hypertrophied siliquae.
6. Hypertrophied primary branches with healthy and hypertrophied abortive siliquae.
7. Healthy primary branches with malformed siliquae possessing white rust mass on and inside the siliquae.
8. The flower of *B. juncea* cv. RH 30 with white rust infection shows (a) normal flower with infected peduncle at different height, (b) hypertrophied flower vs. white rust infected peduncle, and (c) normal siliquae with minor infection of white rust on peduncle (Fig. 2.22).

Fig. 2.18 Tips of main raceme converted into long hypertrophied malformed structure with *white* pustules in linear longitudinal fashion

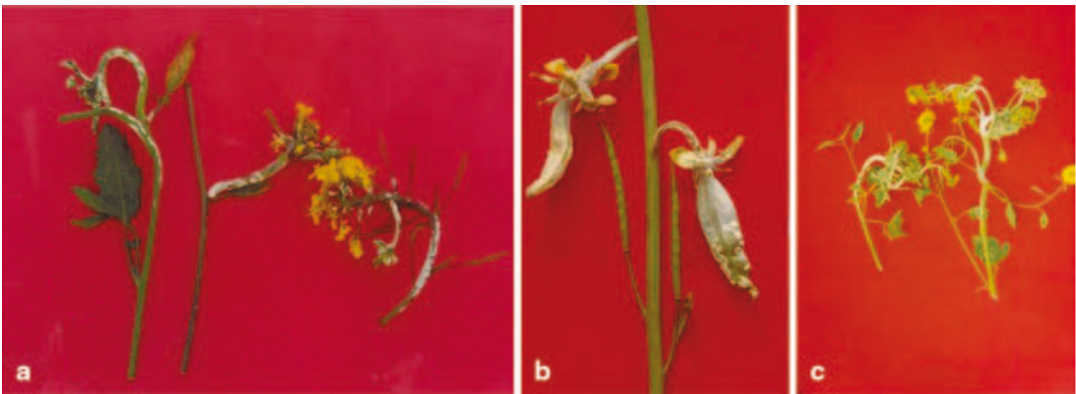

Fig. 2.19 Variation in stagheads of different species of oilseed Brassica infected with *white* rust

9. Variation in the symptoms of white rust on different flower parts: (a) calyx (b) corolla (c) androecium (d) gynoecium and (e) peduncle in *B. juncea* (Fig. 2.23).
10. Flower develops normal siliquae but their sepals become thick, dark green, succulent with linear white pustule.
11. Distorted green, swollen, and fleshy florets which persist up to maturity (Fig. 2.24).
12. Systemically-infected blighted leaf with white pustules on both abaxial and adaxial surfaces (Fig. 2.25).
13. Systemic infection on the leaf and its different parts: (a) on leaf petiole; (b) on leaf blade towards the leaf petiole, and (c) on leaf blade towards leaf apex (Fig. 2.26).
14. The white pustule girdles the axis of main raceme.
15. Variation in the symptoms on distorted *B. juncea* (a) without branches; (b) with single primary branch bearing normal flower and siliquae, and (c) with stunted and thickened growth of plants with white pustule on its surface (Fig. 2.27).

The extent of systemic infection varies in different oilseed *Brassica* (Table 2.2). The length and width of *B. juncea* staghead ranges from 1–60 cm and 0.2 to 1.6 cm, respectively. However, the average length and width observed is 11.2 and 0.5 cm, respectively. The length of stagheads in *B. nigra* ranges between 5–15.0 cm with

Fig. 2.20 *White* rust infection on stem of *B. juncea* cv. RH 30. **a** Twisted and swelling appearance on the stem. **b** *White* rust infection on the axil of branch of *Brassica juncea*. **c** *White* rust linear pustules on the stem

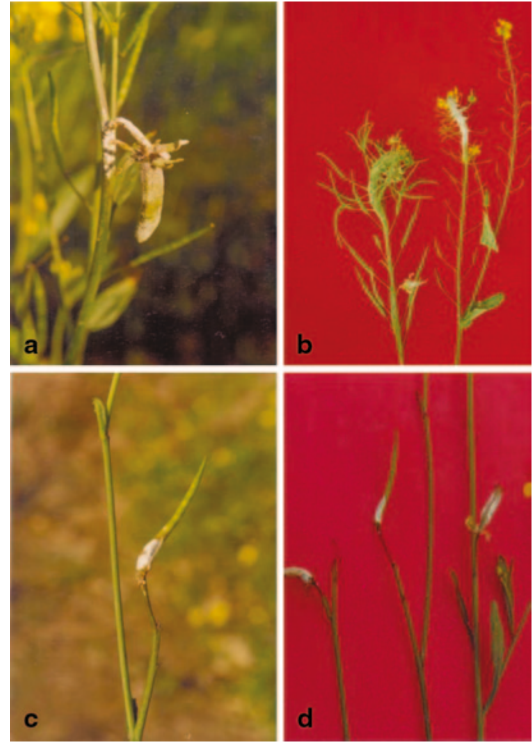

Fig. 2.21 *White* rust infected siliquae of *Brassica juncea* cv. RH 30. **a** Single siliquae infected on whole plant. **b** Two siliquae infected on whole plant. **c** Siliquae showing *white* rust pustules covering 1/3 portion. **d** Siliquae showing *white* rust pustule covering whole and 1/3 portion

a mean of 7.5 cm; width ranges from 0.2–0.8 cm with a mean of 0.4 cm. Similarly, in *B. rapa* var. Toria length and width of stagheads range from 2–25 cm and 0.2 to 1.6 cm with an average of 10.6 cm and 0.8 cm, respectively. Only three oilseed *Brassica* species, *B. juncea; B. nigra* and *B. rapa* var. Toria, produce stagheads under field conditions. All other species including *B. chinensis, B. tournefortii, B. pekinensis, B. rapa* var. Brown Sarson and *B. rapa* var. Rapa show WR symptoms on leaves only.

2.4.3 Symptoms Specificity

Pathogen-isolate specific symptoms have been recorded by Gupta and Saharan (2002). The symptoms variability on the leaves of *B. juncea* genotypes infected with different isolates of *A. candida* is observed in the form of small, pinhead size, creamy-white pustules, 1 mm in diameter, regular margin, raised in appearance on abaxial surface, and pinhead type green island without halo zone on the corresponding abaxial surface. Another type of pustules are small but slightly bigger than pinhead size, bright white, 1–2 mm in diameter, raised in appearance on the abaxial surface, and no green island on the corresponding adaxial surface. Similarly, medium-size, creamy-white pustules with one concentric ring and pinhead type dot in the centre, 3–4 mm in diameter, regular margin, raised in appearance on the abaxial surface, and light-yellowish green island without halo zone on adaxial surface. Fourth types of pustules are medium size, circular, creamy-white with two concentric rings on the abaxial surface, and violet island without halo-zone on adaxial surface. The symptoms produced by an isolate on artificially-inoculated plants were similar to those observed

Fig. 2.22 White rust infection on flower of *Brassica juncea* cv. RH 30. **a** Normal flower with infected peduncle at different height. **b** Hypertrophied flower with infected peduncle. **c** Closed flower bud with infected peduncle. **d** Normal siliquae with little infection on peduncle

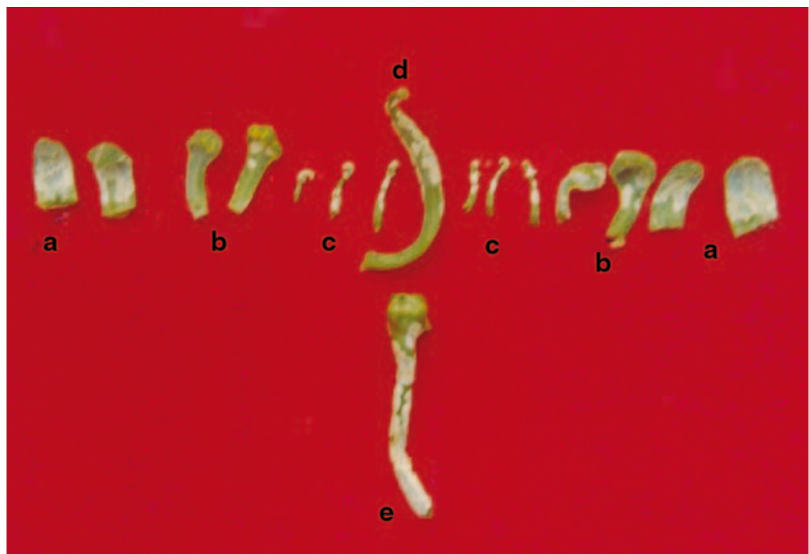

Fig. 2.23 *White* rust infection on flower parts of *Brassica juncea* cv. RH 30. **a** Calyx. **b** Corolla. **c** Androecium **d** Gynoecium. **e** Peduncle

Fig. 2.24 Distorted malformed *green*, swollen, and fleshy florets

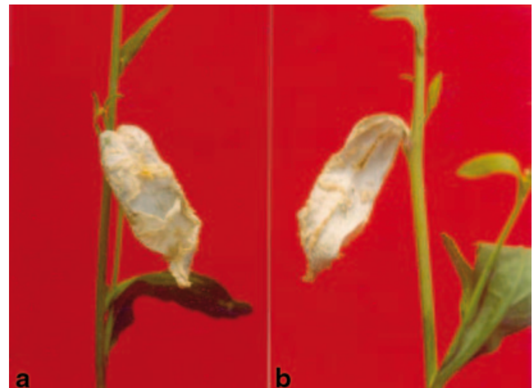

Fig. 2.25 Systemically-infected blighted leaf of *Brassica juncea* cv. RH 30 **a, b**

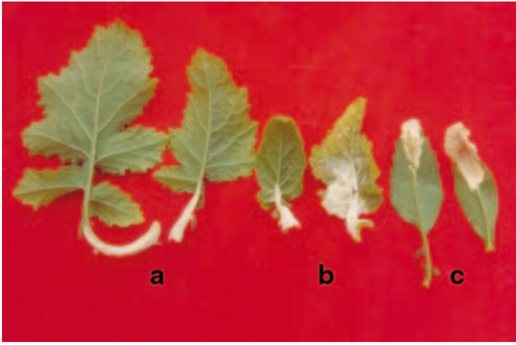

Fig. 2.26 Progress of systemic infection on leaf of *Brassica juncea* cv. RH 30. **a** *White* rust infection on leaf petiole. **b** Progress of *white* rust infection from leaf petiole to leaf blade. **c** Progress of *white* rust infection from leaf apex to leaf blade

on naturally-infected plants. These results suggest that the symptom variability between isolates was due to isolates specificity and not because of host differentials. Except reports by Jat (1999), there is no published record of such stable symptom variability between *A. candida* isolates.

2.5 Disease Assessment

White rust disease (*Albugo*-host-interaction) interaction phenotypes (IP) on different cruciferous crops have been assessed at cotyledonary, true leaves and staghead formation stages. Different disease scoring scales are used by researchers to categorize host genotypes into resistant and susceptible categories. The disease incidence and severity indices are used to assess losses caused by the disease in different host crops, genotypes and varieties. The progression of the disease in relation to nutrition, plant population, spacing, environmental interaction, and chemical control has been measured through infection rates (r) and area under disease progression curves (AUDPC); per cent disease control is calculated with the application of a formula. The disease assessment scoring scales developed, and in use, amongst researchers are as follows:

2.5.1 Assessment of Albugo-Crucifer Interaction Phenotype (IP)

2.5.1.1 Pound and Williams (1963) 0–9 Disease Scoring Scale

0 = No symptoms on either leaf surface
1 = Small, pinpoint to larger brown necrotic flecks under inoculation point on upper surface, occasionally necrosis extending to lower epidermis. No sporulation
3 = Very sparse, 1-few, minute scattered pustules on upper surface, none to very few pustules on lower surface
5 = Few to many scattered pustules on upper surface. None to few scattered pustules on lower surface
7 = Many to few pustules on upper surface. Many scattered small to larger pustules on lower surface
9 = Very few to no pustules on upper surface. Many large-coalescing pustules on lower surface

$$\text{Disease index} = \text{DI} = \frac{\sum_{i=0}^{9} (\text{ixj})}{\text{n}}$$

Whereas:
n = Total plants
i = IP class
j = Number plants/class
Selection: Susceptible controls, IP = 7–9 by 7 days; PI; partial resistance, IP = 3–5; and resistant plants, IP = 0–1.

2.5.1.2 Leckie et al. (1996) 0–7 Disease Scoring Scale

Leckie et al. (1996) also developed 0–7 disease scoring system for assessment of both the host plant response, and the growth of the pathogen in which disease is recorded 14 days after inoculation. Accordingly, seedlings were categorised into eight interaction phenotype classes (Tables 2.3, 2.4; Fig. 2.28).

Fig. 2.27 Variation in distorted plant of *Brassica juncea* cv. RH 30 by systemic infection of *Albugo candida*. **a** Distorted plant without branching. **b** Single primary branching. **c** Stunted and thickened growth of whole plant bearing *white* rust pustules on its surface

Table 2.2 Type of infection and variability in the staghead phase infection of different crucifers. (Jat 1999)

Host	Type of infection		Length of staghead (cm)	Average length of staghead (cm)	Diameter of staghead (cm)	Average diameter of staghead (cm)	Nature of staghead infection
	Leaf phase	Staghead					
Brassica juncea	+	+	1–60	11.2	0.2–1.6	0.5	WR only
B. nigra	+	+	5–15.0	7.5	0.2–0.8	0.4	WR only
B. chinensis	+	–	–	–	–	–	–
B. tournifortii	+	–	–	–	–	–	–
B. pekinensis	+	–	–	–	–	–	–
B. rapa var. Brown Sarson	+	–	–	–	–	–	–
B. rapa var. Yellow Sarson	–	–	–	–	–	–	–
B. rapa var. Toria	+	+	2–25	10.6	0.2–1.6	0.8	WR only
B. rapa var. rapa	+	–	–	–	–	–	–
B. napus	–	–	–	–	–	–	–
B. carinata	–	–	–	–	–	–	–
Raphanus sativus	–	–	–	–	–	–	–
B. oleracea	–	–	–	–	–	–	–

+ Infection, – No infection

2.5.2 A Two-Way Analysis of Variance

A two-way analysis of variance was performed on the log-transformed data. Use of a visual scale in selection for components of partial resistance to the WR disease has been suggested by Fox and Williams (1984); WR rating scale on *B. rapa* is based on proportion of cotyledon area with necrotic flecks, or WR pustules 7-days after the adaxial surface was inoculated with two 10-µl droplets of a 10^5 zoospore/ml *A. candida* suspension. The interaction phenotype symptoms ratings on adaxial/abaxial cotyledon surface are:

0 = Nothing/nothing
1 = Necrotic flecks/nothing
3 = Few small pustules/nothing

Table 2.3 Scoring system for *Brassica* spp. response to *A. candida*. (Leckie et al. 1996)

<table>
<tr><td>Host response</td><td>No</td><td colspan="7">Yes</td></tr>
<tr><td>Necrotic flecking</td><td></td><td>(F)N</td><td colspan="4">F</td><td colspan="2"></td></tr>
<tr><td>Chlorosis</td><td colspan="2"></td><td colspan="6">Ch</td></tr>
<tr><td>Tissue collapse</td><td colspan="6"></td><td colspan="2">Tc</td></tr>
<tr><td>Pathogen growth</td><td colspan="3">No</td><td colspan="5">Yes</td></tr>
<tr><td>Type of sporulation</td><td colspan="3"></td><td>mp</td><td>FP</td><td>MP</td><td>LP</td><td>CP</td></tr>
<tr><td>Interaction phenotype</td><td>NN</td><td>(F)N</td><td>FN</td><td>S1</td><td colspan="2">S2</td><td colspan="2">S3</td></tr>
<tr><td>Numerical categorisation</td><td>0</td><td>1</td><td>2</td><td></td><td>4</td><td>5</td><td>6</td><td>7</td></tr>
</table>

Table 2.4 Host response, pathogen growth and scores of different interaction phenotype classes. (Leckie et al. 1996)

Interaction Phenotype	Host response	Pathogen growth	Disease score
NN	No response	No sporulation	0
(F)N	Light necrotic flecking	No sporulation	1
FN	Heavy necrotic flecking	No sporulation	2
S1	Any host response	Minute pustule on upper surface of cotyledon (mp)	3
S2	Any host response	Few (FP) or numerous pustules (MP) on lower surface of cotyledon	4 5
S3	Any host response	Large scattered (LP) or coalescing pustules (CP) on lower surface of cotyledon	6 7

Interaction phenotypes (IP): *0–2* resistant, *3–5* moderately susceptible (tolerant), *6–7* susceptible

5 = Many small pustules/few small pustules
7 = Few small pustules/many larger pustules
9 = Nothing/large coalescing pustules (Fox and Williams 1984)

2.5.3 Jat's (1999) 0–5 Disease Scoring Scale for Leaf Phase

The individual plants are divided into three equal parts (bottom, middle, and top) and disease is scored as follows:

Grade/numerical scale	Description/percentage of leaf area affected	Disease reaction
0	No symptom on leaf	Highly resistant
0.1–1.0	Small, few raised, scattered pustules on leaf covering upto 5 % leaf area	Resistant
1.1–2.0	Raised pustules covering upto 10 % leaf area	Moderately resistant
2.1–3.0	Raised pustules covering upto 25 % leaf area	Moderately susceptible
3.1–4.0	Raised pustules extensive on whole leaf covering up to 40 % leaf area	Susceptible
4.1–5.0	Numerous pustules giving blighted appearance covering above 40 % leaf area	Highly susceptible

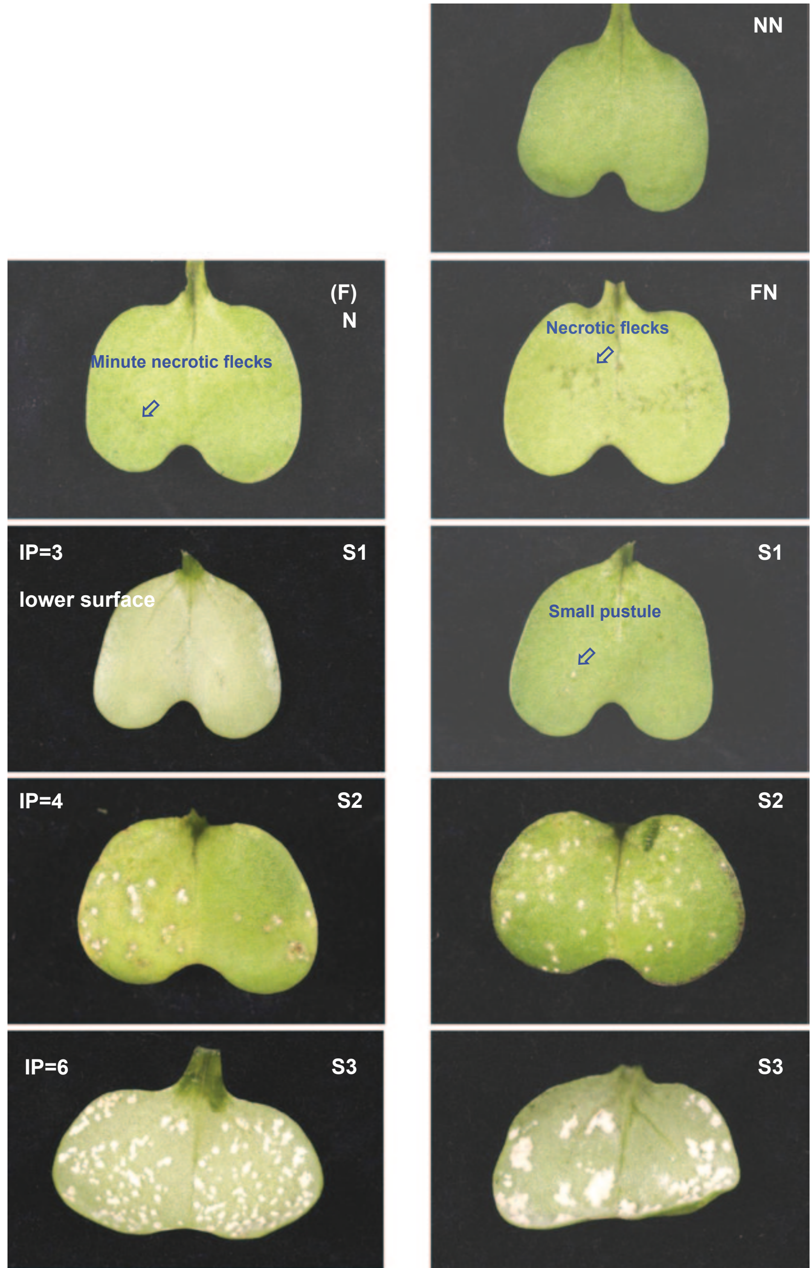

Fig. 2.28 Different interaction phenotypes at cotyledon stage. Interaction Phenotypes (*IP*): *0–2* Resistant, *3–5* Moderately susceptible (tolerant), *6–7* Susceptible. (Leckie et al. 1996)

2.5.4 Jat's (1999) 0–5 Disease Scoring Scale for Staghead Phase

Scoring Scale: Staghead Phase

Grade/numerical scale	Description/percentage of leaf area affected	Disease reaction
0	No staghead infection	Highly resistant
0.1–1.0	Few to 5 % pods transformed into staghead	Resistant
1.1–2.0	Up to 10 % pods or 2 to 3 inflorescence twigs transformed into staghead	Moderately resistant
2.1–3.0	Up to 25 % pods or 4 to 10 inflorescence twigs transformed into staghead	Moderately susceptible
3.1–4.0	Up to 40 % pods or 11 to 15 inflorescence twigs transformed into staghead	Susceptible
4.1–5.0	More than 40 % pods or more than 16 inflorescence twigs transformed into staghead	Highly susceptible

2.5.5 Gupta and Saharan's (2002) 0–5 Disease Scoring Scale at Both Leaf (Vegetative) and Silique (Reproductive) Phases

Scoring Scale: Leaf Phase (Gupta and Saharan 2002)

Graded numerical scale	Description/percentage of leaf area affected	Disease reaction
0.0	No symptoms on leaf	Highly resistant
0.1–1.0	Small, few raised, scattered pustules on leaf covering up to 5 % leaf area	Resistant
1.1–2.0	Raised pustules covering up to 10 % leaf area	Moderately resistant
2.1–3.0	Raised pustules covering up to 25 % leaf area	Moderately susceptible
3.1–4.0	Raised pustules extensive on whole leaf covering up to 40 % leaf area	Susceptible
4.1–5.0	Numerous pustules giving blighted appearance covering above 40 % leaf area	Highly susceptible

Scoring Scale: Staghead Phase (Gupta and Saharan 2002)

Graded numerical scale	Description/percentage of siliquae/inflorescence twigs transformed into staghead	Disease reaction
0.0	No staghead infection	Highly resistant
0.1–1.0	Few to 5 % siliquae transformed into staghead	Resistant
1.1–2.0	Upto to 10 % siliquae or 2 to 3 inflorescence twigs transformed into staghead	Moderately resistant
2.1–3.0	Upto to 25 % siliquae or 4 to 10 inflorescence twigs transformed into staghead	Moderately susceptible
3.1–4.0	Upto to 40 % siliquae or 11 to 15 inflorescence twigs transformed into staghead	Susceptible
4.1–5.0	More than 40 % siliquae or more than 16 inflorescence twigs transformed into staghead	Highly susceptible

2.5.6 Assessment of White Rust Disease Severity

White rust severity expressed as percentage of total plant tissue area affected (area of plant tissue affected) on foliage has been assessed using the following 0–5 disease scoring grades for both foliage and staghead phases (Lakra and Saharan 1990; Saharan 1992a, b):

(i) Foliage Infection

Grade	Description	Per cent disease severity
0	No symptoms on leaf	0.0
1	Small, few raised scattered pustules/colonies covering 1–5 % leaf area	1–5
2	Raised pustules/downy growth covering 6–10 % leaf area	6–10
3	Raised pustules/downy growth covering 11–25 % leaf area	11–25
4	Raised pustules/downy growth covering 26–40 % leaf area	26–40
5	Numerous pustules/downy mildew covering more than 40 % leaf area	41–100

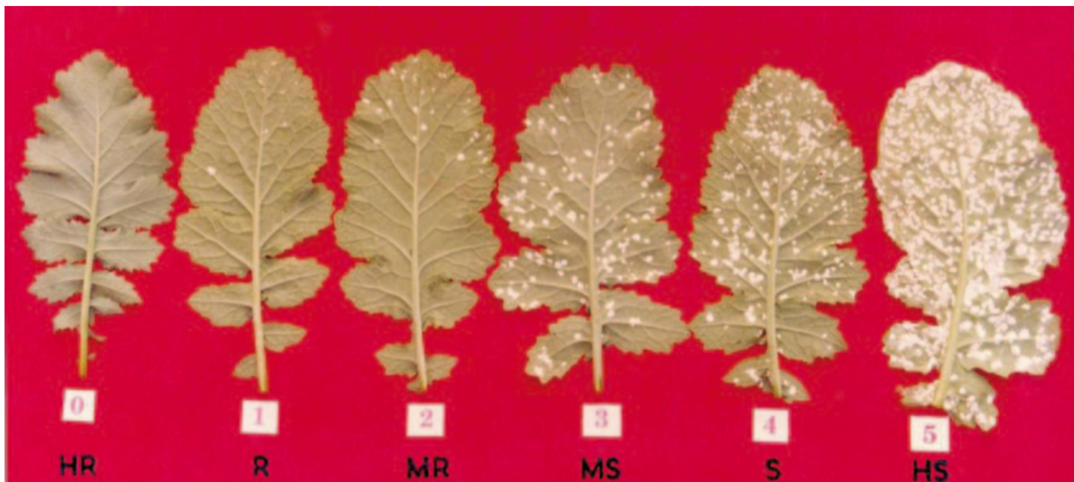

Fig. 2.29 *White* rust rating scale (0.5) on leaves

The disease indices were calculated by using McKinney (1923) formula:

$$\text{Disease index (\%)} = \frac{\text{Sum of all numerical rating}}{\text{Total No. of leaves assessed}} \times \frac{100}{\text{Maximum grading}}$$

(ii) Staghead/malformation infection

The disease incidence of staghead/malformation was calculated by counting both the diseased and total number of plants in a plot. The disease severity scores of the malformed inflorescence (the length of area covered with growth of the pathogens) were recorded and converted into following scores:

Length of staghead (cm)	Score
0–3	1
3–6	2
6–9	3
9–12	4
More than 12	5

(iii) Per cent disease control

Per cent disease control was calculated by using the following formula:

$$\text{Disease control (\%)} = \frac{\text{Disease index in contol} - \text{Disease index in treatment}}{\text{Disease index in control}} \times 100$$

(iv) Area Under Disease Progression Curve (AUDPC)

The AUDPC was calculated by using formulae as suggested by Shaner and Finney (1977), and Campbell and Madden (1990).

$$\text{ADPC} = \sum_{i=1}^{n} \{(x_i + 1 + x_1)/2\}\{t_i + 1 - t_i\} = i = 1$$

Where:

n = Total no. of observations

x_i = Proportion of tissue affected at the i th observations

t = Time (days) of the inoculation at the i th observation

2.5.7 A Pictorial Key (0-5 scale)

A disease scoring pictorial key was developed and used by researchers (Verma, Saharan, Jat and Sudheer Kumar unpublished) to categorise host-pathogen interaction phenotypes (IP) into HR, R, MR, MS, S, and HS (Fig. 2.29).

2.5.8 The Rate of Disease Progression Method (Vander Plank 1963)

The rate of disease progression is computed with the method suggested by Vander Plank (1963).

Table 2.5 Analysis of variance for disease intensity of white rust (*A. candida*) in Indian mustard (*B. juncea*) genotypes under normal-and-late-sowing dates. (Gupta et al. 2002b)

Source of variance	d.f.	Date of sowing	Mean squares	
			WRL	WRS
Replications	2	N	0.02	0.01
		L	1.09	0.7
Genotypes (G)	13	N	6,025.3[a]	8.0[a]
		L	7,988.5[a]	33.5[a]
Environment (E)	2	N	4,551.8[a]	12.9[a]
		L	5,080.4[a]	49.8[a]
GXE	26	N	157–9[a]	1.2
		L	262.2[a]	3.9[a]
Error	82	N	0.7	0.02
		L	0.7	0.1
CV		N	2.7	21.4
		L	2.1	12.9

N normal, *L* late, *d.f.* degree of freedom, *WRL* white rust at leaf phase, *WRS* white rust at inflorescence phase
[a] Significant at $P = 0.05$

$$R = \frac{2.3}{t_2 - t_1} \log \frac{X}{X_0}$$

Where:

$t_2 - t_1 = t$ = Time interval beween X and X_0
t_2 = Time when X was recorded
t_1 = Time when X_0 was recorded
X_0 = Initial amount of disease at time t_1
X = Final amount of disease at time t_2
r = Apparent infection rate per period

2.5.9 Disease Scoring Scale 0–9 for Assessing White Rust Reaction Singh et al. 1999, 2002

Singh et al. (1999, 2002) developed 0–9 disease scoring scale for assessing WR reaction on both cotyledons and true leaves of crucifers which was modified from Pound and Williams 1963 .

0 = No symptoms or sign of *A. candida* infection
1 = Pinpoint necrotic flecks at inoculation site, no sporulation
2 = Larger necrotic flecks at inoculation site, no sporulation
3 = Sparse sporulation, upto 5 % of surface covered with pustules
4 = 6–10 % of leaf area covered with pustules
5 = 11–20 % of leaf area covered with pustules
6 = 21–30 % of leaf area covered with pustules
7 = 31–50 % of leaf area covered with pustules
8 = 51–75 % of leaf area covered with pustules, and
9 = >75 % of leaf area covered with pustules.

2.5.10 Gupta et al. (2002a, b) and Saharan (1992a, b) 0–5 Disease Scoring Scale for Assessing White Rust Severity on B. juncea Under Genotype-Environment Interactions

The analysis of variance for WR disease intensity, at leaf (LP), and inflorescence phases (SP), in various Indian mustard genotypes in different environments under normal (22nd October 1999) and late-sown (7th November 1999) dates (Table 2.5) revealed that genotypes, environments and genotype X environment interactions were significant for both phases under normal-and-late-sown-dates except for SP under normal-sown conditions only (Table 2.6). Significant mean squares amongst different genotypes showed considerable genetic variations for the disease at both phases. Significant mean squares due to environments indicated considerable variations between the natural-and-artificially-inoculated environments. Significant mean squares due to genotype x environment interactions in-

Table 2.6 White rust (*A. candida*) disease intensity on Indian mustard (*B. juncea*) genotypes under-normal-sowing dates. (Gupta et al. 2002b)

Genotypes	Disease intensity (%)							
	Leaf phase (LP)				Inflorescence phase (SP)			
	WR				WR			
	N	A	C	M	N	A	C	M
EC-129126-1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
RH 781	69.1	75.2	48.7	64.3	0.9	1.0	0.0	0.6
PHR-1	35.4	36.8	12.6	28.2	0.3	0.3	0.0	0.6
Varuna	70.2	72.4	42.5	61.7	2.6	3.0	0.0	0.2
Kranti	61.5	63.2	34.2	52.9	1.0	1.2	0.2	0.8
RH 8113	34.2	37.0	14.2	28.4	3.9	5.0	0.9	3.2
RL 1359	44.3	46.4	21.1	37.2	1.7	1.8	0.3	1.2
Rajat	50.6	53.8	28.5	44.3	1.1	1.2	0.0	0.7
HR 30	80.2	82.4	49.0	70.5	1.6	1.8	0.2	1.2
Pusa bold	54.3	57.2	23.7	45.0	1.2	1.2	0.0	0.8
PR-8805	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
RC 781	13.2	14.8	2.5	10.1	0.0	0.0	0.0	0.0
ZEM-1	2.4	2.25	0.0	1.5	0.0	0.0	0.0	0.0
Shiva	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	36.8	38.6	19.7	31.7	1.0	1.2	0.2	0.8
CD 5% (G)				0.8				0.2
CD 5% (E)				0.4				0.1
CD 5% (GxE)				1.4				0.3
CV				2.7				21.4

Significant at $P = 0.05$
WR white rust, *N* natural diseased environment, *A* artificially pathogen-inoculated-environment, *C* disease-controlled environment, *M* mean, *G* genotype, *E* environment

dicated that the genotypes interacted differently under different environments. The comparison of mean squares due to genotypes and environments revealed that mean squares due to environments were higher than the genotypes, indicating that the disease intensity variation was less between genotypes than under different environments. The low coefficient of variance suggested adoption of effective local control on both dates of sowing.

Comparison of mean squares for normal-and-late-sown conditions indicated that the mean squares for genotypes, environments, and genotype x environment interactions were significantly higher under late-sown than under normal-sown conditions.

Disease intensity in various genotypes of Indian mustard under normal date of sowing (Table 2.6)

A critical perusal of the table revealed that *B. juncea* genotypes EC-129126-1, Shiva and PR-8805 showed no incidence of WR at both the phases indicating high degree of resistance against this fungus; RC 781 and ZEM-1 also showed 0% disease intensity at SP.

The low mean values of disease intensity at both phases were recorded where recommended fungicides were used. In general, addition of pathogen inoculum for creating disease pressure considerably helped to increase disease intensity. It is evident, that higher average disease intensity was observed under artificially-inoculated than natural-diseased environment. RH 30 showed highest disease intensity at LP, whereas RH-8113 contracted higher disease intensity at SP.

Disease intensity in various genotypes of Indian mustard under late-sown conditions (Table 2.7)

Table 2.7 White rust (*A. candida*) disease intensity on Indian mustard (*B. juncea*) genotypes under late-sown date (Gupta et al. 2002b).

Genotypes	Disease intensity (%)							
	Leaf phase				Inflorescence phase			
	WR				WR			
	N	A	C	M	N	A	C	M
EC-129126-1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
RH 781	83.1	87.4	60.2	76.8	1.8	2.5	1.1	1.8
PHR-1	43.9	47.0	25.4	38.7	0.6	1.3	0.4	0.7
Varuna	80.2	85.8	56.9	74.3	4.4	6.1	1.8	4.1
Kranti	71.8	75.5	47.8	65.0	2.9	4.0	0.4	2.4
RH 8113	42.0	37.0	14.2	28.4	3.9	5.0	0.9	3.2
RL 1359	44.3	47.0	27.0	38.6	7.4	9.9	2.6	6.6
RAJAT	60.2	68.2	39.3	55.9	2.6	3.7	0.5	2.2
RH 30	90.9	91.9	34.1	72.3	2.1	3.6	0.3	1.8
Pusa bold	62.1	69.4	34.1	55.2	1.9	3.1	0.3	1.8
PR-8805	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
RC 781	22.8	25.0	12.7	20.1	0.0	0.0	0.0	0.0
ZEM-1	0.09	0.03	0.0	0.0	0.0	0.0	0.0	0.0
Shiva	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	43.6	46.8	26.3	38.9	1.9	2.8	0.6	1.7
CD 5% (G)				0.8				0.2
CD 5% (E)				0.4				0.1
CD 5% (GxE)				1.3				0.4
CV				2.1				12.9

Significant at $P = 0.05$

WR white rust, *N* natural diseased environment, *A* artificially-pathogen-inoculated environment, *C* disease-controlled environment, *M* mean, *G* genotype, *E* environment

The perusal of this table indicated that both *B. juncea* genotype EC-129126-1 and cultivar Shiva were completely free from WR incidence at both phases irrespective of the environment. RH 30 recorded highest WR disease intensity at the LP under natural, artificially-inoculated, and disease-controlled environments, whereas, RH 781 recorded lowest disease intensity only under controlled-environment. Although, the use of recommended fungicides considerably reduced the incidence, complete control was not achieved. The higher mean values of disease intensities in artificially-inoculated plants suggest that spray of inoculum increased disease pressure.

Higher disease intensity and greater genetic variations were observed under late-sown conditions. Similar results have also been reported by Kolte (1985a, b); Saharan (1980, 1984, 1992a, b) and Saharan et al. (1988). Results of WR disease intensity, both at LP and SP, under normal-sown conditions revealed that EC- 129126-1, Shiva and PR-8805 were completely free from WR incidence indicating high degree of resistance against *A. candida* (Gupta et al. 2002a), which has also been reported earlier by Saharan (1995). RC 781 and ZEM-1 also showed 0.0% disease intensity at SP. These findings confirm the results of Gupta and Singh (1994), and Saharan et al. (1988). The lower mean disease intensity values at both the phases in the disease-controlled environment were attributed to the use of recommended fungicides ridomil-MZ and mancozeb (Kumar 1996). In general, the spray-inoculation of the pathogen at vegetative phase increased the disease intensity considerably, and adversely affecting the performance of different genotypes including seed yield.

2.5.11 Li et al. (2007, 2008) 0–6 disease scoring scale for assessing both disease incidence and severity (modified from Singh et al. 1999 and Mukherjee et al. 2001)

Critical information in the assessment of disease are determination of both the percentage of the total plant population diseased (*disease incidence*), and the percentage of the plant surface area that is affected (*disease severity*).

Disease incidence

0 = no symptoms or sign of pustules; 1 = 1–10 %; 2 = 11–20 %; 3 = 21–30 %; 4 = 31–50 %; 5 = 51–75 %; 6 = > 75 % of leaf area with white rust pustules.

Disease severity

1 = 1–10 %; 2 = 11–20 %; 3 = 21–30 %; 4 = 31–50 %; 5 = 51–75 %; 6 = > 75 % of leaf area covered by pustules.

The AUDPC was calculated separately for disease incidence and disease severity (Campbell and Madden 1990).

$$Y = \sum \frac{[x_i + x_i + 1]}{2} \left(t_i + 1 - t_i \right)$$

Where y is the AUDPC, X_i the disease incidence/severity of the i th evaluation $x_i + 1$ is the disease incidence/severity of the i + 1th evaluation, and $t_i + 1 - t_1$ is the number of days between two evaluations.

2.6 Yield Loss

Earlier researchers (Chupp 1925; Klemm 1938; Loof 1960) have reported only minor yield losses from WR in several cultivated crops. However, with the passage of time WR has become a major problem in cruciferous crops in many countries; the SP (hypertrophies of inflorescence) causes substantial yield losses depending on the intensity of infection. Yield losses in rapeseed in north central Alberta, Canada were 1–2 % in 1971 (Berkenkamp 1972), while in Manitoba, Canada, losses of 30–60 % were reported in severe WR-infected fields (Bernier 1972). Petrie and Vanterpool (1974) reported yield losses of up to 60 % due to *A. candida* staghead infections in rapeseed in Saskatchewan, Canada. Under field conditions, Harper and Pittman (1974) determined the relationship between the severity of systemic infection of the stem and the seed yield of rapeseed using an equation Y, 'yield loss' = 0.952 X % stems infected systemically. In Canada, the loss estimates from hypertrophied inflorescences in *B. rapa* were 1.68, 4.13 and 2.43 million $ for 1970, 1971 and 1972, respectively. In Western Australia, Barbetti (1981) estimated annual yield losses of 5–10 % due to stagheads in rapeseed (Table 2.9). Mixed infections of *Albugo* and Hyalo*peronospora* in *B. juncea* cause yield losses of 17–32 % in India (Bains and Jhooty 1979, 1985). Losses in yield due to mixed infections of DM and WR in *B. rapa* var. Toria is about 34 % if the average length of the individual hypertrophied racemes is 10 cm (Kolte et al. 1981). To estimate the losses due to mixed infections of WR and DM, or WR alone, Kolte et al. (1981) suggested the following formula:

$$Q = A - (BXC)/Ax100$$

Where: Q = % yield loss; A = average actual or expected yield of a healthy plant; B = average expected yield from the diseased raceme, which is equal to actual average yield from the corresponding length of a healthy raceme, and C = number of diseased racemes per plant.

Saharan et al. (1984), Saharan and Lakra (1988) and Lakra and Saharan (1989a) estimated yield losses of 23–54.5 % in Indian mustard cultivars RH-30 and Prakash (Table 2.8). In their study yield losses were correlated with intensity of infections at LP and SP combined, and in isolation. This study clearly showed the effect of each disease severity category on different parameters contributing to yield loss; e.g., number of primary and secondary branches/plant, number of siliqua/branch, siliquae length, number of seeds/siliqua, 1000-seed weight, and total yield per plant. Yield losses of 27.4 and 62.7 % were attributed to plants having the highest disease severity values at both LP, and SP; combined se-

Table 2.8 Effect of WR disease intensity categories on yield and yield components of *B. juncea*. (Lakra and Saharan 1989a)

Categories of infection	Branches/plant	Pods/branch	Pod length (cm)	Seeds/pod	1,000 seed weight (g)	Yield/plant (g)
Healthy plant	34.0	31.0	4.6	13.8	3.3	46.0
1 LP	33.5	30.5	4.5	13.7	3.2	45.1
2 LP	32.9	30.2	4.4	13.6	3.2	44.0
3 LP	32.0	29.8	4.4	13.5	3.2	41.6
4 LP	30.0	28.6	4.3	13.3	3.2	36.8
5 LP	28.1	28.0	4.3	13.0	3.2	33.5
1 LP+1 SP	32.1	30.0	4.4	13.7	3.2	42.3
2 LP+2 SP	30.1	28.2	4.4	13.4	3.2	38.1
3 LP+3 SP	27.5	26.5	4.3	11.5	3.1	27.7
4 LP+4 SP	23.1	20.2	3.5	9.0	3.0	15.6
5 LP+5 SP	9.7	14.1	3.2	8.0	2.8	4.7
CD at 5%	1.3	1.8	0.03	1.0	0.1	1.2

LP leaf phase infection, *SP* staghead phase infection

Table 2.9 Yield losses in *Brassica* crops due to WR

Brassica crops	Yield loss (%)	Location	Year	Reference
Rapeseed	1–2	Alberta, Canada	1971	Berkenkamp (1972)
Rapeseed	30–60	Manitoba, Canada	1971	Bernier (1972)
Rapeseed	60	Saskatchewan, Canada	1974	Petrie and Vanterpool (1974)
Rapeseed	5–10	W. Australia	1981	Barbetti (1981)
B. juncea	17–32	India	1979	Bains and Jhooty (1979)
B. rapa var. Toria	34	India	1981	Kolte et al. (1981)
B. juncea var. RH 30	23–54.5	India	1984	Saharan et al. (1984)
B. juncea var. Prakash	89.8	India	1988	Saharan and Lakra (1988); Lakra and Saharan (1989a)
B. juncea var. Varuna	60	India	2002	Meena et al. (2002b)

vere infection at both phases reduced the yield by 89.8%. To estimate the yield losses, a prediction equation has been suggested:

$Y = a_1x_1 + a_2x_2$, where Y = % loss in yield, $a_1 = 0.437x_1$ and $a_2 = 1.176x_2$ is constants, and x_1 and x_2 are disease indices on LP and SP, respectively.

Meena et al. (2002b) observed that the yield losses in Indian mustard due to WR was higher in treatments under irrigation compared to other cultural practices including nutrition, date of sowing, and cropping systems. For predicting yield (y), equations (y=2573.6–82** wrsp; y=2634.8–82.2** wrsv; y=2550.4–22.3** sh) were fitted well on per cent WR disease severity on leaves, and the number of stagheads per plot using cv. Varuna (wrsv), and cv. PCR-7 (wrsp).

With delay in planting time, disease intensity increases rapidly in both LP and SP, and yield reduces drastically (Table 2.8, Fig. 2.30). Delayed-planting exposes host plants for a longer duration to high amount of inoculum pressure coupled with congenial weather variables. Although, early-sown crop gets the infection first, but lack of congenial weather conditions hinders rapid disease progression. The number of opportunities for secondary infection in a host plant affects the severity of epidemics via multiple secondary infection points per leaf or per inflorescence. The late-sown crops have more opportunities for multiple secondary infections, and thus produce maximum stagheads and the lowest yield. Lakra and Saharan (1990) and Kolte et al. (1986) also reported least staghead infection in mustard, Yel-

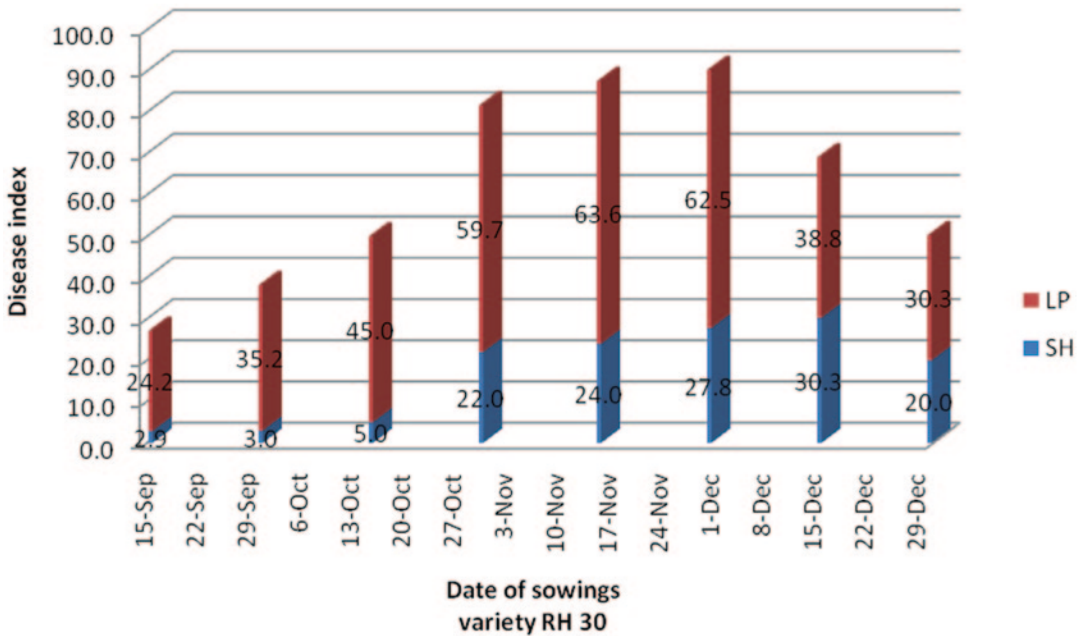

Fig. 2.30 Effect of date of sowing on WR incidence and yield of Indian mustard. (Lakra and Saharan 1990)

low Sarson and Toria crops planted in first week of October. Barbetti (1981) also observed less incidence of both LP and SP infections in normal-planting dates, and suggested that level of seed contamination affected final staghead incidence. Maximum stagheads were produced due to secondary infection, and their incidence depended on initial disease severity and congenial environmental factors prevalent during inflorescence initiation, and flower bud opening. Dew/rain/fog/high humidity provides high amount of condensation in the form of water droplets which serves as a generation house for secondary infection when temperature is low (12–14 °C) by facilitating sporangial germination and hence infection. Medium rainfall coupled with lower temperature range (14.3–17 °C) in the crop season have also been reported congenial for WR under Punjab conditions (Bains and Jhooty 1979, 1985).

The wide range of yield losses in *Brassica* crops (Table 2.9) estimated (1 to 90 %) by different workers in the world indicates the presence of an array of disease tolerance genes in the host (Saharan 2010). There is a need to assess these tolerance genes in each crop under suitable disease stress environmental conditions. Gupta et al. (2002b) recorded diversity in disease intensities of *A. candida* in different Indian mustard genotypes (Table 2.10, 2.11; Figs 2.31, 2.32, 2.33, 2.34) under-normal-and-late-sown crops in naturally-infected, artificially-inoculated, and disease-controlled environments.

Disease stress tolerance index (DSTI) was found to be effective selection criteria for assessing genotypes for their higher disease stress tolerance and yield potential. The genotypes Rajat, Kranti and RH 781 under normal-sown, and Rajat (PCR-7), RL 1359 and Kranti under late-sown conditions performed with uniform superiority in both non-disease-stress and disease-stress environments. Both, normal-sown conditions, and disease-controlled-environments (Yp) were found significantly positively correlated with yield under disease-stress-environment (Ys), mean productivity (MP), disease tolerance (TOL), geometric mean productivity (GMP), and disease stress tolerance index (DSTI), whereas, under late-sown conditions and non-disease-stress environment (Yp), the potential yield had significant positive association with yield under disease-stress-environment (Ys), MP, GMP, and DSTI (Gupta et al. 2002a).

Table 2.10 Under normal date of sowing estimates of disease stress tolerance attributes from the potential yield under disease-stress-environment (*DSI* SI0.195) in genotypes of Indian mustard. (Gupta et al. 2002c)

Genotypes	YP	YS	MP	TOL	DSSI	GMP	DSTI
EC-129126-1	2,508	2,030	2,272	472	1.0	2,260	0.9
RH 781	2,635	2,063	2,349	572	1.1	2.332	0.9
PHR-1	2,034	1,753	1,894	281	0.7	1,888	0.6
Varuna	2,586	2,087	2,337	499	1.0	23.23	0.9
Kranti	2,738	2,217	2,478	521	1.0	2,464	1.0
RH 8113	2,318	1,886	2,102	432	1.0	2,091	0.8
RL 1359	2,680	2,025	2,353	655	1.3	2,330	0.9
Rajat	2,807	2,234	2,524	573	1.1	2,504	1.1
RH 30	2,769	1,897	2,333	872	1.6	2,292	0.9
Pusa bold	2,463	1,856	2,160	607	1.3	2,138	0.8
PR-8805	2,107	1,826	1,967	281	0.7	1,961	0.7
RC 781	1,838	1,781	1,810	57	0.2	1,757	0.6
ZEM-1	1,973	1,564	1,769	409	1.1	1,757	0.5
Shiva	2,413	2,027	2,220	386	0.8	2,212	0.8
Mean	2,419	1,947	2,183	473	1.0	2,169	1.0
CD (P=0.05)	183.07	106.87	139.06	111.92	0.2	136.04	0.1

Date of sowing is 25th October
Significant at P=0.05
YP yield under disease-controlled-environment (kg/ha), *YS* yield under artificially-pathogen-inoculated-environment, *DSI* disease stress intensity, *MP* mean productivity, *TOL* disease tolerance, *DSSI* disease stress susceptibility index, *GMP* geometric mean productivity, *DSTI* disease stress tolerance index

Table 2.11 Under late date of sowing estimates of disease stress tolerance attributes from the potential yield under disease stress environment (*DSI* SI0.209) in genotypes of Indian mustard. (Gupta et al. 2002c)

Genotypes	YP	YS	MP	TOL	DSSI	GMP	DSTI
EC-129126-1	1,691	1,149	1,420	542	1.5	1,393	0.7
RH 781	1,656	1,281	1,469	375	1.1	1,456	0.8
PHR-1	1,576	1,294	1,435	282	0.9	1,428	0.7
VARUNA	1,865	1,483	1,674	382	1.0	1,663	1.0
KRANTI	1,768	1,463	1,616	305	0.8	1,608	0.9
RH 8113	1,625	1,493	1,559	132	0.4	1,557	0.9
RL 1359	1,865	1,481	1,673	384	1.0	1,662	1.0
RAJAT	1,849	1,160	1,727	244	0.6	1,723	1.1
RH 30	1,796	1,452	1,624	344	0.9	1,615	0.9
Pusa bold	1,699	1,358	1,529	341	1.0	1,519	0.8
PR-8805	1,598	1,005	1,302	593	1.8	1,267	0.6
RC 781	1,575	1,201	1,388	374	1.1	1,375	0.7
ZEM-1	1,440	1,125	1,283	315	1.1	1,272	0.6
Shiva	1,357	1,094	1,226	263	0.9	1,218	0.5
Mean	1,669	1,320	1,494	348	1.0	1,482	0.8
CD (P=0.05)	89.0	105.3	91.6	66.4	0.2	93.7	0.1

Date of sowing is 8th November
Significant at P =0.05
YP yield under disease controlled environment (kg/ha), *YS* yield under artificially-pathogen-inoculated-environment, *DSI* disease stress intensity, *MP* mean productivity *TOL* disease tolerance, *DSSI* disease stress susceptibility index, *GMP* geometric mean productivity *DSTI* disease stress tolerance index

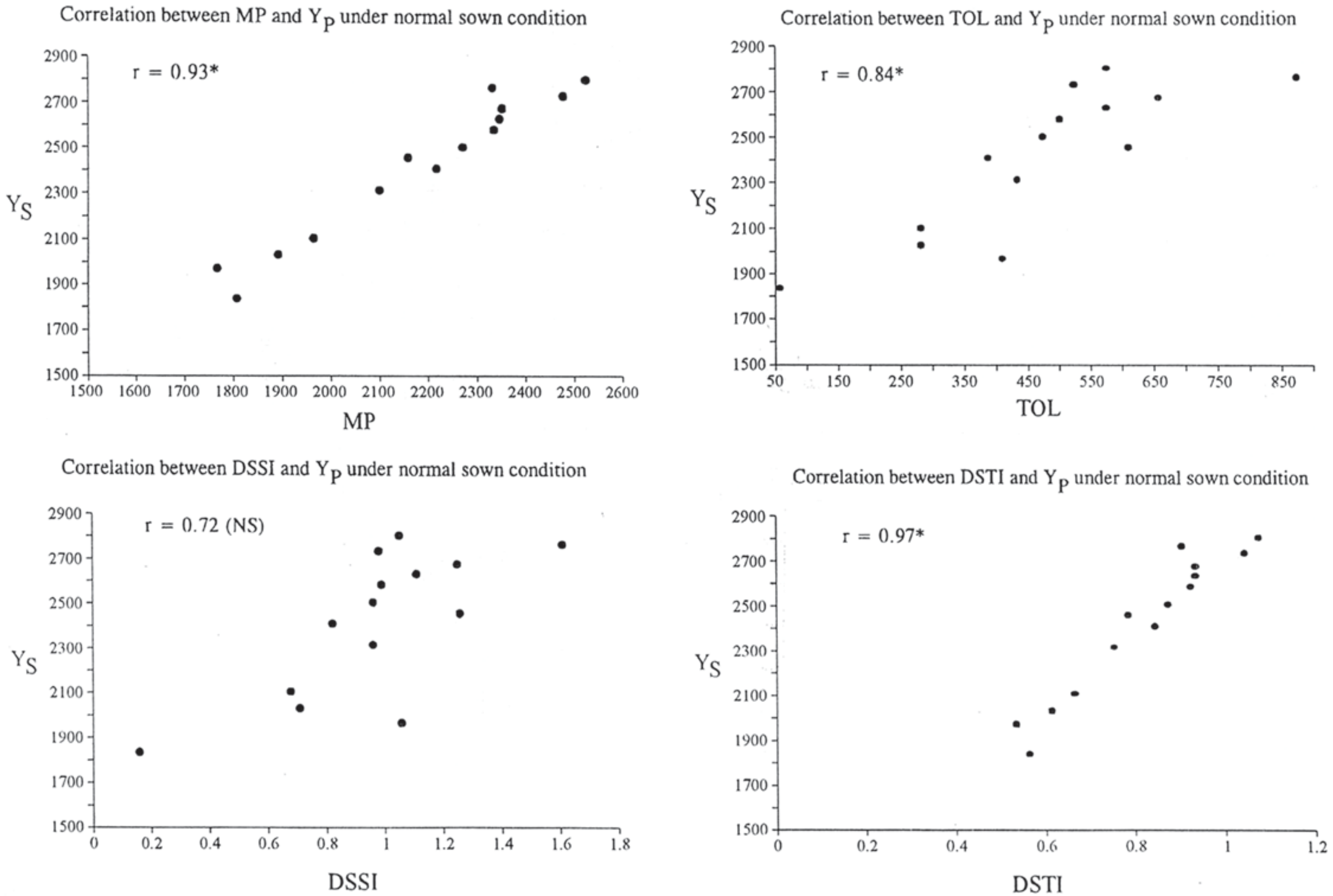

Fig. 2.31 Correlation between potential yield (*Yp*) and other stress tolerance attributes under normal date of sowing. (Gupta et al. 2002c)

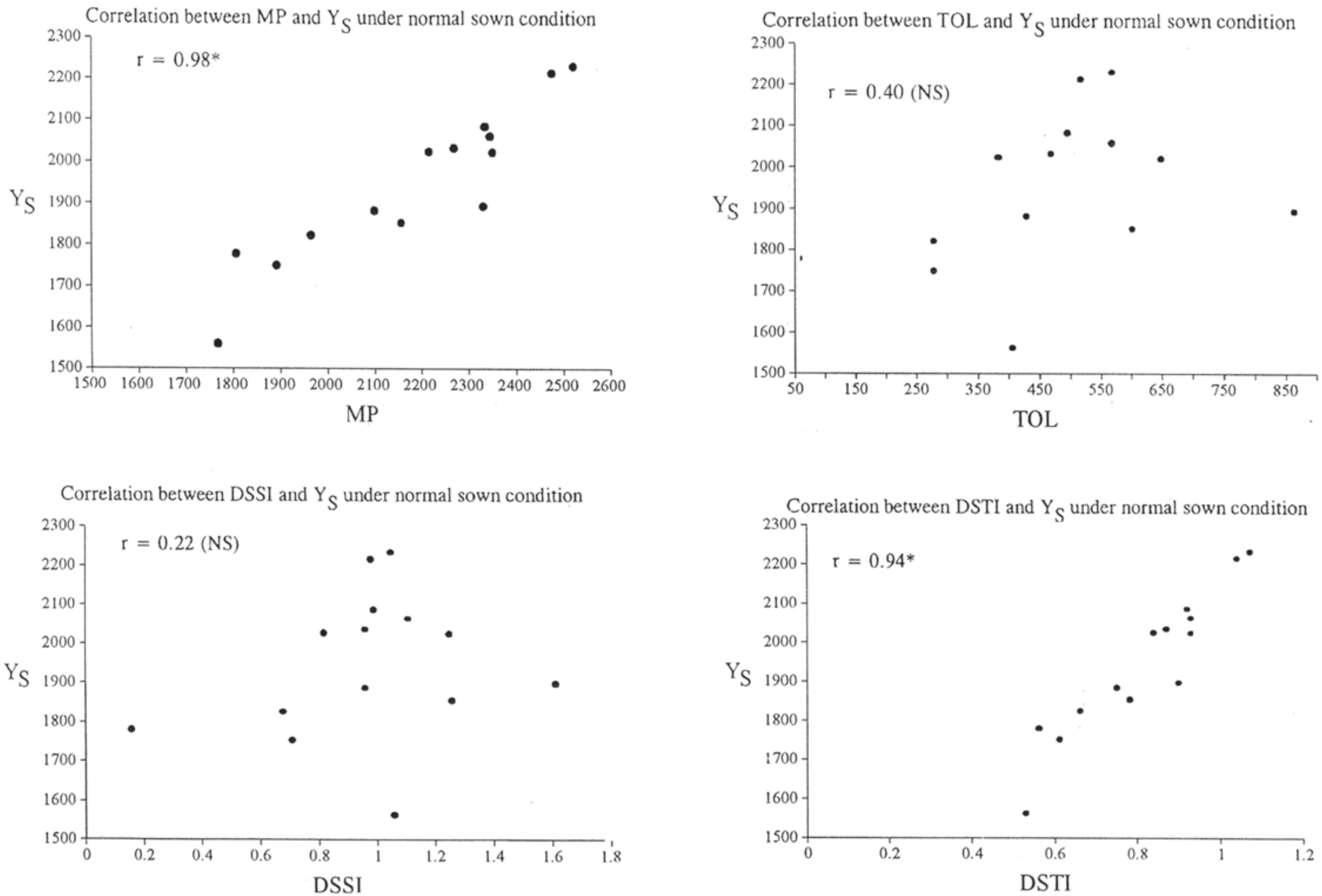

Fig. 2.32 Correlation between disease stress yield (*Y*) and other stress tolerance attributes under late date of sowing. (Gupta et al. 2002c)

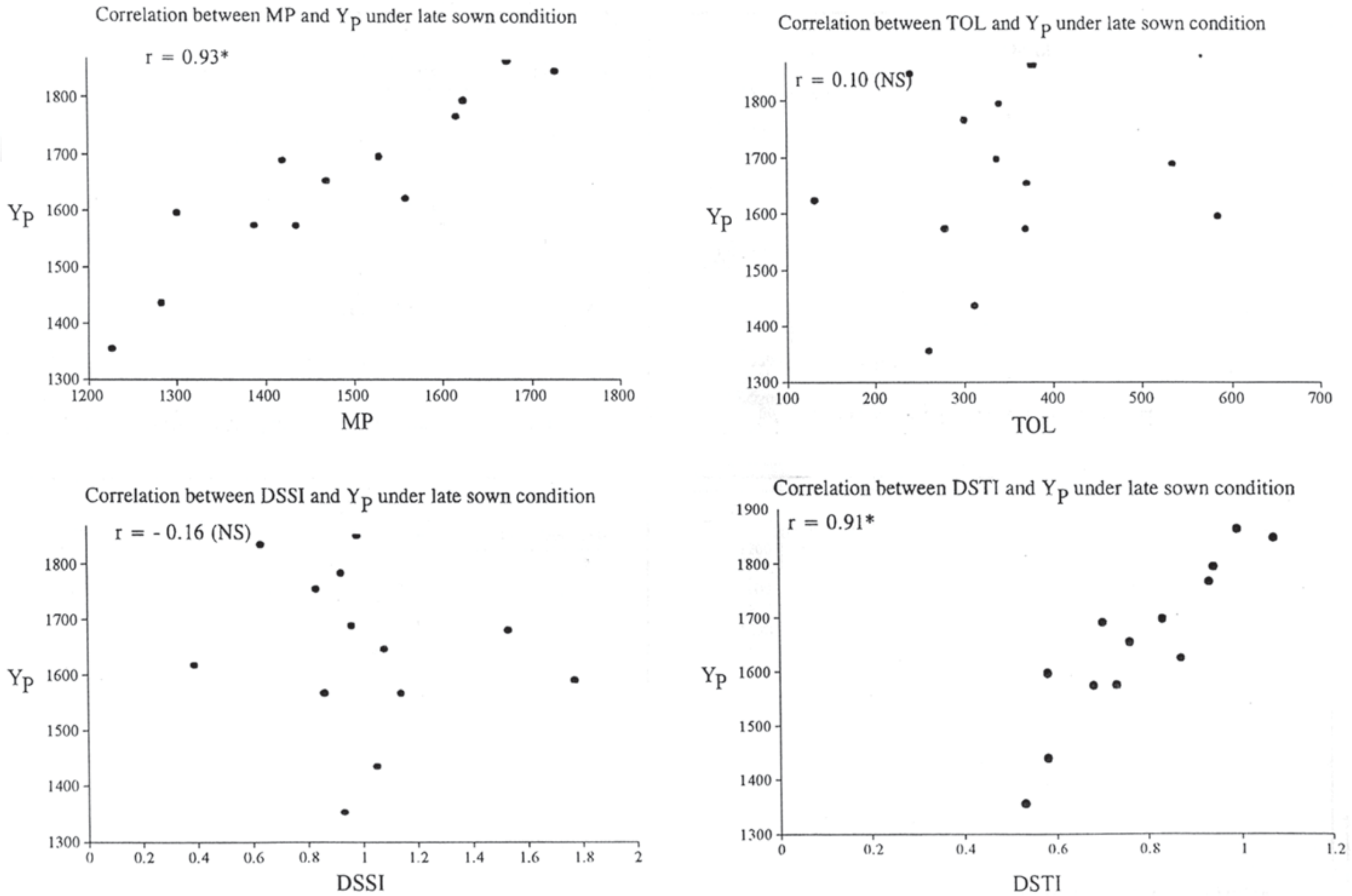

Fig. 2.33 Correlation between potential yield (*Y*) and other stress tolerance attributes under late date of sowing. (Gupta et al. 2002c)

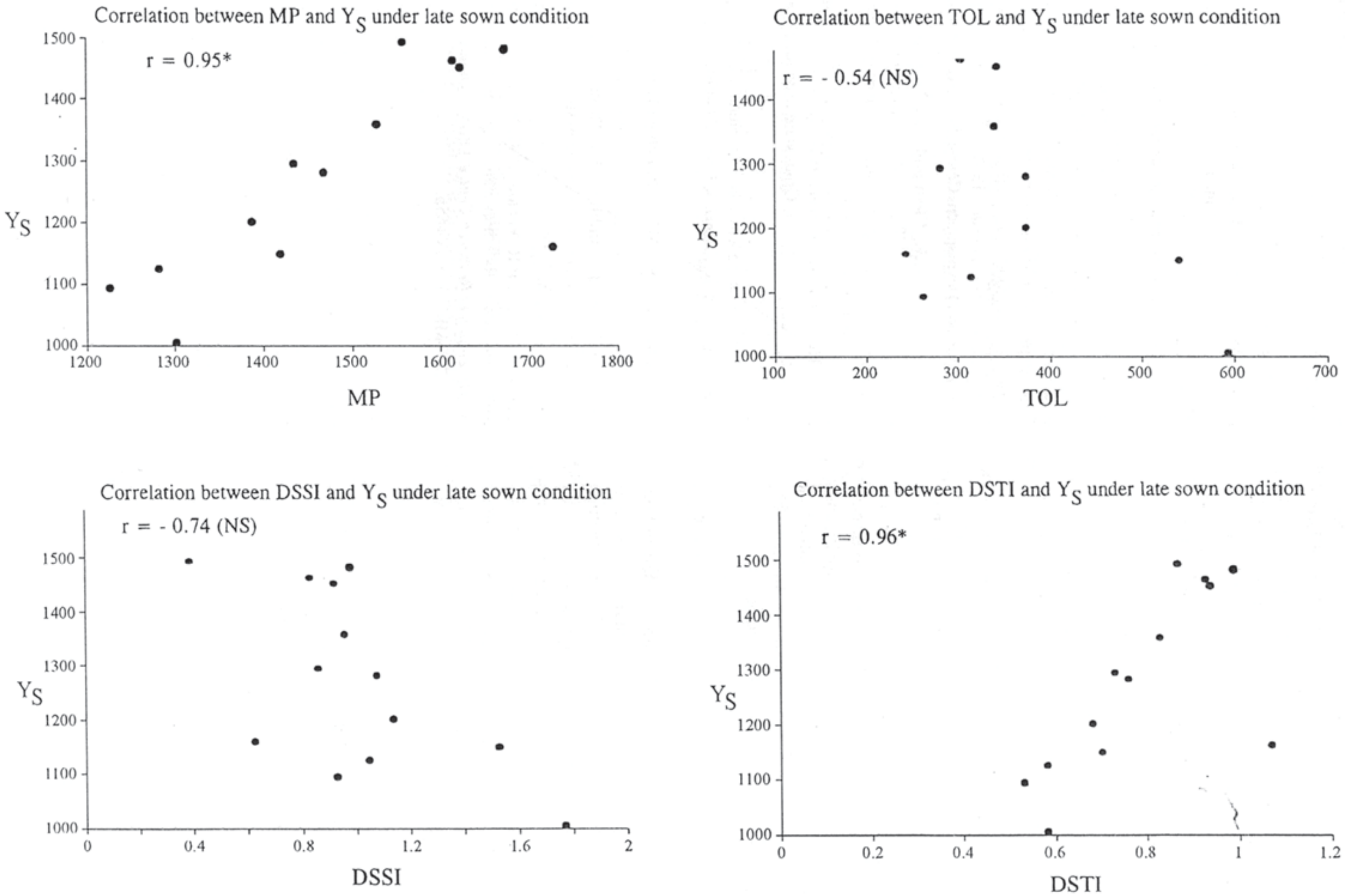

Fig. 2.34 Correlation between disease stress yield (*Ys*) and other stress tolerance attributes under late date of sowing. (Gupta et al. 2002c)

2.7 Association or Mixed Infection

The association or mixed infections, or WR-DM-disease-complex, or simultaneous occurrence of *A. candida* and *Hyaloperonospora parasitica* on leaves, stems, inflorescence, and siliquae of crucifers in nature is very common (Saharan and Verma 1992; Saharan 2010). White rust pathogen, *A. candida,* is frequently associated with the DM pathogen, *H. parasitica*. Numerous instances are known of considerable damage from combined infection (Magnus 1894; Butler 1918; Ocfemia 1925; Savulescu and Rayss 1930; Butler and Jones 1949, 1961; Vanterpool 1958; Sansome and Sansome, 1974). In India, mixed infections of DM and WR are common on *B. juncea* (Bains and Jhooty 1985; Saharan 1984). *A. candida* appears first on leaves, which probably predisposes the host to infection by *H. parasitica* (Bains and Jhooty 1985). The intensity of mixed infections varies from 0.5–35 %, depending on local weather conditions (Bains and Jhooty 1979; Saharan 1984). The hypertrophied and malformed inflorescences of *B. juncea* infected with *A. candida* are usually covered heavily with the white powdery growth of the DM spores (Chaurasia et al. 1982; Saharan 1984). *A. candida* alone, on artificial inoculation of flower buds, induces typical hypertrophy of the inflorescence (Verma and Petrie 1980). However, whether *H. parasitica* alone induces hypertrophy of the inflorescence in *B. juncea* or other hosts remains to be confirmed. The role(s) played by *A. candida* and *H. parasitica* in induction of mixed infections of inflorescences in the field is also yet to be determined. Under field conditions, *A. candida* is reported to elevate both incidence and severity of infection by *H. parasitica* in crucifers (Constantinescu and Fatehi 2002). A similar situation has been described for *H. arabidopsis* in *A. thaliana* (Holub et al. 1991) and *B. juncea* (Cooper et al. 2008) after pre-inoculation with *A. candida*. After simultaneous co-inoculation of *B. juncea* plants, Singh et al. (2002) showed that while infection with a virulent isolate of *H. parasitica* inhibited or adversely affected the development of a virulent isolate of *A. candida,* an avirulent isolate of *A. candida* induced host resistance to *H. parasitica*. Over 20 species of fungi, including several pathogens of crucifers, have been reported in association with *A. candida* produced hypertrophies of the inflorescence (stagheads), stem, and pod blisters on turnip rape (*B. rapa*), wild mustard (*B. kaber*), and false flax (*Camelina microcama*). The most prevalent fungal associates of *Albugo* are *H. parasitica, Alternaria alternata, Fusarium roseum, F. accuminatum, F. equiseti, Alternaria raphani, A. brassicae,* and *Cladosporium* sp. (Petrie 1986). *Alternaria, Cladosporium,* and *Fusarium* spp. reported here seem to be common saprophytes under humid conditions. Kaur et al. (2011b, c) reported that the presence of asymptomatic systemic colonization of *H. parasitica* in a resistant host increases susceptibility to WR disease.

Fig. 2.35 *Albugo candida* produces pustules, and *H. parasitica* produces a coating of fine *white* mass of sporangia on the malformed floral parts

2.7.1 Symptoms

Symptoms of mixed infection of WR and DM diseases of Brassicas can be seen at both LP and SP of plant growth. On leaves WR pustules are surrounded by white or creamy white sporulation of DM pathogen. On the malformed floral parts, *A. candida* produces pustules, and *H. parasitica* produces a coating of fine white mass of sporangia (Fig. 2.35). At crop maturity and under high humidity, malformed inflorescence may be covered with saprophytes turning into dark brown to black colour (Saharan 1992a, b; Saharan and Mehta 2002).

Table 2.12 Interaction between *A. candida* and *H. parasitica* during pathogenesis of *B. juncea*. (Mehta et al. 1995)

Treatment	Incubation period (days)		Latent period (days)	
	HP	AC	HP	AC
HP-AC (24 h)	7	5–6	12	12
HP alone	7	–	12	–
AC-HP (24 h)	7	5–6	12	12
AC alone	–	5–6	–	12
HP+AC	9–10	5–6	12	12
Control (without pathogen)	–	–	–	–

HP Hyaloperonospora parasitica, *AC Albugo candida*

2.7.2 Yield losses

Mixed infection of WR and DM causes loss of 17–32 % in *B. juncea* under Punjab conditions (Saharan et al. 1997; Bains and Jhooty 1979). In *B. rapa* var. Toria, yield loss of 34 % has been estimated if the average length of hypertrophied raceme is 10 cm (Kolte et al. 1981). Lakra and Saharan (1989a) observed that combined infection of WR and DM may cause yield loss upto 89.8 % during epidemic years.

2.7.3 Pathogenesis

The nature of association of mixed infection, or simultaneously occurrence, or WR-DM disease-complex, seems to be synergistic, since the magnitude of effect on host plant is increased manifold. The sequence of events during the pathogenesis of *Hyaloperonospora* and *Albugo* in Indian mustard cv RH-30 has been explained by Mehta et al. (1995; Table 2.12).

In the treatment where *Hyaloperonospora* was inoculated 24 h prior to *Albugo* (HP-AC), the infection by *Hyaloperonospora* was recorded after 7-days, and by *Albugo* after 5–6 days. Similarly, in reverse treatment i.e. (AC-HP), the incubation period of *Hyaloperonospora* and *Albugo* was same. Like-wise, the incubation period was same when both pathogens were inoculated separately. But in the treatment where both the pathogens were inoculated together (HP+AC), there was a delay in incubation period of *Hyaloperonospora*. However, *Albugo* did not show variation in its incubation period. The latent period of each pathogen was recorded 12 days in all the treatments irrespective of their time of inoculations (Table 2.12). The results showed that when both pathogens were inoculated together, there was a delay in incubation period of *Hyaloperonospora* for 2–3 days only.

2.7.4 Histopathology

In histopathology studies, the transverse sections of inoculated leaves collected at different intervals revealed that the epidermal layer was broken at many places. Generally, there was an overall deformation in the size and shape of mesophyll cells in the inoculated leaves compared to the sections of the healthy leaves.

The anatomical examinations of leaves collected 24 h after inoculation with either of the pathogen, or combination of both, did not show any penetration; germinated conidia or sporangia were seen on the leaf surface (Mehta et al. 1995). The samples collected after 2 days of inoculation indicated that the initiation of infection confined mainly to epidermis. The pathogen penetrated up to 1/3 of mesophyll cells in samples obtained 3 days after inoculation (Fig. 2.36a); 6 days after inoculation, pathogen invaded deeper into the tissue. In the treatment where *H. parasitica* was inoculated prior or after *Albugo*, mycelium could be seen in the intercellular spaces with globose to knob like haustoria in the mesophyll cells (Fig. 2.36b). Similarly, in the treatments where *Albugo* was inoculated alone or in combination with *H. parasitica*, the pathogen penetrated up to lower epidermis, and

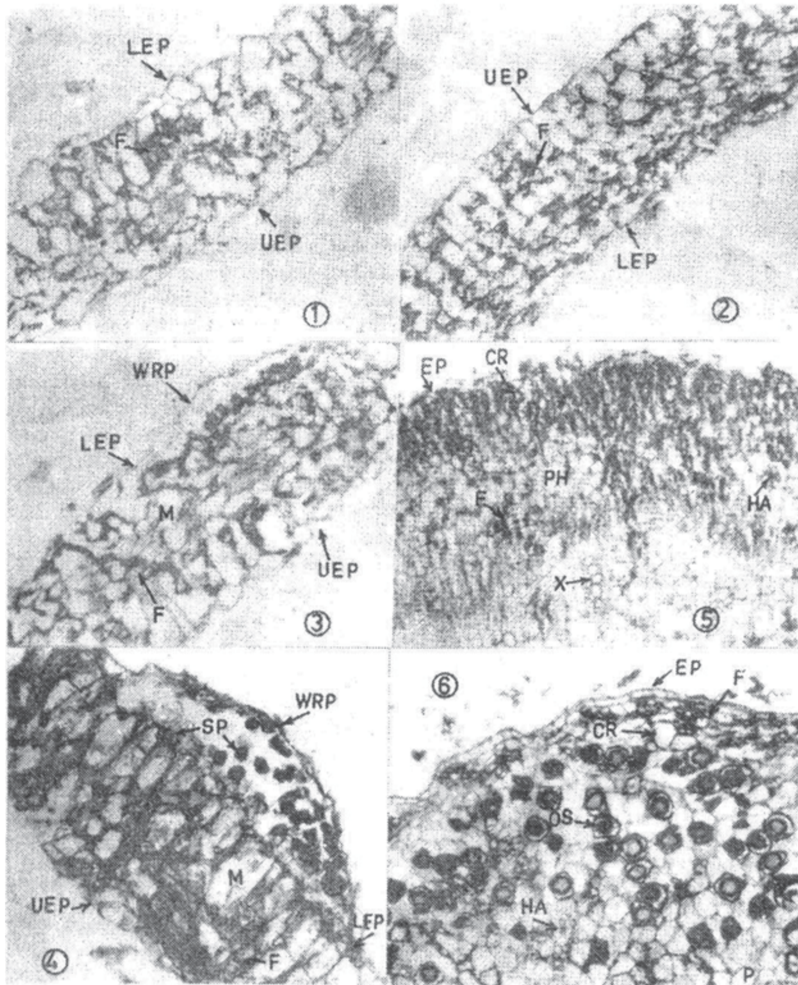

Fig. 2.36 a Transverse section of leaf 3 days post inoculation AC-HP or HP-AC showing mycelium in the intercellular spaces. *UEP* Upper epidermis layer, *LEP* Lower epidermis layer, *F* Fungus GMS x 66 x 8 Approx. (Mehta et al. 1995). b Transverse section of mustard leaf 6 days post inoculation (AC-HP) showing mycelium in the intercellular spaces. *UEP* Upper epidermis layer, *LEP* Lower epidermis layer, *F* Fungus GMS x 66 x 8 Approx. c Transverse section of mustard leaf 6 days post inoculation (AC alone) showing mycelium in the inter-cellular spaces and developing white rust pustules. *UEP* Upper epidermis layer, *F* Fungus, *M* Mesophyll cells, *WRP* White rust pustules GMS x 66 x 8 Approx. d Transverse section of mustard leaf 9 days post inoculation (AC alone) showing fungal mycelium, white rust pustules with sporangia/sporangiophores on abaxial surface. *UEP* Upper epidermis layer, *F* Fungal mycelium, *M* Mesophyll cells, *SP* Sporangia/sporangiophores GMS x 66 x 8 Approx. e Transverse section of DM-infected mustard inflorescence depicting fungal mycelium and haustoria in the cortical cells and pith. *EP* Epidermis layer, *F* Fungus, *CR* Cortex cells, *X* Xylary vessels, *Ph* Phloem, *P* Pith, *HA* Haustoria; GMS x 66 x 8 Approx. f Transverse section of white rust infected mustard inflorescence showing fungal mycelium, haustoria and oospores in the cortical, xylary vessels. *OS* Oospores, *P* Pith, *HA* Haustoria, *F* Fungus; GMS x 66 x 8 Approx. (Mehta et al. 1995)

there was development of pustules on the lower epidermis (Fig. 2.36c). Similar interaction was observed with *H. parasitica* showing necrosis in the mesophyll cells. However, when both the pathogens were inoculated together (HP+AC), the infection was confined to the upper layer of mesophylls, and there was a limited colonization of the cells, with a limited number of haustoria or mycelium in the intercellular spaces. Leaves collected after 6-days of inoculation with either

Fig. 2.37 Downy mildew growth on or around the WR pustules on the abaxial surface of mustard leaf. (Mehta et al. 1995)

Fig. 2.38 Downy mildew *white* sporulation on malformed inflorescence of mustard infected with WR. (Mehta et al. 1995)

of the pathogen inoculated alone, or in combination, showed characteristic disease symptoms (Mehta et al. 1995). The *Albugo* pustules showed hyaline sporangiophores bearing globose to oval shaped sporangia in chain (Fig. 2.36d). The *H. parasitica* mycelium was intercellular with lobe shaped haustoria in the distorted tissues of leaves (Fig. 2.36e). When both the pathogens were inoculated together, infection was extended to mesophyll cells, and there was a development of pustules below the epidermis (Fig. 2.36f). The leaves samples collected 12 days after inoculation in all the treatments showed complete colonization of the pathogen as evident from the development of necrotic zone by *H. parasitica* and bursting of pustules in case of *Albugo* releasing sporangia.

The sections of diseased inflorescence showed deep colonization of *H. parasitica*. The mycelium passed through the epidermis, hypodermis, cortex, and finally reached the pith region. The fungal mycelium was in the cortex and produced conidiophores bearing conidia above the epidermis layer. Similarly, where *Albugo* was inoculated, numerous sporangiophores bearing sporangia were observed below the epidermis layer in the form of pustules and knob. In the samples where both the pathogens were inoculated together, deep colonization of the tissues was observed, but the individual pathogen could not be distinguished in the host tissues. These studies revealed that the pathogens could invade deeper into the tissues inter-cellularly, and *Albugo* had faster growth in terms of incubation period than the *H. parasitica*. In colonized tissues, both the pathogens could not be distinguished on the basis of their somatic morphology (Mehta et al. 1995).

The incubation period of *H. parasitica* was 7 days in all the treatments, except in case where both the pathogens were inoculated together. The incubation period of *A. candida* was 5–6 days in all the treatments. These results indicate that *Albugo* colonizes the host tissue earlier than *Hyaloperonospora*. The DM colonies appear on or around the WR pustules on leaves and malformed inflorescence under field conditions (Figs. 2.37, 2.38). The *Albugo* mycelium while developing in the intercellular spaces, and by disturbing the metabolism of the cell, may create congenial situation for *Hyaloperonospora* to colonize at later stages. This could be a possible reason why incubation period was delayed in case of *H. parasitica* where mixed inoculations were done. Another possibility could be the competition between the two pathogens for the same site of infection, and *H. parasitica* may get limited site for infection and development. But once the fungus penetrated and developed, there was no delay in the production of asexual spores, since the same latent period was recorded in all the treatments (Mehta et al. 1995). Liu and Rimmer (1990) reported 5–6 days of incubation period for *Albugo,* but contrarily, *H. parasitica* has been reported to colonize the cotyledonary leaves within 4 days of inoculation (Kluczewski and Lucas 1982). The longer incubation period in case of *H. parasitica*

may be attributed to the fact that inoculation was made on true leaves than the cotyledonary leaves. In addition, the weather parameters prevailing at the time of infection may affect the time required for infection. The latent period of 12 days was recorded in all the treatments when inoculated with both the pathogens with different combinations. Kolte (1985a) observed a period of 10 days for release of sporangia in case of *Albugo* under Pantnagar conditions. The slight variation in latent period in the investigations by Mehta et al. (1995) may be due to host influence and effect of micro and macro climate prevailing around the plant (Bains and Jhooty 1985). The sections of the leaves collected at different intervals after inoculation with either of the pathogen, or in combination of both, revealed that penetration of the host started 24 h after inoculation, and complete infection and colonization took place by the third day. Showed that sporangia penetrated the cotyledonary leaves of crucifers within 24 h of inoculation. Longer incubation period reported by Mehta et al. (1995) may be attributed to the fact that inoculations were made on the true leaves. However, Kluczewski and Lucas (1982) reported colonization of *H. parasitica* on oilseed rape (*B. napus*) within 4 days of inoculation on cotyledonary leaves, whereas mycelium development continued upto 5th day accompanied by abundant sporulation. Mehta et al. (1995) reported that sections of leaves obtained after 6 days of inoculations also show production of conidiophores and conidia. The development of necrosis and complete infection of the inoculated leaves after 9 and 12 days of inoculation are in accordance with the findings of Kluczewski and Lucas (1982).

In case of *Albugo,* the sori (pustules) development was started 3-days after inoculation. According to Verma et al. (1975), *Albugo* produced the haustorium in *B. juncea* cotyledonary leaves after 16–18 h of inoculation, and the mycelium occupied the intercellular spaces within 3 days of inoculation. After 4 days, almost all the intercellular spaces of the inoculated tissues were occupied by the mycelia, and WR pustules were visible 5–6 days after inoculation (Liu et al. 1989). Similar observations have also been made by Mehta et al. (1995).

However, according to Kaur et al. (2011b, c) pre-inoculation with *Hyaloperonospora* reduces incubation period, and increases severity of WR disease in *B. juncea* variety resistant to DM. White rust symptoms appear 4 days earlier and are more severe when DM-resistant but highly-WR-susceptible *B. juncea* variety is first inoculated with *Albugo* followed 10 days later with *Hyaloperonospora.* DNA extraction of tissues, indicated that *Hyaloperonospora* has colonized the asymptomatic plants systemically. Studies of Singh et al. (2002) showed that the infection of *B. juncea* with a virulent isolate of *H. parasitica* inhibited or adversely affected the development of a virulent isolate of *A. candida.*

Under field conditions, *A. candida* can elevate both incidence and severity of infection by *H. parasitica* in crucifers (Constantinescu and Fatchi 2002). Cooper et al. (2008) observed broad-spectrum suppression of host defense by *A. candida* in *A. thaliana* and *B. juncea.* It seems that genes governing the virulence of *A. candida* in *Brassica* system eludes the plant defense mechanism, through reduction of phytoalexin biosynthetic pathway, and by producing metabolites preferred as food by the pathogen *Hyaloperonospora* for colonization of host tissues. The compatibility genes of both the pathogens may be same or situated on the same locus as alles or tightly linked or epistatic. However, detailed study is required on these aspects (Saharan 2010).

2.7.5 Epidemiology

Development of WR and DM disease complex in Indian mustard in relation to planting time and environmental interaction was investigated by Mehta and Saharan (1998). There was no staghead formation in the crops sown during September (Tables 2.13 and 2.14), but significantly higher staghead incidence was observed in crops planted late in October and November. The crops sown in mid-November although had less incidence of staghead but their intensity was signifincatly higher. Staghead length was comparatively shorter in crops planted in December, but it had higher DM incidence. The maximum sta-

Table 2.13 Effect of planting dates on the development of staghead due to DM and WR disease complex in Indian mustard cv. RH-30 during 1991–92 crop season. (Mehta and Saharan 1998)

Observation recording (week)	Date of sowing											
	8th September		24th September		19th October		29th October		4th December		Mean	
	I	L	I	L	I	L	I	L	I	L	I	L
1	–	–	–	–	0	0	5	3	3	1	2.5	1.5
2	–	–	–	–	6	2	17	11	3	2	8.8	5.1
3	–	–	–	–	7	2	17	11	5	5	10.0	6.4
4	–	–	–	–	7	2	17	11	6	6	10.2	6.6
Mean	–	–	–	–	5.2	1.6	14.2	9.5	4.2	3.5	–	–
LSD (0.05)			I = Incidence (%)				L = Length (cm)					
Date of sowing (D)			3.81				1.22					
Interval (I)			4.39				1.41					
D × I			7.62				2.45					

Table 2.14 Effect of planting dates on the development of staghead due to DM and WR disease complex in Indian mustard cv. RH-30 during 1992–1993 crop season. (Mehta and Saharan 1998)

Observation recording (week)	Date of sowing													
	15th September		30th September		15th October		5th November		18th November		4th December		Mean	
	I	L	I	L	I	L	I	L	I	L	I	L	I	L
1	–	–	–	–	18	7	29	11	16	8	14	14	13.0	6.7
2	–	–	–	–	18	8	29	11	16	8	14	15	13.0	7.1
3	–	–	–	–	18	8	29	11	16	9	14	15	13.0	7.2
4	–	–	–	–	18	9	29	11	16	9	14	15	13.0	7.2
Mean	–	–	–	–	18.0	7.8	29.0	11.0	16.0	8.5	14.0	15.0	–	–
LSD (0.05)			I = Incidence (%)					L = Length (cm)						
Date of sowing (D)			4.74					2.16						
Interval (I)			NS					NS						
D × I			NS					NS						

Table 2.15 Prediction equations for progression of WR and DM complex in relation to environmental factors during 1991–1992 and 1992–1993 crop seasons. (Mehta and Saharan 1998)

Diseases	Year	R^2	$Y = a_1$	+	b_1x_1	+	b_2x_2	+	b_3x_3	+	b_4x_4	+	b_5x_5	+	b_6x_6
White rust	1991–1992	0.66	56.119	+	1.017	−	4.086	+	8.702	−	2.015	+	2.723	+	0.890
	1992–1993	0.16	25.041	−	4.790	+	3.834	+	3.429	+	1.237	−	1.146	+	4.270
Downy mildew	1991–1992	0.21	−0.511	−	0.82	+	0.157	−	0.075	+	0.040	−	0.038	−	0.005
	1992–1993	0.36	−32.744	+	0.095	+	0.316	+	1.316	+	0.127	+	0.221	−	0.038
Malformation (incidence)	1991–1992	0.26	−0.261	+	0.048	−	0.323	+	1.460	−	0.202	+	0.342	−	0.123
	1992–1993	0.23	−18.687	−	2.858	+	2.539	+	4.540	+	0.621	−	0.268	+	1.056
Malformation (length)	1991–1992	0.28	−0.784	+	0.079	−	0.271	+	1.078	−	0.172	+	0.257	−	0.094
	1992–1993	0.26	−17.485	−	1.598	+	1.571	+	2.644	+	0.413	−	0.170	−	1.022

x_1 Temp (Maximum) °C, x_2 Temp (Minimum) °C, x_3 Sunshine (h), x_4 RH (Morning) %, x_5 RH (Evening) %, x_6 Rainfall (mm), x 0.05 level of significance, y Expected disease estimate

Table 2.16 Correlation coefficient between WR-DM disease complex and meteorological parameters. (Mehta and Saharan 1998)

Weather parameters	White rust		Downy mildew	
	1991–1992	1992–1993	1991–1992	1992–1993
Temperature (maximum)	−0.52	−0.25	0.30	0.45
Temperature (minimum)	−0.35	−0.18	0.30	0.42
Sunshine	−0.24	−0.08	0.16	0.21
RH (morning)	0.26	−0.08	−0.13	0.01
RH (evening)	0.54	−0.10	−0.24	0.16
Rainfall	0.44	0.22	−0.15	−0.12

ghead incidence was recorded from first week of January to mid-February for successive 2 years.

During the time of inflorescence formation in the September-planted crop, the weather was not favourable for staghead development. However, fewer incidences of stagheads in the December-sown crop may be attributed to improper growth of the crop. Kolte et al. (1986) reported that early-sown crop either escaped or showed least staghead incidence. The late-sown crop, however, had higher incidence and severity of stagheads. High RH and low temperature favoured staghead formation (Lakra et al. 1989; Lakra and Saharan 1990). Bains and Jhooty (1979) found that temperature of 14.3 °C and 152 mm rainfall resulted in high infection.

2.7.6 Disease Forecasting

Correlation between weather parameters and disease progression have revealed that the combination of six independent variables accounted for more than 66 % variation in WR during 1991–1992, and only 16 % during 1992–1993, although disease intensity was more (Table 2.15). Similarly, these variables accounted for 21 and 36 % variation in DM intensity during 1991–1992 and 1992–1993, respectively. All the test variables influenced floral malformation incidence and intensity to the extent of 26 and 28 %, respectively, in 1991–1992, and to the extent of 23 and 26 % respectively, in 1992–1993 crop seasons (Mehta and Saharan 1998).

The analysis of six test independent variables that fit the analysis best revealed that each variable played an important role in disease development in addition to other unknown factors which were not included in the study. The prediction equations which showed maximum R^2 value are presented in Table 2.15. Results revealed that variable evening RH played a significant role in WR development. Similarly, in case of DM, maximum and minimum temperatures played a significant role in disease development.

The correlation coefficients between diseases and meteorological parameters showed that morning and evening RH, and rainfall had positive correlation for WR, whereas, temperature had positive correlation for DM development during the two years of study (Table 2.16). The infection and progression of WR and DM on rapeseed-mustard was affected by variations in the weather factors.

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3 The Pathogen

Albugo (Pers.) Gray, 1821
= *Uredo albugo* Persoon, 1801
= *Elysibe sensu* Walker, 1833
= *Cystopus Leveilla*, 1847
= *Cystopus* deBary, 1863

Biga (1955) re-examined the morphology of the sporangia and constructed a key, in Italian, to differentiate species of genus *Albugo*. Wilson (1907) described the North American species, Savulescu (1946a, b) the Romanian species, and Wakefield (1927) gave a historical note on the genus in her account of the species in South Africa. Morphological details of *Albugo* on crucifers were given by Togashi and Shibasaki (1934), and Togashi et al. (1930, 1931). Baker (1955) upheld the validity of *Albugo* against *Cystopus*. Waterhouse (1975) described the species of *Albugo* of the Aizoaceae in the UK. An account of Indian species was given by Butler and Bisby (1934) and Safeefulla (1952a, b). Burdyukova (1980) described the Russian species of *Albugo*. Choi and Priest (1995) have given a key to the genus *Albugo.* A key to *Albugo* species parasitic to Brassicales based on oospore characteristics has also been prepared by Choi et al. (2011a, b, c) (Table 3.1). A revision of genus *Albugo* has recently been presented by Thines and Spring (2005).

Historical Account

A detailed historical account of genus *Albugo* through research developments after its discovery as a pathogen has been nicely given by Thines and Spring (2005) in chronological order. The genus *Albugo* (Pers.) de Roussel (1806) comprises ca. 50 species that are obligate biotrophic parasites of Dicotyledonae (Biga 1955; Choi and Priest, 1995). Some species, like *Albugo occidentalis, Albugo portulacearum, Albugo candida*, and *Albugo tragopogonis,* have economic relevance, causing diseases known as 'white rusts' (WR) in various crop plants. The genus *Albugo* had first been placed in the *Protomyceae* by Gray (1821). Later, deBary (1863) transferred *Albugo* to the *Peronosporaceae* under the generic name *Cystopus* Lév., which had been introduced by Léveilla (1847) after the sexual form of *Albugo* had been discovered. Through the use of *Cystopus* by deBary, this generic name became widespread and was adopted in the Sylloge Fungorum (Berlese and de Toni 1888 and proceeding volumes), and also by Wakefield (1927). Biga (1955) pointed out that the name *Albugo* was in consent with the International Code of Botanical Nomenclature, which allows the further use of *Albugo* although the sexual form had been described under the generic name *Cystopus*.

In 1893, Schröter introduced the family *Albuginaceae* to accommodate the genus *Albugo*. The monogeneric *Albuginaceae* has traditionally been placed within the order *Peronosporales* (downy mildews) (Kirk et al. 2001; Dick 2001). However, recent molecular phylogenetic studies do not support this placement (Petersen and Rosendahl 2000; Cooke et al. 2000; Riethmüller et al. 2002), but placed *Albugo* basal to the *Pythiales*. Moreover, Hudspeth et al. (2003) even doubted a closer relationship between the WR (which were found to be basal to the *Rhipidiales*

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Table 3.1 Key to *Albugo* species parasitic to *Brassicales* based on oospore characteristics. (Choi et al. 2011c)

Oospore characteristic code	Oospore characteristics	*Albugo* spp. specialized lineages
1.	Warts of wall omamentation in oospores often connected forming ridges and often branched	2
	Warts tuberculated and isolated without ridges and not branched	4
2.	Wall ornamentation pseudo-reticulated; warts mostly connected and branched	*A. hohenheimia*
	Wall ornamentation tuberculate; warts isolates or connected	3
3.	Size of oospores mostly exceeding 45 µm iam; wall omamentation mostly obvious	*A. candida*
	Size of oospores less than 45 µm diameter; wall omamentation often unclear	*A. laibachii*
4.	Warts densely and regularly distributed, blunt or rounded; oospores often exceeding 50 µm diameter	5
	Warts irregularly to regularly distributed, variable in shape; oospores mostly less than 50 µm in diameter	6
5.	Oospores yellowish to brownish; size of oospores 44–55 (av. 48.4) µm diameter	*A. voglmayrii*
	Oospores pale yellowish; size of oospores 46–61 (av. 53.6) µm diameter	*A. resedae*
6.	Warts regularly distributed, often more than 5 µm wide; tips of warts mostly blunt or rounded	7
	Warts irregularly distributed, mostly less than 4 µm wide; tips of warts obtuse, echinulate or truncate	8
7.	Warts small; 2–3.5 µm long, 2–5 µm wide	*A. lepidii*
	Warts large; 4–6 µm long, 3–6 (–7) µm wide	*A. hesleri*
8.	Warts very rarely connected, slightly curved, often elongated up to 6 µm	*A. koreana*
	Warts often connected, variable in size and shape, mostly less than 4 µm	*A. rorippae* sp. nov.

in their survey), and the Downy mildew (DM), and thus suggested that *Albugo* should be raised to ordinal level. Wolf and Wolf (1947) probably noticed the distinctness of this group as they mentioned an order *Albuginales,* but provided no description and, in addition, did not include the order in their key to the orders of the Oomycetes.

As a unique feature within the *Peronosporales,* sporangia in *Albugo* are formed basipetally in chains beneath the epidermis of the host plant, whereas in all other obligate biotrophic pathogens within the *Peronosporales,* single sporangia are formed almost simultaneously on the ultimate branchlets of sporangiophores, which emerge through the stomata of the host plant. Furthermore, the way of sexual reproduction, especially as described for the multinucleate oospore formation of *Albugo portulacae* and *Albugo bliti* (Stevens 1899, 1901a, b, c, 1904), points to the unrelatedness of the WR and the DM. The unique mode of oospore germination, producing zoospores without prior formation of a germ tube (deBary 1863; Schröter 1893), also clearly separates *Albugo* from the rest of the *Peronosporales*.

Due to the seemingly morphological uniformity of *Albugo,* the phylogenetic studies have been mainly restricted to molecular phylogenetics, *A. candida* being the only species examined. Although characters like the structure of sporangia or oospore have been used to distinguish between species or species groups within *Albugo,* they have mostly been applied to construct identification keys (Migula 1910; Biga 1955; Choi and Priest 1995), and have not been critically evaluated as possible tools for separating monophyletic groups within *Albugo*. Other useful characters, like metabolic pathways, chemical characters or ultrastructural features, which have been brought into consideration (Hall 1996; Spring and Thines 2004), have not yet been taken into account.

Since the studies of deBary (1863), the presence or absence of an equatorial wall thickening of sporangia has been noticed and used to divide *Albugo* into the sections *Annulatae* and *Aequales* for species with or without equatorial wall thickening. The first section consisted of *A. tragopogonis* and *A. bliti,* the latter of the rest of *Albugo* species recognised at that time. These two sections have proven to be polyphyletic by the molecular phylogenetic analyses of Riethmüller et al. (2002).

Another feature, which has been used to distinguish between groups of *Albugo* species, is the presence or the absence of a distinctive primary or terminal sporangium. Such sporangia, differing from the other sporangia in size, type of germination, colour, wall thickness and wall structure, are reported from *Albugo* parasitic on Asterids and Caryophyllids, but it was claimed that they are lacking in *Albugo* parasitic on *Brassicaceae,* where all sporangia are reported to be similar (deBary 1863; Berlese and de Toni 1888). However, this dimorphism is encountered in all species of *Albugo*.

This genus contains at least three groups of species that deserve generic level. Taking into account the unique features of these organisms, the subclass Albuginomycetidae and the order *Albuginales* are introduced. The genus *Albugo* is emended, and two new genera, *Pustula* and *Wilsoniana,* are introduced for the WR of Asteridae and Caryophyllidae, respectively. Based on morphological, ultrastructural and molecular grounds, eight new combinations are proposed: *Pustula chardiniae*,Pustula hydrocotyles, Pustula tragopogonis, Wilsoniana achyrantis, *Wilsoniana bliti, Wilsoniana platensis, Wilsoniana amaranthi,* and *Wilsoniana portulacae*.

A New Proposal by Thines and Spring (2005)

The organisms presently included in *Albuginaceae* deserve to be placed in an order and a subclass of their own, due to their unique mode of sporangiogenesis, structural differences of the sporangiophores and oospores (deBary 1863; Schröter 1893; Wilson 1907), apparent differences in their sexual life cycle (Stevens 1899, 1901a, b, c, 1904; Davis 1900, 1904; Krüger 1910), as well as evident differences in (LSU), internal transcribed spacer (ITS) and CoxII sequences (Cooke et al. 2000; Petersen and Rosendahl 2000; Riethmüller et al. 2002; Hudspeth et al. 2003). Furthermore, the genus *Albugo,* as now understood, contains at least three distinct groups which warrant generic rank.

In the following sections, a new taxonomic hierarchy is proposed which includes descriptions of a new subclass, one new order, three new genera, and eight new combinations. The kingdom Chromista (Cavalier-Smith 1986) is adopted for the introduction of the new taxa below, because recent findings support the monophyly of the Chromista and the view that plastids were acquired before the formation of the straminipilous flagellum (Yoon et al. 2002; Bhattacharya et al. 2003; Harper and Keeling 2003; Keeling 2004). Also, the presence of genes typical for autotrophic organisms in *Phytophthora* (Andersson and Roger 2002) supports this view. Therefore, the kingdom Straminipila is abandoned in favour of the larger, previously described kingdom Chromista.

Albuginomycetidae Thines
Albuginales Thines
Albuginaceae J. Schröt.
Albugo (Pers.) Roussel
Pustula Thines
Wilsoniana Thines

Albuginomycetidae **Thines, subclassis nova**
Chromista, Peronosporomycetes. Hyphae intercellulares, haustoria intracellularia, plerumque parva globosa. Sporangiophora hyalina, fasciculis in sori aggregata, simplices, leves, cylindracea vel clavata, singula apica sporangiorum seriem moniliformem gerentes. Sori epidermide planta nutricis candidi vel lutescentes, primo tecti, dein epidermidem dirumpentes et sporangia matura dispergentes. Sporangia irregularia, conformibus et hyalina aut difformibus, sporangia hyaline solita zoosporipara, sporangia luteola solita sterilia. Oogonia globosa vel irregularia, brunneola. Oosporae globosae, luteolae vel brunneae, zoosporipara.

Typus: *Albugo* (Pers.) Roussel, Flore du Calvados, 2e Ed.: 30 (1806)

Chromista, Peronosporomycetes. Hyphae intercellular, haustoria intracellular, small, mostly globose vesicles. Sori formed beneath the epidermis of the host plant, white to yellowish, first covered by the epidermis of the host plant. Powdery mass of mature sporangia dispersed through ruptured epidermis of the host plant. Sporangiophores colourless, agglomerated in large fascicles or sori, unbranched, clavate to cylindrical.

Sporangia produced basipetally, in chains, at the top of the sporangiophore. Sporangia variously

shaped, either of one type, colourless, on germination usually producing zoospores, or a second type, yellowish, usually sterile, also present. Oogonia globose to irregular, oospores globose, yellowish to brownish, producing zoospores on germination.

Albuginales **Thines, ordo nov.** = *Albuginales nom. nud.* (Wolf and Wolf 1947)
Chromista, Peronosporomycetes. Hyphae intercellulares, haustoria intracellularia, plerumque parva globosa. Sporangiophora hyalina, fasciculis in sori aggregata, simplices, leves, cylindracea vel clavata, singula apica sporangiorum seriem moniliformem gerentes. Sori epidermide planta nutricis candidi vel lutescentes, primo tecti, dein epidermidem dirumpentes et sporangia matura dispergentes. Sporangia irregularia, conformibus et hyalina aut difformibus, sporangia hyaline solita zoosporipara, sporangia luteola solita sterilia. Oogonia globosa vel irregularia, brunneola. Oosporae globosae, luteolae vel brunneae, zoosporipara.

Typus: *Albugo* (Pers.) Roussel, Flore du Calvados, 2e Ed.: 30 (1806) Chromista, Peronosporomycetes. Hyphae intercellular, haustoria intracellular, small, mostly globose vesicles. Sori formed beneath the epidermis of the host plant, white to yellowish, first covered by the epidermis of the host plant. Powdery mass of mature sporangia dispersed through ruptured epidermis of the host plant. Sporangiophores colourless, agglomerated in large fascicles or sori, unbranched, clavate to cylindrical.

Sporangia produced basipetally, in chains, at the top of the sporangiophore. Sporangia variously shaped, either of one type, colourless, on germination usually producing zoospores, or a second type, yellowish, usually sterile, also present. Oogonia globose to irregular, oospores globose, yellowish to brownish, producing zoospores on germination.

Albuginaceae J. Schröt., Nat. Pflanzenfamilien 1(1): 110 (1893)

Typus: *Albugo* (Pers.) Roussel, Flore du Calvados, 2e Ed.: 30 (1806)

Albugo **(Pers.) Roussel, emend.** *Chromista, Albuginales. Hyphae intercellulares, haustoria intracellularia, plerumque parva globosa. Sporangiophora hyalina, fasciculis in sori aggregata, simplices, leves, cylindracea vel clavata, singula apica sporangiorum seriem moniliformem gerentes. Sori epidermide planta nutricis candidi vel lutescentes, primo tecti, dein epidermen disrumpentes et sporangia matura dispergentes. Sporangia primo formis ceteris similis, sporangia hyalina, globosa vel subglobosa, verrucosa, paries circumcirca aequalis vel paries aequatorialis incrassatis modestis, plerumque zoosporipara. Oogonia globosa vel irregularia, brunneola. Oosporae globosae, luteolae vel brunneae, zoosporipara.*

Species typica: *Albugo candida* (Pers.) Roussel, Flore du Calvados, 2e Ed.: 41 (1806)

Chromista, *Albuginales*. Hyphae intercellular, haustoria intracellular, small, mostly globose vesicles. Sori formed beneath the epidermis of the host plant, white to yellowish, first covered by the epidermis of the host plant. Mature sporangia dispersed through ruptured epidermis of the host plant. Sporangiophores colourless, agglomerated in large fascicles or sori, unbranched, clavate to cylindrical. Sporangia produced basipetally, in chains, at the top of the sporangiophore. Sporangia first formed similar to the others, in contrast to *Wilsoniana* and *Pustula,* sporangia colourless, globose to subglobose, wall of uniform thickness, or slightly thickened in the equatorial part, commonly forming a broad annulus, in contrast to *Pustula,* where the annulus is usually thicker and narrow; surface appearing verrucose in scanning electron microscopy, the warts forming lined to reticulate structures; on germination usually producing zoospores. Oogonia globose to irregular; oospores globose, yellowish to brownish, on germination producing zoospores.

Pustula **Thines, gen. nov.** *Chromista, Albuginales. Hyphae intercellulares, haustoria intracellularia, plerumque parva globosa. Sporangiophora hyalina, fasciculis in sori aggregata, simplices, leves, cylindracea vel clavata, singula apica sporangiorum seriem moniliformem gerentes.*

Sori epidermide planta nutricis candidi vel lutescentes, primo tecti, dein epidermidem dirumpentes et sporangia matura dispergentes. Sporangia difformibus, sporangia primo formis ceteris plerumque majoribus, lutescentes, depresse globosa, sterilibus, paries valde crassa, ceteris hyalina, subglobosa vel cylindracea, reticulata vel striata, irregularia, brunneola. Oosporae globosae, luteolae vel brunneae, zoosporipara.

Species typica: *P. chardiniae* (Bremer and Petr.) Thines

Etymology: From Latin *pustula* (blister)

Chromista, *Albuginales*. Hyphae intercellular, haustoria intracellular, small, mostly globose vesicles. Sori formed beneath the epidermis of the host plant, white to yellowish, first covered by the epidermis of the host plant. Mature sporangia dispersed through ruptured epidermis of the host plant. Sporangiophores colourless, agglomerated in large fascicles or sori, unbranched, clavate to cylindrical. Sporangia produced basipetally, in chains, at the top of the sporangiophore. Sporangia of two types, the first produced yellowish, mostly larger, sterile with an overall thickened wall, in contrast to *Albugo*; the subsequent sporangia are colourless, subglobose to cylindrical, wall surface appears reticulate to striate in scanning electron microscopy, in contrast to *Wilsoniana,* where the surface ornamentation is never reticulate; equatorial part of the wall significantly thickened, the annulus usually narrow, in contrast to *Albugo* and *Wilsoniana*; on germination commonly producing zoospores. Oogonia globose to irregular, oospores globose, yellowish to brownish, on germination producing zoospores.

New Combinations P. chardiniae (Bremer and Petr.) Thines, **comb. nov**
Basionym: Albugo chardiniae Bremer and Petr., Sydowia 2(1): 248 (1947)
Pustula hydrocotyles (Petr.) Thines, **comb. nov**.
Basionym: Albugo hydrocotyles Petr., Sydowia 9: 559 (1955)
Pustula spinulosa (deBary) Thines, **comb. nov.**
Basionym: Cystopus spinulosus deBary, Ann. Sci. Nat., ser. 4, 20:133 (1862)
Replaced synonym: Albugo spinulosus (deBary) Kuntze, Revis. gen. pl. (Leipzig) 2: 658 (1891)
P. tragopogonis (Pers.) Thines, **comb. nov.**
Basionym: Uredo candida ß tragopogi Pers., Syn., Meth. Fung. p. 223 (1801)
Replaced synonym: A. tragopogonis (Pers.) Gray, Nat. Arr. Brit. Pl. (London) 1: 540 (1821)

***Wilsoniana* Thines, gen. nov.** *Chromista, Albuginales. Hyphae intercellulares, haustoria intracellularia, plerumque parva globosa. Sporangiophora hyalina, fasciculis in sori aggregata, simplices, leves, cylindracea vel clavata, singula apica sporangiorum seriem moniliformem gerentes. Sori epidermide planta nutricis candidi vel lutescentes, primo tecti, dein epidermidem dirumpentes et sporangia matura dispergentes. Sporangia difformibus, terminalibus ceteris plerumque minoribus vel majoribus, lutescentes, globosa, sterilibus, paries circumcirca valde crassa, ceteris hyalina, ovoidea vel cilindrico-ovoidea vel pyriformibus, striata irregularis vel punctata irregularis, zoosporiparis, paries circumcirca aequalis vel paries aequatorialis incrassatis modestis. Oogonia globosa vel irregularia, brunneola. Oosporae globosae, luteolae vel brunneae, zoosporipara.*

Species typica: Wilsoniana portulacae (DC. ex Duby) Thines

Etymology: Dedicated to the American mycologist G. W. Wilson.

Chromista, *Albuginales*. Hyphae intercellular, haustoria intracellular, small, mostly globose vesicles. Sori formed beneath the epidermis of the host plant, white to yellowish, first covered by the epidermis of the host plant. Mature sporangia dispersed through ruptured epidermis of the host plant. Sporangiophores colourless, agglomerated in large fascicles or sori, unbranched, clavate to cylindrical. Sporangia produced basipetally, in chains, at the top of the sporangiophore. Sporangia of two types, the terminal ones yellowish, smaller or larger than the subsequent, with overall thickened wall, usually sterile, in contrast to *Albugo,* the others colourless, ovoid to cylindrical ovoid to pyriform in contrast to *Pustula,* where pyriform sporangia are usually not

present; surface wall irregular striate to punctate, as seen in scanning electron microscopy.

The wall of sporangia either uniformly thick, or the wall is thickened to a moderate degree in the equatorial part, the annulus usually broad; on germination commonly produce zoospores. Oogonia globose to irregular shaped, oospores globose, yellowish to brownish, on germination producing zoospores.

New Combinations Wilsoniana achyranthis (Henn.) Thines, **comb. nov.**
Basionym: Cystopus bliti (Biv.) de Bary f. achyranthis Henn., Engl. Bot. Jahrb. 28:259 (1901).
Replaced synonym: Albugo achyrantis (Henn.) Miyabe, Trans. Sapporo nat. Hist. Soc. 14(1): no. 19 (1935).
Wilsoniana bliti (Biv.) **Thines**, **comb. nov.**
Basionym: Uredo bliti Biv., Stirp. rar. Sicilia 3: 11 (1815).
Replaced synonym: Albugo bliti (Biv.) Kuntze, Revis. gen. pl. (Leipzig) 2: 658 (1891).
W. platensis (Speg.) **Thines**, **comb. nov.**
Basionym: Cystopus platensis Speg., Rev. Arg. Hist. Nat. 1: 32 (1891).
Replaced synonym: Albugo platensis (Speg.) Swingle, J. Mycol. 7(2): 113 (1894).
W. portulacae (DC. ex Duby) **Thines comb. nov.**
Basionym: Uredo portulacae DC., Fl. France 5: 88 (1815).
Replaced synonym: A. portulacae (DC. ex Duby) Kuntze, Revis. gen. pl. (Leipzig) 2: 658 (1891).

Albugo caryophyllacearum (Wallr.) Cif. & Biga was excluded from the new combinations because of significant differences in oospore development, as described by Ruhland 1904, although the morphological characters suggest that this species should be transferred to *Wilsoniana*. For further notes on the synonyms see Wilson (1907) and Biga (1955).

3.1 Taxonomy and Morphology

The systematic position of the pathogen described by Ainsworth et al. (1973) is as:

Kingdom:	Mycota
Division:	Eumycota
Subdivision:	Mastigomycota
Class:	Oomycetes
Order:	Peronosporales
Family:	Albugonacae
Genus:	*Albugo*
Species:	*candida*

Albugo candida (Pers. Ex Lev.) Kuntze = (*Albugo cruciferarum* S.F. Gray). It is an obligate parasite. However, recently Thines and Spring (2005) while revising *Albugo,* have elevated it to Chromista with the class: Peronosporomycetes, and order: Albuginales. Historical and current phylogenetic taxonomy of *Albugo* causing WR is as follow (Fig. 3.1):

3.1.1 Species Concept in Albugo Parasitic to Brassicaceae s.l.

Traditionally, species in the *Albuginaceae* were thought to be host family specific, especially in *Brassicaceae* and *Asteraceae*. This view is, however, asserted by recent molecular phylogenetic studies (Thines and Spring 2005; Voglmayr and Riethmüller 2006; Choi et al. 2006), which revealed that several distinct lineages are present on the same host family and that at least some species are possibly host genus specific.

Several species of *Albugo* parasitic to *Brassicaceae* have been described, but are mostly treated as synonyms of *A. candida,* amongst which the most widely known binominals are *Uredo cruciferarum* DC., *Cystopus sphaericus* Bonord., *Caeoma candidum* Schltdl., *Albugo wasabiae* Hara, *Albugo macrospora* (Togashi) S. Ito, *Albugo lepidii* N.A.S. Rao, *Uredo cheiranthi* Pers. and *Uredo thlaspi* Sowerby. Listing all binominals, including combinations and varieties would not serve practical purpose. This is due to the fact that *A. candida s.l.* is parasitic to a wide range of *Brassicaceae,* and that WR incidence is very common on some of the most widely distributed *Brassicaceae*. After listing a few synonyms, Fischer (1892) mentioned that

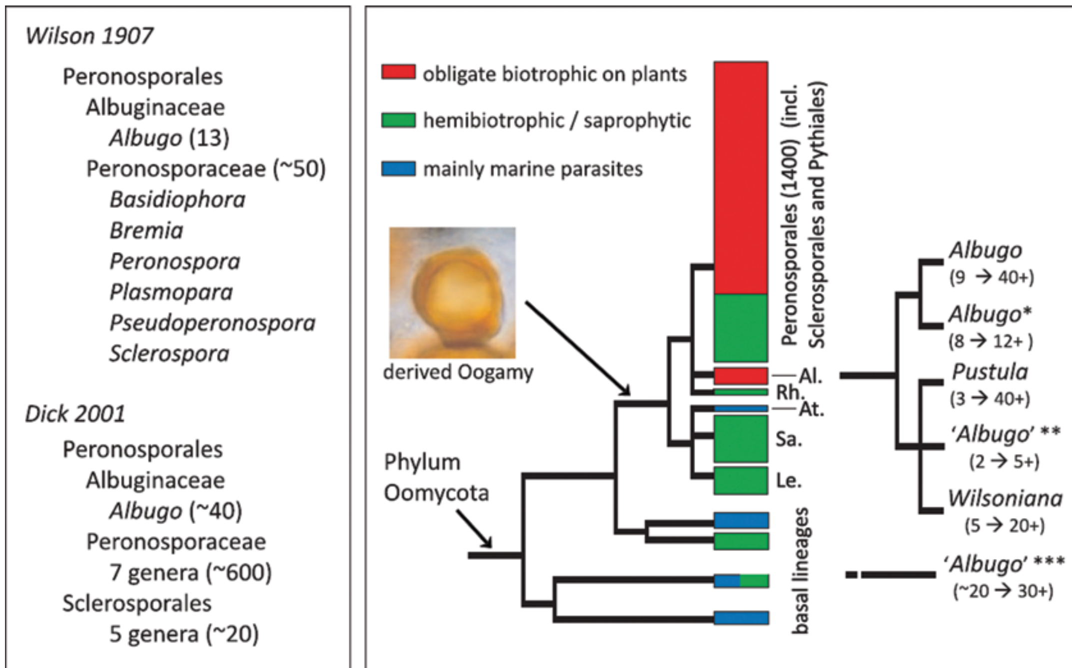

Fig. 3.1 Historical and current taxonomy of white blister rusts. Species numbers at *left* are published species numbers, followed by conservative species estimates of the actual diversity. **Albugo s.l.* on Convolvulaceae, ** *Albugo s.l.* on some Caryophyllales, *** unsequenced species of *Albugo* with uncertain phylogenetic affinity. *Rh*. Rhipidiales, *At*. Atkinsiellales (informal designation), *Sa*. Saprolegniales, *Le* Leptomitales. (Thines 2010)

there are numerous synonyms of this '*vielbenannten Pilzes*' (fungus named many times). Also in the brassicaceous hosts, formerly assigned to the *Capparaceae,* two species of *Albugo* have been described. The type material of one of these, *Albugo chardonii* W. Weston, is indistinguishable from *A. candida* in molecular phylogenetic reconstructions, and therefore, needs also to be considered a synonym of or at least a species closely related to *A. candida s.str*. The slight wall thickening at the base and sides given in the key of Choi and Priest (1995) is a feature present in some sporangia of almost every collection of *A. candida* (Thines and Spring, 2005). The other species described in *Capparaceae* is *Albugo capparis* (deBary) Kuntze, which has been doubted to be a distinct species almost since its description. deBary (1863), who formally described the species, which was given as a variety of *A. candida* by Rabenhorst (1844), noted this species to be *in speciminibus C. candida omnino similia*. Later, Pirotta (1884, as cited in Fischer 1892) too was not able to distinguish between *A. candida* and *A. capparis*. This finding was also confirmed in Saccardo's Sylloge Fungorum (Berlese and deToni 1888), who stated this species to be *A C. candida non v. vix distinguendus*. Biga (1955) included this species in his key, which is starting at the host family level, but the features given by him are identical to *A. candida* var. *macrospora,* comparing both sporangia and oospore features. Therefore, it is interesting that Choi and Priest (1995), who unfortunately did not mention any material they might have studied, cited the two references mentioned above (Biga 1955; Saccardo 1888) to give the oospore ornamentation as 'tuberculate', a feature that would indicate that this species might be related to *A. lepidii,* and also not to be included in *A. candida s. str*. Specimens of *Albugo* from *Capparaceae* sequenced so far are either placed within *A. candida s.str*., or in a sister clade to *A. lepidii,* amongst other specimens from *Brassicaceae* (Figs. 3.4, 3.5). Whether, this clade contains several distinct

species, which could include *A. capparis* and *U. cheiranthi,* or not, has to be elucidated by thorough investigation of Rabenhorst's material. *Albugo* from *Capsella* in Korea is placed sister to the clade comprising *A. lepidii,* and several other distinct lineages, indicating that describing a new species for the pathogen on *Capsella* from Korea, which is distinct from the *Capsella* infecting *Albugo candida s.str*., is highly warranted (Choi et al. 2007).

3.1.2 Lectotypification of *A. candida*

Albugo candida has first been described as an independent species by Persoon in Gmelin (1792) as *Aecidium candidum* Pers. Later, several species of *Aecidium* were transferred to *Uredo* to which numerous species were added by Persoon (1796, 1797, 1800). However, *Aecidium candidum* is first mentioned in 1801 in the *Synopsis Fungorum* (Persoon 1801), where it is transferred to *Uredo* as *U. candida* (Pers.) Pers. It is noteworthy that an allusion is made to an earlier publication (Persoon 1796), where *Botrytis parasitica* (*Hyaloperonospora parasitica* (Pers.) Constant.) is described to occur on *Capsella bursa-pastoris* also exhibiting symptoms of infection with *A. candida*. In the *Synopsis Fungorum,* Persoon (1801) gives three varieties of the species. The first variety, *U. candida* α *thlaspeos,* the third variety, *U. candida* γ *alyssi* and also a separate species, *U. cheiranthi,* are undoubtedly members of the *A. candida* complex. The second variety, *U. candida* β *tragopogi* is now placed in *Pustula* (*P. tragopogonis* (Pers.) Thines). In 1806, Roussel described *Albugo* as genus separate from *Uredo,* and combined *U. candida* (Pers.) Pers. into this genus, also adopting the broad host range given by Persoon (1801) for this pathogen. Later, Kuntze (1891) listed the *Albugo* species known to him; amongst them were *A. candida* and *A. tragopogonis*. Kuntze (1891) typified the genus *Albugo* with *A. candida,* based on *U. candida,* and gave, amongst others, *A. cruciferarum* (DC). Gray as a synonym, which, in the sense of Gray (1821), and the authors of the basionym, *U. cruciferarum* DC. (Lamarck and Candolle 1805) is confined to hosts in the *Brassicaceae*.

To avoid nomenclatural confusion, *Ae. candidum* Pers. needs to be typified with a specimen that agrees with the original description, stabilises the current taxonomy, and makes further name changes in *A. candida s.str*. unnecessary. In the National Herbarium of the Netherlands, section Leiden (L), Persoon's herbarium is deposited. However, no specimen filed under *Ae. Candidum,* without giving a variety, is preserved. This is most likely due to later relabeling of the specimens. Several specimens of *U. candida* are preserved, but no collection details are given, which makes it impossible to determine with certainty, which specimen is the oldest, or was considered to be typical for *Ae. candidum* by Persoon. In addition, to the specimens filed under *U. candida,* several other specimens of WR on various *Brassicaceae* are preserved. Among these are one labelled *U. lactea* var. *cheiranthi incani* on *Cheiranthus incanus* (L 910263-325), with a second label as *Ae. candidum* β *cheiranthi*, possibly the specimen upon which Persoon has described *U. cheiranthi* Pers.; *U. thlaspeos bursae pastoris* (L 910264-213), which is possibly the specimen on which *U. candida* γ *thlaspeos* Pers. was based, and a specimen labeled *U. amoraciae* (L 910264-938) on *Armoracia rusticana*. In addition, one specimen labeled *U. candida*, showing WR on both *Arabis* sp. and *C. bursapastoris* (L 910263-177), and two specimens labelled *U. candida* γ *alyssi* (L 910263-159, L 910263-161), as well as two labelled *U. candida* β *tragopogi* (L 910263-156, L 910263-157—the first one being a doublet of the second one) are preserved.

The type specimen for *Ae. candidum* is chosen amongst the two exsiccati which contains the most widely known and distributed host–pathogen association, i.e. white blister rust on *C. bursa-pastoris*. As a definite solution, the specimen L 910264-213, *lectotypus hic designatus,* containing only *Capsella* as host plant is chosen as lectotype for *Ae. Candidum* Pers., which is now known as *A. candida* (Pers.) Roussel. In the material allowed to take for oospore investigations in the National Herbarium of the Netherlands, only two immature oospores of about 50 µm in

diameter were found. Therefore, although oospore ornamentation was found to be similar to recent collections from *Capsella* in Europe, no explicit statements can be made about the oospore ornamentation in this specimen. However, the size of the oospores renders it highly likely that the species on the specimen examined is indeed *A. candida,* and not the species now discovered in *C. bursa-pastoris* from Korea (Choi et al. 2007).

3.2 Species of Albugo

(i) *A. candida* **(Pers. ex. Hook.) O. Kuntze**

= *Aecidium candidum* Pers.

= *Uredo candida* thlaspeas Pers.

= *Uredo candida* (Pers.) Fr.

= *Cystopus candidus* (Pers.) Lev.

Mycelium intercellular with small globose to knob-like haustoria, one to several in each host cells of white to rarely pale yellow, prominent, deep seated, variable in size and shape, often confluent. Sporangiophores are hyaline, clavate, thick walled, especially towards the base, 30–45 × 15–18 µm diameter. Sporangia are arranged in a basipetal chain, globose to oval, hyaline with uniform thin wall, 12–18 µm diameter. Oospores globose, chocolate-brown, 30–55 µm (generally 45 µm with oogonial wall up to 60 µm), epispore thick, verrucose to tuberculate, or with low blunt ridges which are often confluent and irregularly branched, and sometimes seemingly smooth (Holliday 1980; Mukerji 1975a, 1975b; Wilson 1907). Biga (1955) divided this species into two varieties on the basis of sporangial size.

(ii) *Albugo ipomoeae* **-panduratae (Schwein.) Swing**

= *Aecidium ipomoeae*-panduratae Schwein.

= *Aecidium ipomoeae* Schwein.

= *Cystopus ipomoeae*-panduratae (Schwein.) Stev.and Swing.

Mycelium intercellular with typical knob-like haustoria, sori amphigenous or caulicolous, white or light yellow, prominent, superficial, 0.5–2.00 mm round, arranged concentrically, often confluent. Sporangiophores hyaline, club shaped, unequally curled at base, 30–40 µm × 12–15 µm. Sporangia hyaline, rectangular, short cylindrical, the terminal more rounded, 12–20 µm × 12–18 µm, wall with distinct equatorial thickening. Oospores caulicolous, spherical, light yellowish-brown, 25–40 µm (sometimes up to 45 µm, 55 µm with oogonial wall), epispore papillate or with irregular, more or less curved ridges (Holliday 1980; Mukerji and Critchett 1975; Wilson 1907). Biga (1955) placed two varieties under this species on the basis of sporangial size.

(iii) *A. tragopogonis* **(DC). S.F. Gray**

= *Uredo candida* spp. tragopogi Pers.

= *Uredo tragopogi* (Pers.) DC.

Mycelium intercellular, hyaline, with globose vesicular haustoria have small stalks. Sori (pustules) hypophyllous or caulicolous, prominent, deep seated, white or yellowish, pulverulent, rounded, or elongate, 1–5 × 1–8 mm. Sporangiophores hyaline, club shaped, 40–55 µm × 12–16 µm. Sporangia hyaline to light yellow, short cylindrical to spherical cuboid, the terminal larger, oval and less angular than the lower ones, wall with an equatorial thickening, 18–24 µm × 12–20 µm. Oospores spherical, dark brown to almost black at maturity, 44–57 µm (sometimes up to 68 µm with oogonial wall), epispore reticulate, densely covered by low, tuberculate or spinulose warts, meshes (areolae) 2–3 pm across (Mukerji 1975b). Savulescu (1946a, b) divided this species into two varieties on the basis of the size and shape of sporangia. Biga (1955) made five varieties on the basis of host and size of sporangia. It is distinguished from other species of *Albugo* in having cylindrical sporangia and oospores with reticulate epispore tuberculate at their angles.

(iv) *Albugo ipomoeae* **-aquaticae Sawada**

Sori hypophyllous and on stem or inflorescence, round to irregular, 1–3 mm diameter, up to 7 mm long, dull white, becoming pulverulent, host sometimes distorted. Sporangiophores hyaline, clavate and 32–72 µm long, 18–23 µm wide in size. Sporangia are catenulate, uniform size, globose to cuboid, hyaline 16–23 × 18–26 (av. 19 × 21) µm, enclosed by a smooth, uniformly thickened membrane. Oogonium globose, subglobose or obvate with irregular patterns on the

inner surface, 52–80 µm diameter when globose, or 64–89 µm × 48-68 µm when otherwise shaped, containing one oospore. Oospore globose, hyaline, smooth, 39–48 µm diameter, the wall originally thin but becoming 6–8 µm thick after conjugation (Edie 1970, Edie & Ho 1970a, b; Holliday 1980; Ito and Tokunaga 1935; Safeefulla and Thirumalachar 1953; Sawada 1919, 1922, 1927). A taxonomical and morphological account of 13 North American species of *Albugo* infecting different hosts, along with a key to the species, has been given by Wilson (1907).

(v) *Albugo rorippae* **Y.J. Choi, H.D. Shin, Ploch and Thines, sp. nov. (Choi et al. 2011b)**

MycoBank accession MB 519654.

Etym: 'rorippae' refers to scientific generic name of host plants examined.

Hyphae sporogenae clavatae vel cylindraceae, (27.5–) 34.5–50.7 (–62.5) (medio 42.6) µm longae, (10–) 10.7–14.4 (–16.3) (medio 12.6) mm diameter. Sporangia hyalina, globosa vel subglobosa, sporangia primaria (12–) 13.6–16.3 (–18) (medio 14.9) µm diameter, parietibus 1.0–1.5 mm crassis, sporangia secundaria (13.5–) 15.0–18.5 (–21.5) (medio 16.8) µm diameter, parietibus uniformibus 0.5–1.0 µm crassis. Oogonia sub-flavida, (45–) 50.9–60.7 (–62.5) (medio 55.8) µm diameter Oospora flavida, irregulariter tuberculata, (35–) 38.8–46.0 (–51) (medio 42.4) µm diameter, inclusae tuberculae. Tuberculae plerumque singulares, variabiles, (1) 3–4 (–5) µm longae, (1–) 2–4 (–6) µm diameter.

Typus: POLAND; Miechowice, on living leaves of *Rorippa palustris* affected by white blister rust disease, Oct. 1884, A. Zalewski (KRAM-F-000130—holotypus). Sequences ex-type: HQ377364 (COX2 mtDNA) and HQ3//3/1 (ITS rDNA).

Sori distinct, round or irregular, rarely confluent, whitish or pale yellowish, 0.5–1.5 (–3) mm diameter, mostly on the lower surface of the leaves, rarely on upper side and stem. Mycelium intercellular, with globose haustoria with narrow and short stalk of 1–3 µm long, 2–5 µm diameter, one or two per host cell. Sporogenous hyphae hyaline, clavate or cylindrical, straight to slightly curved, (27.5–) 34.5–50.7 (–62.5) (av. 42.6) µm long, (10–) 10.7–14.4 (–16.3) (av. 12.6) µm wide (n = 50), mostly grouped, thick walled, especially towards the base up to 6 µm. Sporangia arranged in basipetal chains, hyaline, primary sporangia similar to the secondary sporangia, but the first exhibit a slightly thicker wall and somewhat smaller size; primary sporangia mostly globose or polyangular due to mutual pressure, (12–) 13.6-16.3 (–18) (av. 14.9) µm diameter (n = 80), with uniformly thin wall of ca. 1.0–1.5 µm; secondary sporangia subglobose to obovoid or globose, (13.5–) 15.0–18.5 (–21.5) (av. 16.8) µm diameter (n = 100), mostly with uniformly thin wall, but rarely slightly thickening (0.5–1.0 µm) as an equatorial ring, tip round, base subtruncate or rounded, pedicel mostly absent. Resting organs often present as yellowish to brown dots on both the upper and lower surfaces of the leaf spots. Oogonia irregular or broadly globose, pale yellowish, (45–) 50.9–60.7 (–62.5) (av. 55.8) µm diameter (n = 70); wall smooth, as observed in both mature and immature oospores, 2–3 µm thick. Oospores plerotic, yellowish, globose, (32–) 35.4–42.0 (–47) (av. 38.7) µm diameter, but (35–) 38.8–46.0 (–51) (av. 42.4) µm diameter (n = 56) when including the height of tubercles; wall 2–3 µm thick, irregularly tuberculate, without ridges. Tubercles mostly single, rarely connected between neighbouring ones, but not branched, with a ripple-like pattern between the ridges, largely variable in shape and size, (1–) 3–4 (–5) µm long, (1–) 2–4 (–6) µm wide; apex mostly obtuse but often echinulate or truncate.

Habitat: In living leaves of *Rorippa amphibia, R. palustris, R. pyrenaica and R. sylvestris* (Brassicaceae).

Taxonomy Due to its distinct phylogenetic placement and morphological characteristics differing from all other *Albuginaceae* hitherto known, a new species is introduced here to accommodate the undescribed species on *Arabidopsis thaliana*.

(vi) *Albugo laibachii* Thines and Y.J. Choi, *sp. nov.*—MycoBank

MB509563; Fig. 3.2

Mycelia intercellularia, haustoria intracellularia, vesicularia. Sori hypophylli, distincti, rotundi vel irregulares, saepe confluentes, albi, 0.5–4(–11) mm diameter. Sporangiophora hyalina,

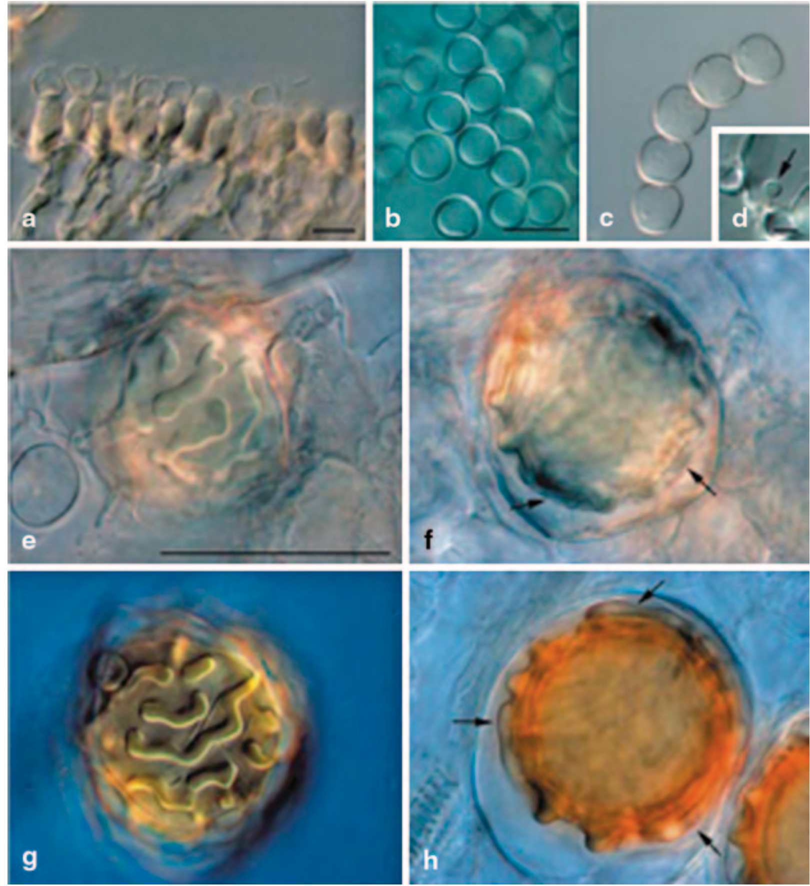

Fig. 3.2 Morphological characteristics of *Albugo* species on *Arabidopsis thaliana*. **a–f**. New species discovered on *Arabidopsis thaliana*; **g**, **h.** *Albugo candida* on *Arabidopsis thaliana*. **a** Sporogenous hyphae **b** primary sporangia **c** secondary sporangia **d** haustorium, e.g. surface ornamentation of oospores **f**, **h** protuberances (*arrows*) as seen in lateral view. Scale bars: **a–c** = 20 µm, **d** = 10 µm, **e–h** = 50 µm. Sources: **a–f** (DAR 73071), **g**, **h** (BP 75214) (Thines et al. 2009)

clavata vel cylindracea, (20–) 23.3–33.9 (–37.5) (av. 28.6) µm long, (10.5–) 11.5–13.8(–15) (av. 12.7) µm diameter (n = 102). Sporangia hyalina, globosa vel subglobosa, sporangia primaria (11.8–) 12.5–14.5(–15.3) (av. 13.5) µm diameter (n = 94), sporangia secondaria (11.5–) 14.3–17.1(–18.5) (av. 15.7) µm diameter (n = 113), parietibus uniformibus. Oogonia in folia, globosa vel irregularia, flavida, (45–) 47.4–54.3(–58) (av. 50.9) µm diameter (n = 63). Oospora luteola vel brunnea, globosa, verruculosa vel tuberculata, (36.8–) 38.3–43.3 (–47) (av. 40.8) µm diameter (n = 34) (Fig. 3.2).

Etymology, dedicated to Friedrich Laibach, who first suggested *A. thaliana* as a model plant for plant genetics.

Mycelium intercellular, *haustoria* knob-like to globose, 3–5 µm diameter, surrounded by thick sheath, with narrow and short stalk, 1–2 µm in length, one to several in each host cell. *Sori* hypophyllous, distinct, rounded or irregular, 0.5–4 (–11) mm diameter, often confluent, whitish, sometimes present in stems and inflorescences. *Sporangiophores* hyaline, clavate or cylindrical, straight to slightly curved, (20–) 23.3–33.9 (–37.5) (av. 28.6) µm long, (10.5–) 11.5–13.8 (–15) (av. 12.7) µm wide (n = 102), mostly grouped, thick walled, especially towards the base up to 6 µm. *Sporangia* arranged in basipetal chains, hyaline, primary sporangia similar to the secondary sporangia, but the former exhibit a slightly thicker wall; primary sporangia globose or polyangular due to mutual pressure, (11.8–) 12.5–14.5(–15.3) (av. 13.5) µm diameter (n = 94), with wall uniformly 1.5 (–2) µm thick; secondary sporangia globose to subglobose, (11.5–) 14.3–17.1 (–18.5) (av. 15.7) µm diameter (n = 113), with uniformly thin wall, tip round, base mostly rounded, but rarely subtruncate, pedicel mostly absent. *Resting organs* rarely present as pale

brown dots on both the upper and lower surface of the leaf spots. *Oogonia* broadly globose or irregular, yellowish, (45–) 47.4–54.3 (–58) (av. 50.9) µm diameter ($n=63$), wall smooth, 1–2 µm thick. *Oospores* plerotic, yellowish to pale brownish, globose, (36.8–) 38.3–43.3 (–47) (av. 40.8) µm diameter including the height of tubercles ($n=34$), wall 2–4 µm thick, irregularly tuberculate, with blunt ridges; tubercles mostly connected, but very rarely single, often branched, up to 4 µm long (Fig. 3.2).

Substratum—Living Leaves of *A. thaliana*

Known distribution—Australia, England, France, Germany.

Specimens examined. Australia, Tasmania, Gretna, 29 Sept. 1980, *D. Morris,* DAR 73071, holotype.

(vii) *Albugo koreana* Y.J. Choi, Thines and H.D. Shin, **sp. nov.**

MycoBank: 510868.

Etymology: 'koreana' refers to the country in which the fungus was first collected.

Chromista, Albuginales. Mycelia intercellularia, haustoria intracellularia, vesicularia.

Sorus hypophyllus, distinctus, rotundibus vel irregularibus, saepe confluentibus, albus vel raroflavus, 0.5–5 mm diameter. *Sporangiophora* hyalina, clavata vel cylindracea, 20–40 × 10–15(−18) µm. *Sporangia* hyalina, globosa vel subglobosa, (13.5–) 15–22 × 12.5–17(−19) µm, parietibus aequalibus. *Oogonia* in folia, globosa vel irregulares, flavida, 37–51 (–63) µm diameter *Oospora*luteola vel brunnea, globosa, verruculosa vel tuberculata, 31–46 µm diameter, verrucis singularis, haudramosis, 2–5 × 3–4 µm.

Mycelium intercellular with small globose to knob-like haustoria with short stalk, one to several in each host cell. *Sori* hypophyllous, distinct, rounded or irregular, often confluent, 0.5–5 mm diameter, white or rarely pale yellow, covering mostly large areas of the lower and rarely upper side of the leaves, stems and inflorescences. *Sporangiophores* hyaline, clavate or cylindrical, straight to slightly curved, 20–40 × 10–15(–18) µm, mostly grouped or sometimes single, thick walled, especially towards the base up to 5 µm.

Sporangia arranged in basipetal chains, hyaline, globose or subglobose, with equal thin wall, (13.5–) 15–22 × 12.5–17(–19) µm, l/w ratio = 0.93–1.27 ($n=100$), tip round, base rounded or subtruncate, verrucose, pedicel mostly absent, often a minute protuberance visible at the point of attachment to the sporangiophores or other sporangia, primary sporangia similar to the secondary sporangia, although the first exhibit a slightly thicker wall. *Oogonia* in leaves, broadly globose or irregular, yellowish to brownish, 37–51(–63) µm diameter ($n=100$), wall smooth. *Oospores* plerotic, globose, yellowish, 31–46 µm diameter ($n=100$), wall thick, verruculate or tuberculate without blunt ridges, warts single, not confluent or branched, 2–5 × 3–4 µm.

Typus: Korea, Seoul, Korea University, on leaves of *C. bursa-pastoris* affected by WR disease, 2 April 1999, H.D. Shin (BPI871286, holotypus; SMK15670, isotypus).

Habitat: On living leaves, stems and fruits of *C. bursa-pastoris*.

Known distribution: Korea.

Other materials examined: Korea, Namyangju, Deokso, 4 May 1997, H.D. Shin (SMK13752); Korea, Chunchon, Gangwon-do Forest Experiment Station, 14 May 1999, H.D. Shin (SMK15802); Korea, Yongin, Mt. Kwanak, 28 April 2000, H.D. Shin (BPI871287; SMK17254); Korea, Hongchon, Experimental Forest of Kangwon National University, 21 May 2005, H.D. Shin and Y.J. Choi (BPI871289; SMK21128); Korea, Wonju, Mt. Chiak, 6 May 2005, H.D. Shin and Y.J. Choi (BPI871288; SMK21090).

Wilsoniana amaranthi (Schwein.) Y.J. Choi, Thines and H.D. Shin, comb. nov.

Basionym: *Caeoma amaranthi* Schwein. (Syn. Fung. Amer. Bor.: 292, 1832).

3.3 A Key to the Genus Albugo (Choi and Priest 1995)

(1) **on Acanthaceae**

Sporangia with uniform wall thickness.

Sporangia 10–20 µm diameter

Oospores reticulate, 45–70 µm diameter, brown.
Albugo aechmantherae Zhang *et* Y.X. Wang
Ref.: Zhang et al. (1984).
Sporangia with equatorial wall thickening.
Spornagia 12–25 µm diamater
Oospores undescribed.
Albugo quadrata (Kalchbe. *et* Cooke) Kuntze
Ref.: (Wakefield 1927).

(2) **on Aizoaceae**
Spornagia 10–20 µm diameter, with uniform wall thickness.
Oospores reticulate, areolae 6–15 (24) µm wide, 40–55 µm diameter, dark brown**.**
Albugo molluginis S. Ito
Ref.: (Ito and Tokunaga 1935; Mayor and Viennot–Bourgin 1951; Biga 1955).
Sporangia 15–25 µm diameter, with uniform wall thickness.
Oospores reticulate, areolae 3–6 µm wide, 50–80 µm diameter, dark brown.
Albugo trianthemae Wilson
Ref.: (Wilson 1908; Baker 1955; Waterhouse 1975).
Oospores densely papillate, 40–60 µm diameter
Albugo austro-africana Sydow
Ref.: (Sydow and Sydow 1912; Wakefield 1927).
A revision of the species on the Aizoaceae was carried out by Waterhouse (1975).

(3) **on Amaranthaceae**
Sporangia with distinct equatorial wall thickening.
Spornagia 10–20 µm diameter
Oospores reticulate, areolae 6–8 µm wide, 45–75 µm diameter, dark brown.
Albugo biliti (Biv.) Kuntze
Ref.: (Saccardo 1888; Wilson 1907, 1908; Wakefield 1927; Biga 1955).
Sporangia with uniform wall thickness.
Sporangia 10–20 µm diameter
Oospore smooth, 75–90 µm diameter, dark brown.
Albugo gomphrenae (Speg.) Cif. *et* Biga
Ref,: (Saccardo and Trotter 1912)
Sporangia 15–25 µm diameter
Oospores undescribed.
Albugo achyranthis (P. Henn.) Miyabe apud Ito *et* Tokunaya
Ref.: (Sawada 1922; Ito and Tokunaya 1935).

(4) **on Apiaceae**
Sporangia 10–20 µm diameter with equatorial wall thickening.
Oospores undescribed
A.hydrocotyles Petrak
Ref.: (Petrak 1955).

(5) **on Asteraceae**
Sporangia 15–25 µm diameter with equatorial wall thickening.
Oospores finely reticulate, 50–70 µm diameter, dark brown.
Albugo solivae Schrot.
Ref.: (Hennings 1896; Biga 1955).
Oospores reticulate, areolae 1–2 µm wide, 45–75 µm diameter, dark brown.
A. chardiniae Bremer et Petrak
Ref.: (Bremer and Petrak 1947).
Oospores reticulate with ridges bearing spines, areolae 2–4 µm wide,
40–70 µm diameter, dark brown.
A. tragopogonis (Pers.) S.F. Gray
Ref.: (Wilson 1907; Baker 1955; Biga 1955; Mukerji 1975b).

Several varieties of *A. tragopogonis* have been erected by Savulescu and Rayss (1930) and Biga (1955) based on host specialization and apparent differences in size of sporangia. Whipps and Cooke (1978b) have suggested that physiologic race rather than varietal distinction is used in *A. tragopogonis*. The full nomenclature has been outlined by Whipps and Cooke (1978a).

(6) **on Boraginaceae**
Sporangia 15–25 µm diameter
Oospores reticulate, areolae 3–4 µm wide 45–55 µm diameter
A. cynoglossi (Unamuno) Cif. *et* Biga
Ref.: (Unamuno 1930; Biga 1955).
The status of *A. sibirica* (Zalew.) Wilson is unclear. Biga (1955) did not include it in his key and Wilson (1907) did not describe it.

(7) **on Brassicaceae**
Sporangia with uniform wall thickness, 10–20 µm diameter
Oospores verrucose with short ridges, 30–55 µm diameter dark brown

A. candida (Pers.) Kuntze
Ref.: (Wilson 1907; Biga 1955; Mukerji 1975a; Zhang et al. 1984).

A. candida has been divided into several forms by Savulescu and Rayss (1930) and two varieties (Biga 1955). Host specificity has been demonstrated by Hiura (1930) and Eberhardt (1904a–d). The division of *A. candida* into two varieties based on conidial size and host (Biga 1955), i.e. var. *candida* and var. *macrospora* Togashi and Shibasake appears to be partly supported by Williams and Pound (1963) and Makinen and Hietajarvi (1965). The species *A. lepidii* Rao described from *Lepidium* (Rao 1979) is synonymous.

(8) **on Capparidaceae**
Sporangia with uniform wall thickness, 10–20 µm diameter
Oospores tuberculate, 30–40 µm diameter
A. capparidis (de Bary) Cif.
Ref.: (Saccardo 1888; Biga 1955).
Oospores verrucose with short ridges,
30–55 µm diameter, dark brown.
A. candida (Pers.) kuntze
Ref.: (Wilson 1907; Biga 1955; Mukerji 1975b; Zhang et al. 1984)
Sporangia with wall thickening across the base and sides,
10–20 µm diameter
Oospores undescribed.
A. chardoni Weston
Ref.: (Chardon and Toro 1930).

(9) **on Caryophyllaceae**
Sporangia with uniform wall thickness, 15–25 µm diameter
Oospores densely papillate—echinulate with short ridges—
50–65 µm diameter, brown.
A. caryophyllacearum (Wallr.) Cif. *et* Biga
Ref.: (Saccardo 1888; Wilson 1907; Biga 1955).

(10) **on Chenopodiaceae**
Sporangia 10–20 µm diameter
Sporangia with equatorial wall thickening, contents yellow.
Oospores reticulate—foveate (i.e. shallowly pitted), areolae approx. 2 µm wide.
40–70 µm diameter, yellowish brown.
Albugo occidentalis Wilson
Ref.: (Wilson 1907).
Sporangia 15–25 µm diameter
Sporangia with thin, indistinct equatorial wall thickening.
Oospores reticulate—tuberculate and echinulate
35–60 µm diameter, brown.
Albugo eurotiae Tranzsch.
Ref.: (Saccardo and Trotter 1926).

(11) **on Convolvulaceae**
Oogonial inner wall plicate to tuberculate thickenings, light brown, often appearing confluent with the oospore wall.
Sporangia with equatorial wall thickening.
Sporangia 10–20 µm diameter
Oospores 25–55 µm diameter
A. ipomoeae-panduranae (Schwein.) Swingle
Ref.: (Wilson 1907; Biga 1955; Mukerji and Critchett 1975; Singh and
Bedi 1965).
Oospores 30–45 µm diameter
Albugo minor (Speg.) Cif.
Ref.: (Biga 1955).
Oospores 35–55 µm diameter
A. ipomoeae-pescaprae Cif.
Ref.: (Trotter 1972).
Sporangia with uniform wall thickness.
Sporangia 10–20 µm diameter
Oospores 30–50 µm diameter
Albugo evolvuli (Damle) Cif. *et* Biga
Ref.: (Damle 1943; Safeeulla and Thirumalachar 1953).
Oospores 22–28 µm diameter
Albugo pestigridis Gharse
Ref.: (Gharse 1964; Rao 1964).
Sporangia 15–25 µm diameter
Oospores 30–40 µm diameter, smooth, hyaline.
A. ipomoeae-hardwickii Sawadw
Ref.: (Sawada 1927).
Oospores 35–50 µm diameter, smooth, hyaline.
A. ipomoeae-aquaticae Sawada
Ref.: (Sawada 1922; Safeeulla and Thirumalachar 1953; Ho and Edie 1969).

Two varieties of *A. evolvuli*; var. *merremiae* Safeeulla and Thirum, (Safeeulla and Thirum-

alachar 1953), and var. *mysorensis* Safeeulle (Safeefulla 1952a), and the species *Albugo pratapi* Damle (Damle 1955) have been described on hosts in the convolvulaceae. All appear to be synonymous with *A. evolvuli.*

The oospores of *A. ipomoeae-panduranae* and *A. ipomoeae-pescaprae* were described as papillate with irregular ridges and tuberculate, respectively. However, we suspect that the tuberculate thickenings of the inner oogonial wall may have been misinterpreted as the oospore ornamentation. Both oospore wall and oogonial wall must be described accurately for any revision of the species on the Convolvulaceae.

(12) **on Crassulaceae**
Sporangia 15–25 µm diameter, with wall thickening across the base and sides.
Oospores undescribed.
Albugo tilleae (Lagerh.) Cif. *et* Biga
Ref.: (Patouillard and de Lagerheim 1891).

(13) **on Fabaceae**
Sporangia 10–20 µm diameter
Oospores undescribed.
Albugo mauginii (Parisi) Cif. *et* Biga
Ref.: (Biga 1955).

(14) **on Fumariaceae**
Sporangia 10–20 µm diameter
Oospores undescribed.
Albugo keeneri Solheim *et* Gilbertson
Ref.: (Solheim and Gilbertson 1977).

(15) **on Gentianaceae**
Sporangia with equatorial wall thickening
Sporangia 10–20 µm diameter
Oospores reticulate, 40–55 µm diameter, deep yellow.
Albugo swertiae (Berl. *et* kom.) Wilson
Ref.: (Saccardo and Trotter 1912).
Sporangia with indistinct equatorial wall thickening.
Sporangia 15–25 µm diameter
Oospores closely reticulate, 50–60 µm diameter, dark brown.
Albugo centaurii (Hansf.) Cif. et Biga
Ref.: (Hansford 1954).

(16) **on Nyctaginaceae**
Sporangia with equatorial wall thickening, 15–25 µm diameter
Oospores reticulate, areolae 3–5 µm wide, 50–80 µm diameter, dark brown.
A. platensis (Speg.) Swingle
Ref.: (Saccardo and Saccardo 1905; Wilson 1907; Spegazzini 1909).

(17) **on Papaveraceae**
Sporangia with uniform wall thickness, 10–20 µm diameter
Oospores undescribed.
Albugo eomeconis Zhang *et* Wang
Ref.: (Zhang and Wang 1981).

(18) **on Peperomiaceae**
Sporangia with uniform wall thickness, 15–25 µm diameter
Oospores verrucose, 30–40 (60) µm diameter
Albugo tropica (Lagerh.) Lagerh.
Ref.: (Patouillard and de Lagerheim 1892; Biga 1955).

(19) **on Portulacaceae**
Sporangia with uinoform wall thickness, 10–20 µm diameter
Contents pale yellow.
Oospores reticulate with a spine in each areola,
Areolae 8–12 µm wide, 40–70 µm diameter, dark brown.
A. portulacae (DC.) Kuntze
Ref.: (Saccardo 1888; Wilson 1907; Wakefield 1927; Baker 1955; Biga 1955).

(20) **on Resedaceae**
Sporangia 10–20 µm diameter
Oospores verrucose, 45–60 µm diameter
Albugo resedae (Jacz.) Cif. *et* Biga
Ref.: (Rayss 1938; Biga 1955).

(21) **on Scrophulariaceae**
Sporangia with equatorial wall thickening, 15–25 µm diameter
Oospores undescribed.
Albugo evansil Sydow
Ref.: (Sydow and Sydow 1912; Wakefield 1927).

(22) **on Solanaceae**
Sporangia 10–20 µm diameter
Oospores undescribed.
Albugo hyoscyami Zhang, Y.X., Wang *et* Fu
Ref.: (Zhang et al. 1986).

(23) **on Urticaceae**
Sporangia with uniform wall thickness, 10–20 µm diameter

Table 3.2 Diversity within the *A. candida* complex. (Choi et al. 2006)

Albugo candida isolates infecting		
Group-I		Group-II
Arabis	*Iberis*	*Capsella*
Autrieta	*Lunaria*	*Descuriania*
Berteroa	*Raphanus*	*Diptychocarpus*
Biscutella	*Sinapis*	*Draba*
Brassica	*Sisymbrium*	*Lepidium*
Cardaminopsis	*Thaldaspi*	
Diplotaxis	*Eruca*	
Erysimum	*Heliophila*	

Oospores smooth slightly wrinkled 30–50 µm diameter, pale brown.
Albugo pileae Tao *et* Y. Qin
Ref.: (Tao and Qin 1983).

3.4 Pathogenic Diversity

Recent molecular studies (Choi et al. 2006; Voglmayr and Riethmüller 2006) have supported not only its wide host range, but also the high degree of genetic diversity within *Albugo* parasitic to Brassicaceae. Subsequently, six new specialised species have been introduced, *A. koreana* from *C. bursa-pastoris* (Choi et al. 2007), *A. laibachii* from *A. thaliana* (Thines et al. 2009b), *Albugo voglmayrii* from *Draba nemorosa* (Choi et al. 2008), *Albugo hesleri, Albugo hohenheimia,* and *Albugo leimonios* from species of the genus *Cardamine* (Ploch et al. 2010). In addition, *A. lepidii* (Choi et al. 2007) and *A. resedae* (Choi et al. 2011b, c) were confirmed as species distinct from *A. candida* while *A. chardonii* from Cleomaceae was revealed to be synonymous with *A. candida* (Choi et al. 2007, 2009). It is believed that more than a dozen distinct species so far regarded to be *A. candida* await discovery (Thines and Voglmayr 2009). On the other hand, the phylogenetic results also demonstrated that the host range of *A. candida* s.str. extends from Brassicaceae to Cleomaceae and Capparaceae (Choi et al. 2007, 2009), but possibly not to Resedaceae (Choi et al. 2011b, c).

The three genera, *Alyssum, Barbarea*, and *Rorippa,* are widely distributed and of these, about 50 species have been recorded as common host plants of *A. candida* in monographic studies (Wilson 1907; Biga 1955; Kochman and Majewski 1970; Constantinescu and Negrean 1983; Novotel'nova and Pystina 1985; Ul'yanishchev et al. 1985; Vanev et al. 1993; Farr and Rossman 2010).

The genetic diversity within the *A. candida* complex from various host plants was investigated by sequence analysis of the ITS region of rDNA, and the cytochrome-*c*-oxidase subunit II (COX2) region of mtDNA (Choi et al. 2006). The aligned nucleotide sequences of *A. candida* shared significantly high distances, up to 20.4 and 8.9 %, in two genes. The phylogenetic trees showed two separate groups that corresponded to the host genera. Group-I included *A. candida* isolates infecting *Arabis, Autrieta, Berteroa, Biscutella, Brassica, Cardaminopsis, Diplotaxis, Eruca, Erysimum, Heliophila, Iberis, Lunaria, Raphanus, Sinapis, Sisymbrium* and *Thlaspi*. Group-II contained all isolates from *Capsella, Descurainia, Diptychocarpus, Draba, Lepidium* (Table 3.2). The genetic similarities between the two genes amongst isolates within Group-I were 99.0–100 % and 99.6–100 % while those within Group-II were 90.4–100 % and 91.1–100 %, respectively, showing considerably lower values for Group-II than for Group-I. The *A. candida* isolates from *C. bursa-pastoris* in Korea are clearly separated by sequence analysis for the two genes compared to those from Wales, England and the USA. Based on the molecular data from the two genes, the existence of high degree of genetic diversity within *A. candida* complexes warrants their division into several distinct species (Choi et al. 2006). *Albugo thaliana, A. candida* and *A.*

laibachii may occupy the same host within the same environment, but are phylogenetically distinct as inferred from analysis of both mitochondrial and nuclear DNA sequences (Thines et al. 2009). The strains obtained from *Brassica juncea* and *Raphanus raphanistrum* (wild radish) are different in their host range. The isolate from *B. juncea* showed significant levels of sporulation on both differential cultivars of *B. juncea,* Vulcan and Commercial Brown (used for differentiating pathotype 2A from 2V) confirming the presence of pathotype 2V in Western Australia. This same isolate was able to infect *Brassica napus* from China (FAN 189), and *Brassica tournefortii* (wild turnip), *Brassica nigra* and *Raphanus sativus*. This serves as a caution to breeders when sourcing resistance against *A. candida* from *B. napus* germplasm. The isolate from *R. raphanistrum*, tested against the same set of cruciferous host differentials, caused significant sporulation on *B. juncea* differential Commercial Brown, *B. napus* (FAN 189), *B. nigra* (90745), *R. raphanistrum* and *R. sativus*. Therefore, the strain from *R. raphanistrum,* while being a direct threat to *B. juncea,* may be a hazard, not only to any *B. napus* germplasm developed from *B. napus* breeding lines from China, but also to *B. nigra* and *R. sativus,* should these species be utilised commercially in Australia (Kaur et al. 2008). Isolates from Alberta and British Columbia in Canada appear to be hybrid pathotypes combining virulence characteristics of pathotypes 2A or 2V with 7A or 7V, since these isolates were pathogenic on both *B. juncea* and *B. rapa* cultivars (Rimmer et al. 2000).

3.5 Phylogenetic Relationship

Many monographic studies have recognized *A. candida* as the sole species infecting *C. bursa-pastoris,* (Saharan and Verma 1992). But Choi et al. (2007) have observed that *A. candida* on this host collected in Korea was differentiated from those in other geographical regions on molecular grounds. However, the study included only a small number of specimens from a limited geographical area. Thirty six *Albugo* collections on *Capsella* specimens comprising 8 from North and South America (Argentina, Canada, USA), 8 from Asia (India, Korea, Palestine), 18 from Europe (England, Finland, Germany, Hungary, Ireland, Latvia, Netherlands, Romania, Russia, Sweden, Switzerland) and 2 from Oceania (Australia), were used for phylogenetic and morphological analysis by Choi et al. (2007).

Several studies have already proven that the sequence analyses of the ITS region of nrDNA (Cooke et al. 2000; Constantinescu and Fatehi 2002; Choi et al. 2003; Voglmayr 2003; Göker et al. 2004; Thines and Spring 2005; Spring et al. 2006; Kaur et al. 2011a), and the mitochondrial COX2 gene, which encodes subunit II of the cytochrome c oxidase complex (Hudspeth et al. 2003; Martin 2000; Martin and Tooley 2003), are useful to resolve closely related species within the *Oomycota*. Molecular analysis based on LSU nrDNA was recently used to uncover the phylogenetic relationship amongst several species of the *Albuginaceae* (Voglmayr and Riethmüller 2006). In morphological analysis, characteristics of the oospore wall, which were previously suggested as useful to identify and compare species of *Albugo* (Choi and Priest 1995; Voglmayr and Riethmüller 2006), were found to be more helpful in distinguishing *Albugo* specimens on *C. bursa-pastoris,* than characters of the sporangiophores and sporangia. The use of morphological and molecular tools to investigate and to correctly identify *Albugo* species infecting *C. bursa-pastoris* and other hosts throughout the world and to help solving the systematic problem of the *A. candida* complex by stabilising the taxonomy of this pathogen by selecting an appropriate type specimen is suggested (Choi et al. 2007).

Phylogenetic maximum parsimony and Bayesian analyses of 60 collections belonging to 12 species of *Albugo* were performed using nuclear LSU ribosomal DNA sequences containing the D1 and D2 regions. These data were supplemented with detailed light and scanning electron microscopical analyses of oospore morphology. Molecular data revealed two main clades: One containing the collections from hosts belonging to the *Caryophyllales* and *Asteraceae,* and the other containing the collections from hosts belonging to the *Brassicaceae* and *Convolvulaceae*.

Separation into these two clades was also corroborated by oospore morphology. Whereas the *Albugo* collections from *Caryophyllales* did not form a monophyletic lineage, the collections originating from *Brassicaceae, Convolvulaceae* and *Asteraceae* each formed highly supported monophyletic clades. According to DNA sequence data and oospore morphology, the host genus *Amaranthus* harbours two distinct species, *Albugo amaranthi* and *A. bliti*. The DNA sequence data further indicate that *A. candida* and *A. tragopogonis* each may consist of several distinct lineages (Voglmayr and Riethmüller 2006).

The sequence analysis of *A. candida s.l.*, based on the COX2 mtDNA, ITS rDNA and 28S rDNA genes, was used to elucidate the phylogenetic structure within this complex. Although *A. candida* is commonly regarded as the sole species causing WR on various plants of the *Brassicaceae,* including *Capsella, A. candida* from *Capsella* was clearly divided into two clades, and several distinct lineages are present in *A. candida s.l*. The phylogenetic partition between Korean and other continental specimens indicated by two nuclear and one mitochondrial locus suggests that there is a reproductive barrier between these groups, meeting the criteria of genealogical concordance phylogenetic species recognition (GCPSR) (Taylor et al. 2000). Therefore, Korean specimens warrant species rank, based on morphological and molecular data. WR pathogens from various brassicaceous plants, including *Capsella* of other continental regions, formed a well-supported group in analysis of both COX2, ITS and 28S rDNA. The majority specimens from the *Brassicaceae* are positioned within this large group (group 1 in Figs. 3.3, 3.4, 3.5).

In a study by Voglmayr and Riethmüller (2006), two clades are apparent in *A. candida s.l.*, which are both parasitic to a variety of *Brassicaceae,* and are supported with high bootstrap values. To this dataset, *Albugo* specimens from *Capsella* in Korea were added, so several interesting points can be addressed. Usually, *Albugo* specimens from the same host genus are grouped closely together, with the exception of *Capsella* (Choi et al. 2006, 2007) and *Sisymbrium* (Voglmayr and Riethmüller 2006). In the corresponding phylogenetic reconstruction, *Albugo* from *Sisymbrium loeselii* is identical in sequence with *Albugo* from *Erysimum cheiranthoides*. This might possibly be explainable by a misidentification of the host, which is not highly unlikely, as *E. cheiranthoides* and *S. loeselii* are quite similar in appearance. In the dataset of Voglmayr and Riethmüller (2006), *Albugo* specimens from *Lunaria, Capsella, Raphanus* and *Berteroa* are grouped closely together, which are in accordance with the study of Choi et al. (2006), who also found the Korean specimens from *Brassica* and *Raphanus,* which have been reported to host *A. macrospora,* nested in the large, homogenous group of *A. candida s.str*. It is notable that the specimen from *Eutrema* originating from Taiwan, included in the first study, is also placed within this group. From this host species *A. wasabiae* has been described from Japan, a species ignored by subsequent authors. The specimens from *Arabidopsis* are placed in the more heterogenous, smaller group in Voglmayr and Riethmüller (2006) and in a similar distinct, heterogenous group in Choi et al. (2006), in the later study, this clade also contained *Albugo* from *Lepidium,* and *Albugo* from the Korean specimens from *C. bursa-pastoris,* which form a homogenous group. In the reanalysis of the dataset of Voglmayr and Riethmüller (2006), to which two *Albugo* specimens from *Capsella* gave similar results, suggesting placement of the Korean specimens from *Capsella* in a third distinct clade (group 3 in Fig. 3.5), which is placed sister to the clade containing *A. lepidii* and several further distinct lineages. Based on the molecular and morphological data available, it is believed that this phylogenetic group is to be regarded as new species, *A. koreana*. It also seems likely that other lineages in *A. candida s.l.* are distinct species (Choi et al. 2007).

The taxonomy of species within the *Albuginaceae* has been based primarily upon differences in the host range (Kochman and Majewski 1970; Vanev et al. 1993), particularly host families. The size of oospores, sporangiophores and sporangia of the *Albuginaceae* may vary according to host plant and climatic factors (Togashi et al. 1931; Makinen and Hietajarvi 1965; Lakra and Saharan 1988; Jat 1999; Saharan 1995). However,

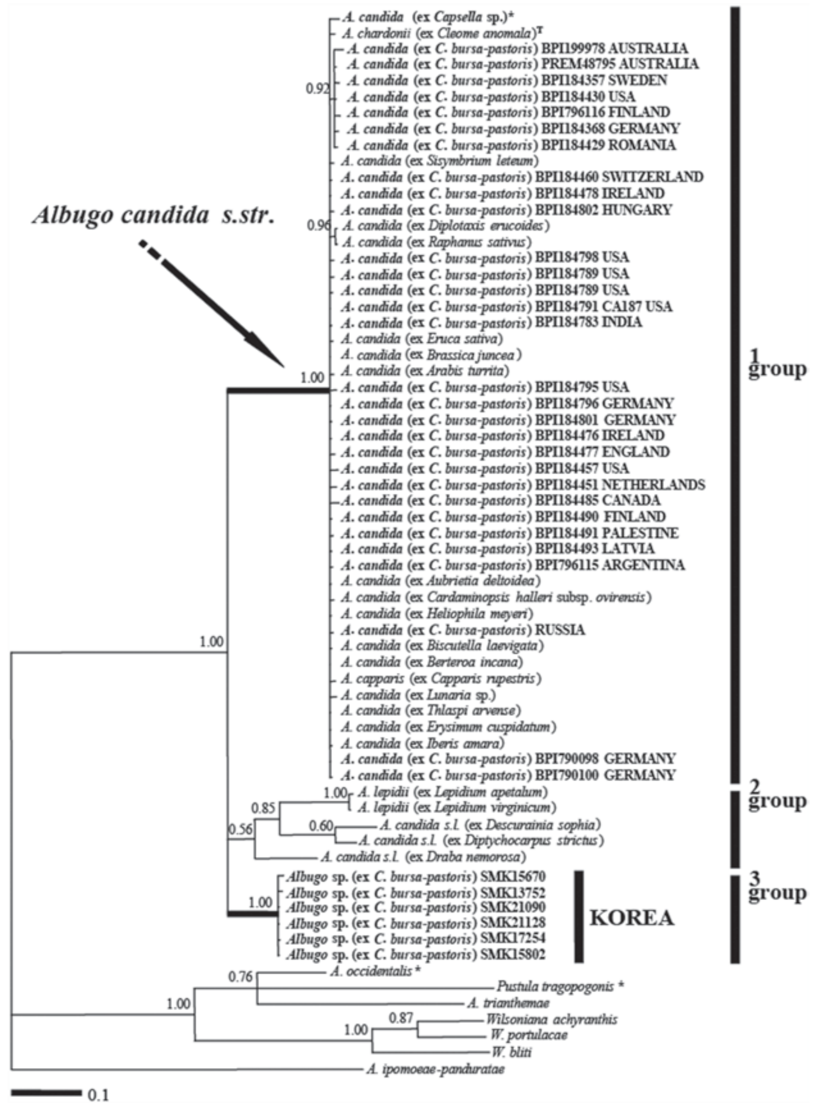

Fig. 3.3 Phylogenetic tree for *Albuginaceae* species from various hosts based on the partial COX2 mtDNA. Bayesian analysis showing mean branch lengths of a 50 % majority-rule consensus tree calculated from trees revealed during MCMC analysis of one-million generations. *Numbers* above the branches are the posterior probability values. The number of nucleotide changes between taxa is represented by branch length and the scale bar equals the number of nucleotide substitution per site. *Albugo* specimens from *Capsella bursa-pastoris* are in *bold*. An *asterisk* (*) shows taxa obtained from the GeneBank. (Choi et al. 2007)

ornamentation of the oospore wall remains the critically important morphological characteristic for distinguishing species of *Albugo* (Biga 1955; Choi and Priest 1995; Voglmayr and Riethmüller 2006). The most useful characteristic for distinguishing *A. koreana* from *A. candida s.str.* is the surface ornamentation of the oospores, which are verrucose or tuberculate without ridges in the first, and with confluent ridges in the later species. As described and illustrated by Wilson (1907), oospores in *A. candida s.l.* can be 'verrucose, or with low blunt ridges which are often confluent and irregularly branched'; judging from his drawings, his statement refers to characters which can be found on the same oospore (Wilson 1907). Corresponding to results of the phylogenetic analysis, WR pathogens that infect *Eruca* and *Heliophila* also had the same oospore ornamentation as *A. candida s.str*. Besides, *A. candida s.str*. from *C. bursa-pastoris*, the fungus from *Armoracia rusticana* (Voglmayr and Riethmüller 2006), *R. sativus* (Wilson 1907), *B. rapa* (Tewari and Skoropad 1977), and *B. juncea* (Nath et al. 2000) has been previously observed to have identical oospore characteristics.

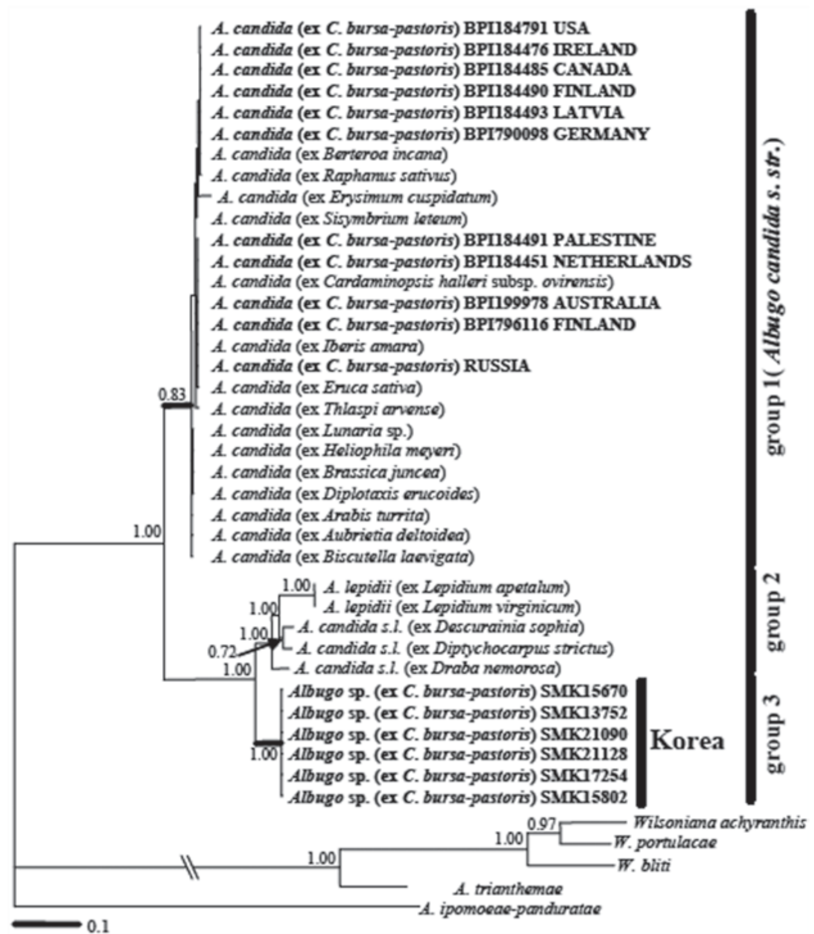

Fig. 3.4 Phylogenetic tree for *Albuginaceae* species from various hosts based on the complete ITS nrDNA. Bayesian analysis showing mean branch lengths of a 50 % majority-rule consensus tree calculated from trees revealed during MCMC analysis of one-million generations. *Numbers* above the branches are the posterior probability values. The number of nucleotide changes between taxa is represented by branch length and the scale bar equals the number of nucleotide substitution per site. *Albugo* specimens from *Capsella bursa-pastoris* are in *bold*. (Choi et al. 2007)

Therefore, oospores with low blunt ridges that are often confluent and irregularly branched might be seen as a morphological characteristic of *A. candida s.str*. infecting various brassicaceous plants, including *Capsella* from countries other than Korea, and oospores without blunt ridges as a characteristic of Korean specimens of *Albugo* from *Capsella*. *Albugo* materials from five genera, *Capsella, Draba, Eruca, Heliophila* and *Lepidium*, from which oospores were successfully observed, were divided into two types on the basis of wall ornamentation; *Albugo* specimens from *Draba* and *Lepidium* were exhibiting some similarity compared to *A. koreana,* while those from *Eruca* and *Heliophila* were identical to those of *A. candida s.str*. Therefore, the three molecular groups of *A. candida s.l*. found by Choi et al. (2006), and in the amended dataset of Voglmayr and Riethmüller (2006), as well as ITS and COX2 molecular phylogenetic reconstructions by Choi et al. (2007), may be morphologically also differentiated by oospore characteristics, with groups 2 and 3, which are grouped with high support in LSU and ITS trees, exhibiting similar oospore ornamentation. Group 1 most likely contains a single species, *A. candida s.str*., group 2 comprises several species, amongst which are *A. lepidii* and several other lineages, which might also warrant species rank, and its sister group (group 3) contains *Albugo* specimens from *Capsella* from Korea, which are assigned to the new species *A. koreana* (Choi et al. 2007).

Molecular data of Choi et al. (2006, 2007) revealed that *Lepidium* WR are differentiated from *A. candida s.str*. and *Albugo* from *Capsella* in Korea. This supports the conclusion of Rao

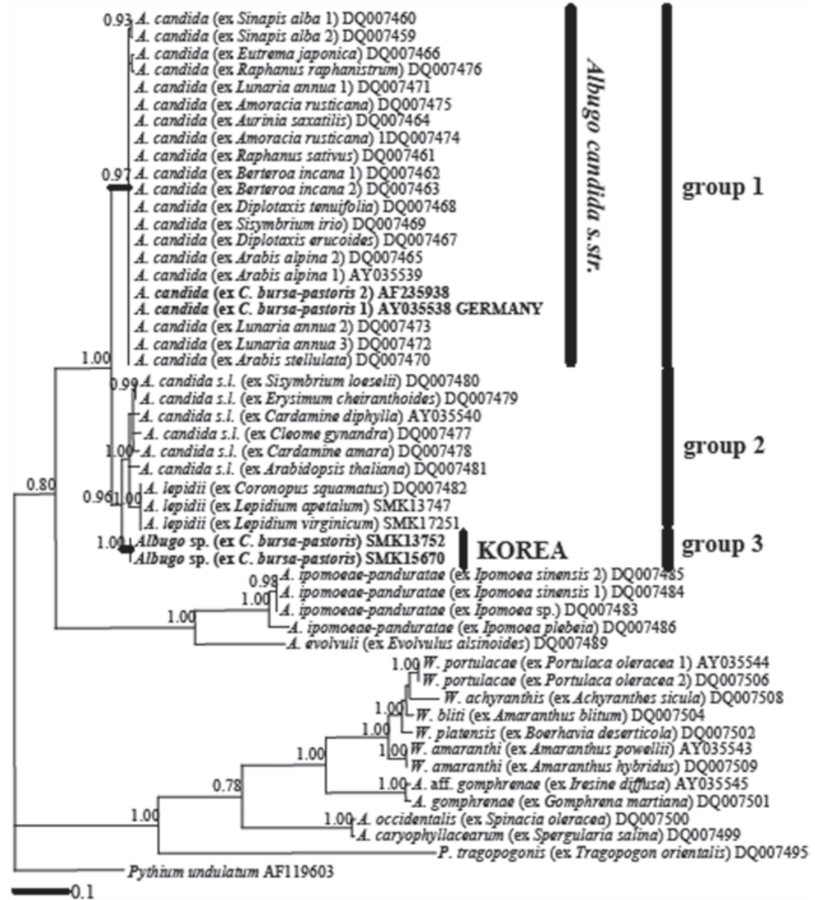

Fig. 3.5 Phylogenetic tree for *Albuginaceae* species from various hosts based on the partial 28S nrDNA sequences alignment of Voglmayr and Riethmüller (2006). Bayesian analysis showing mean branch lengths of a 50 % majority-rule consensus tree calculated from trees revealed during MCMC analysis of one-million generations. *Numbers* above the branches are the posterior probability values. The number of nucleotide changes between taxa is represented by branch length and the scale bar equals the number of nucleotide substitution per site. *Albugo* specimens from *Capsella bursa-pastoris* are in *bold*. (Choi et al. 2007)

(1979), who described *Albugo* specimens from *Lepidium* as *A. lepidii* A. N. S. Rao. The presence of several species on the same host genus, based on molecular and morphological properties, was also shown by Voglmayr and Riethmüller (2006), who found that two distinct species were parasitic to *Amaranthus,* based on nrLSU-DNA sequences and oospore ornamentation. As this species is clearly embedded within *Wilsoniana* Thines, both morphologically and based on molecular phylogeny, as also shown by Voglmayr and Riethmüller (2006), and a reinvestigation of the dataset of Choi et al. (2007), *A. amaranthi* (Schwein.) Kuntze is combined into that genus below, to avoid nomenclatural confusion. *A. gomphrenae,* which is most likely also a member of that genus, as molecular data suggests (Voglmayr and Riethmüller 2006), is not transferred to *Wilsoniana* (Choi et al. 2007). *A. candida* races (9) reported from Western Australia are similar phylogenetically. However, the isolate obtained from *B. tournefortii* was phylogenetically most distinct (Petkowski et al. 2010; Kaur et al. 2011b). *Albugo* from *Alyssum montenum, Barbarea vulgaris* and *Rorippa* species was placed in three phylogenetically distinct clades, but closer to *A. candida* s. str. (Choi et al. 2011b, c).

3.6 Structures and Reproduction

The members of the Albuginaceae are distinguished from those of related families by the formation of the asexual sporangia in basipetal chains.

3.6.1 Mycelium and Haustoria

The non-septate and intercellular mycelium of *Albugo* species feeds by means of globose or knob-shaped intracellular haustoria. The details of haustorial formation and development have been given by Berlin and Bowen (1964a, b), Coffey (1975, 1983), Davison (1968), Fraymouth (1956) and Wager (1896). Fine structures detailed information is given in sect. 11.

3.6.2 Sporangiophore and Sporangia

The mycelium soon organizes the characteristic groups of sporangiophores which develop beneath the epidermis, raising it to make whitish pustules or extended blister-like areas due to the merging of adjacent sori. As soon as the covering epidermis ruptures, the sporangia are set free (Heald 1926; Wager 1896). After a pustule or sorus forms, numerous short sporangiophores arise from the mycelium in a closely compacted palisade layer beneath the epidermis and at a right angle with it. The first sporangium is formed at the tip of the sporangiophore, and others are formed successively beneath. Pads of gelatinous material formed between successive sporangia function as disjunctors. The sporangiophores are short, basally branched, club shaped and give rise to simple chains of sporangia. The number of sporangia produced is indefinite. They are formed in basipetal succession; that is, the sporangiophore forms a cross-wall or septum, cutting off that portion which is to become a sporangium. The sporangiophore increases in length, a second sporangium is cut off and the process continues, resulting in the simple chains of multinucleate sporangia. As sporangial production continues, the older, terminal portions of the chain break, releasing the individual sporangia. The sporangia are hyaline and nearly spherical and germinate by the formation of zoospores and, on rare occasions, by means of a germtube (Heald 1926; Wager 1896; Walker 1957).

Depending on the hosts and locations, the size of sporangia ranged between 13.6–21.8 µm (Table 3.3): the size of sporangia on *B. rapa* var. Brown Sarson from Hisar, India ranged from 13.6 to 17.0 µm indicating the presence of mostly microsporangia while sporangia formed on *B. rapa* var. Toria at Hisar, India forms only macrospores of 17.5–21.3 µm. The sporangia forming dotted pustules on *B. juncea* under Madhya Pradesh, India had two distinct forms:

a. Microsporangia: size 14.0–16.0 µm
b. Macrosporangia: size 17.0–21.0 µm

This is in accordance with the observations of Togashi and Shibasaki (1934), who included sporangia with a size of 14.5–15.5 µm in microspores, and 18–20 µm as macrospores. However, (Table 3.3) at large did not support the theory of macro and microspores since in majority of samples a clear cut distinction could not be established. The range of sporangial dimension varied from 13.6 to 21.8 µm. Holliday (1980) reported 15–18 µm, Kolte (1985) reported the sporangial diameter of 12–18 µm. Lakra and Saharan (1989b), besides supporting the work of Biga (1955) and Togashi and Shibasaki (1934) for the existence of macro and micro sporangia, indicated that sporangia existed in four distinct groups.

1. Presence of micro and macro sporangia.
2. A continuous range without any demarcation of above two forms.
3. Presence of only microsporangia having average size < 16 µm.
4. Presence of only macrosporangia having average size.

The above four categories of sporangia forms are a function of distinct pathological race which should be established on the basis of pathogenicity on a standardized set of host differentials by taking single pustule. On the critical observations, one can find mostly small sized sporangia as spherical while bigger size sporangia are elongated to globular. Variation in per cent germination was observed from 72.7 to 82.1 which may be due to age of sporangia and differential temperature responses to a pathotype formed on specific host species.

Table 3.3 Morphological variations in sporangia of *A. candida* in different crucifers from different locations. (Lakra and Saharan 1988)

Categories of pustules on different crucifers	Size of sporangia (µm)	Shape of sporangia	Percent germination at 13.5±0.5 °C after 8 h
Circular (1–3 mm diameter) having halo from *B. juncea*	13.9–20.6 (18.7)	Globular	79.6
Circular (3–5 mm diameter) having halo from *B. juncea*	13.8–20.6 (18.6)	Globular	81.0
Pin head size pustules from *B. juncea*	13.6–20.0 (71.0)	Spherical	82.1
Circular (5–7 mm diameter) from *B. juncea*	13.8–20.6 (18.6)	Globular	80.3
Irregular (0.5–2.0 mm diameter) with thick raised mass from *B. juncea*	19.0–12.8 (20.0)	Slightly elongate to globular	80.4
Small (1–3 mm diameter) from *B. nigra*	15.6–21.7 (19.1)	Spherical	78.5
Dark green bordered (1–2 mm diameter) from *B. pekinensis*	14.0–12.5 (18.8)	There are two types of spores (a) small circular (b) big elongated to globular	75.0
Circular (3–5 mm diameter) from *B. juncea* (Karnataka)	16.7–19.0 (17.8)	Spherical	–
Broad circular (9–12 mm) from *B. juncea* (Gujarat)	16.0–20.1 (17.3)	Circular	–
Pin head (1 mm diameter) from *B. nigra* (IARI, N. Delhi)	16.4-21.0 (18.4)	Globular	–
Pin headed (1–2 mm diameter) *B. juncea* having yellow halo (West Bengal)	16.5–19.8 (18.3)	Globular	–
Irregular broad type pustules up to 2 cm diameter from *B. juncea* (Rajasthan)	14.9–21.0 (18.0)	Globular	–
Circular (3–5 mm diameter) from *B. juncea* (Rajasthan)	16.0–20.0 (17.8)	Spherical	–
Small dots on *B. juncea* (Tamil Nadu)	16.5–18.9 (17.0)	Spherical	–
Small sized (1–2 mm) from *B. juncea* (Punjab)	16.6–20.7 (18.4)	Globular	–
Medium sized (3–5 mm) from *B. juncea* (Punjab)	16.0–20.9 (18.0)	Globular	–
Dotted (1–1.5 mm) on *B. juncea* (Madhya Pradesh)	14.0–21.0 (17.8)	(a) Micro circular (14.0–26.0) (b) Macro slightly elongated (17.0–21.0)	–
Samples of staghead infection of *B. juncea* (HAU, Hisar)	18.0–21.4 (19.4)	Globular only macrospores more than 16 µm	76.3
Samples of staghead infection of *B. rapa* var. Toria (Hisar)	17.5–21.3 (19.0)	Globular only macrospores more than 16 µm	74.9
White rust pustules 1–3 mm diameter from *B. chinensis* (Hisar)	13.9–19.0 (15.9)	Sherecal	82.1
White rust pustules 1–3 mm diameter from *B. juncea* (Uttar Pradesh)	14.0–19.5 (16.5)	Globular	–
White rust pustules (1–2 mm) from *B. juncea* (Gujarat)	14.6–20.0 (16.6)	Globular	–
Pin head pustules 1–2 mm diameter from *B. rapa* var. Brown Sarson (Hisar)	13.6–17.0	Globular only microspores present	72.7

( ) mean of 100 sporangia
– No germination could be observed because sporangia were dead

The morphological variability in sporangia of *A. candida* isolates (single pustule) infecting different oilseed *Brassica* and their genotypes collected from different geographical locations in India was determined in terms of shape and size (Jat 1999). The size of sporangia was in two extreme ranges (Table 3.4).

(i) The sporangia of isolate ACjun-5, ACjun-7 and ACjun-9 were in the group of having no extreme range in length and width (13.8–13.8 µm).
(ii) The sporangia of remaining 31 single pustule isolate (SPI) had similar extreme range (13.8–27.5 µm) in length and width.

The average dimensions of length based on 100 sporangia per isolate fall between 25.9 µm in isolate ACjun-8, and 13.8 µm in isolates ACjun-5, ACjun-7 and ACjun-9. Likewise, their width ranges from 22.3 µm in isolate ACjun-20, and 13.8 µm in isolate ACjun-5, ACjun-7 and ACjun-9. However, the sporangia of all the isolates of *A. candida* collected from different genotypes of *B. juncea, B. rapa* and *B. nigra* were grouped into nine morphological forms on the basis of average dimensions, as under:

(i) The sporangia of isolate ACjun-5, ACjun-6, ACjun-7, ACjun-9 and ACjun-27 possessed the average dimension of 13.8–14.3 × 13.8–14.3 µm.
(ii) The sporangia of isolate ACjun-2 and ACjun-16 were in the average dimension of 16.5–17.9 × 14.3–15.1 µm.
(iii) The sporangia of isolate ACjun-l, ACjun-30, ACrap-2 and ACnig-1 possessed the average dimension of 18.4–18.7 × 16.8–17.3 µm.
(iv) The sporangia of isolate ACjun-3, ACjun-17, ACjun-24 possessed average dimension of 19.36–19.8 × 14.3–14.8 µm.
(v) The sporangia of isolate ACjun-18, ACjun-22, ACjun-26, ACjun-29 and ACrap-l were in between the average dimension of 20.1-20.6 × 14.9-18.9 µm.
(vi) The sporangia of isolate ACjun-15, ACjun-25 and ACjun-31 possessed an average dimension of 21.2–21.7 × 14.3–18.4 µm.
(vii) The sporangia of isolate ACjun-l1, ACjun-14, ACjun-20, ACjun-21 and ACjun-28 had average dimension of 22.0–22.8 × 16.0–22.3 µm.
(viii) The sporangia of isolate ACjun-13, ACjun-19 and ACjun-23 had average dimension in between 23.4–23.7 × 14.0–14.6 µm.
(ix) The sporangia of isolate ACjun-4, ACjun-8, ACjun-10 and ACjun-12 possessed an average dimension of 24.8–25.9 × 14.0–21.5 µm.

Further, the average dimensions of sporangia of isolates collected from leaf of *B. juncea* varied from 13.8–25.9 × 13.8–22.3 µm in comparison to isolates collected from systemically infected leaf, inflorescence and flower parts which showed average sporangial dimension of 19.8–24.8 × 14.0–21.4 µm.

While taking into consideration the average dimension of sporangia, marked differences were observed amongst the sporangial dimension of isolates collected from different location. Isolates collected from *B. juncea* genotypes had greater variability in average sporangial dimension (13.8–25.9 × 13.8–22.3 µm), whereas those from *B. rapa* and *B. nigra* had similar average sporangial dimension (18.4–20.6 × 17.1–18.9 µm). The smaller dimension of sporangia on Kranti was observed in comparison to those on cv. RCC-4, collected from the same location (Kangra, Himachal Pradesh). However, sporangia collected from the leaves of Kranti possessed marked differences in the average dimension at different locations (Kangra, Bharatpur and Navgaon). The sporangia of isolates ACjun-20 and ACjun-21 collected from leaves of RL 1359 (Navgaon) had no marked variation in average length, but differed in average width as well as in shape. The sporangia of isolate ACjun-26, ACjun-27 and ACjun-28 collected from DS-7, R.S. Pura, (Jammu and Kashmir) possessed marked differences in average dimensions with respect to host tissue infected and had spherical and ellipsoidal shape.

The isolates of Nawalganj, Uttar Pradesh having pinhead and medium-sized pustules collected from leaves of Varuna (ACjun-ll) and Rohini

Table 3.4 Morphological variations in sporangia of *A. candida* infecting oilseed *Brassica* at different geographical regions of India. (Jat 1999)

Host		Isolate code	Shape of sporangia	Dimension of sporangia (µm)			
Genotype	Tissue			Range		Average*	
				Length	Width	Length	Width
RH 30	Leaf	ACjun-1	Ellipsoidal	13.8–27.5	13.8–27.5	18.7	168
RH 30	Leaf	ACjun-2	Spherical	13.8–27.5	13.8–27.5	16.5	15.1
RH 30	Leaf	ACjun-3	Ellipsoidal	13.8–27.5	13.8–27.5	19.8	14.3
RH 30	Inflorescence	ACjun-4	Ellipsoidal	13.8–27.5	13.8–27.5	24.8	14.0
RH 30	Leaf	ACjun-5	Spherical	13.8–13.8	13.8–13.8	13.8	13.8
EC 182925	Leaf	ACjun-6	Spherical	13.8–27.5	13.8–27.5	14.3	14.0
DYS-7-3-1	Leaf	ACjun-7	Spherical	13.8–13.8	13.8–13.8	13.8	13.8
Candle	Leaf	ACrap-1	Spherical	13.8–27.5	13.8–27.5	20.6	18.9
Span	Leaf	ACrap-2	Spherical	13.8–27.5	13.8–27.5	18.7	17.1
Local	Leaf	ACnig-1	Spherical	13.8–27.5	13.8–27.5	18.4	17.3
RL-91-191	Leaf	ACjun-8	Ellipsoidal	13.8–27.5	13.8–27.5	25.9	16.0
RL-91-191	Leaf	ACjun-9	Spherical	13.8–13.8	13.8–13.8	13.8	13.8
RH 9119	Leaf	ACjun-10	Ellipsoidal	13.8–27.5	13.8–27.5	25.9	16.8
Varuna	Leaf	ACjun-11	Spherical	13.8–27.5	13.8–27.5	22.8	21.5
Rohini	Leaf	ACjun-12	Spherical	13.8–27.5	13.8–27.5	25.0	21.5
RH 30	Leaf	ACjun-13	Ellipsoidal	13.8–27.5	13.8–27.5	23.7	21.4
RH 30	Leaf	ACjun-14	Spherical	13.8–27.5	13.8–27.5	22.8	14.3
RCC-4	Leaf	ACjun-15	Ellipsoidal	13.8–27.5	13.8–27.5	21.7	14.3
Kranti	Leaf	ACjun-16	Ellipsoidal	13.8–27.5	13.8–27.5	17.9	15.4
RK-9501	Leaf	ACjun-17	Ellipsoidal	13.8–27.5	13.8–27.5	19.3	14.9
Kranti	Leaf	ACjun-18	Ellipsoidal	13.8–27.5	13.8–27.5	20.6	14.6
Kranti	Leaf	ACjun-19	Ellipsoidal	13.8–27.5	13.8–27.5	23.7	22.3
RL 1359	Leaf	ACjun-20	Spherical	13.8–27.5	13.8–27.5	22.6	16.0
RL 1359	Leaf	ACjun-21	Ellipsoidal	13.8–27.5	13.8–27.5	22.8	16.2
BIO 902	Leaf	ACjun-22	Ellipsoidal	13.8–27.5	13.8–27.5	20.6	14.0
RH-30	Gynoecium	ACjun-23	Globose	13.8–27.5	13.8–27.5	23.4	14.8
RH-30	Sepal	ACjun-24	Ellipsoidal	13.8–27.5	13.8–27.5	19.8	18.4
RH-30	Stamen	ACjun-25	Ellipsoidal	13.8–27.5	13.8–27.5	21.2	18.4
DS-7	Leaf (SI)	ACjun-26	Ellipsoidal	13.8–27.5	13.8–27.5	20.1	14.3
DS-7	Leaf	ACjun-27	Spherical	13.8–27.5	13.8–27.5	14.3	19.8
DS-7	Staghead	ACjun-28	Ellipsoidal	13.8–27.5	13.8–27.5	22.0	15.4
RH-30	Leaf	ACjun-29	Ellipsoidal	13.8–27.5	13.8–27.5	20.4	16.8
RH-30	Leaf	ACjun-30	Spherical	13.8–27.5	13.8–27.5	18.6	18.4
RH-30	Leaf	ACjun-31	Globose	13.8–27.5	13.8–27.5	21.4	–

ACjun = *A. candida* isolate derived from *B. juncea*; ACrap = *A. candida* isolate derived from *B. rapa* and ACnig = *A. candida* isolate derived from *B. nigra*; * Average of 100 sporangia

(CACjunTl-12) had greater difference in average length of sporangia, but similar in average width and spherical shape. The pinhead-sized pustule isolate of Berhampur, West Bengal obtained from leaves of *B. juncea* genotype RK-9501 possessed an average size of 19.3 × 15.4 µm. The clear cut demarcation between micro and macro sporangia could be established only in isolates ACjun-5, ACjun-6, ACjun-7, ACjun-9 and ACjun-27 which all fall in the average dimension range of 12.5–15.0 µm and grouped as *A. candida* microspora. The isolates ACjun-2 and ACjun-16 were in the average dimension range of 15–17.5 µm, and grouped as *A. candida* or macrospora while remaining 27 isolates were in none of the above groups.

In general, most of the sporangia of different isolates were spherical, ellipsoidal and globose in shape. The small-sized and young sporangia were spherical in shape while large and old spo-

rangia were spherical, ellipsoidal and globose. A critical perusal of data revealed that the morphological variability in the shape and size of sporangia could not establish any relationship with the pathogenic variability.

3.6.3 Zoospores

The production of zoospores was first recorded by Prevost (1807). Tulasne (1854) and Hoffmann (1859) were unable to confirm observation of Prevost and described germination by germ tubes. Melhus (1911) mentioned that deBary (1860) described for the first time the details of zoospore formation. Sporangia absorb water and swell, vacuoles develop in the granular protoplasm, and finally 4–12 uninuleate polyhedral portions of the protoplasm are delineated by fine lines. In the meantime, an obtuse papilla is formed at one side of the sporangium, which now swells and opens. The zoospores, still immobile, emerge usually one by one, with final cleavage following complete emergence of the sporangium's contents. The flagella soon become apparent by an oscillatory motion of the entire zoospore mass. These single-nucleated spores (zoospores) formed in sporangia are released only in aqueous environment. The slightly concave–convex zoospore contains a disc-like vacuole on one side, near which are attached two flagella, one short and one long, by which the zoospore soon detaches itself from the mass and swims away if liquid is present. They have one tinsel flagellum, and one whiplash flagellum. Only the tinsel flagellum has distinctive flagellar hairs. Zoospore formation occurs within minutes and is considered one of the fastest developmental processes in any biological system. Once released from the sporangium, zoospores exhibit chemotactic, electrotaxis and autotaxis or autoaggregation to responses to target new hosts for infection (Walker and West 2007). Zoospores soon come to rest, retract their flagella, encyst and germinate by the formation of a germ tube. If germination occurs on a susceptible host, the germ tube penetrates through stomata to form an intercellular mycelium (Heald 1926; Wager 1896; Walker 1957).

3.6.4 Sexual Organs

The oogonia and antheridia are formed from the mycelium in the intercellular spaces of the host, particularly in a systemically invaded tissue (Wager 1896). Oogonia are globose, terminal or intercalary, each containing up to 100 nuclei and its contents clearly defined into a peripheral zone of periplasm, and a single central oosphere. Antheridia are clavate, each containing 6–12 nuclei, and are applied to the sides of an oogonium (Heald 1926; Heim 1959; Walker 1957).

3.6.5 Gametogenesis, Fertilization and Oospore Formation

The classical information on the process of gametogenesis, fertilization and oospore formation in different species of *Albugo* has come from the exhaustive studies of Wager (1896); Davis (1900); Stevens (1899, 1904) and Thirumalachar et al. (1949). One or more antheridia come to occupy a position close to an oogonium. There are two types of egg organization within an oogonium. In certain species (*A. candida*), the protoplast becomes differentiated into a peripheral or external zone, the periplasm, which contains many nuclei, and a central mass, the egg cell or ooplasm, which contains a single nucleus. In other species (*A. bliti, A. portulacae, A. tragopogonis*), the central ooplasm remains multinucleate. The antheridium, which is a multinucleate cell, produces a short, tube-like outgrowth, the fertilization tube, which penetrates the periplasm and comes in contact with the egg cell or ooplasm. The antheridial or male nuclei are discharged through this tube into the egg cell. In the uninucleate egg, the female nucleus fuses with a single male nucleus, whereas in the multinucleate egg, female and male nuclei fuse in pairs. This nuclear union constitutes the process of fertilization (Heald 1926; Walker 1957). Following fertilization, the egg is gradually transformed into a thick-walled oospore. The periplasm is absorbed, the oospore wall darkens and thickens, and develops a characteristic external ridge, reticulations or knobs while the interior of the oospore fills with an

abundance of reserve food in the form of oily or fatty globules. The fully developed oospore lies within the old empty oogonial cell. The oospores are released only by weathering and decay of the host tissues (Heald 1926).

Little is known about the sexual reproduction and genetics of the fungus due to the difficulty in determining the factors responsible for induction of the sexual reproductive phase. The effect of temperature on in vitro germination of oospore has been reported (Verma and Petrie 1975b), however, information on the optimum temperature and the time required for production of oogonia, antheridia and mature oospore in leaf tissue would assist in designing experiments for the study of oogenesis, fertilization and karyogamy. The detached leaf culture technique was used to determine effect of temperature and incubation period on progressive development of oospores of *A. candida* race 2V in *B. juncea* leaves (Goyal et. al. 1995).

The progressive development of *A. candida* oospores in detached leaves of *B. juncea* was largely dependent on incubation temperature (Goyal et. al. 1995). Oogonia and oospore production occurred over the entire range of incubation temperatures of 10–27 °C. The earliest development of oogonia was observed at 25 °C, 7 days after inoculation and incubation. The largest number of oospores was formed at 21°, 23°, 24° and 25 °C treatments were observed 12 days post-inoculation and their numbers decreased thereafter. At lower and higher temperatures, development of oospores occurred later. Maximum numbers of oospores were recorded after 17 days at 15 °C. Mature oospores were observed 12 days after incubation at 23 and 24 °C. The number of mature oospores was still increasing at 17 days post-inoculation at all temperatures. Mature oospores developed later and more slowly at lower and higher incubation temperatures.

The production of *A. candida* oospores in leaf tissues could be important in disease perpetuation. Hypertrophied tissues (staghead) are quite resistant to decomposition and the release of oospores could take up to 3–4 years. Leaf tissues are quick to decompose, and thus oospore release from such material could be expected the following year. In naturally infected leaves, oospores are produced in the later part of the season when temperatures are warm (Verma 1989). Warm temperatures hasten leaf senescence, which in turn enhances tissue decomposition and early release of oospores. The knowledge of an optimum temperature and time for the development of oospores in detached leaves in this study (Goyal et al. 1995) makes it possible to compare the sequential events of oogenesis, fertilization and karyogamy in various *Albugo* species at the earliest stages of their development. These, comparative investigations in *Albugo* species could also be useful in fungal taxonomy. The detached leaf culture technique for oospore development could also be used to determine the heterothallic nature of *A. candida.*

3.6.6 Oospores

The characteristics of oospores are useful criteria for distinguishing species of *Albugo*. According to Wilson (1907), the species fall into two well-defined groups. The first group includes *A. candida, A. ipomoeae-panduratae, A. lepigoni, A. sibirica* and *A. tropica,* in which the epispore of the oospore is tuberculate or ridged. Zalewski (1883) and Stevens (1901a, b, c) confirmed that this is the more specialized group, where there is complete development of the epispore with cytological phenomena. The second group is characterized by a reticulate epispore and contains the remaining species in which oospores are known. In *A. bliti* and *A. platensis* the reticulations are very evident, and the areolae are deep and unoccupied by elevations. The pattern is often imperfectly developed. In *A. tragopogonis* and *A. swertiae* the areolae are not as deep and the reticulations are crested at their angles with more or less prominent tubercles. In *A. portulacae* tubercles form within the areolae while the reticulations themselves are similar to those of *A. bliti*. Unique within the genus is *A. occidentalis,* in which the epispore is finely reticulate and the areolae shallow as to give the impression of pits rather than reticulations (Wilson 1907). According to Zalewski (1883), the reticulate oospores have a less perfectly developed epispore which

Table 3.5 Size of oospores of *A. candida* on different host/genotypes. (Saharan 1995)

Host/genotypes	Size of oospores (µm)	
	Length	Breadth
B. rapa var. Brown Sarson	50.0–75.0 (52.0)	37.5–62.5 (51.5)
B. rapa var. Parkland	50.0–75.0 (67.0)	50.0–62.5 (54.5)
B. rapa var. Tobin	50.0–75.0 (66.4)	50.0–62.5 (55.5)
B. rapa var. Torch	62.5–75.0 (67.0)	50.0–62.5 (54.8)
B. rapa var. Yellow Sarson	58.0–87.0 (71.2)	53.0–78.0 (61.7)
B. carinata	50.0–87.5 (71.4)	50.0–75.0 (58.0)
B. juncea	48.8–68.3 (60.5)	43.9–63.4 (55.3)
B. nigra	50.0–75.0 (69.2)	50.0–62.5 (58.3)
B. parkinensis	62.5–75.0 (68.3)	50.0–62.5 (58.8)
B. tournifortii	50.0–75.0 (68.2)	37.5–62.5 (54.5)
Sinapis alba	50.0–75.0 (57.7)	50.0–62.5 (51.0)

Figures in parentheses are average of 125 observations

reaches its highest development in *A. tragopogonis*. Stevens (1901a, b, c) confirmed this arrangement of species.

Saharan (1995) reported that the size of oospores of *A. candida* varies with host plant infected. The length of the oospore ranges from 50.0 to 87.8 µm on 11 differential host/genotypes. On an average, maximum length of oospore was 71.4 µm in case of *B. carinata,* whereas maximum breadth was 61.7 µm in case of *B. rapa* var. Yellow Sarson. On an average, minimum length was 52 µm in *B. rapa* var. Brown Sarson, and the minimum breadth was 51.0 µm in case of *B. alba* (Table 3.5).

3.7 Life Cycle of the Pathogen

The symptoms of general or systemic infection of *A. candida* are distortion, hypertrophy, hyperplasia and sterility of inflorescences (stagheads) of the host plant. When ripe, stagheads are almost entirely composed of brown, thick-walled oospores which are known to survive during storage for a period of over 20 years (Verma and Petrie 1975b). Oospores, carried on seed, or overwintered in soil, are an important source of initial infection in the field (Verma and Petrie 1975b). After a resting stage, oospores germinate by the production of one or two simple or branched germ tubes, or by release of 40–60 zoospores from either sessile or terminal vesicles (Petrie and Verma 1974a,b; Verma and Petrie 1975b). When the vesicle wall bursts, the zoospores are liberated, they swarm, encyst, and are finally germinated by germ tubes. Germ tubes from zoospores penetrate the host cotyledons/leaves through stomata. The mycelium is intercellular and feeds by means of small, capitate, globose or knob-shaped intracellular haustoria which penetrate the host cell walls through minute perforations. Hyphae grow around palisade mesophyll cells as a downward spiral penetrating the individual cells with as many as 14 haustoria per cell (Verma et al. 1975). The coenocytic mycelium branches profusely and eventually fills all the available intercellular spaces. At a certain stage of maturity, short, club-shaped sporangiophores are developed from a dense layer of mycelium which shows a characteristic branching pattern not common in earlier stages of vegetative mycelial growth (Verma et al. 1975). The sporangiophores are borne in close proximity to one another in solid layers immediately below the epidermis of the host. Each sporangiophore gives rise to several sporangia in succession with the oldest at the tip of the chain and the youngest at the base. Both the growth of the mycelium and the production of an enormous number of sporangiophores and sporangia exert pressure from below on the host epidermis causing it to break. The released sporangia form a white crust on the

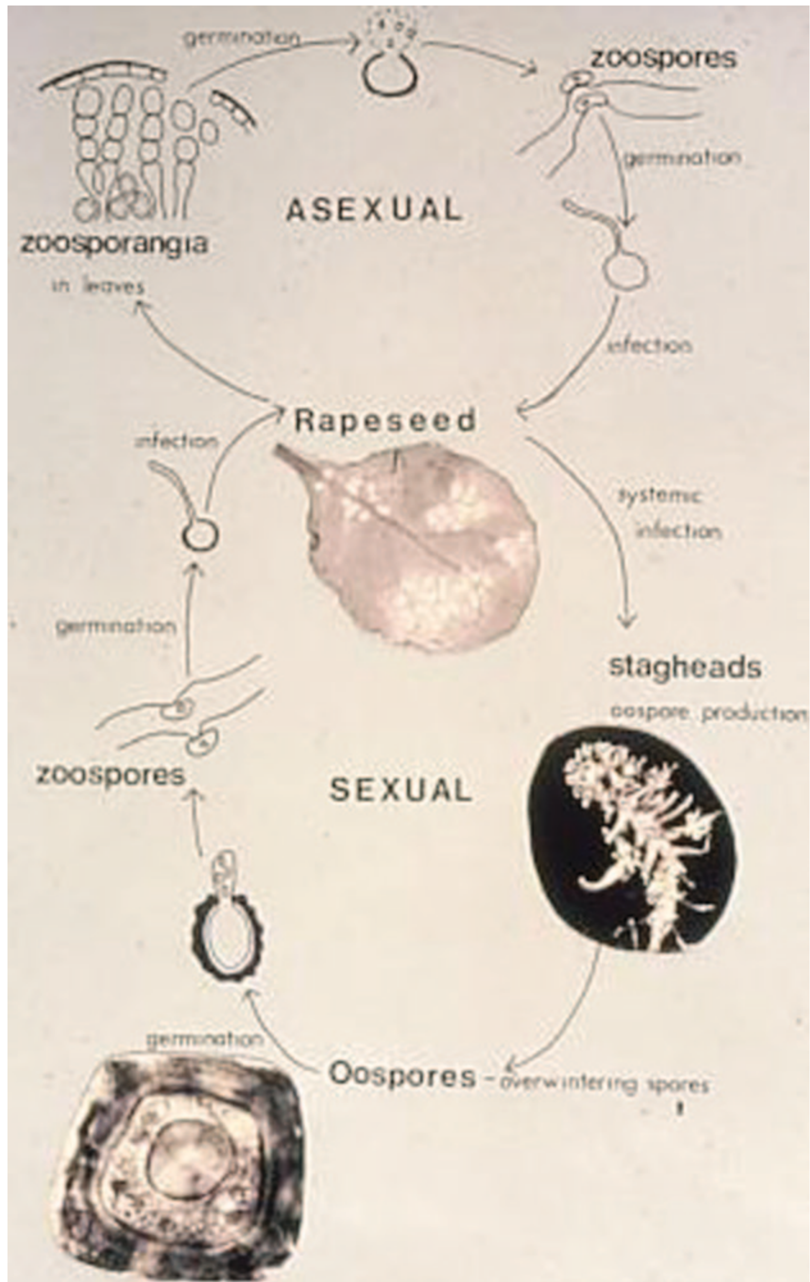

Fig. 3.6 Life cycle of *A. candida* (Saharan and Verma 1992)

surface of the host. The sporangia are disseminated by wind, by water or by other means onto the host surface. The sporangium, when germinating, extrudes 4–12 zoospores into a sessile or terminal vesicle. The zoospores, on germination, infect new plants (Fig. 3.6). Thus, the asexual cycle is repeated many times during the season. Sporangia are important only in the spread of the disease during the growing season, but play no important role in the survival of the pathogen during the noncrop season. The oospores, however, are important both for initiation of the disease, as well as for the survival of the pathogen in the absence of the host (Saharan and Verma 1992).

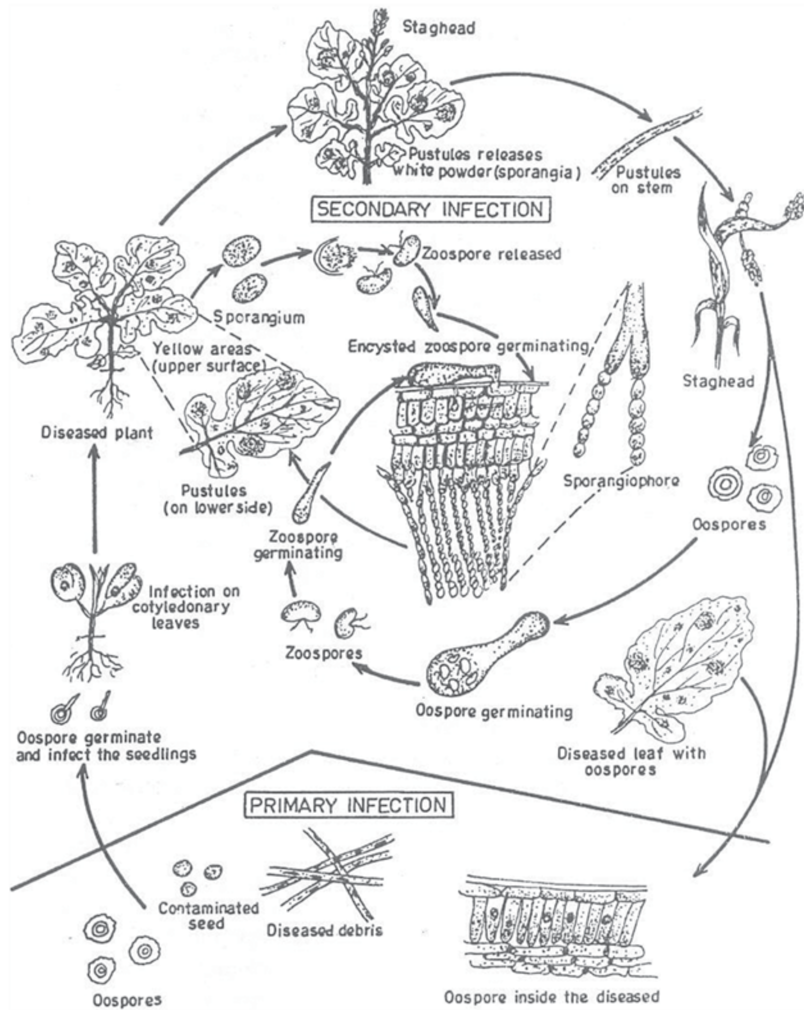

Fig. 3.7 Disease cycle of white rust on Brassicas. (Saharan and Mehta 2002)

3.8 Disease Cycle

The pathogen perpetuates through the oospore formed in the hypertrophied tissues lying in the soil, in diseased plant debris, or moving with diseased host pieces along with the seeds as contaminant. The oospores can survive in diseased host tissues under dry storage conditions for more than 20 years (Verma and Petrie 1975b). Perennial weed hosts may also serve as a source of primary inoculum. Oospores germinate by producing zoospores at suitable temperature (10–20ºC) and relative humidity (> 70 %) and cause primary infection in the host leaves when the germ tube enters the host through stomata. The secondary spread of the disease is through sporangia and planospores formed in the diseased pustules (Fig. 3.7). The sporangia are readily carried by air currents after breaking open the mature pustules. Moisture on the host surface is essential for germination and infection through sporangia and planospores. Motile planospores swim for a short time, become quiescent and produce germ tubes. Invasion takes place through stomata. At maturity stage of the crop, oospores are formed in the hypertrophied plant to serve as source of primary inoculums. Since, the flower bud infections have repeatedly been proved to be the major source of staghead formation (systemic infection) (Verma and Petrie 1980; Goyal et al. 1996), the old-theory regarding staghead production (systemic infection) from primary infection of cotyledons or primary leaves is highly questionable. During

the harvesting operations, pieces of malformed tissues which contain numerous oospores of the pathogen either get mixed with the seeds or fall on the ground. The seed infestation also explains the appearance of this disease in mustard and rapeseed fields that are relatively weed free. It is believed that in perennial hosts such as horseradish, the mycelium is capable of perpetuating in the infected crowns and lateral roots. The mycelium remains dormant during the off season, but resumes its activity and grows into new shoots that the host produces in the crop season.

In a study, Lakra and Saharan (1989d) observed that sporangia of *A. candida* can survive for 4–5 days at 15ºC on detached-infected *B. juncea* leaves, but lose their viability after 18 h if separated and incubated without host tissues. However, sporangia can be stored for 105 days at 40ºC as a dry powdered mass. Oospores are formed in the hypertrophied tissues (leaves, stems, inflorescence and siliqua) of infected host plants. Over-summered oospores in infected plant debris in soil function as the source of primary inoculum of the pathogen. Oospores have also been observed in naturally infected senesced leaves of *B. juncea* and *B. rapa* var. Toria (Lakra and Saharan 1989b). Lakra and Saharan (1989b) estimated 8.75×10^5 oospores in 1 g of hypertrophied cup-shaped leaves, and 21.85×10^5 oospores in 1 g of hypertrophied staghead. Oospores can remain viable for 20 years under dry storage conditions (Verma and Petrie 1975b). The oospores are differentiated into five layered cell wall, and their greater longevity is probably due to the heavily fortified cell wall. There is a possibility of survival and spread of oospores carried externally on seed (Saharan and Verma 1992; Saharan 1997).

In nature, WR has been recorded on *B. rapa* var. Yellow Sarson, Brown Sarson, Toria, *B. alba, B. nigra, B. rugosa, B. napus, B. juncea, B. tournefortii, B. chinensis, and Eruca sativa.* In addition to the above rapeseed-mustard group of crops, it is common on *R. sativus, R. raphanistrum, B. oleracea* var. *Capitata, B. oleracia* var. *Botrytis, A. lapthifolia, Cheiranthus cheiri, Matthiola incana, Rorippa islandica, Cardamine hirsute, Cleome spinosa, Malcoimia maritime, Lepidium oleracium, L. sativum, Rorippa nasturtium, Sisymbrium officinale, Alliara officinalis, C. bursa-pastoris* and *Erysium hieracifalium. A. candida* infects a large number of host plants in Aizoaceae, Capparidaceae, Coleomaceae, Cruciferae and Amaranthaceae families which may serve as collateral hosts of the pathogen (Saharan and Mehta 2002).

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4 Survival of Pathogen

The survival of a pathogen between cropping seasons and its effective dispersal to healthy plants are crucial aspects of the plant disease cycle. If either of these is prevented, the disease will not occur. Most pathogens possess mechanisms to survive intercrop periods or periods of unfavourable environmental conditions. The spread of inoculum can be airborne, soil borne, water borne, seed or clone borne, or vector borne. Airborne inoculum can travel for great distances, even across oceans, while soil-borne inoculum is rarely spread any great distance. Many pathogens are dispersed by more than one mechanism. These fungi are able to form resting structures that enable them to survive long periods without a suitable host or when environmental conditions are unfavourable.

4.1 Mycelium

It is believed that in perennial hosts such as horseradish, the mycelium is capable of overwintering in the infected crowns and lateral roots (Endo and Linn 1960; Kadow and Anderson 1940; Walker 1957). Remaining dormant during the winter, the mycelium resumes its activity and grows into new shoots that the host produces in spring.

4.2 Sporangia

Sporangia of *Albugo bliti* attached to host tissues remain viable for 15 days at 3–10 °C, whereas detached sporangia remain viable only for 24 h within this temperature range (Mishra and Chona 1963). At 30 °C, viability is lost after 4 h when attached and within 2 h when detached from host tissues. Lakra and Saharan (1989d) observed that sporangia of *Albugo candida* can survive for 4.5 days at 15 °C on detached infected *Brassica juncea* leaves, but lose their viability within 18 h if separated and incubated without host tissues. However, sporangia can be stored for 105 days at −40 °C as a dry powdered mass (Table 4.1 and 4.2). Bartaria and Verma (2001) studied that the zoosporangia of *A. candida* (Indian isolate) on *B. juncea* infected leaves can survive up to 4 days at 20 °C and for 2 days at 25 °C while Canadian race 2 up to 3 days at 20 °C and 8 h at 25 °C.

4.3 Oospores

Oospores are formed in the hypertrophied tissues (leaves, stems, inflorescences, pods, roots) of infected host plants. Overwintered oospores in infected plant debris in soil function as the source of

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Table 4.1 Viability of *A. candida* under different storage conditions. (Lakra and Saharan 1989d)

Storage conditions	Survival period (days)
Dry sporangial mass in lab conditions[a]	0.75
Dry sporangial mass at 10 °C	0.92
Sporangia on detached leaf in lab conditions	4.50
Dry sporangial mass at 0 °C	21.00
Dry sporangial mass at −20 °C	85.00
Dry sporangial mass at −40 °C (defreeze dry)	105.00

[a] Mean laboratory temperature was 15 °C

Table 4.2 Sporangial thermal death point of *A. candida.* (Lakra and Saharan 1989d)

Temperature (°C)	Sporangial germination	
	When attached with leaf	When detached from leaf
20	++	++
22	++	++
24	++	++
26	++	++
28	++	+
30	+	+
32	+	–
34	–	–

++ more than 50 % sporangia germinated, + less than 50 % sporangia germinated, – no germination

primary inoculum of the pathogen (Butler 1918; Butler and Jones 1961; Chupp 1925; Kadow and Anderson 1940; Verma et al. 1975; Verma 2012; Walker 1957). Oospores have also been observed in naturally infected senesced leaves of *B. juncea* and *Brassica rapa var.* Toria. Lakra and Saharan (1989b) estimated 8.75×10^5 oospores in 1 g of hypertrophied cup-shaped leaves and 21.85×10^5 oospores in 1 g of hypertrophied staghead portions (Table 4.3). The high percentage of oospore production has been recorded in detached cotyledon culture (> 73 %) and naturally senescing cotyledons (> 92 %) by Liu and Rimmer (1993) (Table 4.4). Meena and Sharma (2012) reported that under delayed sowing conditions, higher disease severity on leaves (32 %), and incidence (43 %) of stagheads can be attributed to higher germination of overwintered oospores in infected plant debris in soil. Disease severity increased with the increased concentration of oospore inoculum both at two-leaf and five-leaf growth stages of *B. juncea*. Verma and Petrie (1975b) found that oospores can remain viable for over 20 years under dry storage conditions. According to Tewari and Skoropad (1977), oospores have a highly differentiated five-layered cell wall and their greater longevity is probably due to the heavily fortified cell wall. Petrie (1975) found more than 1,500 oospores per gram of rapeseed seed and reported the possibility of survival and spread of the pathogen by means of oospores carried externally on seeds. However, information regarding production of oospores inside the seed and their possible importance in the survival of the pathogen is lacking.

Table 4.3 Location and estimation of oospores of *A. candida* in infected plant parts of mustard. (Lakra and Saharan 1989b)

Type of infected sample	Presence/absence of oospores	Size of oospores[a] (μm)	No. of oospores/g of infected crushed material
Leaves with fresh white rust pustules	–	–	–
Leaves turning yellow with white rust pustules	–	–	–
Diseased decaying leaves with saprophytic fungal growth	–	–	–
Hypertrophied cup-shaped decaying leaves with saprophytic fungal growth	+	52.6 (SD=4.54)	875000 (8.7×10^5) ($SD=1.67\times10^5$)
Stagheads	+	52.6 (SD=4.54)	2,185,000 (21.85×10^5) ($SD=2.18\times10^5$)

+ oospores are present, – oospores are absent

[a] Mean of 100 oospores vary from 45–60 µm

Table 4.4 Percentage of infected cotyledons of *B. juncea* var. Burgundy and *B. rapa cv*. Torch. (Liu and Rimmer 1993)

Method I[a]		Method II[b]	
Race 2 in Burgundy	Race 7 in Torch	Race 2 in Burgundy	Race 7 in Torch
Experiment 1			
72.4 (6.3)[c]	69.8 (5.2)	889.5 (3.1)	85.3 (2.1)
Experiment 2			
67.8 (7.1)	73.1 (6.0)	88.1 (4.9)	92.2 (3.3)

[a] Production of oospores through detached cotyledon culture; values represent the mean of ten plates with 15 cotyledons per plate

[b] Production of oospores in naturally senescenced leaves; values represent the mean of three flats with 100 cotyledons per flat

[c] Values in parenthesis are standard deviations. There is a highly significant difference between the methods ($P<0.01$), but no significant difference between the two races or between the two experiments ($P>0.05$)

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5 Spore Germination

The fungus *Albugo* produces three kinds of spores, viz., oospores, sporangiophores and zoospores. The information generated on the germination of oospores and sporangiospores by various researchers is given below. However, very little is known about germination of zoospores.

5.1 Oospores

DeBary (1866) first observed germination of *Albugo* oospore via a sessile vesicle. Vanterpool (1959) confirmed this and described a second mode of germination by means of a terminal vesicle; however, maximum germination was only 4 % and its occurrence was unpredictable. Petrie and Verma (1974a, 1974b) described a very reliable and reproducible technique for germination of *Albugo candida* oospores. Verma and Petrie (1975) found that maximum germination of *A. candida* oospores from hypertrophied inflorescence of *Brassica rapa* was 88 % at 13 °C. Oospores germinated by the production of one or two simple or branched germ tubes, by the release of zoospores from vesicles formed at the ends of germ tubes (terminal vesicles), and by the release of zoospores from sessile vesicles (Fig. 5.1). Germination by sessile vesicles was the most common. Verma and Bhowmik (1988) observed that the treatment of oospores with 200 ppm $KMn0_4$ for 10 min induced increased germination. Oospores do not appear to require any dormancy period. Verma and Petrie (1975) and Verma (2012) were able to germinate 71 % of the oospores within 2 weeks of collection from the field. High percentage (> 80 %) germination of oospores formed in hypertrophied stems, and floral parts was consistently obtained by agitating oospores for up to 24 h in sterile distilled water containing a 1–2 % mixture of β-glucuronidase and acrylsulfatase followed by 3 days of washing on a rotary shaker at room temperature and 15 h of chilling at 13 °C (Liu and Rimmer 1993; Meena and Sharma 2012).

5.2 Sporangia

Sporangial germination in *A. candida* was studied by Prevost (1807), Tulasne (1854), Hoffmann (1859), DeBary (1860), Melhus (1911), Napper (1933) and Endo and Linn (1960). In 1911, Melhus reviewed the earlier work on sporangial germination. Prevost and de Bary found that sporangial germination occurs via the production of zoospores. Tulasne and Hoffmann stated that the sporangia germinate only by the production of germ tubes. Harter and Weimer (1929) stated that sporangia may germinate by the direct production of germ tubes, but germination via zoospores was more frequent. Eberhardt (1904a, 1904b, 1904c, 1904d), Melhus (1911) and Napper (1933) found that sporangia of *A. candida* germinate invariably by the production of zoospores, which was confirmed by Lakra and Saharan (1988) and Lakra et al. (1989). DeBary (1860)

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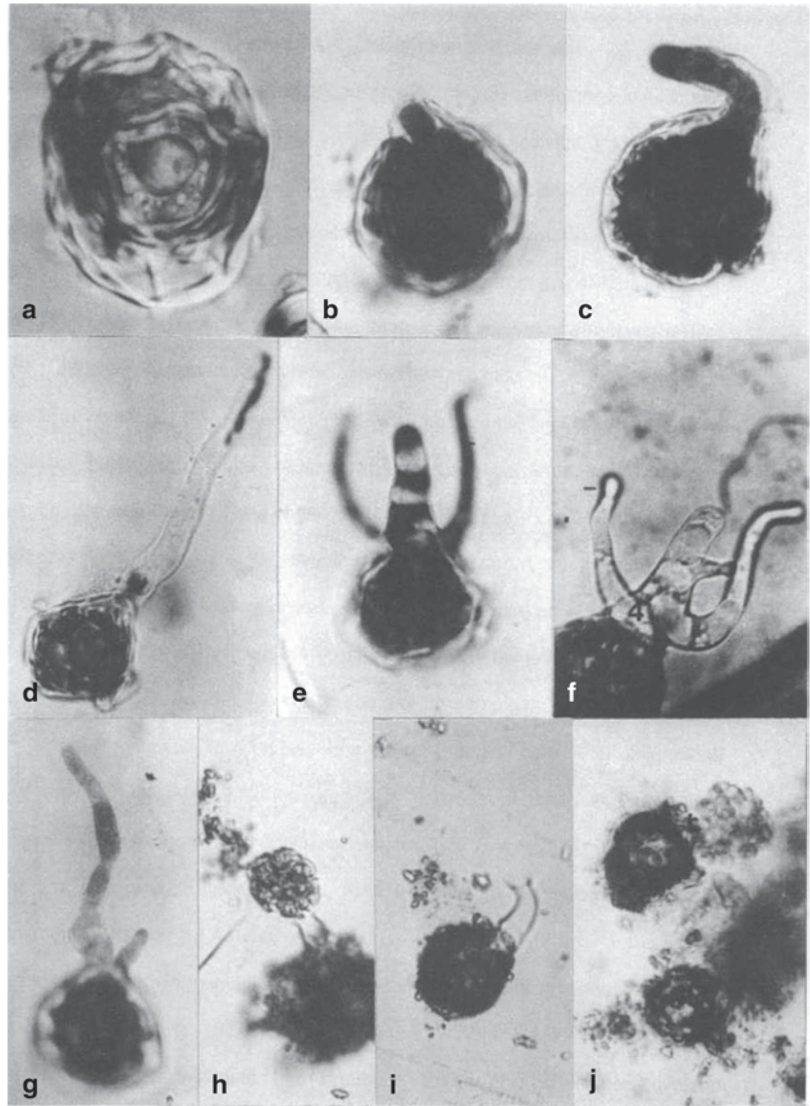

Fig. 5.1 **a**–**j** germination of oospores of *A. candida* race 7. **a** Mature oospore showing thick wall and well-developed central globule. **b** Germ tube initial emerging from oospore and penetrating oogonial wall. **c** Young germ tube. **d** Long, less densely stained germ tube with some constriction at the exposure wall. **e**–**g** Branches arising from the main germ tube. **h** Terminal vesicular mode of germination. **i** An empty exit tube with a circular opening at its end. **j** Sessile vesicular mode of germination (zoospores at *top*) (Verma and Petrie 1975)

and Melhus (1911) reported that sporangia did not germinate above 25 °C or below 0 °C; the best germination was at lower temperatures. Napper (1933) did not observe sporangial germination above 20 °C. Melhus (1911) suggested 10 °C as the optimum temperature for sporangial germination, but Napper (1933) found that germination takes place readily between 1–18 °C. Endo and Linn (1960) reported optimum temperature range of 15–20 °C, with maximum germination occurring between 0–28 °C. However, Lakra and Saharan (1988), and Lakra et al. (1989) observed >75 % sporangial germination at 12–14 °C after 8 h incubation (Table 5.1), and sporangia ceased to germinate below 6 °C and above 22 °C; germination started after 4 h and reached their maximum 8 h after incubation. A quadratic equation, $Y-103.16+26.99x-1.01\times 2$ ($R^2=0.99$), where $Y=$% sporangial germination and $x=$ temperature in °C was proposed to estimate the frequency of sporangial germination of *A. candida* from *Brassica juncea* at any known temperature. The variation in the cardinal temperatures for sporangial germination among different studies is probably due to the involvement of different host-specific biological races of *A. candida*. Germination of

Table 5.1 Effect of light and temperature on per cent germination of *Albugo candida* sporangia. (Lakra et al. 1989)

Temperature (°C)	4 h		5 h		6 h		7 h		8 h		Mean	
	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Y	Y[a]
6	3.0	2.5	9.8	9.0	15.7	14.5	21.3	20.7	22.5	22.0	22.2	22.4
8	22.5	20.9	32.8	32.0	40.0	39.2	46.4	45.9	48.2	47.6	47.8	48.1
10	40.4	40.0	50.7	49.0	58.0	57.4	63.5	63.3	65.7	65.1	65.3	65.7
12	46.5	46.0	60.5	60.0	67.8	65.1	74.3	73.9	76.5	75.9	76.2	75.2
14	47.1	46.3	63.1	62.7	70.4	69.9	76.7	76.2	77.2	76.8	76.9	76.7
16	41.0	40.3	53.5	58.8	61.2	60.9	68.0	67.2	70.0	69.7	69.8	70.1
18	24.1	23.3	42.0	41.3	47.0	46.5	53.2	52.6	55.5	55.0	55.2	55.4
20	13.6	13.0	20.3	19.8	27.2	26.7	31.1	30.8	32.8	32.1	32.4	32.6
22	0.5	0.3	0.8	0.6	1.0	0.0	1.9	1.5	2.3	1.9+	2.0	1.7

Critical Difference (CD) at 1 % for temperature = 0.2467
CD at 1 % for light = 0.1147
CD at 1 % for incubation period = 0.2026
CD at 1 % for temperature *x* incubation period = 0.6042
Y = Observed sporangial germination at 8 h
$Y = a + bx + cx^2$; where, *Y* = % sporangial germination; *x* = temperature; *a, b* and *c* are constants
[a] Estimated sporangial germination at 8 h

A. candida sporangia from naturally-infected *B. juncea* and *Brassica rapa* var. Toria leaves occurred within 1 h at 13 °C (Lakra et al. 1989).

Although, Melhus (1911) and Holliday (1980) reported that sporangial germination is not affected by light or darkness, Lakra et al. (1989) demonstrated that exposure to light of 150 $\mu EM^{-2}s^{-1}$ slightly delays sporangial germination. Although Melhus (1911) found that sporangia germinated readily in both saturated and nonsaturated atmospheres, Lakra and Saharan (1988) and Lakra et al. (1989) observed that a film of free water is essential. Melhus (1911) and Napper (1933) found that chilling and a reduction of 30 % water content in sporangia were essential for germination. Lakra et al. (1989), however, states that it is not a prerequisite, since up to 75 % of sporangia germinated without chilling or dehydration. According to Uppal (1926), sporangia of *A. candida* require oxygen for germination. Takeshita (1954) reported that sporangia of *A. candida* from horse radish germinated best at pH 4.5–7.5 at 10–20 °C; light did not affect germination. However, Endo and Linn (1960) reported that *A. candida* sporangia from horseradish require pH range of 3.5–9.5, with an optimum pH of about 6.5, and temperature range of 15–20 °C. Only a few studies have been carried out on sporangial germination of species other than *A. candida*. Edie and Ho (1970) demonstrated that although the sporangial germination in *Albugo ipomoeae-aquaticae* is nearly identical with that of other *Albugo* species, it requires a slightly higher germination temperature in the range of 12–30 °C, with an optimum of about 25 °C. However, Safeefulla and Thirumalachar (1953) mentioned that sporangia germinated at 15 °C, but not at 24 °C. Sporangia of *A. ipomoeae-panduratae* germinate between 8–25 °C (Harter and Weimer 1929), with an optimum of 12–14 °C. Sporangia of *Albugo tragopogonis* germinate at 4–35 °C, with an optimum range of 4–15 °C. Encysted zoospores germinate best at 10 °C (Kajomchaiyakul and Brown 1976). Sporangial germination of *A. tragopogonis* from *Senecio squandus* occurs at 5–15 °C, with an optimum of 10–15 °C, and very little germination occurs at 20 °C (Whipps and Cooke 1978a, 1978b). *Albugo bliti* sporangia germinate at a temperature range of 2–25 °C, with an optimum near 18 °C (Mishra and Chona 1963). Chilling increases sporangial germination, but mature sporangia from just-opened pustules, or those from naturally infected detached leaves, germinated best. Sporangia of *Albugo occidentalis* germinate at 2–25 °C, with an optimum near 12 °C (Raabe and Pound 1952a, 1952b). Light, water content of sporangia and pH also have little effect on sporangial germination.

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6 Infection

In all species of *Albugo*, oospores are the primary source of inoculum (Butler 1918; Butler and Jones 1961; Chupp 1925; Heald 1926; Verma & Petrie, 1975, 1980; Walker 1957). In perennial hosts like horseradish, the mycelium persists in the crowns, and occasionally in the lateral roots (Kadow and Anderson 1940). Secondary infection and spread of disease during the growing season is by means of sporangia, which are readily carried short distances by splashed water droplets or, to a certain degree, by air currents. Moisture on the host surface is essential both for sporangial germination and infection by zoospores. The most likely primary infection sites are the emerging cotyledons (Verma et al. 1975). The zoospores derived from germinating oospores are also capable of causing infection (Verma et al. 1975), but no evidence exists of infection arising directly by germ tubes produced from germinating oospores (Verma and Petrie 1975; Verma 2012).

On germination, each sporangium and oospore releases about 4–12 and 40–60 biflagellate zoospores, respectively (Verma and Petrie 1975). Raabe and Pound (1952) reported a persistent terminal bead on one flagellum of *Albugo occidentalis* zoospores. In *Albugo tragopogonis*, Whipps and Cooke (1978) observed the appearance and subsequent disappearance of flagellar beads during zoospore differentiation, and the absorption of beads by encysting zoospores. After swimming for a time, a zoospore encysts and forms a germ tube, which enters the host through a stoma (Verma et al. 1975). Encystment of zoospores, penetration of germ tube through a stoma and formation of a haustorium within 8 h after inoculation were similar in both resistant (R) and susceptible (S) hosts (Verma et al. 1975). In the former, mycelial growth of the pathogen ceases in the substomatal chamber, and within 72 h after inoculation, a marked encapsulation forms around each single haustorium (Fig. 6.1-6, Verma et al. 1975). In the congenial or S host, however, the mycelium advances intercellularly with the production of several haustoria per infected cell (Liu and Rimmer 1990; Liu et al. 1989; Napper 1933; Verma et al. 1975; Walker 1957). According to Liu and Rimmer (1990), the host genotype, inoculum concentration and incubation temperature, as well as the interactions among these factors, had significant effects on infection. The day/night temperatures of 22/17 °C were more favourable for fungal growth than 15/10 °C. Infection levels increased with the concentration of inoculum on each line/cultivar at both the two-leaf and five-leaf growth stages of *Brassica napus* and *Brassica rapa*.

While studying the histopathology of compatibility and incompatibility between *B. rapa* and *Albugo candida* race 7, Liu et al. (1989) observed haustorium formation in the palisade mesophyll cells adjacent to the substomatal chambers 8 h after inoculation. In the R cultivars, most primary hyphae produced only one haustorium, necrosis of the invaded host cells occurred within 12 h and mycelial growth ceased 72 h after inoculation (Liu et al. 1989). The death of host cells was largely restricted to the penetration site; the adjacent non-penetrated cells remained apparently

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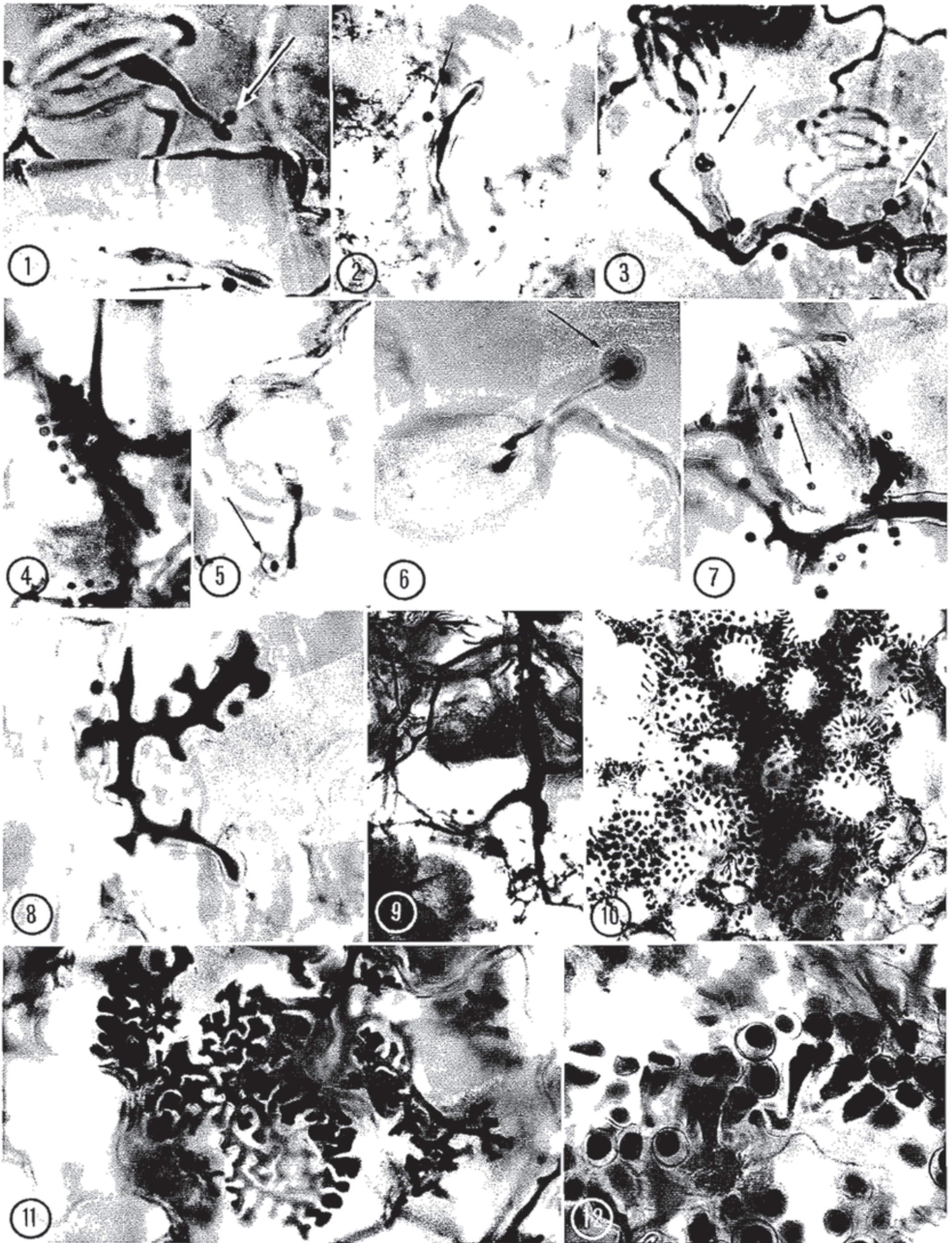
unaffected. In the S hosts, necrosis of infected cells occurred only infrequently, and hyphal growth continued unabated, resulting in mycelial ramifications into the mesophyll. Numerous haustoria per infected cell were produced. The presence of as many as 14 haustoria in a single cell in 'green island' tissue of artificially infected *Brassica juncea* cotyledons have been reported (Fig. 6.1, Verma et al. 1975).

Lakra and Saharan (1988a, b) found that in host varieties RH-30, RLM-1357, Kranti and RC-781, the pathogen colonizes within 76.8, 74.4, 79.2 and 204.0 h of inoculation, and with an incubation period of 98.4, 91.2, 124.8 and 283.2 h, respectively (Table 6.1). In RC-781, exceptionally long periods of colonization and expression of symptoms were required due to its inherent R character. The pathogen could not establish in the host tissues of variety RH-8541 due to its high degree of resistance. In the S cultivars RH-30, RLM-1357 and Kranti, a shorter incubation period was required for both colonization and expression of symptoms. The decision between compatible (susceptible) and incompatible (resistant) reactions of *Albugo–B. juncea* system is made within 80 h of inoculation (Verma et al. 1975). Colonization in the leaves of all S cultivars occurred within 80 h of inoculation compared with 204 h in the R cultivar RC-781. Verma et al. (1975) found that up to the formation of the first haustorium, there is no difference in the infection process in R and S hosts of *B. juncea, B. rapa*, *B. nigra* and *B. napus*. However, Napper (1933) found that *A. candida* entered through the stomata of an R host as readily as those of an S host. The delay in colonization and incubation period of the R host formed a practical base to identify host resistance under both field and laboratory conditions. Besides, age of the plant, age of the leaf involved, prevailing environmental conditions and nutrients applied may also influence the incubation periods. Coffey (1975) observed an incubation period of 192 h in cabbage cotyledons under favourable conditions of high humidity and a temperature of 15 °C. Under field conditions, longer incubation periods in highly tolerant and R cultivars (Lakra and Saharan 1988a), therefore, suggest that growing of such cultivars will help to reduce buildup of primary inoculum (oospores) in the soil.

Progression of *A. candida* infection was studied in cotyledons of S (*B. rapa, B. juncea*), moderately resistant (*B. hirta*) and immune (*B. napus*) hosts (Verma et al. 1975). Cotyledons of all four *Brassica* species were inoculated with zoospores of *A. candida* produced from germinating oospores or zoosporangia. At different times after inoculation, whole cotyledons were fixed in 95 % ethanol–acetic acid (v/v) solution, cleaned in 70 % lactic acid for 3–4 days and stained with cotton blue in lactophenol. The preparation was examined under the compound microscope.

Generally, the sequence of events from zoospore encystment to formation of the first haustorium was the same in all hosts, although under field conditions, *B. hirta* is moderately resistant and *B. napus* is essentially 'immune'. In *B. juncea*, the first haustorium was observed 16–18 h after inoculation (Fig. 6.1-1), while in *B. rapa*, *B. hirta* and *B. napus*, the first haustorium was observed about 48 h after inoculation (Fig. 6.1-4, Verma et al. 1975).

Fig. 6.1 Development of *Albugo candida* in the cotyledons of several *Brassica* spp. with increasing time after inoculation. *1 Brassica juncea* 18 h after inoculation (× 100). Note the penetration of stomata by germ tube and formation of first haustorium (*arrows*). *2 Brassica rapa* 48 h after inoculation (× 530). Haustorium is indicated by *arrows*. *3 Brassica juncea* 46 h after inoculation (× 770). Haustoria are indicated by *arrows*. *4 Brassica rapa* 9 days after inoculation (× 640). Note the several haustoria in one cell. *5 Brassica napus* 4–5 days after inoculation (× 530). Encapsulation is indicated by the *arrow. 6 Brassica napus* 4–5 days after inoculation (× 820). Encapsulation is indicated by the *arrow*. *7 Brassica rapa* 9 days after inoculation (× 640). Note the encapsulation around one haustorium (*arrow*). *8 Brassica rapa* 3 days after inoculation (× 650). Note typical branching of coenocytic mycelium. *9 Brassica rapa* 6 days after inoculation (× 300). Note the varying thickness of individual hyphae. *10 Brassica hirta* 9 days after inoculation (× 130). Note the massive amount of intercellular fungal thallus and production of zoosporangia from club-shaped sporangiophores. *11 Brassica campestris* 9 days after inoculation (× 800). Note the characteristically branched mycelium from which sporangiophores develop. *12 Brassica rapa* 9 days after inoculation (× 900). Note the club-shaped sporangiophores and production of chains of zoosporangia. (Verma et al. 1975)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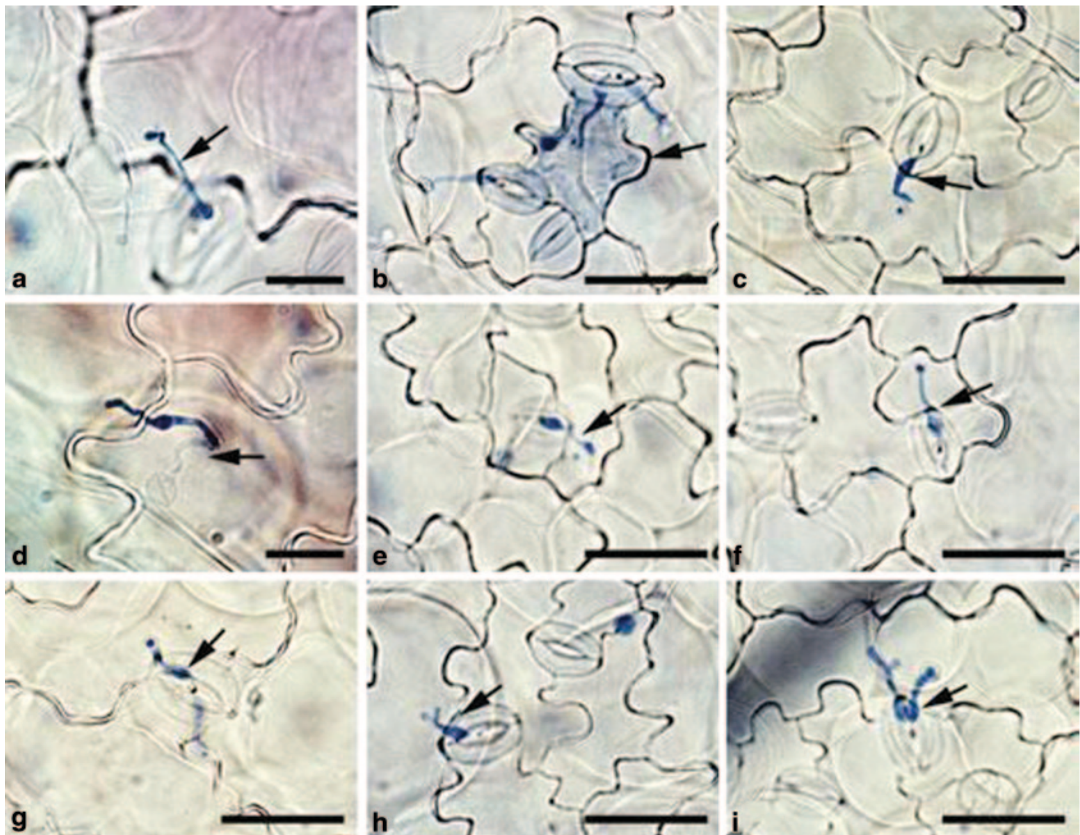

Fig. 6.2 Histology of host–pathogen interaction of *Albugo candida* race 7v on *Brassica napus*, *B. rapa* and *B. juncea* after 1 day of inoculation. **a** Spore germination on the stomatal opening of the partially resistant genotype *B. napus* A00-63N. **b** Spore germination and hypersensitive response of the host epidermal cell of resistant *B. napus* A00-65N genotype. **c** Spore germination on the stomatal opening. **d** Spore germination on the epidermal cell. **e** Spore germination and penetration through the epidermal cell of moderately susceptible *B. napus* 88-1409K, and **f**, **g** through the stomatal opening of susceptible *B. rapa* 'Torch'. **h**, **i** Spore germination on the stomatal opening of resistant *B. napus* 'Westar', and *B. juncea* 'Commercial Brown'. In each figure, an *arrow* points to the feature indicated. **a**, **d** Bar = 0.02 mm; **b**, **c**, **e–i** Bar = 0.05 mm. (Bansal et al. 2005)

Table 6.1 Colonization and incubation period of *A. candida* in resistant and susceptible varieties of mustard. (Lakra and Saharan 1988a)

Varieties	Colonization in host tissues (h)	SD	Incubation period (h)	SD
RH-30 (S)	76.8	4.6	94.4	4.8
RLM-1357 (S)	74.4	3.8	91.2	3.6
Kranti (S)	79.2	5.8	124.8	5.8
RC-781 (R)	204.0	5.7	283.2	6.5
RH-8541 (R)	–	–	–	–

R resistant to white rust, *S* susceptible to white rust, *SD* standard deviation, – no infection

In the S hosts, after the formation of the first haustorium, the hyphae grew rapidly and produced variable number of haustoria in each cell (Fig. 6.1-1-4). The profusely branched, coenocytic mycelium appeared to fill all available intercellular spaces, and in 5–6 days after inoculation, the club-shaped zoosporangia developed from a dense layer of mycelium (Fig. 6.1-7-12).

In the immune host, usually only one haustorium was formed, after which the hyphae ceased to elongate. At about 72 h after inoculation, a fairly thick, densely stained encapsulation was usually detected around each haustorium, and later only 'ghost' outline of hyphae and haustorium were observed (Fig. 6.1-5-6. Encapsulations were not observed around haustoria of S hosts. From these observations (Verma et al. 1975), it seems very probable that zoospores derived from germinating oospores constitute the primary inoculum for infection of cotyledons of susceptible *Brassica* species. No evidence of direct infection by the simple or branched germ tubes from germinating oospores was seen (Verma and Petrie 1975).

The establishment and maintenance of a compatible relationship between *A. candida* and its hosts hinges on the successful formation of the first haustorium. A similar sequence of events in both the S and immune hosts up to this point suggests that there appears to be no morphological barrier to zoospore encystment, germination and subsequent penetration through stomata. In the incompatible combination, however, it is not clear whether the parasite fails to produce a functional haustorium, or whether a viable haustorium is formed within the host cell and is subsequently killed by the host's defence mechanism. The fairly dense, thick encapsulation observed around haustorium of immune host tissue suggests that the later may be the case. In any event, it does seem that the decision between compatibility and incompatibility is made within 48 h after inoculation (Verma et al. 1975).

6.1 Host–Pathogen Interaction

Histopathology with light and electron microscopes has revealed detailed fine structures during the process of host–pathogen interaction. Cotyledons of *B. napus*, *B. rapa* and *B. juncea* inoculated with zoospores of race 7v were observed under light microscope at 1, 3 and 7 days after inoculation (Bansal et al. 2005). One day after inoculation, zoospore germination was generally high and exhibited a similar behaviour on all three host genotypes (Fig. 6.2). In most cases, germ tubes penetrated stomata after forming appressoria, but in a few cases, the germ tubes penetrated directly without forming appressoria. Occasionally, more than one germ tubes penetrated each stoma. In general, the frequency of zoospore germ tube penetration without appressoria was lower in the R and partial resistant (PR) than in the moderately susceptible (MS) and S genotypes. A hypersensitive response was also observed in the resistant-host–pathogen interaction (Fig. 6.2h). Haustorium formation was observed 1 day after inoculation in the S, and occasionally, also in the MS, PR and R genotypes.

Three days after inoculation, most histological features of the pathogen (Fig. 6.3) were similar to those formed 1 day after inoculation (Fig. 6.3). In the S control 'Torch', while mycelium was present in the deeper mesophyll layers of the cotyledonary leaves (Fig. 6.2), in the R, PR and MS genotypes, most of the fungal growth was restricted only to the first layer of mesophyll cells (Fig. 6.3).

Seven days after inoculation, the intercellular mycelium was observed in two mesophyll cell layers in the PR and MS genotypes, but in three mesophyll cell layers in the S genotype. In the R and PR genotypes, guard cells of stomata through which penetration occurs appeared shrunken and necrotic, and this hypersensitive response was more frequent in these than in the MS genotypes. There was no hypersensitive response observed in the guard cells of the S genotype, and the hyphal growth was more profuse and penetrated more deeply than in any of the other genotypes. Seven days after inoculation, generally, the histology of host–pathogen interactions remained

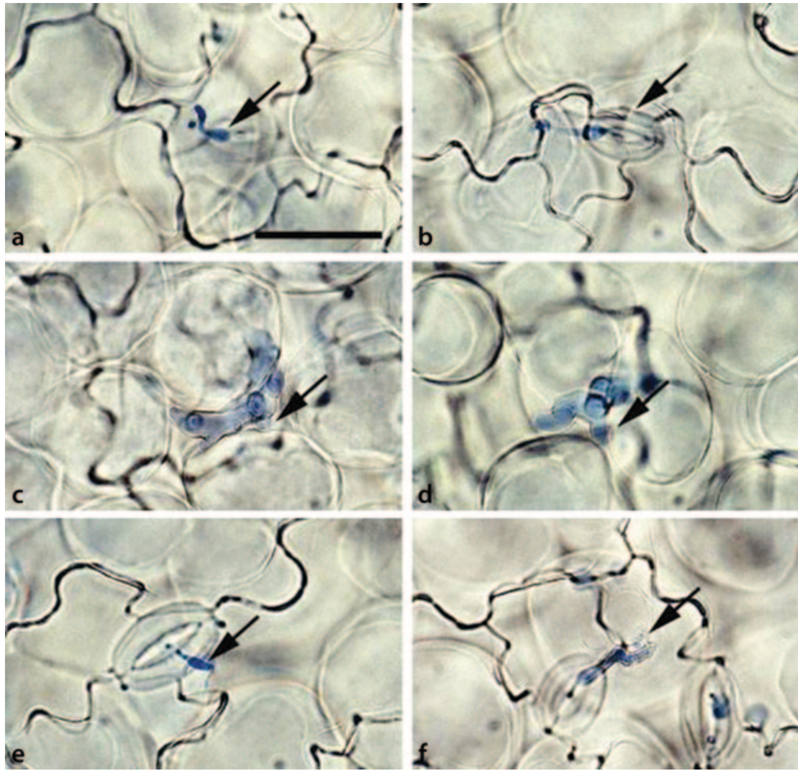

Fig. 6.3 Histology of host–pathogen interaction of *Albugo candida* race 7v on *Brassica napus*, *B. rapa* and *B. juncea* 3 days after inoculation. **a** Spore germination on the stomatal opening of the partially resistant genotype *B. napus* A00-63N and **b** resistant genotype *B. napus* A00-65N. **c** Subepidermal fungal mycelium in the mesophyll cell layer of moderately susceptible *B. napus* 88-1409K and **d** in the intercellular spaces of mesophyll tissue of susceptible *B. rapa* 'Torch'. **e** Spore germination on the stomatal opening of resistant *B. napus* 'Westar'. **f** Spore germination with some mycelial growth close to the stomatal opening of resistant *B. juncea* 'Commercial Brown'. In each figure, an *arrow* points to the feature indicated. Bar = 0.05 mm. (Bansal et al. 2005)

essentially the same as in 3 days after inoculation (Bansal et al. 2005). Mesophyll tissue was colonized occasionally, but only up to one cell layer. Hypersensitive response and shrinking of guard cells were also noticed (Bansal et al. 2005).

In general, greater numbers of haustoria were observed in the S than in the MS, PR and R genotypes. Localized cell necrosis (hypersensitive reaction) of cells in the area of infection was observed in the R, PR and MS genotypes, and the extent of necrosis among the genotypes was MS < PR < R. Pustule development appeared to be inversely correlated with the level of necrosis. The pustules were few, diminutive and poorly sporulating in the PR host, 7 days after inoculation. In contrast, the S hosts produced copious quantities of pustules with high levels of sporulation. In the R genotypes, no pustules were observed in this investigation except in 'Commercial Brown', where a small number of seedlings developed a few small pustules. The histological observations clearly indicated that fungal growth in the PR genotype was more restricted than in the MS and S genotypes. The differences in the extent of mycelial growth and host invasion correlated well with the final phenotypic expression of the disease symptoms (Bansal et al. 2005).

Pidskalny and Rimmer (1985) have reported that races 7 and 2 from *B. rapa* and *B. juncea*, respectively, are not cross-pathogenic. But, mycelial growth sometimes developed in intercellular spaces of the first mesophyll cell layer of

the highly R genotype 'Commercial Brown', and very small pinhead-size pustules, were occasionally noticed on some of these plants (< 5 %). This may reflect partial resistance in this genotype (Bansal et al. 2005).

Transverse section of the hypertrophied inflorescence axis revealed a single-layered epidermis with idioblasts at some places, 4–5-layered cortex having parenchymatous and chlorenchymatous cells and conjoint collateral vascular bundles with 2–5-layered cambium and parenchymatous pith. In hypertrophied axis, epidermal cells were elongated because of the pressure exerted by the developing fungal oospores within the host cells. The cortical cells were penetrated by fungal haustoria. The vascular cylinder was stretched due to the development of the antheridia and oogonia in the cortex and pith.

The xylem elements increased in length and became thin walled. There was poor lignification of the xylem elements after invasion of *Albugo cruciferarum.* Thus, the hypertrophied tissue represents an example of hypoplasty. The malformation is mainly due to hypertrophy and hyperplasia of the infected cortical and pith cells. The differentiation of tissues in the infected inflorescence axis appeared of low order, but cell enlargement and cell division were very conspicuous (Maheshwari et al. 1985a, b).

Histopathology of compatibility and incompatibility between oilseed rape and *A. candida* has been studied by Liu et al. (1989). Germination of encysted zoospores occurred 2–3 h after inoculation; infection was initiated with germ tubes penetrating through stomata; and haustorium formation was first observed 8 h after inoculation in the palisade mesophyll cells adjacent to the substomatal chambers.

Only after the establishment of the first haustorium did the compatible and incompatible interactions begin to differentiate. In the R cultivar, most primary hyphae produced only single haustorium. Necrosis of the invaded host cell was first observed 12 h after inoculation followed by cessation of fungal growth. The death of host cells was largely restricted to the penetration site; the adjacent non-penetrated cells remained apparently unaffected. In the S hosts, necrosis of infected cells occurred only infrequently, and hyphal growth continued unabated, resulting in mycelial ramification into the mesophyll. Numerous haustoria per invaded host cell were produced (Liu et al. 1989).

Histological studies showed that the earliest event distinguishing a compatible from an incompatible interaction occurred after formation of the first haustorium, and that resistance was not manifested until the host mesophyll cells had come into contact with the first haustorium.

Transmission electron microscopy was used to examine details of the host–pathogen interface in *Arabidopsis thaliana* cotyledons infected with *A. candida* (Soylu 2004; Soylu et al. 2003). After successful entry through stomatal pores, the pathogen developed a substomatal vesicle and subsequently produced intercellular hyphae. Observations revealed that coenocytic intercellular hyphae ramified and spread intercellularly throughout the host tissues forming several haustoria in host mesophyll cells. Intracellular haustoria were spherical and 4.5 µm in diameter. Each haustorium was connected to intercellular hyphae by a narrow, slender haustorium neck. The cytoplasm of the haustorium included the organelles characteristic of the pathogen. No obvious response was observed in host cells following formation of haustoria. Most of the mesophyll cells contained normal haustoria, and the host cytoplasm displayed a high degree of structural integrity. Absence of host cell wall alteration and cell death in penetrated host cells suggested that the pathogen exerts considerable control over basic cellular processes, and in this respect, response to this biotrophic oomycete differs considerably from responses to other necrotrophic pathogens. Modification of the host plasma membrane (PM) along the cell wall, and around the haustoria, was detected by applying the periodic acid-chromic acid-phosphotungstic (PACP) staining technique. After staining with PACP, the host PM adjacent to the host cell wall and the distal region of the haustorial neck was found to be intensely electron dense. By contrast, the extrahaustorial membrane, where the host PM surrounded the haustorium, was consistently very lightly stained (Soylu et al. 2003; Soylu 2004).

6.2 Molecular Mechanism of Host–Pathogen Interaction

To gain insight into the molecular mechanisms underlying activation of complex plant defence responses that occur in R and S varieties of *B. juncea*, challenged with *A. candida*, a time-course protein profiling experiment followed by transcript studies was conducted (Kaur et al. 2011). The latter studies investigated whether the observed proteome changes were substantiated by changes in mRNA levels. Results revealed a clear differentiation between R and S interactions and led to the identification of six differentially regulated proteins potentially involved in defence reactions in the R variety. A seventh differentially expressed protein is potentially involved in suppressing the host defence system, as it was detected only in the S variety.

Plant defence responses often begin with gene-for-gene recognition of the pathogen. Production of virulence effectors by a pathogen leads to its recognition by a host plant (Jones and Dangl 2006). Recognition results in the rapid activation of defence responses usually accompanied by an oxidative burst, that is, the rapid production of reactive oxygen species (ROS). ROS production is also required for the hypersensitive response (HR), a type of programmed cell death thought to limit the access of the pathogen to water and nutrients (Glazebrook 2005).

The plastid-localized red chlorophyll catabolite reductase (RCCR) protein was found to be down-regulated in the pathogen-inoculated compared with the mock-treated R cultivar. The protein was only detected from 8 hpi onwards in the mock-treated seedlings and, to a lesser extent, the pathogen-inoculated R seedlings. RCCR transcript levels, however, showed a transient up-regulation in both R and S varieties under both treatments at 2–8 hpi suggesting a diurnal control of gene expression. This different trend in the 2-D gel-based proteome analysis compared with the observed changes in transcript abundance recorded by quantitative reverse transcription polymerase chain reaction may be due to post-transcriptional or post-translational modifications that may have led to the altered steady-state protein amounts in the two cultivars.

Pheophorbide (Pheide) a oxygenase and RCCR catalyse the key reaction of chlorophyll catabolism, the porphyrin macrocycle cleavage of Pheide to a primary fluorescent catabolite (Wuthrich et al. 2000). Disruption of RCCR activity is likely to result in the accumulation of RCC and Pheide, which are phototoxic and cause cell death by the light-dependent production of free oxygen radicals (Mach et al. 2001). Alternatively, accumulation of RCC itself may cause a specific signal that triggers cell death (Mach et al. 2001). Cell death and chlorophyll breakdown have been related to plant senescence and disease progression (Rodoni et al. 1997; Wuthrich et al. 2000; Mach et al. 2001; Pruzinska et al. 2007).

The Arabidopsis *ACD2* gene encodes RCCR and was found to suppress the spread of disease symptoms caused by *Pseudomonas syringae* (Mach et al. 2001). Inoculated plants that lack RCCR/ACD2 activity show spreading cell death beyond the initial containment zone around the initial lesion. On the other hand, plants expressing high levels of the RCCR/ACD2 protein showed higher tolerance to infection with a virulent *P. syringae* strain. This suggests that an increase in RCCR/ACD2 protein amount may alter the flux of chlorophyll catabolites that normally accumulate during disease and trigger cell death (Mach et al. 2001). The inability to detect RCCR protein at any stage of the infection cycle in the S variety might point towards the inability of these seedlings to initiate a successful HR during the early stages of pathogen attack.

The production of ROS presents a challenge to a plant because of the potential of these compounds to damage cellular components. The plant must therefore maintain a balanced system that produces ROS for defence at the same time as it produces antioxidants to protect against ROS-mediated oxidative damage. One of the critical metabolites for maintaining redox balance in the face of oxidative stress is glutathione (GSH), which is a ubiquitous supplier of reducing power for cellular processes (Noctor and Foyer 1998). GSH is a conjugate of glutamate, cysteine and

glycine. The last two steps in cysteine biosynthesis are catalysed by a bi-enzyme complex of serine acetyltransferase (SAT) and cysteine synthase, also called O-acetylserine (thiol) lyase (OAS-TL). Kaur et al. (2011) found that a *B. juncea* homologue of the plastid-localized isoform AtOAS-TL B was present in the R variety, but not detectable in the S variety. The plastid-localized isoform of OAS-TL B contributes to up to 65 % of the total OAS-TL enzyme activity in wild-type *Arabidopsis* plants (Heeg et al. 2008).

Cysteine is incorporated into GSH by a two-step enzymatic reaction catalysed by *c*-glutamylcysteine synthetase, an enzyme localized exclusively to the plastids, and GSH synthetase, an enzyme that shows dual targeting to both the cytosol and plastids (Wachter et al. 2005). GSH biosynthesis is strongly regulated by cysteine availability (Strohm et al. 1995). Overexpression of a plastidic OAS-TL isoform or an inactive SAT isoform caused moderate to very high increases of cysteine and GSH content in tobacco, respectively (Noji et al. 2001; Wirtz and Hell 2007). The pool size and redox status of GSH are important for determining a plant's ability to control ROS production and combat microbial pathogens (Noctor and Foyer 1998).

Both primary and secondary sulphur metabolism were found to be induced at the transcriptional, translational and metabolite levels upon pathogen attack in *Arabidopsis* when grown under sufficient sulphate supply, while basal resistance against necrotrophic fungi was compromised under sulphate-limiting conditions (Kruse et al. 2007). An increase in GSH content was also reported to occur in leaves attacked by avirulent biotrophic pathogens (El-Zahaby et al. 1995; Fodor et al. 1997; Vanacker et al. 1998, 1999). This suggests that a boost in cysteine and GSH levels is important for the incompatible interaction between *A. candida* and *B. juncea* (Kaur et al. 2011).

One of the processes consuming GSH could involve the *B. juncea* GSTF9 homologue. This GST is encoded by one of 13 phi-class *GST* genes in *Arabidopsis*. In *B. juncea*, GSTF9 was detected only in the R variety and increased in abundance as the infection progressed. The plant-specific phi-class *GST*s are related to fungal and bacterial proteins, and little is known about their substrate specificities (Dixon et al. 2010). The *B. juncea* peptides identified closely match a jasmonic acid-inducible *B. juncea GST*, with close homology to the cytosolic dimeric GSTF9 protein from *Arabidopsis*. Abundance of GSTF6, F9, U5 and U13 transcripts were found to be methyl jasmonate responsive in *Arabidopsis* (Jost et al. 2005). A quadruple knock-out line of *Arabidopsis* lacking GSTF6, F7, F9 and F10 showed no impairment in growth or development even under numerous stress conditions, but the subtle metabolic changes observed suggest an impaired response to oxidative stress conditions (Sappl et al. 2009). The endogenous products of oxidative damage, including membrane lipid peroxides and products of oxidative DNA degradation, are highly cytotoxic. GSTs conjugate GSH with such endogenously produced electrophiles, resulting in their detoxification (Pickett and Lu 1989; Dudler et al. 1991; Bartling et al. 1993; Berhane et al. 1994). Some GSTs also function as GSH peroxidises to detoxify oxidative products directly (Williamson and Beverley 1987; Bartling et al. 1993), and there are many reports that suggest a central role for GSH in plant defence activation (Marrs 1996; Dixon et al. 2010).

Kaur et al. (2011) also detected two plastid-localized isoforms of superoxide dismutase (SOD) in the R *B. juncea* variety that were absent from the S variety. One of these SOD isoforms, the FSD1 homologue, showed an early and sustained increase in abundance only in the R *B. juncea* variety on both transcript and protein level. Interestingly, the other isoform, the CSD2 homologue, was induced to a similar extent on transcript level in both varieties under pathogen attack, but protein accumulation was only detectable in the R cultivar upon pathogen challenge. This again suggests strong post-transcriptional regulation of this particular isoform that is resulting in different outcomes in the R and S varieties. SODs are known to play an important role in protecting cells against the toxic effects of ROS produced in various subcellular compartments (Fridovich 1986; Halliwell and Gutteridge 1989). An oxidative burst is the key feature underlying

successful pathogen recognition at the site of the initial infection (Alvarez et al. 1998; Van Breusegem and Dat 2006). Rapid induction of SOD and accumulation of hydrogen peroxide (H_2O_2) are characteristic early features of the HR following perception of avirulence signals from the pathogen (Lamb and Dixon 1997).

ROS-detoxifying enzymes such as SOD catalyse the dismutation of ROS such as superoxide and peroxide (O_2^-) to H_2O_2. H_2O_2 itself can trigger cell death, but at the same time functions as a messenger in cellular communication demanding a finely tuned adjustment of its cellular concentration (Foyer and Noctor 2000). SODs therefore play an important role in ameliorating stress during conditions such as pathogen infection where overproduction of ROS is induced (De Gara et al. 2003).

Various ROS have similar deleterious effects on plant cells, but they each activate different signalling pathways (Laloi et al. 2007). Moreover, the cellular compartment in which ROS are generated determines the nature of this signal (Miller et al. 2007). Kaur et al. (2011) could find two plastid-localized SODs responding differently to *A. candida*. The CSD2 protein seems to accumulate transiently in the R variety upon pathogen inoculation, while the FSD1 protein is very strongly induced and keeps increasing throughout. There was a transient increase in both FSD1 and CSD2 transcript abundances upon pathogen inoculation in the S variety, but these are not sustained at the protein level.

Recently, a cyclophilin was found to be necessary for host–pathogen recognition in *Arabidopsis* (Coaker et al. 2005). The peptidyl-prolyl cis-trans isomerase subfamily of cyclophilins is known to have chaperone function assisting in protein folding and the assembly of large protein complexes (Kromina et al. 2008). One of the seven cytosolic cyclophilin isoforms of *Arabidopsis*, ROC1, is the closest homologue of human cyclophilin A and yeast CPR1, and is required for activation of the *P. syringae* effector protein AvrRpt2 (Coaker et al. 2005). However, many of the 29 cyclophilins in *Arabidopsis* are targeted to the plastid. While most of these reside in the thylakoid lumen, one, CYP20-3/ROC4, is found in the chloroplast stroma. However, CYP20-3 protein was detected only in the S *B. juncea* variety, and this was confirmed by a very low CYP20-3 transcript abundance in the R variety. This suggests that the *B. juncea* CYP20-3 protein might play an important role in the compatible interaction between *A. candida* and *B. juncea*. However, its subcellular localization makes it less likely that it is a direct target for pathogen-derived effector proteins (Kaur et al. 2011). Earlier studies suggest that its activity might be sensitive to changes in redox balance and that it is induced by light. It was recently demonstrated in *Arabidopsis* that CYP20-3 may play a role in the regulation of cysteine biosynthesis in the chloroplast stroma through its interaction with the plastid isoform SAT1. This interaction seems to be required for optimal enzyme activity under stress conditions, most likely by stabilizing the assembly of one SAT trimer and three OAS-TL dimers into the cysteine synthase complex (CSC). It was shown that CYP20-3 mutants do not show an increase in thiol content in plants subjected to salt stress or high light conditions (Dominguez-Solis et al. 2008). The authors suggest that ROS elicit the transcriptional activation of SAT1 as well as the oxidation of CYP20-3 in a peroxiredoxin-dependent manner. Thioredoxin would then be needed to regenerate active CYP20-3 (Motohashi et al. 2003). It is interesting to note that the *B. juncea* CYP20-3 protein could only be detected in leaves of the S, but not the R, cultivar. This could indicate that oxidized CYP20-3 accumulates in this cultivar due to a redox imbalance in the chloroplast rendering the CSC inactive, but this needs to be investigated further (Kaur et al. 2011).

Resistance to pathogens is associated with the activation of signalling pathways that lead to the expression of certain pathogenesis-related (PR) proteins (Selitrennikoff 2001). While the precise mechanism of action of the PR-5 protein is not yet completely understood (Richardson et al. 1987; Wang et al. 1996; Coca et al. 2000; Ibeas et al. 2000), it is most likely involved in the degradation of the pathogen cell wall. Several PR-5 proteins have been reported to cause cell

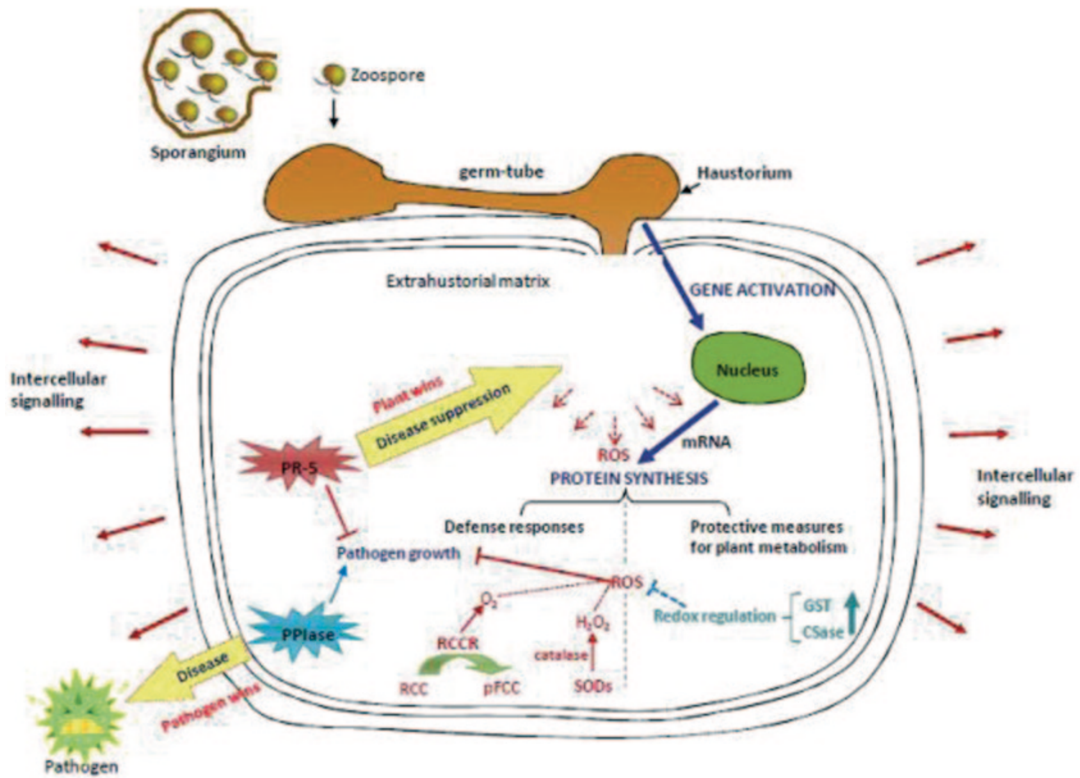

Fig. 6.4 Major elicitor-induced changes during the interaction of *Brassica juncea* and *Albugo candida.* (Kaur et al. 2011)

permeability changes in fungal cells with intact cell walls (Roberts and Selitrennikoff 1990). The maize PR-5 protein zeamatin inhibits growth of suspension cultures of *Candida albicans*, *Neurospora crassa* and *Trichoderma reesei* by causing rapid cell lysis, even at low temperatures (Roberts and Selitrennikoff 1990). PR-5 proteins may have a direct role in cell wall degradation, as they bind to (1, 3)-b-glucans and have in vitro (1, 3)-b-glucanase activity (Grenier et al. 1993; Trudel et al. 1998). The induction of the PR-5 protein following inoculation with *A. candida* in the R *B. juncea* variety suggests an anti-pathogenic role. This role is further indicated by its absence from the proteome profiles of the S variety and by the lower transcript abundance in the S variety compared with the R variety. The delayed induction of PR-5 transcript accumulation in the S line compared with the R line suggests that PR-5 needs to be present in the early stages of infection to exert its anti-pathogenic effect. Thus, in the context of disease progression in the *A. candida–B. juncea* pathosystem, the timing of the induction of PR-5 in R and S varieties appears to be crucial for mounting an effective defence response to this pathogen.

All but two of the candidate proteins identified by Kaur et al. (2011) reside in the chloroplast stroma and are directly or indirectly involved in redox homeostasis. This is quite remarkable given that chloroplasts are increasingly being regarded as sensors of environmental changes and mediators of plant stress responses (Fernandez and Strand 2008). Defence against pathogens might therefore require the activation of photoinhibitory ROS production combined with the balancing power of the reactive oxygen regulatory network of the chloroplast (Mittler et al. 2004; Dietz et al. 2006). This may be important for generating an oxidative burst that limits the

uncontrolled spread of cell death once an HR has been triggered by the presence of a pathogen (Belhaj et al. 2009). This is the first time that the host–pathogen interaction of the *B. juncea–A. candida* pathosystem has been dissected on a molecular level to differentiate between compatible and incompatible interactions. These results demonstrate that the timing of the expression of defence-related genes plays a crucial role during pathogenesis and incompatible interactions and that the redox balance within the chloroplast may be of crucial importance for mounting a successful defence response. These findings also indicate that synergistic and conserved strategies are utilized by the R host to fight off the *A. candida* attack. Determining the changes in protein expression patterns elicited by *A. candida* in an R versus an S variety of *B. juncea* has not only led to the likely identification of key molecular components of this specific host–pathogen interaction, but now also opens the way for developing genetic markers that could be used to screen for resistance within *B. juncea* germplasm collections. Such studies may also open novel avenues for engineering durable resistance to this pathogen. Kaur et al. (2011) identified steps in the metabolic processes and defence-related proteins that are required for mounting a successful defence response in *B. juncea* against *A. candida*. Looking at functional relationships between different genes of interest using transgenic overexpression and RNAi lines will probably be the next step in the characterization of this pathosystem (Fig. 6.4).

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7 Disease Development (Epidemiology)

Incidence and severity of white rust (WR) varies from year to year on different crops. Factors affecting initiation and development of the disease have not been studied in detail. On spinach, sporangial production is profuse at low temperatures but oospore production is very abundant at higher temperatures (Raabe and Pound 1952a, b). On water spinach in Hong Kong, during the main growing season (May to October), temperatures of 24–29 °C with heavy dew formation is ideal for infection and disease development (Edie and Ho 1970). On sunflower in Australia, WR development is greatest between 20–25 °C (Kajomchaiyakul and Brown 1976). Sempio (1938, 1940) reported that the optimum temperatures for the development of WR on potted radish plants were 16–18 °C, with a range of 12–21 °C; the disease did not develop below 6–7 °C or above 28–29 °C. Relative humidity (RH) of 60–80 % was more favourable than a saturated environment, CO_2 between 70 and 80 mm, and Hg (9.6–11 %) inhibited disease development. The fungus was highly sensitive to the effect of ultraviolet light; the optimum dose for inhibition of disease was 25–30 min. WR of horseradish is most favoured at 15–20 °C (Takeshita 1954). On *Amaranthus* species, ideal condition for the spread of WR around Delhi (India) occurs from October to the end of February. During this period, warm days with low humidity facilitate desiccation of sporangia while cool temperatures and heavy dew deposition at night provide optimum conditions for sporangial germination and infection (Mishra and Chona 1963). WR development on *Brassica juncea* in the field in relation to environmental conditions in India has been studied (Lakra and Saharan 1988d, 1990; Saharan 1984, 1992a, b; Kolte 1985a, b; Saharan et al. 1988; Verma and Bhowmik 1989; Mehta and Saharan 1998). WR pustules increase at a faster rate when the mean temperature is 11.5–12.5 °C, mean RH is > 75 %, and cloudy weather coupled with precipitation, and wind velocity is 2.6 km/h. The infection rate of WR on different genotypes of mustard varies from 0.1 to 0.5 (Fig. 7.1; Table 7.1).

Delay in sowing until after October 20 increases the WR disease intensity in *Brassica* species. Late planting (3rd week of October or first week of November) causes high incidence (10–43 %) and severity (13–32 %) of stagheads in *Brassica rapa* var. Toria and mustard (Kolte 1985a, b; Kolte et al. 1986). These authors also reported that 2–6 h of sunshine per day, concomitant with a mean minimum, and a mean maximum temperatures 6–10 °C, and 21–25 °C, respectively, and rainfall up to 160 mm during the flowering and pod formation are favourable for development of disease in *B. rapa* var. Toria and Indian mustard (Kolte et al. 1986). In their 2 years of field experiments in India, Verma and Bhowmik (1989) showed that a mean temperature of < 16 °C and a mean RH of > 60 % were necessary for WR development in *B. juncea*. Infection rate was the highest (0.574) at a mean temperature of 14.7 °C, and a RH of 73.3 %; infection rate decreased with the increase in mean temperature, and decrease in mean RH. Rainfall of 9 inches coincided with the highest infection rate. Duration of sunshine

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hours did not appear to have any marked effect, although prolonged periods coincided with poor disease development. In a laboratory study, Verma et al. (1983) found that the medium aged-detached leaves of *B. rapa* cv. Torch inoculated with *Albugo candida* 7 produced maximum pustules at 18.5 °C.

Role of host resistance and planting time on progression of WR and yield of mustard indicated that WR progressed rapidly on susceptible cultivar RH 30 when there was RH ≥ 65 % and average temperature ≤ 15 °C. Staghead initiation required higher RH besides low temperature. On cv. RH 30, delay in planting time from 15 September to 30 November 1985, sharply increased infection of both leaf as well as staghead phases (LP, SP). Planting in December were more favourable for staghead formation. The resistant cultivar RC 781 sustained infection even during epidemics (Fig. 7.2; Table 7.2). Lower yield losses in early sown crops are attributed due to high temperature (> 15 °C), low RH (< 60 %), and absence of rainfall, which were unfavourable for pathogen but very favourable for plant growth (Lakra and Saharan 1990).

Generally, older (lower) leaves were more susceptible than younger (upper) leaves; older leaves showed symptoms, 4 days after inoculation with disease intensity of 11.2 %, compared to younger leaves where symptoms appeared after 6 days with a disease intensity of 5.7 %. Symptoms appeared 3 days after inoculation on the lower surface with disease intensity (10 days after inoculation) of 14.6 %, compared to the upper surface where symptoms appeared after 5 days with a disease intensity of 10.5 %. One g/pot of oosporic inoculum was found to be optimum for disease development. White rust symptoms appeared after 23 days when oosporic inoculum was placed at a depth of 7.5 cm in soil, compared to 56 days at a depth of 10 cm. Late-sown crops suffered the most from WR infection (Biswas et al. 2011).

The optimum temperature for disease development ranged from 12 to 18 °C. Only 3 h of wetness was required for disease development at 12–22 °C. A minimum wetness period of 6–12 h was required for disease development at suboptimal temperatures (Sullivan et al. 2002). WR severity was higher in late-sown crops under low-bright sunshine hours (2 h/day) coupled with high morning RH (90 %) during the early hours of the day (Srivastava et al. 2005). First appearance of WR pustules on leaves, and silique (staghead formation) of mustard occurred between 36–131 and 60–123 days after sowing, respectively. Severity of disease on leaves was favoured by > 40 % minimum afternoon, and > 97 % maximum morning RH, and 16–24 °C maximum daily temperature. Staghead formation, on the other hand, was significantly and positively influenced by maximum daily temperature of 20–29 °C, and > 97 % morning RH (Chattopadhyay et al. 2011).

Studies by Mehta and Saharan (1998) determining effect of planting dates on WR-and-DM-disease-complex of rapeseed-mustard showed significantly increased WR infection and development with the delay in date of sowing during the two crop seasons. However, in the crop planted on two different dates in September, WR intensity was statistically at par. Observations revealed that WR infection was delayed by 40–50 days in early sown crop compared to crop sown in October onwards, where it generally appeared on 20 to 30-day-old plants depending on the time of sowing (Fig. 7.3 and 7.4). The maximum development of WR was recorded during December and January. In the early sown crops, higher temperatures prevailed for longer duration compared to the late-sown crops where temperature below 10 °C was congenial for the disease development. During November to end of December, the maximum and minimum temperatures were about 15–6 °C, respectively, resulting in higher disease incidence and severity in the 19 October 1992-planted crop (Table 7.3 and 7.4) (Mehta and Saharan 1998).

The development of WR on rapeseed-mustard varieties, Culture-1, CSR-721, and LWR-7 was measured in terms of increase in size (mm), and numbers of pustules in relation to the prevailing environmental conditions (Saharan 1995). Increase in WR pustules on variety Culture-1 was negatively correlated with minimum temperature (X_2), sunshine hours (X_5), and average rainfall (X_6), whereas positive correlation was observed

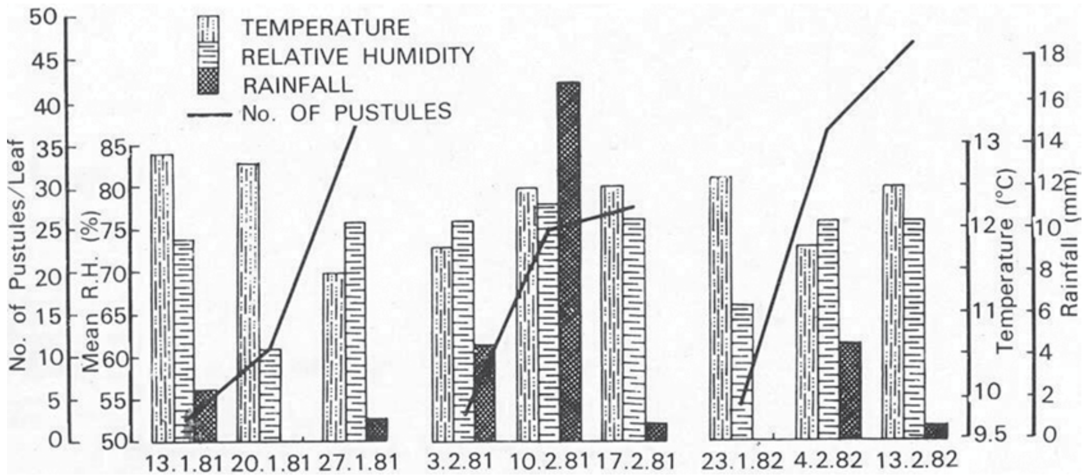

Fig. 7.1 Progress of white rust pustules on *B. juncea* cultivar Prakash in relation to environment. (Saharan et al. 1988)

Table 7.1 Relative resistance of rapeseed-mustard cultivars to white rust. (Saharan et al. 1988)

Cultivars	No. of pustules	Size of pustules	Infection rate (r)
Tower	0.0	0.0	0.0
RC-781	7.0	1.2	0.1
RH-30	9.0	1.5	0.3
YRT-3	10.6	1.5	0.3
CSR-741	13.6	1.5	0.4
CSR-448	15.9	1.6	0.4
CSR-142	20.0	1.7	0.4
Prakasha	29.2	2.0	0.5

between maximum temperature (X_1), and morning (X_3) and evening RH (X_4). In other two germplasm lines, CSR-721 and LWR-7, increase in size and number of WR pustules was positively correlated with maximum temperature (X_1) and morning RH (X_3) while the minimum temperature (X_2), evening RH (X_4), sunshine hours (X_5), and the average rainfall (X_6) were negatively correlated. The periodical progression of WR pustules was significant on cv. CSR-721 with R^2 value of 0.85; in CSR-721, Culture-1 and LWR-7, the maximum temperature (X_1) and morning RH (X_3) were positively correlated while the other factors were negatively correlated. The rainfall is negatively correlated with the development of WR under field conditions. The effect of weather variables on WR development was more apparent on CSR-721 with R^2 value of 0.88 followed by the R^2 values of 0.81 and 0.75 in Culture-1 and LWR-7, respectively. In Culture-1 and CSR-721, maximum temperature (X_l), and in case of LWR-7 minimum temperature (X_2), were positively correlated; weather variables were negatively correlated in other five cultivars.

Periodical progression of number of WR pustules was maximum on cv. CSR 721 under maximum temperature 20–25 °C, minimum temperature 5.1 °C, morning RH >94 %, evening RH>43 %, and sunshine hours 5.45 (Saharan 1995).

Progression of staghead formation on rapeseed-mustard genotypes DYS-25-10, NDIC-90-1, CSR-721, RH-8686, and RWDR-8411 were recorded from February 22 to March, 1994 in relation to prevailing environmental conditions by Saharan (1995). Cumulative and periodical

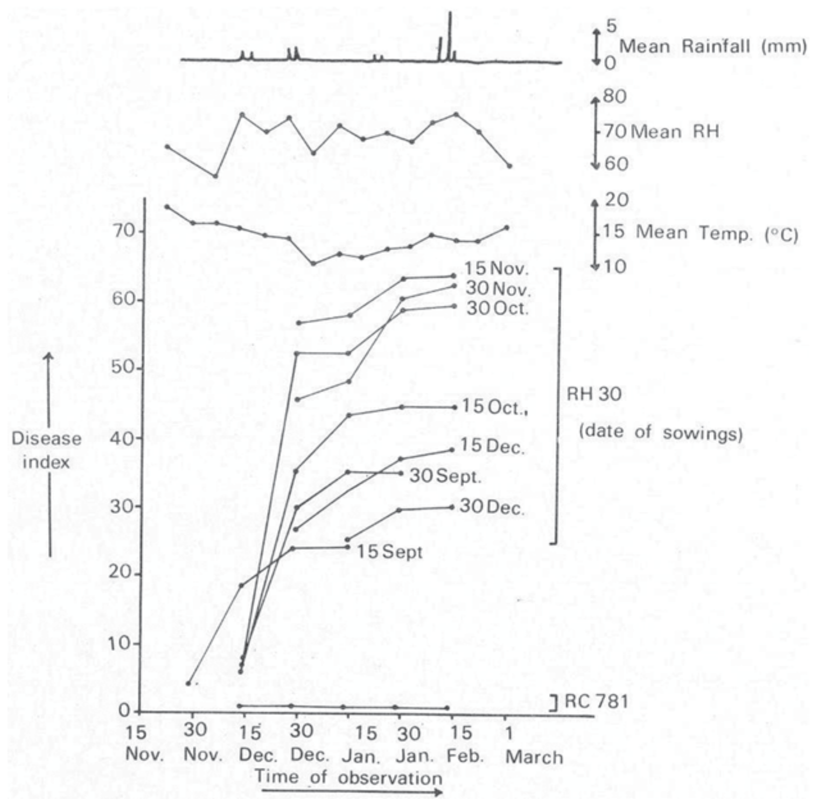

Fig. 7.2 Effect of date of sowing, environment factors, and host resistance on development of white rust in mustard. (Lakra and Saharan 1990)

Table 7.2 Effect of date of sowing and host resistance on the progression of white rust in mustard. (Lakra and Saharan 1990)

Date of observations	Date of sowings															
	15 Sep		30 Sep		15 Oct		30 Oct		15 Nov		30 Nov		15 Dec		30 Dec	
	LP	SH	SP	SH	LP	SH	LP	SH	LP	SH	LP	SH	LP	SH	LP	SH
RH-30																
30 Nov	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	X	X	X	X	X	X
15 Dec	18.5	0.0	8.3	0.0	7.5	0.0	6.3	0.0	0.0	0.0	0.0	0.0	X	X	X	X
30 Dec	23.9	0.0	29.6	0.0	35.4	0.0	55.0	0.0	56.8	0.0	45.7	0.0	26.8	0.0	X	X
15 Jan	24.2	2.9	35.1	1.6	43.7	1.0	55.2	0.9	58.1	0.0	48.4	0.0	32.4	0.0	25.1	0.0
30 Jan	X	X	35.2	3.0	44.9	4.0	58.9	6.0	63.4	3.2	60.4	0.0	37.2	0.0	29.6	0.0
15 Feb	X	X	X	X	45.1	5.0	59.7	14.5	63.3	18.6	62.5	11.8	38.8	0.0	30.3	0.0
01 Mar	X	X	X	X	X	X	N.O	22.0	N.O	24.9	N.O	27.8	N.O	30.3	N.O	20.0
RC-781					1.0	0.0	1.0	0.0	1.0	0.0	1.0	0.0	1.0	0.0	1.0	0.0

In RC-781, only final data was taken because only leaf phase of 1 disease index existed and no staghead infection was observed

X either crop was not sown or was harvested, *N.O.* no observation was taken, *LP* leaf phase infection, *SH* staghead infection

increase in staghead formation (Table 7.5 and 7.6) in relation to weather variables was analyzed statistically using multiple regression analysis. Predictive equations were statistically significant for periodical progression of stagheads on CSR-721, and there was a strong correlation between the increase in staghead formation and environmental variables. Prediction equations explained

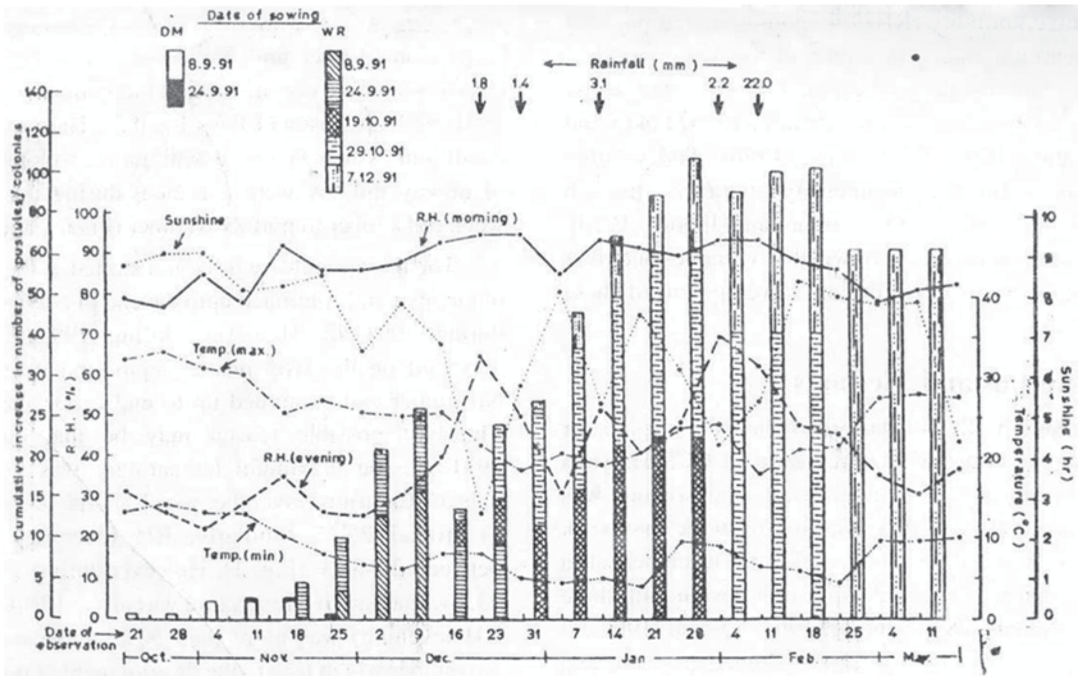

Fig. 7.3 Progression of white rust and downy mildew of mustard sown on different dates in relation to environmental variables during 1991–1992. (Mehta and Saharan 1998)

56 % variability in staghead formation as influenced by weather variable used. The maximum periodical increase in staghead formation was observed on 26th February, 1994 when there was 24.4 °C maximum temperature, 6.5 °C minimum temperature, 87 % morning RH, 39 % evening RH, and 9.4 h of sunshine.

A. candida staghead formation in *B. juncea* in relation to plant age, inoculation sites, and incubation conditions has been studied by Goyal et al. (1996). Maximum staghead formation was obtained in 26-day-old (growth stage (GS) 3.1) plants by inoculating differentiating flower buds with a zoospore suspension of *A. candida* race 2 V; exposing apical meristem tissues by opening the flower buds with forceps proved more conducive to staghead formation. Inoculation of 35- and 45-day-old plants (GSs 4.1 and 5.0) produced fewer hypertrophies mainly in isolated flowers. Inoculation of 7- and 13-day-old plants (GSs 1.0 and 2.1) did not produce any hypertrophied flowers but did result in the production of hypertrophied branches at the first node on the main stem. In general, hypertrophies were initiated more readily under greenhouse conditions than in the growth chamber (Table 7.8). Other *Brassica* hosts inoculated with *A. candida* race 2 V or 7 V at GS 3.1 showed similar rankings for staghead formation and leaf infection (Table 7.7 and 7.8; Fig. 7.5).

7.1 Disease Forecasting Models

Survival of the pathogen on diseased seeds or in infected plant debris in tropical or subtropical India has been ruled out (Verma and Bhowmick 1988), unlike the situation in temperate conditions (Humpherson-Jones 1991). In India, Indian mustard is sown from September to November, depending on the prevailing temperature and availability of soil moisture for seed germination. Harvesting occurs from February to May. Off-season crops are grown in nontraditional areas from May to September and this, coupled with harbouring of the fungal pathogen by oilseed,

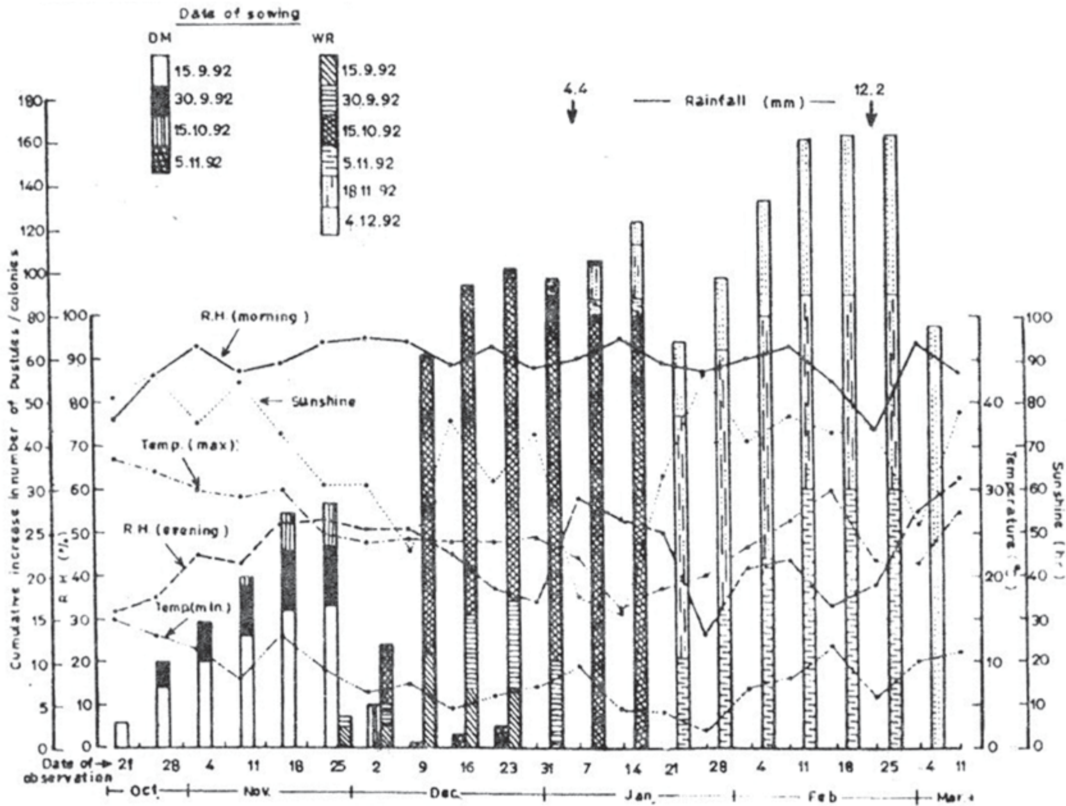

Fig. 7.4 Progression of white rust and downy mildew of mustard sown on different dates in relation to environmental variables during 1991–1992. (Mehta and Saharan 1998)

vegetable *Brassica* crops and alternative hosts can initiate carryover infection of *A. candida* from one crop-season to another (Kolte 1985a, b). Efficient, economical, and environmental friendly control of the WR disease may be obtained through knowledge of its timing of infection in relation to weather factors, which may enable growers to use timely fungicidal sprays for an efficient crop management. Weather is an important factor in the initiation, incidence, and severity of WR in Indian mustard. Empirical models have been developed to relate occurrence of WR on Indian mustard to temperatures, RH and sunshine hours (Bains and Jhooty 1979, 1985; Kolte et al. 1986; Saharan et al. 1988; Hegde and Anahosur 1994; Mehta and Saharan 1998; Sangeetha and Siddaramaiah 2007). However, the available information provides no insight into quantitative prediction of the disease in different parts of India. Accurate forecast of the crop age at first appearance of the WR and the risk of a WR epidemic would enable farmers in deciding appropriate timing for spraying of fungicides, and to avoid unnecessary pesticide application. Chattopadhyay et al. (2011) developed forecasting models for determining exact age of crop important for (a) first appearance of WR symptoms, and (b) production of highest (=peak) disease severity only on leaves; although malformed inflorescence (stagheads) were recorded until harvest, their data were not used in generating models.

For developing forecasting models in India, Chattopadhyay et al. (2011) planted seeds of susceptible cultivars Varuna and Rohini on ten dates at weekly intervals at Bharatpur, New Delhi, and

Table 7.3 Effect of sowing dates on downy mildew (DM) and white rust (WR) development on mustard leaves (cv. RH-30) during 1991–1992 crop season. (Mehta and Saharan 1998)

Disease recording week	Cumulative increase in number of DM colonies/white rust pustules with respect to date of sowing											
	Sept 8		Sept 24		Oct 19		Oct 29		Dec 7		Mean	
	DM	WR	DM	WR	DM	WR	DM	WR	DM	WR	DM	WR
1	0	0	0	0	–	0	–	0	–	1	0.0	0.3
2	0	0	0	0	–	1	–	0	–	9	0.0	1.9
3	0	0	0	0	–	1	–	1	–	19	0.0	4.1
4	0	0	1	0	–	1	–	9	–	28	0.6	7.8
5	2	0	1	4	–	3	–	15	–	43	1.3	13.1
6	2	0	1	6	–	6	–	21	–	50	1.5	16.7
7	2	0	0	8	–	12	–	29	–	63	1.1	22.3
8	0	3	0	9	–	16	–	39	–	64	0.0	26.4
9	0	13	0	9	–	22	–	41	–	64	0.0	29.7
10	0	15	0	9	–	23	–	41	–	64	0.0	30.5
Mean	0.7	3.1	0.2	1.5	–	8.1	–	9.7	–	40.5	–	–
		DM	WR					DM			WR	
LSD (0.05)	D/S	0.15	1.53					a=20.10.91			25.10.91	
	Interval	0.34	2.17					b=19.11.91			18.11.91	
	DM	0.49	4.86					c=no disease			18.11.91	
								d=no disease			16.12.91	
								e=no disease				
Data recorded 30 days after								f=disease did not appear				

Table 7.4 Effect of sowing dates on downy mildew (DM) and white rust (WR) development on mustard leaves (cv. RH-30) during 1992–1993 crop season. (Mehta and Saharan 1998)

Disease recording week	Cumulative increase in number of DM colonies/white rust pustules with respect to date of sowing													
	Sept 15		Sept 30		Oct 15		Nov 5		Nov 18		Dec 4		Mean	
	DM	WR	DM	WR	DM	WR	DM	WR	DM	WR	DM	WR	DM	WR
1	0	0	3	0	4	0	0	0	9	0	–	0	4.4	0.0
2	3	0	5	0	4	0	2	0	–	0	–	2	3.4	0.3
3	7	0	6	0	5	1	2	2	–	3	–	9	5.0	2.6
4	10	0	7	0	5	7	0	4	–	16	–	20	5.5	8.1
5	13	0	7	0	0	51	0	7	–	26	–	33	5.0	19.6
6	16	0	0	2	0	80	0	9	–	38	–	55	4.0	30.5
7	17	2	0	6	0	80	0	10	–	49	–	73	4.1	37.1
8	0	3	0	9	0	80	0	16	–	58	–	75	0.0	40.0
9	0	4	0	10	0	80	0	22	–	58	–	75	0.0	41.7
10	0	7	0	10	0	80	0	30	–	58	–	75	0.0	43.4
Mean	6.6	1.6	2.7	3.8	1.5	46	0.5	10.1	–	30.5	–	41.7	–	–
		DM	WR					DM			WR			
LSD (0.05)	D/S	0.53	2.48	Disease appeared on				a=21.10.92			25.11.92			
Interval		0.81	3.21					b=28.10.92			25.11.92			
	DM	1.69	7.86					c=11.11.92			25.11.92			
								d=9.12.92			23.12.92			
								e=no disease			23.12.92			
								f=no disease			07.01.92			
						g=disease did not appear								

Table 7.5 Cumulative progression of staghead formation (white rust–downy mildew complex) in different germplasm lines of rapeseed-mustard sown on 3rd November, 1993. (Saharan 1995)

Germplasm lines	Number of staghead (white rust–downy mildew complex) on different dates						
	22/2	23/2	24/2	25/2	26/2	27/2	28/2
DYS-25-10	28	41	55	62	71	74	74
NDIC-90-1	35	46	58	62	78	78	83
CSR-721	13	13	13	16	23	23	23
RH-8686	6	6	6	8	10	10	10
RWDR-8411	18	29	29	35	42	42	42

Table 7.6 Periodical progression of staghead formation (white rust–downy mildew complex) in different germplasm lines of rapeseed-mustard sown on 3rd November, 1993. (Saharan 1995)

Germplasm lines	Number of staghead (white rust–downy mildew complex) on different dates						
	22/2	23/2	24/2	25/2	26/2	27/2	28/2
DYS-25-10	-	13	14	7	9	3	0
NDIC-90-1	-	11	12	4	16	0	5
CSR-721	-	0	0	3	7	0	0
RH-8686	-	0	0	2	2	0	0
RWDR-8411	-	11	0	6	7	0	0

Table 7.7 Mean deviation ratios of the proportion of plants in cultivars with stagheads and leaf pustules (±SE) obtained by artificial inoculation with *A. candida* race 7V and 2V on apical meristems and leaves at growth stage 3.1. (Goyal et al. 1996)

Genus species	Cultivar	Apical meristem inoculation		Leaf inoculation	
		Stagheads	Leaf pustules	Stagheads	Leaf pustules
		Race 7V			
Brassica rapa	Torch	0.79 ± 0.06	0.26 ± 0.06	0.10 ± 0.06	0.93 ± 0.04
B. rapa	Tobin	0.77 ± 0.06	0.12 ± 0.05	0.06 ± 0.03	0.84 ± 0.05
B. rapa	Parkland	0.44 ± 0.07	0.04 ± 0.03	0	0.53 ± 0.07
B. napus	ZSN045	0	0	0	0.08 ± 0.04
B. napus	ZSN046	0	0	0	0
B. napus	Excel	0	0	0	0
		Race 2V			
B. juncea	Comercial brown	0.82 ± 0.05	0.25 ± 0.06	0.03 ± 0.02	0.98 ± 0.00
B. juncea	Cutlass	0.93 ± 0.03	0.11 ± 0.04	0.07 ± 0.03	0.95 ± 0.02
B. napus	ZSN046	0.14 ± 0.05	0	0	0.19 ± 0.06
B. napus	Excel	0	0	0	0
B. napus	ZSN045	0	0	0	0

Kangra in India. First appearance of WR disease occurred between 36 and 131 days on leaves, and between 60 and 123 days on pods (staghead), respectively. Severity of WR disease on leaves was favoured by >40 % minimum afternoon, >97 % maximum morning RH, and 16–24 °C maximum daily temperature. Staghead formation was significantly and positively influenced by 20–29 °C maximum daily temperature, and was further aided by >12 °C minimum daily temperature

Table 7.8 Percentage (±SE) of hypertrophied plants of *B. juncea* cv. Commercial Brown artificially inoculated with *A. candida* race 2V in green house and growth chamber. (Goyal et al. 1996)

Growth stage (age in days) at inoculation	Part of plant inoculated	Hypertrophied axillary branches on stem		Hypertrophied flower buds (stagheads)	
		Green house	Growth chamber	Green house	Growth chamber
1.0 (7)	Apical meristem	72±7	50±8	0	0
2.1 (13)	Apical meristem	60±8	35±8	0	0
3.1 (26)	Green flower buds	0	0	70±3	51±3
4.1 (35)	Yellow flower buds	0	0	41±4	14±3
5.0 (45)	Flower	0	0	25±3	5±2

and > 97 % maximum morning RH. Regional and cultivar specific models thus devised can predict the crop age at which WR would be expected to first appear, attainment of highest WR severity on leaves, and formation of highest number of stagheads at least 1 week ahead of first appearance of the disease on the crop.

Field trials were seeded on ten dates at weekly intervals (1, 8, 15, 22, 29 October, 5, 12, 19, 26 November and 3 December) at Bharatpur (27° 12′N; 77° 27′E), New Delhi (28° 39′N; 77° 13′E), and Kangra (32° 4′N; 76° 16′E) in nine (1999–2000 to 2007–2008) post-rainy (rabi) crop seasons with cvs. 'Varuna' and one locally important cultivar 'Rohini' at Bharatpur, 'BIO-902' at New Delhi, and 'RCC-4' at Kangra. Each plot measured 1.5 × 5 m with 30 × 10 cm planting spacing. The recommended doses of only N and P fertilizers were applied. Insect–pest protection practices were undertaken including seed treatment with Imidacloprid at the rate of 7 g/kg, and spray of oxydemeton methyl at the rate of 0.025 % a.i. at 15-day intervals. No attempts were made to control any diseases.

Occurrence of higher severity of WR on leaves and greater number of stagheads in late-sown crops matched with earlier findings of Tomar et al. (1992) and Meena et al. (2002). Mathur (1993) indicated higher disease severity during 35–70 d.a.s., which was further confirmed in this investigation. Our findings on range and correlation of different weather parameters with WR severity in the field matched with some earlier findings under laboratory conditions (Lakra and Saharan 1988b, d; Mathur 1993), with minor disagreement regarding range of temperatures favouring WR severity. The information on conditions favouring severity of WR on leaves, and formation of stagheads can be useful in providing predictions related to the disease (Gilles et al. 2000); delayed-sowing results coincide with the vulnerable growth stage of the crop and favourable weather conditions (high morning and afternoon RH, 16–24 °C maximum daily temperature, and > 12 °C minimum daily temperature). The presence of such favourable weather conditions decides the longevity of the WR attack period, and further build-up of inoculum on the crop, which consequently produces heavy disease infection, and adversely affects yield (Table 7.9). The damage caused to a crop by WR is likely to be related to sowing date, i.e. late sowing results in higher rust severity (Meena et al. 2002). Thus, under Indian conditions, it would be advisable to plant the crop at the earliest possible time to enable escape or noncoincidence of the flowering stage with favourable temperature and humidity factors. The late-sown crop matures quicker than the timely sown crop due to higher temperature towards the end of the crop season, It was noted that the early sown crops may escape WR on leaves but produce higher number of stagheads due to coincidence of favourable weather factors at vulnerable crop growth stage (Kolte et al. 1986).

Fig. 7.5 Hypertrophied inflorescence and branches in *Brassica juncea* cv. Commercial Brown plants artificially inoculated with *Albugo candida* race 2 V at growth stages 1.0–5.0 in the growth chamber/greenhouse. **a** Hypertrophied axillary branches from the first node on the main stem in plants inoculated at the apical meristem at GS 1.0. **b** Hypertrophied and stunted main stem in plants inoculated at the apical meristem at GS 2.1. **c** Completely hypertrophied inflorescences with apical growth terminating in stagheads on plants inoculated on differentiating flower buds at GS 3.1. **d** Hypertrophied inflorescence axis with pronounced stunting in plants inoculated on differentiating flower buds at GS 3.1. **e** Healthy (*N*) and hypertrophied (*H*) pods on the inflorescence of plants inoculated at GS 5.1. (Goyal et al. 1996)

7.1.1 Weather Data Recording

Weather data for maximum and minimum daily temperatures, morning (07:00 h) Local Apparent Time (LAT), RH were calculated on the basis of longitude of a location as per standard norms of the World Meteorological Organization (WMO) (Doorenbos 1976; Ghadekar 2002); afternoon (14:00 h LAT), RH, sunshine hours, and wind speed were recorded from standard meteorological observatories at New Delhi and Kangra. Meteorological observatories at these two locations were 110–170 m from the experimental site, and the data recording instruments were installed as per standard specifications of the WMO (Doorenbos 1976; Ghadekar 2002). At

Table 7.9 Effect of date of sowing on highest white rust severity on leaves of Indian mustard in 2002–2003 at different locations. (Chattopadhyay et al. 2011)

Date of sowing	Per cent WR severity on leaves of cultivars of Indian mustard at different locations					
	Bharatpur		New Delhi		Kangra	
	'Varuna'	'Rohini'	'Varuna'	'BIO-902'	'Varuna'	'RCC-4'
1 October	0.0	0.0	12.2	4.3	24.2	23.2
8 October	0.0	0.0	14.2	5.8	29.6	24.2
15 October	0.3	0.0	14.9	6.3	30.4	28.8
22 October	7.6	13.2	17.9	12.4	33.6	35.2
29 October	11.3	16.3	18.4	12.6	34.4	36.4
5 November	13.8	17.2	19.0	13.4	37.6	38.4
12 November	15.3	21.3	21.0	13.7	38.4	39.2
19 November	18.2	28.5	24.6	14.6	42.2	40.0
26 November	32.7	33.0	29.9	14.9	44.0	45.6
3 December	34.7	42.2	31.4	15.9	50.4	47.2

Bharatpur, the weather data were recorded using automatic weather station, located 35 m from the experimentation site, where apart from recording the weather variables, data for leaf wetness were also recorded. The sensors were installed as per standard specifications (Doorenbos 1976; Ghadekar 2002).

7.1.2 Disease Assessment

All experiments relied entirely on natural incidence of the disease. Data for initial date of appearance of WR, temporal progression of the disease on leaves, and staghead formation were monitored at each location. Observations for per cent disease severity (PDS) were recorded twice a week (on Tuesday and Friday) until harvest from ten randomly tagged plants in each plot on leaves and pods following the scale of Conn et al. (1990). For each assessment date, PDS on leaves and staghead formation in 10 tagged plants from each plot were averaged to give respective single values.

7.1.3 Forecasting Models

Different ranges of weather variables of 1 week preceding the assessment date were used as independent variables to identify the boundary and favourable weather conditions that influenced the dependent variables or disease severity on leaves and staghead formation, through regression analysis. Correlations of timing (days after sowing or d.a.s.) of first appearance of the WR symptoms on leaves, the highest severity of WR pustules on leaves, staghead numbers, and crop age with weather variables were analyzed. Linear prediction models based on the weather parameters as independent variables, and crop age (d.a.s.) at the time of first appearance of the disease on leaves, staghead on the crop, highest severity of the disease on leaves, highest number of stagheads in the season, and crop age at peak severity of the disease at each week starting from week of sowing as dependent variables, were fitted by multiple stepwise regression (Draper and Smith 1981) using data of the initial 8 years separately. Based on correlation coefficients between dependent variables under study with the respective weather parameter (i) in different weeks, a composite weather variable (zi) was developed as the weighted sum of the weather variable in different weeks starting from the pre-sowing week up to the week of prediction (Agrawal et al. 1986; Desai et al. 2004; Chattopadhyay et al. 2005). Similarly, interaction terms (zij) were developed as weighted sums of product between two weather variables, weightings being correlation coefficients of the dependent variable under study with products of weather variables in respective weeks. The important weather indices

Table 7.10 Models to forecast crop age (Y_1) at first appearance of white rust on leaves. (Chattopadhyay et al. 2011)

Location	Cultivar	Crop age (week) of prediction	Model	R^2
Bharatpur	Varuna	4	$Y_1 = -70.35 + 0.03\ z_{maxtmp \times aftRH}$	0.92
Bharatpur	Rohini	4	$Y_1 = -363.26 + 0.18\ z_{maxtmp \times aftRH} + 0.038\ z_{maxtmp \times mornRH}$	0.95
New Delhi	Varuna	4	$Y_1 = -18.86788 + 0.00335897 \times z_{maxtmp \times mornRH}$	0.99
New Delhi	B10-902	4	$Y_1 = -17.30825 + 0.003228 \times z_{maxtmp \times mornRH}$	0.96
Kangra	Varuna	2	$Y_1 = 47.89 + 0.081\ z_{maxtmp \times aftRH} - 0.16\ z_{maxtmp \times mintmp}$	0.82
Kangra	RCC-4	3	$Y_1 = -89.50 + 0.97\ z_{mornRH}$	0.67

maxtmp maximum daily temperature, *mintmp* minimum daily temperature, *morn* morning, *aft* afternoon, *RH* relative humidity

were selected through stepwise regression (Chattopadhyay et al. 2011).

Models were fitted for prediction of the dependent variables, viz. highest disease severity, or crop age (d.a.s.) at highest disease severity, or crop age (d.a.s.) at first appearance of the disease on weekly basis starting from the time of sowing, second week after sowing and so on (f = 1, 2, …). The dependent variables were related with weather parameters in different weeks (Table 7.10). The interactions of weather parameters were also found to be significant. The models were developed in the following format (Chattopadhyay et al. 2011):

$$Y = a_0 + \sum_{i=1}^{p} a_i z_i + \sum_{i \neq j}^{p} b_{ij} z_{ij} + e \qquad ((7.1a))$$

$$= a_0 + a_{max\,tmp} z_{max\,tmp} + a_{min\,tmp} z_{min\,tmp} + \ldots + a_{ssh} z_{ssh} + b_{max\,tmp} \times {}_{min\,tmp} z_{max\,tmp} \times \text{min tmp} + \ldots + b_{aft} RH \times ssh z_{aft} RH \times ssh + e \qquad ((7.1b))$$

where,

$$Z_i = \sum_{w=1}^{f} r_{iw} x_{iw} \qquad ((7.2a))$$

$$Z_{ij} = \sum_{w=1}^{f} r_{ijw} x_{iw} x_{jw} \qquad ((7.2b))$$

with *Y* being the dependent variable, x_{iw} the value of *i*th weather parameter in *w*th week, r_{iw} the value of correlation coefficient between *Y* and *i*th weather parameter in *w*th week, r_{ijw} the correlation coefficient between *Y* and product of x_i and x_j in *w*th week, *p* the number of weather variables, f the week after sowing when predicted and e the error term. Weather indices based on summation of weightings of different meteorological factors as per correlation coefficients in different weeks after sowing until the forecast was provided were taken into account. The ninth (2007–2008) crop season was used to validate the models for forecasting the targeted parameters at different locations based on the models developed in each of the initial 8 years for each of the parameter, viz. crop age at first appearance of the disease (Y_1), crop age at peak disease severity (Y_2) and highest disease severity (Y_3); paired *t*-test was used to assess the difference among predicted and observed values. The severity of WR on leaves and the number of stagheads (diseased pods) were higher in late-sown crops. First appearance of WR disease on leaves of mustard occurred between 36 and 131 d.a.s., being highest at 54, 71, 50, 53, 57, 55, 58, 53 and 54 d.a.s. during 1999–2008, respectively. First appearance of the disease on pod (staghead formation) occurred between 60 and 123 d.a.s. (Chattopadhyay et al. 2011).

Table 7.11 Models to forecast crop age (Y_2) at highest white rust severity on leaves. (Chattopadhyay et al. 2011)

Location	Cultivar	Crop age (week) of prediction	Model	R^1
Bharatpur	Vamna	3	$Y_2 = -98.50 + 0.0056\ z_{mornRH \times aftRH} + 1.66\ z_{maxtmp}$	0.98
Bharatpur	Rohini	2	$Y_2 = -5.36 + 0.01\ z_{maxtmp \times mornRH} - 0.30\ z_{maxtmp \times ssh}$	0.98
New Delhi	Varuna	4	$Y_2 = 1.72577 + 0.11375\ z_{maxtmp}$	0.98
New Delhi	BIO-902	5	$Y_2 = 0.7446 + 0.10568\ z_{maxtmp}$	0.94
Kangra	Varuna	5	$Y_2 = 467.85 + 0.02\ z_{maxtmp \times mornRH} - 0.02\ z_{maxtmp \times aftRH} + 1.86\ z_{mornRH}$	0.94
Kangra	RCC-4	5	$Y_2 = 657.36 + 2.48\ z_{mornRH} - 1.54\ z_{maxtmp}$	0.85

maxtmp maximum daily temperature, *mintmp* minimum daily temperature, *morn* morning, *aft* afternoon, *RH* relative humidity, *s.s.h* sun shine hours

Correlation study of the data from the relevant centres revealed that WR severity on leaves was positively correlated to >40% minimum afternoon RH (R^2: 0.92), >97% maximum morning RH (R^2: 0.89), >72% daily mean RH (R^2: 0.8), >10 °C daily mean temperature (R^2: 0.79), and 16–24 °C maximum daily temperature (R^2: 0.83). Staghead formation was significantly and positively correlated to 20–29 °C maximum daily temperature (R^2: 0.81) and further aided by >12 °C minimum daily temperature (R^2: 0.84), >97% maximum morning RH (R^2: 0.89), and >72% daily mean RH (R^2: 0.85). Empirically, a look at weather data available from the automatic weather station indicated >10 h of leaf wetness during the preceding 3 days which favoured the progress of WR severity on leaves, and formation of stagheads (Chattopadhyay et al. 2011).

Crop age (d.a.s.) at first appearance of the WR on the crop (Y_1), crop age (d.a.s.) at peak severity of the rust (Y_2), and highest disease severity (Y_3) were related with weather variables in different weeks including pre-sowing week, and the interactions were found significant. Regional and cultivar-specific models devised using data of the initial 8 years predicted the crop age at which white rust first appears on the leaves (Table 7.10), crop age at highest rust severity on leaves (Table 7.11), the peak rust severity on leaves, and number of stagheads (Table 7.12). Weather indices based on summation of weightings of different meteorological factors as per correlation coefficients in different weeks after sowing until the forecast was provided were taken into account. Most of the models saw entry of variable maximum and minimum temperature, morning RH, afternoon RH, and sunshine hours also getting entered in some cases. Proper monitoring of disease progress during recording of observations in experiments could enable to devise models for providing accurate forecasts a few weeks after sowing, about crop age at first appearance, crop age at highest severity, and highest level of disease severity. The predictions were possible at least 1 week ahead of first appearance of the disease on the crop, thus allowing growers to undertake timely sprays. The disease was never found to appear before 36 d.a.s. or beginning of the sixth week after sowing while the prediction for crop age at first appearance of rust on leaves was possible for most of the locations in the beginning of the fifth week (29 d.a.s.). Only at New Delhi, the prediction for highest severity of the disease on cv. ‘BIO-902’ was delayed till beginning of the eighth week after sowing, where the disease on the plant parts reached its peak much later. Common models for the targeted three parameters with the mustard cv. Varuna at all the three locations were attempted but due to low R^2 values they were not considered for validation. The models were validated in the ninth (2007–2008) year, wherein the observed and predicted values matched closely with low residual values. Out of the models developed in each of

Table 7.12 Models to forecast highest white leaf rust severity and staghead numbers (Y_3). (Chattopadhyay et al. 2011)

Location	Cultivar	Crop age (week) of prediction	Model	R^2
Bharatpur	Varuna/leaf	4	$Y_3 = 133.13 + 19.87\ z_{maxtmp}$	0.82
Bharatpur	Rohini/leaf	4	$Y_3 = 96.4 + 12.03\ z_{maxtmp}$	0.87
Bharatpur	Varuna/staghead	3	$Y_3 = AA7 + 0.69\ z_{maxtmp}$	0.82
Bharatpur	Rohini/staghead	3	$Y_3 = 4.17 + 0.58zmaxtmp$	0.87
New Delhi	Varuna/leaf	4	$Y_3 = -96.929 + 0.0222\ z_{maxtmpRH} + 0.0203\ z_{momRH \times ssh}$	0.99
New Delhi	BIO-9027/leaf	8	$Y_3 = 38.6277 + 0.02713 \times z_{momRH \times ssh} - 0.01637\ z_{aftRH} + 0.02282\ z_{aftRH \times ssh}$	0.99
Kangra	Varuna/leaf	5	$Y_3 = 77.18 + 0.27\ z_{aftRH}$	0.91
Kangra	R CC-47/leaf	5	$Y_3 = -117.73 + 0.62\ z_{momRH}$	0.80

maxtmp maximum daily temperature, *morn* morning, *aft* afternoon, *RH* relative humidity, *ssh* sun shine hours

the 4 initial years for forecasting each of the targeted parameters, only those models have been presented in the Tables 7.10–7.12, wherein the observed and predicted values matched closely with low or even no residual values (Tables 7.13 and 7.14) or difference between predicted and observed values in different cases were not significant ($P < 0.05$). Models devised for forecast of crop ages at (a) first appearance of staghead, (b) at peak census of staghead for all locations, and (c) highest staghead numbers, at New Delhi and Kangra, had low R^2 values and hence were not considered for validation. Model to forecast peak staghead number on the crop was devised for Bharatpur only, which enabled early assessment of the risk involved on the crop at the location.

The application of the first fungicidal spray is critical and differs with seasons and regions (Sansford et al. 1995) for different diseases. Hence, accurate region and cultivar-specific forecast for the crop age at first appearance of the disease assumes importance, keeping in view the fact that sowing date is controlled by several factors including available soil moisture, time of withdrawal of monsoon, ambient temperature, and availability of field for sowing. Thus, based on the predictions of the time of first appearance of the rust on the crop (d.a.s.) available at least 1 week before the actual appearance of the disease and the risk involved on the crop as related to the disease, the growers could undertake timely fungicidal sprays for better efficacy and avoid unnecessary ones. Though the Indian mustard is grown across a large part of India and that the WR disease is also found to be a problem at several of the cropping areas, their conditions for crop culture vary widely along with the specific conditions that favour the disease at different locations. There also could be variation in pathotype of *A. candida* across sites (Lakra and Saharan 1988c). These can be the reasons for different weather parameters getting entered in to the models for different locations. Most of the models saw the entry of variable maximum daily temperature with minimum daily temperature, morning and afternoon RH, and sunshine hours in some cases. Further investigation can provide the importance of the different weather factors favouring WR infection, and progress in order of their priority. Though in the present study, relationship of *A. candida* biology with some weather variables, viz. sunshine or light hours, were not considered, the models based on weather factors and rust severity on Indian mustard crop could provide effective prediction about the crop age as related to their time of appearance, peak disease severity on leaf, staghead number, and crop age at highest disease severity on leaves, and highest staghead numbers. Further, in all the cases, the models invariably included temperature among the weather factors. Weather indices based on accumulated weightings of different meteorological factors as per correlation coefficients in different weeks after sowing until the forecast were

Table 7.13 Validation of models for different dependent variables and cultivars at Bharatpur. (Chattopadhyay et al. 2011)

Cultivar	Dates of sowing (2007–2008)	Crop age (days after sowing) at first appearance of white rust (Y_1)		Crop age (days after sowing) at highest severity of while rust (Y_2)		Highest per cent white rust severity on leaf/no. of stagheads per plot (Y_3)	
		Predicted	Observed	Predicted	Observed	Predicted	Observed
Varuna/leaf	1 October	86	87	133	133	0.35	0.7
	8 October	81	81	126	131	3.3	3.0
	15 October	75	74	119	124	4.9	5.1
	22 October	71	70	118	118	8.3	6.9
	29 October	66	63	108	107	14.4	166
	5 November	47	45	105	106	22.6	28.6
	12 November	35	35	96	96	39.0	38.5
	19 November	33	33	84	83	39.0	38.7
	26 November	33	33	72	72	40.3	41.8
	3 December	30	3 1	70	70	47.5	47.0
Rohini/leaf	1 October	87	88	133	133	0.1	1.0
	8 October	8I	81	128	129	2.2	1.3
	15 October	75	76	119	119	3.3	2.6
	22 October	70	70	112	109	5.9	6.2
	29 October	63	63	112	107	10.1	11.1
	5 November	49	49	112	102	16.2	16.6
	12 November	35	36	101	100	20.0	19.5
	19 November	35	35	88	93	25.2	22.9
	26 November	35	34	86	86	29.4	28.5
	3 December	32	31	78	79	34.3	35.7
Varuna/ stagheads per plot	1 October	121	123	135	136	20.0	21.0
	8 October	118	119	130	132	20.0	21.0
	15 October	111	112	120	125	29.0	28.0
	22 October	105	105	115	118	29.6	30.0
	29 October	98	98	107	111	31.9	34.0
	5 November	92	91	107	111	36.9	38.0
	12 November	84	84	107	108	60.0	61.0
	19 November	77	77	102	104	130.0	132.0
	26 November	70	70	102	104	131.0	132.0
	3 December	63	63	95	97	139.7	133.0
Rohini/ stagheads per plot	1 October	121	123	135	135	20.0	19.0
	8 October	119	119	130	134	20.0	20.0
	15 October	112	112	130	134	31.9	33.0
	22 October	105	105	130	132	36.5	36.7
	29 October	98	98	130	132	39.6	38.0
	5 November	91	91	122	125	49.9	52.0
	12 November	8I	84	118	118	75.4	77.0
	19 November	78	77	112	111	98.1	100.0
	26 November	70	70	102	104	105.0	112.0
	3 December	63	63	97	97	124.0	120.0

taken into account. Proper monitoring of disease progress during recording of observations in experiments could help devise models for providing accurate forecasts of crop age at first appearance, crop age at highest severity, and highest staghead numbers. The importance of studying the spore biology of *A. candida* in relation to weather factors cannot be undermined, such in-depth stud-

Table 7.14 Validation of models for different dependent variables and cultivars at New Delhi and Kangra. (Chattopadhyay et al. 2011)

Location/ cultivar	Dates of sowing (2007–2008)	Crop age (days after sowing) at first appearance of white rust (Y_1)		Crop age (days after sowing) at highest severity of white rust (Y_2)		Highest per cent white rust severity on leaf/no. of stag heads per plot (Y_3)	
		Predicted	Observed	Predicted	Observed	Predicted	Observed
New Delhi/ Varuna	1 October	93	94	135	136	7.3	8.8
	8 October	85	87	131	132	10.7	11.2
	15 October	78	80	122	125	11.2	11.4
	22 October	70	69	112	114	12.0	11.9
	29 October	63	66	110	111	12.4	12.5
	5 November	63	63	105	108	15.0	15.4
	12 November	56	59	105	108	17.5	18.3
	19 November	56	56	102	101	26.0	25 2
	26 November	49	49	100	101	26.0	25.6
	3 December	49	48	92	94	26.5	26.1
New Delhi/ BIO-902	1 October	94	94	121	122	5.9	6.3
	8 October	86	87	112	115	7.5	7.4
	15 October	81	80	110	112	9.0	9.1
	22 October	70	69	106	108	12.5	13.6
	29 October	66	66	105	105	14.5	14.3
	5 November	63	65	105	104	18.0	17.8
	12 November	58	59	96	98	18.9	19.2
	19 November	57	58	92	91	23.1	22 5
	26 November	49	51	84	84	24.5	24.3
	3 December	42	41	77	77	32.8	33.0
Kangra/ Varuna	1 October	41	40	139	136	33.8	33.2
	8 October	40	40	132	132	38.0	38.0
	15 October	40	40	129	125	38.7	38.0
	22 October	39	40	127	122	38.8	38.0
	29 October	36	37	121	119	38.9	38.0
	5 November	36	37	118	118	39.3	39.0
	12 November	36	37	116	116	40.4	40.0
	19 November	33	33	115	114	40.6	40.0
	26 November	30	30	112	111	41.3	40.5
	3 December	29	29	108	110	44.1	44.0
Kangra/ RCC-4	1 October	44	44	143	140	38.8	38.9
	8 October	43	44	132	132	39.9	38.9
	15 October	42	40	132	131	10.6	40.0
	22 October	41	40	128	129	43.4	43.0
	29 October	38	37	117	118	46.9	45.2
	5 November	37	37	117	117	50.0	50.7
	12 November	37	37	117	117	52.8	51.7
	19 November	33	33	111	112	52.9	51.3
	26 November	31	30	108	110	54.2	51.7
	3 December	29	29	103	104	55.8	55.0

ies as done in Australia, Canada, and Europe for diseases of oilseed Brassicas (Gilles et al. 2000; West et al. 2001) could improve the accuracy of the models presented here as that could provide the grower with an advance warning of the risk of the disease, which may allow a period of several weeks in which to make a decision about fungicide applications and the risk can be updated later (Gilles et al. 2000). But, in an effort to cater to the need for providing real-time regional and cultivar-specific forecasts of the WR menace in India, it is a beginning by-passing the study of weather and spore biology relationship, which avoids the risk of inaccuracy, especially at the cultivar-specific level as it is based on fewer relationships which do not fully describe the dynamics of the biological processes influencing the disease epidemics (Gilles et al. 2000).

Using these models in combination with crop planting dates and standard meteorological data, it would be possible to provide necessary forecasts for the time being centrally from the Directorate of Rapeseed–Mustard Research (ICAR) at Bharatpur, India. Although the models could be improved with further detailed study on pathogen inoculum and spore biology, there would be a need to provide a simple computer package to enable any user to get a robust, accurate forecast on the internet. The models presented here are expected to guide growers efficiently for making fungicidal sprays more effective. However, the forecasters must take into consideration the other information regarding boundary and favourable weather conditions for disease severity on leaves, and stagheads incidence along with the output of location and cultivar specific models. The available literature indicates association of several weather factors with WR severity (Bains and Jhooty 1979; Kolte et al. 1986; Saharan et al. 1988; Lakra and Saharan 1988d; Hegde and Anahosur 1994; Mehta and Saharan 1998; Sangeetha and Siddaramaiah 2007) but is silent about providing forecast of the disease. In years of appearance of WR on crop before the decision week, growers may be advised about the risk expected. Further, the forecasts need to account for the margin of error in order to maintain the confidence of resource-poor mustard growers of India in the forecast system. A high priority over the next decade should be the collation of accurate disease and weather data, and development of models to forecast the effects of climate change on other plant diseases (Evans et al. 2008).

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8 Physiologic Specialization (Pathogenic Variability)

8.1 Historical Development

Physiologic specialization has long been known in *Albugo candida*. Eberhardt (1904a, b, c, d) recognized two specialized groupings of *Albugo,* one attacking *Capsella, Lepidium,* and *Arabis,* and the other attacking *Brassica, Sinapis,* and *Diplotaxis*. He was, however, hesitant to use the phrase "biological forms" for this fungus. Later, Melhus (1911) also suggested the existence of specialization in *A. candida*. Pape and Rabbas (1920) demonstrated that this fungus on *Capsella bursa-pastoris* should be considered a distinct form. Pfister (1927) found several biological forms in *Albugo tragopogonis*. Taubenhaus (1923) and Ciferri (1928) reported that distinct races occurred on *Ipomoea batatas* and on *Ipomoea* spp. in the USA and West Indies. Ciferri (1928) divided *Albugo ipomoeae-panduratae* into two biologic species. Savulescu and Rayss (1930) distinguished eight morphological forms within *A. candida*. Later, Savulescu (1946a, b) made ten varieties of *A. candida* based on host specialization and morphology. Hiura (1930) distinguished three biologic forms of *A. candida,* the first on *Raphanus sativus,* the second on *Brassica juncea,* and the third on *Brassica rapa var. Chinensis*. Napper (1933) described 20 races of *A. candida* in Britain. Togashi and Shibasaki (1934) found that sporangia of *Albugo* from *Brassica* and *Raphanus* were 20 × 18 μM in size, while those from *Cardamine, Capsella, Draba,* and *Arabis* measured 15.5 × 14.5 μM; these were classified as macrospora and microspora, respectively. Results of this Japanese study suggested that five distinct biological forms of *Albugo* were present. Subsequently, Ito and Tokunaga (1935) elevated the forms with the larger spores to the rank of the species *Albugo macrospora* (Togashi) Ito. Biga (1955) recognized two morphological taxa: *A. candida macrospora* and *A. candida microspora,* as proposed by Togashi and Shibasaki (1934), but renamed them *A. candida microspora* and *A. candida,* respectively. On the basis of sporangial measurements from 63 species, Biga (1955) reported that *A. candida microspora* (15–17.5 μM diameter.) is restricted to *Armoracia, Brassica, Erucastrum, Raphanus,* and *Rapistrum,* whereas *A. candida* (12.5–15 μm diameter.) has a wide range of cruciferous hosts. In addition, he identified two intraspecific taxa of *A. ipomoeae-panduratae,* and five of *A. tragopogonis. A. tragopogonis* was considered by Savulescu (1946a, b) to have two varieties differing in spore shape, each of which included morphological forms differing in size. Biga (1955) distinguished five morphological varieties in this species. Endo and Linn (1960) reported one race of *Albugo* on *Armoracia rusticana*.

8.2 Nomenculture of Races/Pathotypes

It is clear that each of the above authors was hesitant in describing specialized races of *A. candida*. Pound and Williams (1963) identified six races of *A. candida*: race 1 from *R. sativus* var. Early

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Scarlet Globe, race 2 from *B. juncea* var. Southern Giant Curled, race 3 from *A. rusticana* var. Common, race 4 from *C. bursa-pastoris,* race 5 from *Sisymbrium officinale,* and race 6 from *Rorippa islandica* (Table 8.1). Race 7 from *B. rapa* Turnip or Polish rapeseed, and race 8 from *Brassica nigra* were added by Verma et al. (1975) and Delwiche and Williams (1977), respectively. In Russia, Novotel'nova (1968), while analyzing intraspecific taxa, established that the *A. candida* species consists of separate morphological specialized forms confined to a particular range of host plants, i.e., to plants of certain species or groups of genera and species. Within the morphological forms, races can be differentiated, while within heterogeneous populations both races and forms can be differentiated. Geographical and climatic conditions leave their distinguishing mark on the processes of form development so that populations of the fungus encountered by investigators in different countries were not identical. Novotel'nova and Minasyan (1970) and Burdyukova (1980) studied the biology of *A. candida* and *A. tragopogonis* in Russia, and conducted an in-depth study of the extent of specialization in *A. candida*. In India, Singh and Bhardwaj (1984) tested 12 *Brassica* species and identified nine races from four hosts, viz., *B. juncea, B. rapa* var. Toria, *B. rapa* var. Brown Sarson, and *B. rapa* var. *Pekinensis.* Lakra and Saharan (1988c) identified five races of *A. candida* on the basis of their reaction on a set of 16 host differentials. They identified two distinct races from *B. juncea* which are different from the previous records. One (race 2) attacks *B. nigra, B. juncea,* and *B. rapa* var. Brown Sarson, and the other (race 3) infects only *B. juncea* and *B. rapa* var. Toria (Table 8.2). According to Petrie (1988), isolates of *A. candida* from *R. sativus, B. juncea, C. bursa-pastoris,* and *B. rapa* represented four distinct races in Saskatchewan, Canada. However, he did not comment on their nomenclature.

Bhardwaj and Sud (1988) tested 26 cultivated and wild cruciferous hosts and identified nine new biological races from nine hosts, viz., *B. rapa* var. Brown Sarson (cv. BSH I), *B. rapa* var. Toria (cv. OK-I), *B. juncea* (cv. Varuna), *Brassica chinensi, B. rapa* var. *Pekinensis* (cv. local), *B. rapa* (cv. PTWG), *R. sativus* (cv. Chin I 'Pink), *Raphanus raphanistrum* (wild radish), and *Lepidium virginicum* (wild). They reported that reaction of nine isolates of *A. candida* differed from each other on 26 differential hosts revealing thereby, that the monotypic pathogen, *A. candida* on crucifers existed in the form of different biological races designated as new biological races or forms 1–9. The concept of races in *A. candida,* as proposed by Pound and Williams (1963), was based on species relationships. Studies have, however, clearly demonstrated that cultivars of *Brassica* crops must be included in a set of host differentials to distinguish isolates of the pathogen within a present accepted race (Burdyukova 1980; Pidskalny and Rimmer 1985). There is an urgent need to standardize host differentials keeping in mind the homogeneity and purity of the species and varieties. Petrie (1988) using North American race 2 and 7 from *B. juncea* and *B. rapa,* respectively, have screened accessions of several *Brassica* species including *B. rapa* var. Yellow Sarson, *B. rapa* var. Brown Sarson, *B. rapa* var. Toria, and *B. juncea* from India. Both yellow and brown sarson were equally highly susceptible to both races, Toria only to race 7, and *B. juncea* only to race 2. A detailed study is needed to determine whether the races of *A. candida* attacking *B. juncea* and several *B. rapa* crops in India are similar to race 2 and 7 from Canada and the USA. Kolte et al. (1991) reported that the white rust isolate obtained from *B. rapa* appeared to be distinct in pathogenicity from the one obtained from *B. juncea* under Indian conditions. Petrie (1994), in Saskatchewan, and Alberta discovered new races, 7v in 1988 and 2v in 1989. Verma et al. (1999) reported two new races of *A. candida* in India, viz., race 12 from *B. juncea* and race 13 from *B. rapa* var. Toria using 14 (including 6 standard) crucifer host differentials (Table 8.3).

Mathur et al. (1995), and Rimmer et al. (2000) collected isolates of *A. candida* from different geographic locations in western Canada and tested for virulence on a number of cultivars and accessions of *Brassica* species to determine variability and distribution of different races in the area. Most isolates were identified as race 7, which could be subdivided into 7a and 7v on the basis of their virulence on *B. rapa* cv. Reward.

Table 8.1 Reaction of a series of crucifers to six isolates of *A. candida,* each from a different host species. (Pound and Williams 1963)

Differential test plants	Host source of *A. candida,* race number, and reaction of differential test plants											
	Race 1 *Raphanus sativus*		Race 2 *Brassica juncea*		Race 3 *Armoria rusticana*		Race 4 *Capsella bursa-pastoris*		Race 5 *Sisymbrium officinale*		Race 6 *Rorippa islandica*	
	+[a]	–	+	–	+	–	+	–	+	–	+	–
R. sativus L. var. Early Scarlet Globe	76	0	0	110	0	118	0	117	0	88	0	84
B. juncea (L.) Czern and Coss. Var. Southern Giant Curled	0	184	92	0	0	160	0	186	0	151	0	155
A. rusticana Gaertn., Mey., and Scherb	0	33	0	41	46	0	0	57	0	65	0	36
C. bursa-pastoris (L.) Medik.	0	144	0	172	0	156	315	0	0	190	0	310
S. officinale (L.) Scop[b]	0	360	0	260	0	284	0	390	218	2	0	325
R. islandica (L.) Bess	0	284	0	170	20	68	3	203	0	120	316	6
B. hirta Moench var. Canadian Yellow	45	0	94	0	59	0	64	0	46	13	8	35
Descurania Sophia (L.) Webb	54	6	78	2	80	0	36	0	25	1	2	73
Erysimum cheiranthoides L.	1	115	0	48	28	0	44	0	0	20	0	47
Eruca sativus Mill.	0	78	0	73	0	62	0	53	0	51	0	56
Nasturtium officinale R. Br.	0	181	0	151	8	128	0	106	0	126	0	163
S. altissimum L.	0	124	0	288	0	294	10	288	0	245	0	156
S. officinale (L.) Scop[c]	70	124	145	3	145	6	52	210	183	3	10	232
Thlaspi arvense L.	0	88	0	102	6	116	20	102	2	103	0	81

The number under each sign represents the actual number of plants responding + or – in that test unit
[a] + Sporulation of the fungus on the host, – no sporulation
[b] Cotyledons on the plants in this series were fully mature
[c] Cotyledons on the plants in this series were very young and not fully enlarged

Isolates 28-7 and 29-1 were less virulent as they were avirulent on all the differentials, except the rapid cycling *B. rapa* CrGCI-I8. *Brassica napus* cv. Tower isolates, 11-6 and 41-4, which could infect cultivars of both *B. rapa* and *B. juncea,* appeared to be hybrids between race 2 and race 7. Wu et al. (1995) studied genetic variation among isolates of *A. candida* using randomly amplified polymorphic DNA (RAPD) with five selected random primers' fingerprint patterns generated for each isolate. Most polymorphism was found between different races than among isolates within a single race. Most Canadian field isolates were grouped as race 7 and could be further subdivided into two groups (7a and 7v). Classification of *A. candida* isolates based on the results from the RAPD analysis was identical to the virulence classification on 10 *Brassica* differentials.

Four distinct and new pathotypes of *A. candida,* viz., AC I4 from RL 1359, AC 15 and AC 16 from Kranti, and AC 17 from RH 30 cultivars of *B. juncea* have been identified on the basis of their differential interactions with 11 host differentials by Gupta and Saharan (2002) (Table 8.4).

Jat (1999) identified 20 distinct pathotypes of *A. candida*; 17 from *B. juncea* (AC 18–AC 34), 2 from *B. rapa* var. Brown Sarson (AC 35–AC 36), and one from *Brassica niger* (AC 37) (Tables 8.5 and 8.6). From Western Australia (WA), Kaur et al. (2008) identified pathotype AC 2A from *B. juncea,* and pathotype AC 2V from *R. raphanistrum*.

The pathogenic variability recorded in *A. candida* in the form of races from all over the world is: 9 from Australia, 20 from Britain, 4 from Canada, 2 from Germany, 49 from India, 8 from Japan, 18 from Rumania, and 7 from the USA. However, nomenclature of *A. candida* races came into practice after the use of host differentials to distinguish races by Pound and Williams (1963). Global virulence of *A. candida* based on primary host is documented in Table 8.7. In

Table 8.2 Reactions of different isolates of *A. candida* from different host species on differential test plants. (Lakra and Saharan 1988c)

Differential test plants	Source of *A. candida,* race number, and reaction of different test plants				
	Race 1 *R. sativus*	Race 2 *B. juncea* (small pustules)	Race 3 *Armoria rusticana* (big pustules)	Race 4 *Capsella bursa-pastoris*	Race 5 *Sisymbrium officinale*
Brassica alba	−	−	−	−	−
B. nigra	−	+ (11)	−	−	−
B. napus	−	−	−	−	−
B. carinata	−	−	−	−	−
B. chinensis	−	−	−	+ (12)	−
B. pekinensis	−	−	−	+ (12)	−
B. tournefortii	−	−	−	−	+ (13)
B. juncea	−	+ (5)	+ (6)	−	−
B. rapa var. *Toria*	−	−	+ (18)	−	−
B. rapa var. Yellow Sarson	−	−	−	−	−
B. rapa var. Brown Sarson	−	+ (10)	−	−	−
B. rapa var. *rapa L.*	−	−	−	−	−
B. oleracea var. *Capitata*	−	−	−	−	−
B. oleracea var. *Botrytis*	−	−	−	−	−
R. sativus	+ (10)	−	−	−	−
Eruca sativa	−	−	−	−	−

Numbers in parentheses indicate incubation period in days + disease, − no disease

total, 117 races/pathotypes of *A. candida* have been reported from different countries (Table 8.8). In *A. candida,* sexual reproduction in the form of oospores is very common and enormous, especially on *B. juncea*. In addition, other mechanisms of variability including recombination, mutation, and heterokaryosis are also in operation in nature. To get the true picture of *A. candida* races and the virulence spectrum, there is an urgent need to standardize host differentials for each crucifer species in the form of isogenic lines at an international level. Standard nomenclature of the races, viz., ACjun I, 2 for *B. juncea* isolates, ACrap 1,2 for *B. rapa* isolates, ACnig 1,2 for *B. nigra* isolates, and ACol 1,2 for *B. oleracea* isolates, etc. may be a very useful beginning (Saharan 2010).

8.3 Virulence Spectrum

As per the gene-for-gene hypothesis, interaction of *Albugo–crucifers* for compatibility and incompatibility phenotype determines number of virulent genes in the pathotype and resistance genes in the host genotype. The virulence of *A. candida* from turnip rape (*B. rapa* var. Turnip Rape) and mustard (*B. juncea*) on various crucifers in Canada has been determined by Pidskalny and Rimmer (1985). It has been observed that pathotypes of *A. candida* from *B. juncea* have wide range of virulent genes. Pathotypes AC 23, AC 24, and AC 17 infect only one, two, and three differential hosts indicating a limited virulence potential. However, pathotypes of wider virulence, viz., AC 29, AC 27, AC 30, AC 18, and AC 21 infected 21, 18, 16, 12, and 10 host differentials, respectively (Jat 1999; Gupta and Saharan 2002). The intensity of virulence by the pathotypes is expressed through the incubation (IP), and latent periods (LP) of pathotypes interacting with the host genotype under the influence of prevailing environmental conditions. Large amount of variability has been discovered in the IP and LP of genotypes from 6 to 17 and 11 to 13 days, respectively in the genotypes–pathotypes interactions (Tables 8.9, 8.10, 8.11, and 8.12).

Table 8.3 Virulence of Indian *A. candida* pathotypes from *B. juncea* and *B. rapa* var. Toria on various cruciferous hosts evaluated at Agricultural Research Station, Morena, MP, India. (Verma et al. 1999)

Host species	Cultivar	*B. juncea* isolate		*B. rapa* var. Toria isolate	
		Infected plants (%)	Disease rating (0–4)	Infected plants (%)	Disease rating (0–4)
Brassica juncea	Commercial brown	97	1.9	97	1.9
	Pusa bold	100	2.8	100	3.4
	Kranti	100	2.8	100	3.4
B. rapa	Candle	0	0.0	33	1.1
	Torch	0	0.0	13	1.0
	Tobin	7	1.0	7	1.0
	Parkland	3	1.0	7	1.0
B. rapa var. Yellow Sarson	R-500	100	1.0	100	2.2
B. rapa var. Brown Sarson	Pusa Kalyani	47	1.9	67	2.8
B. rapa var. *Toria*	Bhawani	77	1.9	93	2.7
B. nigra	–	92	1.7	64	2.3
B. carinata	Dodola	7	1.0	0	0.0
B. napus	Westar	0	0.0	0	0.0
	Regent	0	0.0	0	0.0
B. alba	Kirby	40	1.1	77	1.4
R. sativus	Cherry belle	0	0.0	0	0.0
E. sativus	Morena local	3	1.0	13	1.0
S. officinale	–	0	0.0	0	0.0
R. islandica	–	0	0.0	0	0.0
C. bursa-pastoris	–	0	0.0	0	0.0

– No cultivar

Table 8.4 Reactions of different isolates of *A. candida* from different cultivars of *B. juncea* on host differentials. (Gupta and Saharan 2002)

Host differentials	Reaction of isolates obtained from				
	RL 1359	Kranti		RH 30	
	AC 14	AC 15	AC 16	AC 17	Number of VI/HD
EC-129126-1	−	−	−	−	0
EC-322090	−	−	−	−	0
EC-322092	−	−	−	−	0
EC-322093	−	−	−	−	0
Varuna	+	+	+	+	4
EC-287711	−	−	−	−	0
ZEM-1		+	−	−	1
RC 781	−	+	+	−	2
RH 30	+	+	+	+	4
RH 8113	+	+	+	−	3
Rajat	+	+	−	+	3
Virulence size	4	6	4	3	17

VI/HD virulent isolates per host differential, + denotes compatible interaction, − denotes incompatible interaction

Table 8.5 Reaction of *A. candida* isolates to host differentials during 1995–1996. (Jat 1999)

Host differentials		Reaction of *A. candida* isolates[a]										
Species/ecotypes	Genotypes	*B. juncea*							*B. rapa*		*B. nigra*	Number of VI/HD
		RH 30					EC	DYS	Candle	Span	Local	
		ACjun-1 (Hi)	ACjun-2 (Hi)	ACjun-3 (Si)	ACjun-4[b] (Gu)	ACjun-5 (Fa)	ACjun-6 (Hi)	ACjun-7 (Hi)	ACrap-1 (Hi)	ACrap-2 (Hi)	ACnig-1 (Ja)	
Brassica rapa var. Brown Sarson	Candle	+	+	−	+	−	−	−	−	+	+	5
	Torch	+	−	−	−	−	−	−	−	+	+	3
	Tobin	+	−	−	−	−+	−	−	−	−	−	2
	Span	+	+	+	−	+	−	+	+	−	−	5
	BSH-1	+	−	−	−	+	−	−	−	−	−	2
B. rapa var. Toria	TL 15	+	+	+	+	−	−	+	+	+	+	9
B. rapa var. Yellow Sarson	YSPb 24	+	−	−	+	−	−	−	−	−	−	2
B. rapa var. Turnip	White-4	−	−	−	−	−	−	−	−	−	−	0
B. pekinensis	Fodder rape	+	+	+	+	−	−	−	−	−	+	5
B. nigra	Local	+	+	+	+	−	+	−	+	+	+	8
B. juncea	RH 30	+	+	+	+	−	−	−	−	+	+	6
B. napus	GSL 1	−	−	−	−	−	−	−	−	−	−	0
	Wester	−	−	−	−	−	−	−	−	−	−	0
B. carinata	HC 2	−	−	−	+	−	−	−	−	−	−	1
B. oleracea var. *Botrytis*	H 1	−	−	−	−	−	−	−		−	−	0
B. oleracea var. *Caulorapa*	Pride of India	−	−	−	−	−	−	−	−	−	−	0
B. oleracea var. *Caulorapa*	Purple Vienna	−	−	−	−	−	−	−	−	−	−	0
B. alba	Ochre	−		−	+	−	−	−	−	−	−	1

Table 8.5 (continued)

Host differentials		Reaction of *A. candida* isolates[a]										
Species/ecotypes	Genotypes	*B. juncea*							*B. rapa*		*B. nigra*	Number of VI/HD
		RH 30					EC	DYS	Candle	Span	Local	
		ACjun-1 (Hi)	ACjun-2 (Hi)	ACjun-3 (Si)	ACjun-4[b] (Gu)	ACjun-5 (Fa)	ACjun-6 (Hi)	ACjun-7 (Hi)	ACrap-1 (Hi)	ACrap-2 (Hi)	ACnig-1 (Ja)	
	Local	+	−	−	−	−	−	−	−	−	−	1
E. sativus	T 27	−		−	−	−		−	−	−	−	0
R. sativus	Scarlet Globe	−	−	−	+	−	−	−	−	−	−	1
B. tournefortii	Local	+	−	+	+	−	−	−	−	−	−	3
Virulence size		12	6	6	10	3	1	2	3	5	6	54

VI/HD virulent isolates per host differential, *ACjun* isolate derived from *B. juncea* cv. RH 30, EC182925, DYS-7-3-1, *ACrap* isolate derived from *B. rapa* var. Brown Sarson, *ACnig* isolate derived from *B. nigra*; +, denotes compatible interaction, − denotes incompatible interaction

Location: *HI* Hisar, *Si* Sirsa, *Gu* Gurgaon, *Ja* Jakhal, and *Fa* Fatehabad

[a] Isolate derived from leaf

[b] Isolate derived from staghead

Table 8.6 Reaction of *A. candida* isolates to host differential during 1996–1997. (Jat 1999)

Host differential		Reaction of *A. candida* isolates derived from *B. juncea*[a]										
Species/ecotypes	Genotypes											
		RCC-4	Kranti			RL 1359		BIO-902	RH-30			Number of VI/HD
		ACjun-15 (Ka)	ACjun-16 (Ka)	ACjun-18 (Bh)	ACjun-19 (Na)	ACjun-20 (Na)	ACjun-21 (Na)	ACjun-22 (Na)	ACjun-23[b](Hi)	ACjun-24[c](Hi)	ACjun-25[d](Hi)	
Brassica rapa var. Brown Sarson	Candle	−	+	−	+	+	+	+	−	−	−	5
	Torch	−	−	+	+	+	+	+	+	+	−	7
	Tobin	−	−	−	+	+	+	+	−	−	−	4
	Span	+	−	+	−	+	+	+	−	−	−	5
	BSH-1	−	−		+	+	+	−	−	−	−	3
B. rapa var. Toria	TL 15	−	−	+	+	+	+	+	−	−	−	5
B. rapa var. Yellow Sarson	YSPb 24	−	−	+	+	+	+	+	−	−	−	5
B. rapa var. Turnip	White-4	−	−	−	−	−	+	−	−	−	−	1
B. pekinensis	Fodder rape	−	−	+	+	+	−	+	−	−	−	4
B. chinensis	Local	+	+	+	+	+	+	+	−	−	−	7
B. nigra	Local	−	+	−	−	−	−	−	−	−	−	1
B. juncea	RH 30	+	+	+	+	+	+	+	−	−	−	7
	Shiva	−	−	−	−	−	−	−	−	−	−	0
	Zem-1	−	−	+	+	+	+	+	−	−	−	5
	Rajat	+	−	+	+	+	+	+	−	−	−	6
	RH-8113	−	+	+	+	+	+	+	−	−	−	6
	EC-129126-1	−	−	−	−	−	−	−	−	−	−	0
	EC-129126-2	−	−	−	−	−	−	−	−	−	−	0
	SSK-1	−	−	+	+	+	−	+	−	+	−	5
	RC-781	+	−	+	+	+	−	−	−	−	−	4
	RH-781	+	−	+	+	+	+	−	+	−	−	6
	Domo-4	+	−	+	+	+	+	+	−	+	−	7
B. napus	GSL 1	−	−	−	−	−	−	−	−	−	−	0
	Wester	+	+	+	+	+	+	+	−	−	−	7
B. carinata	HC 2	−	−	−	−	−	−	−	−	−	−	0
B. oleracea var. *Botrytis*	H 1	−	−	−	−	−	−	−	−	−	−	0

Table 8.6 (continued)

Host differential		Reaction of *A. candida* isolates derived from *B. juncea*[a]										
Species/ecotypes	Genotypes											
		RCC-4	Kranti			RL 1359		BIO-902	RH-30			Number of VI/HD
		ACjun-15 (Ka)	ACjun-16 (Ka)	ACjun-18 (Bh)	ACjun-19 (Na)	ACjun-20 (Na)	ACjun-21 (Na)	ACjun-22 (Na)	ACjun-23[b](Hi)	ACjun-24[c](Hi)	ACjun-25[d](Hi)	
B. oleracea var. *Capitata*	Pride of India	–	–	–	–	–	–	–	–	–	–	0
B. oleracea var. *Caulorapa*	Purple Vienna	–	–	–	–	–	–	–	–	–	+	1
B. alba	Ochre	–	–	–	–	–	–	+	–	–	–	1
	Local	–	–	+	–	+	–	+	–	–	–	3
E. sativus	T 27	–	–	–	–	+	–	–	–	–	–	1
R. sativus	Scarlet Globe	–	–	–	–	–	–	–	–	–	–	0
B. tournefortii	Local	+	+	+	+	+	+	+	–	–	–	7
Virulence size		9	7	18	18	21	16	18	2	3	1	113

VI/HD virulent isolates per host differential, *ACjun* isolate derived from *B. juncea* cv. RCC-4, Kranti, RL 1359, BIO-902, and RH 30, + denotes compatible interaction, – denotes incompatible interaction

Location: *Ka* Kangra, *Bh* Bharatpur, *Na* Navgaon, *H* Hisar

[a] Isolate derived from leaf

[b] Isolate derived from gynoecium

[c] Isolate derived from sepal

[d] Isolate derived from stamen

Table 8.7 Global virulence of *A. candida* pathotypes. (Saharan 2010)

Designate pathotype	Country	International primary host	Reference
AC 1	North America	*R. sativus*	Pound and Williams (1963)
AC 2	North America	*B. juncea*	Pound and Williams (1963)
AC 2V	North America	*B. napus*	Petrie (1994)
AC 3	North America	*A. rusticana*	Pound and Williams (1963)
AC 4	North America	*C. bursa-pastoris*	Pound and Williams (1963)
AC 5	North America	*S. officinale*	Pound and Williams (1963)
AC 6	North America	*R. islandica*	Pound and Williams (1963)
AC 7	North America	*B. rapa*	Verma et al. (1975)
AC 7V	North America	*B. rapa* cv. Reward	Petrie (1994)
AC 8	North America	*B. nigra*	Delwiche and Williams (1977)
AC 9	North America	*B. oleracea*	Williams (1985)
AC 10	North America	*B. alba*	Williams (1985)
AC 11	North America	*B. carinata*	Williams (1985)
AC 12	India	*B. juncea*	Verma et al. (1999)
AC 13	India	*B. rapa* var. Toria	Verma et al. (1999)
AC 1 to AC 9	India	*Brassica* species	Singh and Bhardwaj (1984)
AC 1 to AC 5	India	*Brassica* species	Lakra and Saharan (1988)
AC 14	India	*B. juncea* cv. RL 1359	Gupta and Saharan (2002)
AC 15	India	*B. juncea* cv. Kranti	Gupta and Saharan (2002)
AC 16	India	*B. juncea* cv. Kranti	Gupta and Saharan (2002)
AC 17	India	*B. juncea* cv. RH 30	Gupta and Saharan (2002)
AC 18 to AC 34	India	*B. juncea* cv. RH 30; EC 182925; DVS 7-3-1	Jat (1999)
AC 35 and AC 36	India	*B. rapa* var. Brown Sarson	Jat, 1999
AC 37	India	*B. nigra*	Jat (1999)
AC 2A	Western Australia	*B. juncea* cv. Vulcan; Commercial Brown	Kaur et al. (2008)
AC 2V	Western Australia	*R. raphanistrum*	Kaur et al. (2008)

Table 8.8 Global number of *A. candida* races/pathotypes

Country	No. of pathotypes	Ref.
Australia	9	Kaur et al. (2008, 2011)
Britain	20	Napper (1933)
Canada	2+2=4	Verma et al. (1975); Delwiche and Williams (1977); Petrie (1994)
Germany	2	Eberhardt (1904a, b, c, d)
India	9+4+20+5+9+2=49	Bhardwaj and Sud (1988); Gupta and Saharan (2002); Lakra and Saharan (1988); Jat (1999); Verma et al. (1999)
Japan	3+5=8	Hiura (1930); Togashi & Shibasaki (1934)
Romania	8+10=18	Savulescu and Rayss (1930)
USA	1+6=7	Endo and Linn (1960); Pound and Williams (1963)

Table 8.9 Incubation and latent period (in days) of *A. candida* isolates on host differentials under controlled conditions. (Gupta and Saharan 2002)

Host differentials	Incubation and latent period (in days) of *A. candida* isolates obtained from hosts							
	RL 1359		Kranti				RH 30	
	AC 14		AC 15		AC 16		AC 17	
	IP	LP	IP	LP	IP	LP	IP	LP
EC-129126–1	–	–	–	–	–	–	–	–
EC-322090	–	–	–	–	–	–	–	–
EC-322092	–	–	–	–	–	–	–	–
EC-322093	–	–	–	–	–	–	–	–
Varuna	9	13	7	12	8	13	7	11
EC-287711	–	–	–	–	–	–	–	–
ZEM-1	–	–	6	11	–	–	–	–
RC 781	–	–	6	11	7	11	–	–
RH 30	17	©	7	11	9	13	6	11
RH 8113	17	©	9	13	7	11	–	–
Rajat	11	14	6	11	–	–	7	13

IP incubation period, *LP* latent period, − no incubation period, © hypersensitive reaction

Availability of virulence variability in pathotypes from *B. juncea* suggested the possibility of identification of more number of resistant genes in the genotypes including identification of loci and alleles. In the absence of isogenic lines, it is not clear whether the races with wider virulence attack the same genes in the host differentials, or genes for susceptibility are different, or situated on different loci or tightly linked (Saharan 2010).

8.4 Phylogenetic Relationship of Races/Pathotypes

Kaur et al. (2011) has established the prevalence of nine races in WA. From comparing the complete internal transcribed spacer (ITS) rDNA nucleotide sequence of the 9 *A. candida* isolates from WA with those of 38 previously collected Australian isolates of *A. candida,* and also of those previously reported from Europe and Asia, it was possible to distinguish distinct groups within these isolates. Despite the small overall sequence divergence, the majority of these races have already been previously reported from Australia (Petkowski et al. 2010); races analyzed by Kaur et al. (2011) were phylogenetically similar to those reported previously. However, the isolate obtained from *Brassica tournefortii* was phylogenetically different from the most distinct isolates analyzed. The phylogenetic relationships identified are expected to help overcome the technical difficulties usually encountered with host range studies, allowing a better understanding of host specialization.

The use of a molecular systematic classification of different strains of *A. candida* in conjunction with the pathogenic behavior on a variety of cruciferous hosts, the *A. candida* isolates from different hosts in WA provide reliability and repeatability in defining races of *A. candida.* Defining the race status of *A. candida* in WA has provided a sound basis for the selection and deployment of new cultivars of both oilseed and horticultural *Brassica* spp. In particular, this new information is critical not only to the planned rapid expansion of the *B. juncea* industry in Australia, but also in increasing interest to develop alternative *Brassica* spp. for industrial oil production (Kaur et al. 2011).

Table 8.10 Incubation and latent periods (in days) of *A. candida* isolates in host differentials under controlled conditions during 1995–1996. (Jat 1999)

Host differentials		Incubation and latent period (days)of *A. candida* isolates[a]																					
Species/ ecotypes	Geno-types	*B. juncea*														*B. rapa*				*B. nigra*		Average	
		RH-30										EC		DYS		Candle		Span		Local			
		ACjun-1 (Hi)		ACjun-2 (Hi)		ACjun-3 (Si)		ACjun-4[b] (Gu)		ACjun-5 (Fa)		ACjun-6 (Hi)		ACjun-7 (Hi)		ACrap-1 (Hi)		ACrap-2(Hi)		ACnig-1 (Ja)			
		IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP
Brassica rapa	Candle	15	[c]	15	[c]	–	–	11	15	–	–	–	–	–	-	-	–	9	[c]	9	[c]	11.8	15
	Torch	15	[c]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10	[c]	9	[c]	11.3	[c]
Brassica rapa var. *Brown Sarson*	Tobin	15	18	–	–	-	-	-	-	16	[c]	–	–	–	–	–	–	-	-	-	–	15.5	18.0
	Span	15	[c]	11	18	8	15	–	–	–	–	–	–	16	[c]	10	[c]	–	–	–	–	12.0	16.5
	BSH-1	15	[c]	-	–	–	–	–	–	16	[c]	–	–	–	–	–	–	–	–	–	–	15.5	[c]
B. rapa var. Toria	TL 15	8	14	11	[c]	15	18	11	18	16	[c]	–	–	16	[c]	16	[c]	9	[c]	9	[c]	12.3	16.6
B. rapa var. Yellow Sarson	YSPb 24	11	15	–	–	–	–	8	14	–	–	–	–	–	–	–	–	–	–	–	–	9.5	14.5
B. rapa var. Turnip	White-4	–	–	-	-	-	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B. pekinensis	Fodder rape	8	15	15	18	15	[c]	11	[c]	-	–	–	–	–	–	–	–	–	–	9	[c]	11.6	16.5
B. nigra	Local	8	14	8	14	8	15	8	13	–	–	16	[c]	–	–	10	[c]	9	16	9	16	9.5	14.6
B. juncea	RH 30	8	14	7	15	8	14	8	13	–	–	–	–	–	–	–	–	9	[c]	9	[c]	8.1	14.0
B. napus	GSL 1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Wester	–	–	–	–	–	–	–	–	–	–	–	–	–	–	-	–	–	–	–	–	–	–
B. carinata	HC 2	–	–	–	–	–	–	18	[c]	–	–	–	–	–	–	–	–	–	–	–	–	18.0	[c]
B. oleracea var. *Botrytis*	H 1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B. oleracea var. *Cau-lorapa*	Pride of India	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B. oleracea var. *Cau-lorapa*	Purple Vienna	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Table 8.10 (continued)

Host differentials		Incubation and latent period (days)of *A. candida* isolates[a]																					
Species/ ecotypes	Geno- types	*B. juncea*														*B. rapa*				*B. nigra*		Average	
		RH-30										EC		DYS		Candle		Span		Local			
		ACjun-1 (Hi)		ACjun-2 (Hi)		ACjun-3 (Si)		ACjun-4[b] (Gu)		ACjun-5 (Fa)		ACjun-6 (Hi)		ACjun-7 (Hi)		ACrap-1 (Hi)		ACrap-2(Hi)		ACnig-1 (Ja)			
		IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP
B. alba	Ochre	–	–	–	–	–	–	18	[c]	–	–	–	–	–	–	–	–	–	–	–	–	18.0	[c]
	Local	11	[c]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	11.0	[c]
E. sativus	T 27	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
R. sativus	Scarlet Globe	–	–	–	–	–	–	8	14	–	–	–	–	–	–	–	–	–	–	–	–	8.0	14.0
B. tourne- fortii	Local	11	15	–	–	11	15	11	[c]	–	–	–	–	–	–	–	–	–	–	–	–	11.0	15.0
Average		11.6	15.0	11.1	16.2	10.8	15.4	10.2	14.5	16.0	–	16.0	[c]	16.0	[c]	12.0	–	9.2	16.0	9.0	16.0		

IP denotes incubation period, *LP* denotes latent period, – denotes no incubation and no latent period, *ACjun* isolate derived from *B. juncea* RH 30, EC182925, DYS-7–3-1, *ACrap* isolate derived from *B. rapa* var. Brown Sarson, *ACnig* isolate derived from *B. nigra*

Location: *H*i Hisar, *Si* Sirsa, *Gu* Gurgaon, *Ja* Jakhal, and *Fa* Fatehabad

[a] Isolate derived from leaf

[b] Isolate derived from staghead

[c] Denotes hypersensitivity

Table 8.11 Incubation and latent periods (in days) of *A. candida* isolates in host differentials under controlled conditions during 1996–1997. (Jat 1999)

Host differentials		Incubation and latent period (days)of *A. candida* isolates[a]																					
Species/ ecotypes	Genotypes	RCC-4 Kranti RL 1359 BIO-092 RH 30																				Average	
		ACjun-15 (ka)		ACjun-16 (ka)		ACjun-18 (Bh)		ACjun-19 (Na)		ACjun-20 (Na)		ACjun-21 (Na)		ACjun-22 (Na)		ACjun-23[c] (Hi)		ACjun-24[d](Hi)		ACjun-25[e](Hi)			
		IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP
Brassica rapa	Candle	–	–	17	[b]	–	–	6	13	11	[b]	13	[b]	7	[b]	–	–	–	–	–	–	10.8	13.0
	Torch	–	–	–	–	8	17	10	[b]	6	17	13	0	8	[b]	12	[b]	13	[b]	-	–	10.0	17.0
Brassica rapa var. *Brown Sarson*	Tobin	–	–	–	–	–	–	7	11	6	11	17	[b]	6	[b]	–	–	–	–	–	–	9.0	11.0
	Span	17	[b]	–	–	8	0	–	–	12	17	11	[b]	7	17	–	–	–	–	–	–	11.0	17.0
	BSH-1	–	–	–	-	8	[b]	8	[b]	7	17	–	–	–	–	–	–	–	–	–	–	7.6	17.0
B. rapa var. Toria	TL 15	–	–	–	–	11	15	7	[b]	12	[b]	16	[b]	11	13	–	–	–	–	–	–	11.4	14.0
B. rapa var. Yellow Sarson	YSPb 24	–	–	–	–	6	17	6	17	6	17	11	[b]	7	13	-	–	–	–	–	–	7.2	16.0
B. rapa var. Turnip	White-4	–	–	–	–	–	–	–	–	–	–	11	[b]	–	–	–	–	–	–	–	–	11.0	–
B. pekinensis	Fodder rape	–	–	–	–	15	18	13	[b]	7	11	–	–	–	–	–	–	–	–	–	–	11.6	14.5
B. chinensis	Local	13	[b]	17	[b]	8	11	6	11	8	11	13	[b]	7	11	–	–	–	–	–	–	10.3	11.0
B. nigra	Local	–	–	14	[b]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	14.0	–

Table 8.11 (continued)

Host differentials		Incubation and latent period (days)of *A. candida* isolates[a]																					
Species/ ecotypes	Genotypes	RCC-4 Kranti RL 1359 BIO-092 RH 30																				Average	
		ACjun-15 (ka)		ACjun-16 (ka)		ACjun-18 (Bh)		ACjun-19 (Na)		ACjun-20 (Na)		ACjun-21 (Na)		ACjun-22 (Na)		ACjun-23[c] (Hi)		ACjun-24[d](Hi)		ACjun-25[e](Hi)			
		IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP
B. juncea	RH 30	17	[b]	17	[b]	7	11	9	13	10	17	13	[b]	6	11	–	–	–	–	–	–	11.3	13.0
	Shiva	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Zem-1	–	–	–	–	6	11	7	11	6	11	16	[b]	7	11	–	–	–	–	–	–	8.4	11.0
	Rajat	17	[b]	–	–	6	11	6	11	6	11	8	[b]	11	17	–	–	–	–	–	–	9.0	12.5
	RH 8113	–	–	17	[b]	9	13	7	11	8	11	8	[b]	11	17	–	–	–	–	–	–	10.0	13.0
	EC-129126–1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	EC-129126–2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	SSK-1	–	–	–	–	8	11	6	11	7	11	–	–	11	17	–	–	11	[b]	–	–	8.6	12.5
	RC-781	11	[b]	–	–	6	11	7	11	6	11	–	–	–	–	–	–	–	–	–	–	7.5	11.0
	RH 781	11	[b]	–	–	7	11	7	11	8	11	11	[b]	–	–	12	[b]	–	–	–	–	9.3	11.0
	Domo-4	11	[b]	–	–	7	11	7	11	8	11	8	[b]	7	11	–	–	11	[b]	–	–	8.4	11.0
B. napus	GSL 1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Wester	13	[b]	11	[b]	7	11	6	11	7	11	13	[b]	6	11	–	–	–	–	–	–	9.0	11.0
B. carinata	HC 2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B. oleracea var. *Botrytis*	H 1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B. oleracea var. *Caulorapa*	Pride of India	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B. oleracea var. *Caulorapa*	Purple Vienna	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	13	[b]	13	–

Table 8.11 (continued)

Host differentials		Incubation and latent period (days)of *A. candida* isolates[a]																					
Species/ ecotypes	Genotypes	RCC-4 Kranti RL 1359 BIO-092 RH 30																				Average	
		ACjun-15 (ka)		ACjun-16 (ka)		ACjun-18 (Bh)		ACjun-19 (Na)		ACjun-20 (Na)		ACjun-21 (Na)		ACjun-22 (Na)		ACjun-23[c] (Hi)		ACjun-24[d](Hi)		ACjun-25[e](Hi)			
		IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP
B. alba	Ochre	–	–	–	–	–	–	–	–	–	–	–	–	11	[b]	–	–	–	–	–	–	11.0	–
	Local	–	–	–	–	7	[b]	–	–	8	17	–	–	6	[b]	–	–	–	–	–	–	7.0	17.0
E. sativus	T 27	–	–	–	–	–	–	–	–	10	[b]	–	–	–	–	–	–	–	–	–	–	10.0	–
R. sativus	Scarlet Globe	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B. tournefortii	Local	17	[b]	13	[b]	6	11	6	11	7	11	17	[b]	8	17	–	–	–	–	–	–	10.6	12.5
Average		14.1	–	15.1	–	7.7	12.6	7.2	11.7	7.9	13.0	12.4	–	8.0	13.8	12.0	–	11.6	–	13.0	–		

IP denotes incubation period, *LP* denotes latent period, – denotes no incubation and no latent period, *ACjun* isolate derived from *B. juncea*, RCC-4, Kranti, RL 1359, BIO-902, RH 30

Location: *Ka* Kangra, *Bh* Bharatpur, *Na* Navgoan, and *Hi* Hisar

[a] Isolate derived from leaf

[b] Denotes hypersensitivity reaction

[c] Isolate derived from gynoecium

[d] Isolates derived from sepal

[e] Isolates derived from stamen

Table 8.12 Virulence spectrum of *A. candida* races. (Jat 1999; Gupta and Saharan 2002)

A. candida race	Virulence spectrum[a]
AC 14	4
AC 15	6
AC 17	3
AC 18	12
AC 21	10
AC 23	1
AC 24	2
AC 25	9
AC 26	7
AC 27	18
AC 29	21
AC 30	16

[a] Number of host differentials infected

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9 The Genetics of Host–Parasite Interaction

Studies on the genetics of host–parasite interactions in white rust (WR) diseases have focused on the level of specificity among races of pathogens and genotypes of related host species. Even within the confines of race-cultivar specificity, the studies have been one-sided in that no genetic information has been generated on *Albugo,* the causal organism. Interest in such studies was stimulated by Hougas et al. (1952), who studied the genetic control of resistance in WR of horseradish.

9.1 Inheritance of Resistance

The exhaustive work of Williams and Pound (1963) clearly demonstrated that resistance to WR in radish (*Raphanus sativus* var. *Caudatus*) cultivar, China Rose Winter (CRW) and Round Black Spanish (RBS) was controlled by a single dominant gene. Histological studies revealed that resistance in CRW is manifested as a hypersensitive reaction, which may be modified to a sporulating-tolerant reaction by environmentally controlled minor genes. Humaydan and Williams (1976) while studying the inheritance of resistance in *R. sativus* var. *Caudatus* to *Albugo candida* race 1, changed the gene designation "*R*" into the more descriptive symbol "*AC*-1," derived from the initials and race number of *A. candida*. The resistance to *A. candida* race 1 in radish is controlled by a single dominant gene, *AC*-1. The resistance gene *AC*-1 and the gene *Pi,* controlling pink pigmentation, was found to be linked with a recombination value of 3.28 %. Bonnet (1981) found that WR resistance in radish variety "Rubiso 2" is also controlled by one dominant gene. Among *Brassica* species, monogenic dominant resistance to *A. candida* race 2 has been found in *Brassica nigra, Brassica rapa, Brassica carinata,* and *Brassica juncea* (Ebrahimi et al. 1976; Delwiche and Williams 1976, 1977; Thukral and Singh 1986a, b). A single dominant gene, *AC*-2, controlling resistance to *A. candida* race 2 in *B. nigra* was identified by Delwiche and Williams (1981). In a study determining quantitatively inherited resistance to *A. candida* race 2 in *B. rapa* cv. CGS-1, Edwards and Williams (1982, 1987) found that variability in reaction among susceptible *B. rapa* strain PHW-Aaa-1 was due to quantitative genetic regulation, and suggested that rapid progress in resistance breeding could be made via mass selection when starting with a susceptible base population.

Canadian cultivars of *B. napus* are resistant to *A. candida,* but many cultivars of this species grown in China are susceptible (Fan et al. 1983). The inheritance of WR resistance in *B. napus* cv. Regent is conditioned by independent dominant genes at three loci, designated as *AC*-7-1, *AC*-7-2, and *AC*-7-3. Resistance is conferred by dominance at any one of these loci while plants with recessive alleles at all loci are susceptible. Results of a study by Verma and Bhowmik (1989) are in part agreement with those of Fan et al. (1983) and suggest that resistance of *B. napus* cv. BN-Sel to the *B. juncea* pathotype of *A. candida* in India is conditioned by dominant duplicate genes. In

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1980, Canadian researcher at the Agriculture Canada Research Station in Saskatoon registered the world's first highly resistant *B. rapa* variety Tobin (canola) against *A. candida* race 7 (Klassen, Verma, Downey, unpublished data). In their inheritance of resistance studies in crosses involving resistant (*B. rapa* accession from Mexico), and an adapted susceptible *B. rapa* cv. Torch (rapeseed), they showed that the reaction of the F_1 was like the resistant parent, indicating that resistance is dominant and controlled by nuclear genes. Backcrosses of F_1 plants to the resistant parent showed the same reactions as that of the resistant parent. Backcrosses of F_1 to the susceptible parent segregated in a 1:1 ratio of resistant and susceptible. The F_2 segregation of resistant and susceptible plants gave a good fit to 3:1 ratio. From the same Agriculture Canada Research Station, study of inheritance of resistance to *A. candida* race 2 in Indian mustard, Tiwari et al. (1988) found that resistance is dominant, monogenic, controlled by nuclear genes, and is easily transferred to adapted susceptible genotypes via backcrossing. In a study evaluating performance of 15 advanced generations (F_6), progenies of two interspecific crosses of *B. juncea* and *B. carinata* against WR, Singh et al. (1988) showed significant differences among the progenies and that all the hybrid progenies gave resistant reaction; five interspecific crosses between *B. juncea* and *B. carinata* revealed that the dominant gene which conferred resistance to WR is located in "C" genome of *Brassica oleracea,* a progenitor of *B. carinata*. Williams and Hill (1986) and Edwards and Williams (1987) have opened unusual potential for resolving many problems relating to host–parasite interactions and breeding for disease resistance through development of rapid-cycling *Brassica* populations. Their preliminary studies demonstrated considerable isozyme variations among individuals in populations which, when inoculated with several pathogens, showed a wide range of plant-to-plant variation in the levels of resistance and susceptibility. This will assist plant breeders in developing cultivars with genetic resistance to plant diseases. These authors and Hill et al. (1988) constructed gene pools of both major and minor genes for resistance to various crucifer pathogens. The occurrence of systemic protection and changes detected in phenylalanine ammonia lyase and total soluble peroxidase activities in inoculated cotyledons, particularly after the induced inoculation, suggested that host-mediated factors may also be involved in the interaction between the two isolates of *A. candida* that were compatible (CO) and incompatible (IN) on a *B. juncea* accession (Singh et al. 1999). Currently, markers are available for the selection of *B. juncea* plants carrying the resistance gene (*Ac*2*A*1) to *A. candida* race 2A (Prabhu et al. 1998).

Singh and Singh (1987) studied that when *A. candida* resistant Ethopian mustard (*B. carinata*) was crossed with *B. juncea,* the interspecific hybrids showed tolerance to *A. candida.* In a study on the inheritance of resistant to *A. candida* race 7 in *B. napus,* Liu et al. (1987) found that, in a digenic type, dominant resistance is conferred by R_1 and R_2 genes. Presence of a dominant allele at either of the two loci will confer resistance to a plant, whereas homozygous recessive at both loci will result in a susceptible phenotype expression. Liu and Rimmer (1992) suggested that resistance to an Ethiopian *A. candida* isolate from *B. carinata* was conditioned by a single dominant resistant gene.

Pal et al. (1991) evaluated the genetic component of variation for WR resistance through a 12×12 diallel crosses involving resistant and susceptible parents of both Indian- and exotic-origin *B. juncea* under four sets of environment, viz., normal sown in natural conditions, normal sown in artificially created epiphytotic conditions, late sown in natural conditions, and late sown in artificially epiphytotic conditions. Based on these results, they suggested that both additive and nonadditive components of variation is significant for WR resistance in all four sets of environments, but an overdominance under late-sown environment. Gadewadikar et al. (1993) in the study of inheritance of resistance to *A. candida* in crosses involving exotic and national promising varieties suggested that resistance was governed by a single dominant nuclear gene pair, and as such, resistance can easily be transferred via backcross breeding. Paladhi et al. (1993) while

conducting the study on inheritance of field reaction to WR in Indian mustard concluded that gene *PI*-15, a resistance source in *B. juncea,* can easily be transferred to susceptible type via backcrossing as resistance was controlled by a single gene.

Bains (1993) reported that resistance in the leaves differed from that of the young flowers; resistance in the leaves was due to the CC genome transferred from sarson, which can be utilized for breeding purposes. Rao and Raut (1994) observed that the susceptibility of *B. juncea* cv. Varuna to the local Delhi pathotype of *A. candida* was conditioned by two genes, with dominant and recessive gene interaction. Interspecific crosses between *B. juncea* and *B. napus* suggested that resistance in genotypes WW 1507 and ISN 114 to *A. candida* was controlled by a single dominant gene (Jat and Saharan 1999). In the study of three interspecific crosses between *B. juncea* and *B. napus,* Subudhi and Raut (1994) revealed digenic control with epistatic interaction and a close association of parental species and different grades of leaf waxiness for WR resistance. In a diallel fashion, Sachan et al. (1995) crossed two WR-resistant mustard cultivars, Domo and Cutlass, with two susceptible cultivars, Kranti and Varuna, and reported that F_1 hybrids, except susceptible × susceptible, were resistant. Segregation pattern for resistance to WR in F_2 and test crosses were under the control of a single dominant gene present in Domo and Cutlass while a recessive gene for susceptibility was observed to be present in Kranti and Varuna. Liu et al. (1996) in Canada developed monogenic lines for resistance to *A. candida* from a Canadian *B. napus* cv. Regent and suggested that these monogenic lines can be used to study the mechanism of resistance response conditioned by the individual genes. These lines also facilitate molecular mapping of the loci in *B. napus* for resistance to *A. candida* race 7. In an inter-varietal cross between susceptible Indian mustard cvs. Pusa Bold and resistant genotype DIRA 313, Mani et al. (1996) suggested that final intensity of rust on plant (FIP), final intensity of rust on leaf (FIL), and area under disease progress curves (AUDPC) showed significant additive × additive interaction (i) along with the association of complimentary epistatic interactions indicating close association between the nature of inheritance for AUDPC on one hand and FIP and FIL on the other. This was also substantiated by a significant correlation of FIP and FIL with AUDPC suggesting ease in selection for lower AUDPC (slow rusting) through FIP or FIL. Sridhar and Raut (1998) reported presence of a monogenic complete dominance in four crosses and absence of monogenic dominance in seven crosses between *B. juncea* and resistance sources from different *Brassica* species. According to Jat (1999), resistance was dominant in all the crosses except susceptible × susceptible where it was recessive. In intraspecific crosses, inheritance of resistance to *A. candida* was governed by one dominant gene, or two genes with either dominant–recessive epistatic interaction, or complete dominance at both gene pairs where either gene was dominant epistatic to other. Results of inoculation studies in F_2 population of crosses between R × R showed that resistant genes may be located on the same locus, or on different loci. In different intraspecific crosses of *B. juncea* and interspecific crosses of *B. juncea* × *B. carinata, A. candida* resistance was dominant in all the crosses; resistance was governed by one dominant gene, or two genes with either as dominant, recessive, or epistatic interaction, or complete dominance at both gene pairs (Saharan and Krishnia 2001). Partial resistance in *B. napus* was controlled by a single recessive gene designated as *wpr* with a variable expression (Bansal et al. 2005). Dominant alleles at three unlinked loci (*AC* 7h, *AC* 7z, and *AC* 7_3) conferred resistance in *B. napus* cv. Regent to *A. candida* race AC 7 (Fan et al. 1983; Liu et al. 1996). Two loci also controlled resistance in *B. napus* to *A. candida* race AC 2 collected from *B. juncea* (Verma and Bhowmik 1989). The Chinese *B. napus* accession 2282-9, which is susceptible to AC 7, has only one locus controlling resistance to *A. candida* isolate from *B. carinata* (Liu and Rimmer 1992). These studies indicated that only one allele for resistance was sufficient to condition an incompatible reaction in this pathosystem (Ferreira et al. 1995). In addition, a single locus controlling resistance to AC 2 in *B. napus* and *B. rapa* was mapped using restriction fragment length polymorphism (RFLP)

Table 9.1 Inheritance of resistance in crucifers to *A. candida*

Crucifers genotypes	Resistance gene	Reference (s)
R. sativus cv. China	Single dominant	Pound and Williams 1963
Rose winter, Round Black Spanish		
R. sativus cv. Caudatus	Single dominant AC-1	Humaydan and Williams 1976
R. sativus cv. Rubiso 2	Single dominant *AC*-1	Bonnet 1981
B. nigra	Single dominant *AC*-2	Delwiche and Williams 1981
B. rapa	Single dominant *AC*-2	Delwiche and Williams 1974
B. juncea	Single dominant *AC*-2	Ebrahimi et al. 1976
B. carinata	Single dominant *AC*-2	Thukral and Singh 1996
B. napus cv. Regent	Dominant gene *AC*-7-1, *AC*-7-2, *AC*-7-3 to race *AC* 7	Fan et al. 1983; Liu et al. 1996
B. napus cv. BN-Sel.	Dominant duplicate gene to race AC 2	Verma and Bhowmik 1989
B. napus	Partial resistant single recessive gene wpr	Bansal et al. 2005
B. napus	Single dominant gene	Liu and Rimmer 1992
B. juncea cv. EC 12749	Additive dominant gene with epistatic effects	Thukral and Singh 1986
B. juncea cv. Domo and Cutlass	Single dominant gene	Sachan et al. 1995
B. juncea cv. EC 333590	One/two dominant genes with epistatic effects	Jat 1999
B. juncea × *B. carinata*	Monogenic dominant	Tiwari et al. 1988
B. juncea × *B. carinata*	Half dominant gene dominant, recessive, epistatic, or complete dominant at both gene pairs	Saharan and Krishnia 2001
B. juncea × *B. napus cv.* ISN 733, WW 1507	Single dominant gene	Jat and Saharan 1999
B. juncea × *B. napus*	Digenic with epistatic interaction	Subudhi and Raut 1994
A. thaliana	WRR 4 gene confers broad-spectrum resistance to races AC 2, 4, 7, and 9	Borhan et al. 2008

marker (Ferreira et al. 1995). A dominant allele at a single locus or two tightly linked loci confer resistance to both *A. candida* races AC 2 and AC 7 (Kole et al. 2002). According to Borhan et al. (2008), a dominant WR-resistant gene, *WRR* 4 encodes a TIR-NB-LRR protein that confers broad-spectrum resistance in *Arabidopsis thaliana* to *A. candida* races AC 2, AC 4, AC 7, and AC 9 (Tables 9.1 and 9.2).

Genetic variation and the possibilities of increasing the level of WR resistance was evaluated in seven families of interspecific cross between *B. juncea* cv. Varuna × *B. carinata* cv PCC-2 (Krishnia et al. 2000). Results revealed that within family, variation in cross Varuna × PCC-2 on leaves were significant. The minimum disease severity index (DSI) was recorded in progenies of R × R (0.96) which was statistically at par with progenies of R-self (1.18) and significantly different from R-open (1.34). The maximum DSI was observed in progenies of S-open (3.51) which was statistically at par with progenies of S × S and self-families. The 18.64 % reduction in disease score was observed in progenies of R × R over R-self. The minimum limit of disease score range was observed in R × R family, whereas, it was maximum in S-self. The highest genotypic coefficient of variance (GCV) (0.210), heritability broad sense (h^2) (75.31), and genetic gain percent as mean (GG) (55.21) were recorded in progenies of R × S, R × R, and R-self, respectively; these indices were nonsignificant in the progenies of S × S. The progenies of all seven families, except R × R and S × S, showed significant variation for WR-SP; between families, variations were significant. The lowest and highest DSI were recorded in progenies of S-self and R × R, respectively. The GCV (0.012) and GG (12.63) were maximum in R-open and minimum in progenies of R × R and S × S families (Table 9.3).

Table 9.2 Mode of segregation for *A. candida* reaction to *B. juncea* × *B. juncea* and *B. juncea* × *B. carinata* F_2 progenies, BC_1 and BC_2. (Saharan and Krishnia 2001)

Crosses	Host reaction		Total no. of plants	Expected ratio (R:S)	χ^2	*P* value
	Resistant (R)	Susceptible (S)				
F_2						
PCR 3 × Shiva	195	75	270	3:1	1.1	0.2–0.3
Pusa Basant × Shiva	172	66	238	3:1	0.9	0.3–0.5
Rajat × Shiva	204	58	262	3:1	1.1	0.2–0.3
				13:3	2.0	0.1–0.2
Pusa Bahart × Domo	156	42	198	3:1	1.5	0.2–0.3
				13:3	0.8	0.3–0.5
Pusa Basant × EC 322092	174	64	240	3:1	0.4	0.5–0.7
Kranti × EC 322092	142	38	180	3:1	1.5	0.2–0.3
Varuna × EC 322092	181	53	234	3:1	0.7	0.3–0.5
				13:3	2.3	0.1–0.2
RH 30 × EC 322093	246	12	258	15:1	1.1	0.2–0.3
RH 30 × HC-1	186	48	234	3:1	2.5	0.1–0.2
				13:3	0.5	0.3–0.5
Varuna × PCC-2	154	58	212	3:1	0.6	0.3–0.5
BC_1 (F_1 × S)						
F_1 (PCR 3 × Shiva) × PCR 3	43	55	98	1:1	1.5	0.2–0.3
F_1 (Pusa Basant × Shiva) × Pusa Basant	45	36	81	1:1	1.0	0.3–0.5
F_1 (Rajat × Shiva) × Rajat	52	44	96	1:1	0.7	0.3–0.5
F_1 (Pusa Bahar × Domo) × Pusa Bahar	38	32	70	1:1	0.5	0.3–0.5
F_1 (Pusa Basant × EC 322092) × Pusa Basant	46	35	81	1:1	0.5	0.2–0.3
F_1 (Kranti × EC 322092) × Varuna	28	36	64	1:1	1.0	0.3–0.5
F_1 (RH 30 × EC 322093) × RH 30	55	15	70	3:1	0.5	0.3–0.5
F_1 (RH 30 × HC-1) × RH 30	34	43	77	1:1	1.1	0.3–0.5
F_1 (Varuna × PCC-2) × Varuna	32	36	68	1:1	0.2	0.5–0.7

BC2 (F1 × R) in all the ten crosses no segregation
R resistant, *S* susceptible

9.2 Slow White Rusting

Rate of infection or disease spread is influenced by both incubation and latent periods of *A. candida* in its compatible host. In WR, the sporangia become visible after the host epidermis is ruptured as a white powdery mass which can readily be dispersed by wind or watersplash to cause secondary infection. According to Liu et al. (1989), WR pustules become visible 5–6 days after inoculation. However, Coffey (1975) observed symptoms after 8 days on the undersurface of the cabbage leaves. Longer incubation and latent periods have been observed in slow-rusting *B. juncea* cvs. Rajat (11/14 days) and cv. RC 781 (11/15 days). Similarly, *B. rapa* cvs. Candle (11/15 days), Tobin (15/18 days), and Span (11/18 days) also have longer incubation and latent periods (Lakra and Saharan 1988b; Jat 1999; Gupta and Saharan 2002). There is a need to identify genotypes with slow-rusting attributes to curb the epidemic development of WR in field.

Table 9.3 Disease score and selection parameters for WR infection in the progenies of seven families of interspecific cross between *B. juncea* cv. Varuna × *B. carinata* cv. PCC-2. (Krishnia et al. 2000)

Families	Disease score		CD	GCV	Selection parameter		
	Mean	Range			PCV	h^2	GG
White rust (leaf phase)							
R-open	1.3	0.4–2.2	0.5	0.121	0.205	59.0	41.1
R × R	1.0	0.5–1.5	0.3	0.061	0.081	75.3	46.1
R-self	1.2	0.3–1.8	0.4	0.138	0.191	72.3	55.2
R × S	2.2	1.4–3.0	0.5	0.210	0.283	74.2	37.7
S-self	3.5	2.9–4.1	0.3	0.108	0.155	69.7	16.3
S × S	3.3	3.1–3.5	NS	–	–	–	–
S-open	3.5	2.8–4.1	0.3	0.084	0.114	73.7	14.6
Mean	2.3						
CD	0.3						
White rust (staghead phase)							
R-open	0.5 (1.2)	0.1–0.9 (1.1–1.4)	0.2	0.012	0.025	48.0	12.6
R × R	0.7 (1.3)	0.6–0.8 (1.3–1.4)	NS	–	–	–	–
R-self	0.5 (1.2)	0.2–0.7 (1.1–1.3)	0.2	0.010	0.022	45.5	11.4
R × S	0.5 (1.2)	0.3–0.7 (1.2–1.3)	0.2	0.009	0.018	50.0	11.3
S-self	0.4 (1.2)	0.2–0.7 (1.1–1.3)	0.2	0.006	0.015	40.0	8.6
S × S	0.5 (1.2)	0.3–0.7 (1.13–1.30)	NS	–	–	–	–
S-open	0.5 (1.2)	0.3–0.8 (1.1–1.3)	0.2	0.007	0.016	43.8	9.4
Mean	0.5 (1.2)						
CD	0.1						

R resistant, *S* susceptible, *GCV* genotypic coefficient of variance, *PCV* phenotypic coefficient of variance, h^2 heritability (broad sense), *GG* genetic gain (percent of mean), figures in parenthesis are transformed values, *NS* not significant, therefore no need to calculate selection parameters

Slow-rusting resistance in crucifer genotypes can be assessed through low infection frequency, low spore production, and a long latent and a short infection period of *A. candida* (Saharan 2010).

9.3 Inheritance of Partial Resistance

The partial resistance in *B. juncea* lines against race 2 is controlled by a single dominant gene that has variable expressions (Bansal et al. 1999). It is similar to that reported in *B. napus, B. rapa,* and *R. sativus* (Williams and Pound 1963; Edwards and Williams 1987; Kole et al. 1996). Traditional and doubled haploid (DH) genotypes of oilseed *Brassica* spp. resistant, partially resistant, moderately susceptible, and susceptible to *A. candida* were compared for both phenotypic development and histology of host–pathogen interaction. The partially resistant genotype showed pinhead-size pustules, mainly on the upper surface of cotyledonary leaves. Relatively, less mycelium was observed in the partially resistant than in the susceptible genotypes. In resistant *B. napus* genotypes, there was neither pustule development nor any mycelial growth. In the moderately susceptible genotype although the pustules were similar to those in the partially resistant genotype except

Table 9.4 Segregation for resistance and susceptibility to *A. candida* race 7v in parents, F_1, F_2, and DH populations in *B. napus*. (Bansal et al. 2005)

Pedigree	Generation and putative genotype	Observed reaction				Expected R:PR ratio	χ^2 calculated	*P*-value
		R	PR	S	Total			
Parent A00-63N (PR)	P_1 *wpr wpr*	0	68	0	68	–	–	–
Parent A00-66N(PR)	P_2 *wpr wpr*	0	63	0	63	–	–	–
Parent A00-65N(R)	P_3 *Wpr wpr*	60	0	0	60	–	–	–
PR (P_1) × R(P_3)	F_1 *Wpr wpr*	61	0	0	61	–	–	–
R (P_3) × PR(P_1)	F_1(rec.) *Wpr wpr*	155	0	0	155	–	–	–
PR (P_2) × R(P_3)	F_1 *Wpr wpr*	58	0	0	58	–	–	–
R (P_3) × PR(P_2)	F_1(rec.) *Wpr wpr*	145	0	0	145	–	–	–
From F_1 of P_1 and P_3	F_2	108	36	0	144	3:1	0.00	1.00
From F_1 (rec.)	F_2	105	39	0	144	3:1	0.33	0.56
From F_1 of P_2 and P_3	F_2	109	35	0	144	3:1	0.04	0.85
From F_1 (rec.)	F_2	106	38	0	144	3:1	0.15	0.70
From F_1 of P_1 and P_3	DH	29	27	0	56	1:1	0.07	0.79
From F_1 (rec.)	DH	25	46	0	71	1:1	6.21	0.01

Rec. reciprocal, *R* resistant, *PR* partial resistant, *S* susceptible, *wpr* recessive partial resistant, *Wpr* dominant resistant, *DH* doubled haploid

that the pinhead-size pustules were occasionally coalescing in the former. In the moderately susceptible genotype, ample mycelial growth in the mesophyll tissue was similar to that in the susceptible control *B. rapa* cv. Torch. In the resistant *B. juncea* cv. Commercial Brown, no pustules were formed although some mycelial growth was observed beneath the epidermal cell layer and in the mesophyll cell layer of the cotyledonary leaf tissue. For inheritance studies, two partially resistant *B. napus* genotypes were crossed with a resistant *B. napus* genotype and plants of F_1, F_1 (reciprocal), F_2, and DHs were inoculated with a zoospore suspension of *A. candida* race 7v (Bansal et al. 2005). Results showed that the partially resistant phenotype appeared to be controlled by a single recessive gene designated as *wpr* with variable expression (Table 9.4). The simple inheritance of partial resistance against WR can very easily be incorporated into elite breeding lines through conventional- and DH-breeding methods (Bansal et al. 2005).

9.4 Inheritance of Virulence

The inheritance of virulence in *Albugo–Brassica* system and inheritance of avirulence and polymorphic-molecular markers in *A. candida* were studied in crosses of isolates MiAc2-B1 or MiAc2-B5 (metalaxyl insensitive and virulent to *B. juncea* cv. Burgonde) of race 2 (AC 2) and isolate MsAc7-A1 (metalaxyl sensitive and virulent to *B. rapa* cv. Torch of race 7 (AC 7) (Adhikari et al. 2003); hybrids were obtained via co-inoculation onto a common susceptible host and putative F_1 progenies were selfed to produce F_2 progeny. The parents and F_1 progeny were examined for virulence on *B. juncea* cv. Burgonde and *B. rapa* cv. Torch. Segregation of avirulence or virulence of F_2 populations was analyzed on cv. Torch. Putative F_1 hybrids were confirmed by random amplified polymorphic DNA (RAPD) markers specific for each parent. A 3:1 ratio of avirulence to virulence in each of the three populations of F_2 progeny in *B. rapa* cv. Torch supported the hypothesis of a single dominant avirulence gene. Amplified fragment length polymorphism (AFLP) markers also segregated in regular Mendelian fashion among F_2 progenies derived from two F_1 hybrids (Cr2-5 and Cr2-7) of Cross-2. This first putative avirulence gene in *A. candida* was designated as *AvrAc*1. These results suggest that a single dominant gene controls avirulence in race AC 2 in *B. rapa* cv. Torch and provides further evidence for the gene-for-gene relationship in the *Albugo–Brassica* pathosystem (Tables 9.5, 9.6 and 9.7).

Table 9.5 Identification of hybrids and selfed progeny from F_1 crosses between isolates of *A. candida* by virulence and metalaxyl insensitivity testing. (Adhikari et al. 2003)

A. candida parental and F_1 isolates	Differential cultivars[a]				Sensitivity[b]	Race or hybrid
	Brassica juncea		*B. rapa*			
	CrGC4-1	Burgonde	CrGC1-18	Torch		
Parents						
MiAc2-B1	V	V	V	A	I	AC 2
MiAc2-B5	V	V	V	A	I	AC 2
MsAc7-B1	V	A	V	V	S	AC 7
Cross-1 (MiAc2-B1 × MsAc7-A1)						
Cr1-1	V	V	V	A	I	AC 2
Cr1-4	V	V	V	A	I	AC 2
Cr1-5	V	V	V	A	I	AC 2
Cr1-6	V	V	V	A	I	AC 2
Cr1-7	V	V	V	A	I	AC 2
Cr1-9	V	A	V	A	I	Hybrid
Cr1-10	V	V	V	A	I	AC 2
Cr1-11	V	A	V	A	I	Hybrid
Cr1-12	V	A	V	A	I	Hybrid
Cr1-13	V	V	V	A	I	AC 2
Cr1-14	V	A	V	A	I	Hybrid
Cr1-15	V	V	V	A	I	AC 2
Cross-2 (MiAc2-B5 × MsAc7-A1)						
Cr2-1	V	V	V	A	I	AC 2
Cr2-2	V	V	V	A	I	AC 2
Cr2-3	V	V	V	A	I	AC 2
Cr2-5	V	A	V	A	I	Hybrid
Cr2-6	V	V	V	A	I	AC 2
Cr2-7	V	A	V	A	I	Hybrid
Cr2-8	V	A	V	A	I	Hybrid
Cr2-9	V	V	V	A	I	AC 2
Cr2-11	V	V	V	A	I	AC 2
Cr2-12	V	A	V	A	I	Hybrid
Cr2-16	V	V	V	A	I	AC 2
Cr2-17	V	V	V	A	I	AC 2

A avirulence if interaction phenotype (IP) = 0–3, *V* virulent if IP = 4, values represent the mean of ratings on 24–30 seedlings, *I* insentivity to metalaxyl, *S* sensitive

[a] CrGC4-1 and CrGC1-18 are common susceptible hosts to races AC 2 and AC 7

[b] Sensitivity to metalaxyl

9.5 Induction of Systemic Resistance

Induction of systemic resistance in *B. juncea* to WR by pre- or co-inoculation with an incompatible isolate of *A. candida* has been observed by Singh et al. (1999). In an investigation of the interaction between two isolates of *A. candida* that were CO and IN on a *B. juncea* accession, the IN isolate induced both local and systemic protection of cotyledons and true leaves against the CO isolate. The extent of the protection was proportional to the zoosporangial concentration used in inducing infection by IN isolate. Protection was greatest locally on cotyledons, and least on true leaves (the most remote tissue from the point of inoculation). Protection occurred only when

Table 9.6 Segregation of avirulance among F_2 population of *A. candida* on *B. rapa* cv. Torch. (Adhikari et al. 2003)

A. candida crosses and F_2 populations	*B. rapa* cv. Torch			
	F_2 ratio (A:V)	Expected ratio	χ^2	Probability
Cross-1 (MiAc2-B1 × MsAc7-A1)				
Crl-9	44:9	3:1	1.87	0.1–0.5
Crl-11	40:11	3:1	0.32	0.5–0.9
Crl-14	54:12	3:1	1.63	0.1–0.5
Cross-2 (MiAc2-B5 × MsAc7-A1)				
Cr2-5	40:8	3:1	1.77	0.1–0.5
Cr2-7	46:10	3:1	1.52	0.1–0.5
Cr2-8	44:9	3:1	1.81	0.1-0.5

A avirulent, *V* virulent

Table 9.7 Segregation of AFLP markers in the F_2 population of *A. candida.* (Adhikari et al. 2003)

	Segregation of F_2 population[b]								
	Cross-2–5				Cross-2–7				
AFLP markers[a]	+	−	Ratio +:−	χ^2	+	−	Ratio +:−	χ^2	Probability
E-AA/M-CT2	29	19	3:1	5.44[c]	44	12	3:1	0.38	NS
E-AA/M-CT4	37	11	3:1	0.11	40	16	3:1	0.38	NS
E-AA/M-CT5	39	9	3:1	1.00	48	18	3:1	3.43	NS
E-AA/M-CT6	36	12	3:1	0.00	42	14	3:1	0.00	NS

NS not significant
[a] Each marker band was scored against 104 progeny pooled from two F_1s of Cross-2 (48 progeny from Cross-2–5 and 56 progeny from Cross-2–7)
[b] Band absent, + band present, Goodness of fit (χ^2) of the ratios of the different markers in F_2 progeny expected for single dominant markers (3:1)
[c] (χ^2) values in excess of 95% significance levels (3.84 for two classes)

the two isolates were inoculated together, and it was maximum when the interval between the IN and CO isolate inoculations was longer. The IN isolate induced only slight protection when it was inoculated after the CO isolate. No induced susceptibility to the IN isolate occurred with any treatment. There was some evidence of competition between CO and IN zoospores for infection sites (stomata). The occurrence of systemic protection, changes in phenylalanine ammonia lyase, and total soluble peroxidase activities in inoculated cotyledons, particularly after the inoculation of IN isolate, suggested that host-mediated factors may also be involved in the interaction between the two isolates (Figs 9.1, 9.2 and 9.3).

However, altered phenotypic response to *Hyaloperonospora parasitica* in *B. juncea* seedlings following prior inoculation with an avirulent or virulent isolate of *A. candida* was observed by Singh et al. (2002a). Prior inoculation with the IN isolate of *A. candida* induced resistance to subsequently inoculated *H. parasitica.* The degree of resistance was proportional to the zoosporangial concentration of the IN isolate, and induced resistance was more marked in the cotyledons receiving the inducing inoculum compared to the opposite cotyledons, and subsequently emerging true leaves which had not been pre-inoculated. Induction of resistance was also observed if the IN isolate of *A. candida* and *H. parasitica* were co-inoculated simultaneously. The effect, however, was greater if the interval period between inoculations was up to 4 days. When the IN isolate of *A. candida* was inoculated 4 h after *H. parasitica,* there was no marked effect on resistance to the latter. In contrast, prior inoculation with the CO *A. candida* isolate increased susceptibility

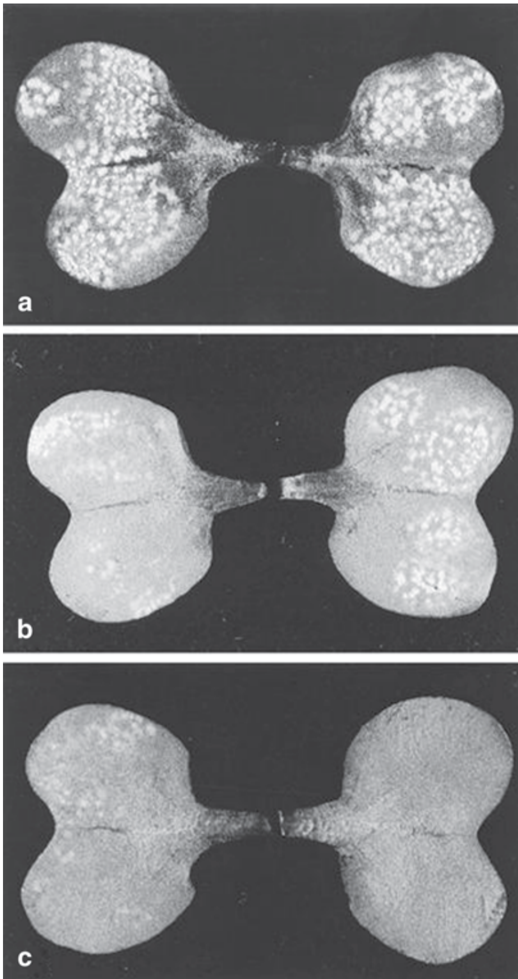

Fig. 9.1 Symptom development on cotyledons of *B. juncea* accession PPBJ-1 at 7 days after inoculation with IN and CO isolates of *A. candida*. **a** CO isolate inoculated on both cotyledons. **b** CO and IN isolates inoculated together on left cotyledon; CO isolate applied alone to right cotyledon. **c** CO and IN isolates inoculated together on left cotyledon; IN isolate applied alone to right cotyledon. (Singh et al. 1999)

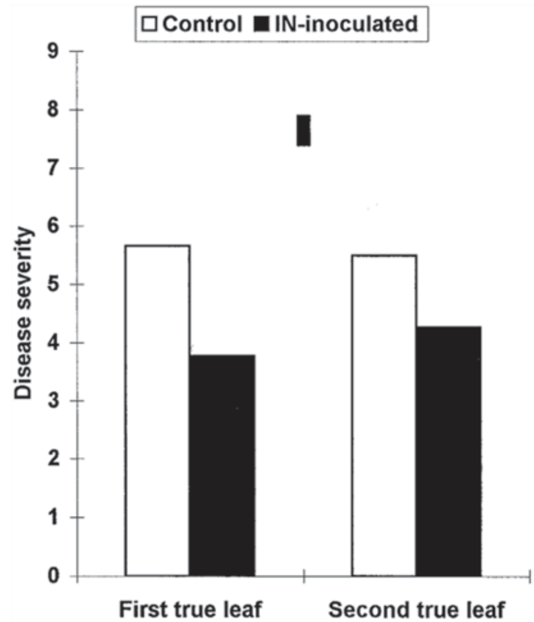

Fig. 9.2 White rust severity on first and second true leaves of *B. juncea* when the compatible *A. candida* isolate was applied 5 days after both cotyledons had been sprayed either with sterile distilled water (control) or inoculated with the IN isolate. *Bar* represents LSD (Least Significant Difference) ($P=0.05$, $df=8$). (Singh et al. 1999)

to *H. parasitica.* Pre- or co-inoculation with *H. parasitica* suppressed the development of the CO *A. candida* isolate. A spectrum of responses was observed when one cotyledon was inoculated simultaneously with both the IN and CO isolates of *A. candida,* and followed subsequently with *H. parasitica* after different time intervals. This range of altered responses appeared to reflect the outcome of the differing kinetics and countereffects of resistance and susceptibility induction.

9.6 Plant Defense Resistant Genes

Plant's defense against colonization by a biotrophic pathogens was thought to be triggered by either direct or indirect interaction between the pathogen and a corresponding plant resistance protein. Several resistance genes (*R* genes) against bacteria, fungi, viruses, and nematodes have been cloned from *A. thaliana* and various crop species (Ellis et al. 1999, 2000; Holub 2001; Hulbert et al. 2001; Takken and Joosten 2000). However, timing of the expression of defense related genes plays a crucial role during pathogenesis and IN interactions, and that the redox balance within the chloroplast may be of crucial importance for mounting a successful defense response. Synergistic and conserved strategies are

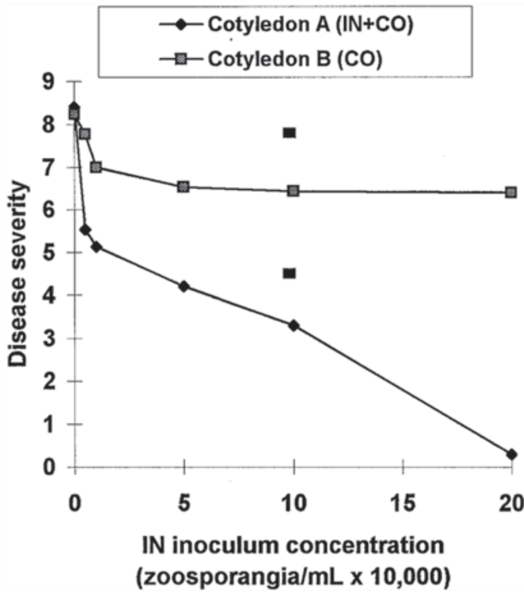

Fig. 9.3 Effect of IN isolate (*A. candida*) inoculum concentration on disease severity on *B. juncea* after inoculation with the CO isolate. On each seedling, cotyledon A was inoculated with both the IN and CO isolates; at the same time, cotyledon B was inoculated with the CO isolate alone. The *top bar* represents LSD (Least Significant Difference) ($P=0.05$, $df=24$) for comparisons at different inoculum concentrations. The *bar* between the *lines* represents LSD (Least Significant Difference) ($P=0.05$, $df=12$) for comparisons of disease reaction on the two cotyledons at a particular IN inoculum concentration. (Singh et al. 1999)

utilized by the resistant host to fight-off the *A. candida* attack (Kaur et al. 2010). A single gene (*Acr*) responsible for conferring resistance to this pathogen was mapped on a densely populated *B. juncea* RFLP map. Two closely linked RFLP markers identified (X42 and X83) were 2.3 and 4 cM from the *Acr* locus, respectively (Cheung et al. 1998).These markers may be useful for marker-assisted selection and map-based cloning of these genes.

WR in natural populations of *A. thaliana* is caused by a distinct subspecies of *A. candida* subsp. *Arabidopsis* (Borhan et al. 2008), which offers an attractive model for investigating the molecular basis of broad-spectrum defense suppression. From previous molecular genetic analyses of downy mildew (DM) resistance, there are numerous examples of receptor-like genes in *A. thaliana* that vary in different modes of defense regulation (Eulgem et al. 2004; Holub 2001; Holub et al. 1995; McDowell et al. 1998, 2000; Tor et al. 2002).The majority of plant *R* genes encode nucleotide-binding site leucine-rich repeat (NB-LRR)-type protein which can be further grouped into two subclasses based on their N-terminal sequence: those containing a coiled-coil (CC) domain (CC-NB-LRR), or those containing a domain with similarity to *Drosophila* toll and mammalian interleukin-1 receptor (TIR) (TIR-NB-LRR) (Hammond-Kosack and Jones 1997; Jones and Jones 1997; Young 2000). Leucine-rich repeats (LRR) are involved in protein–protein interactions and occur in a number of proteins with different function (Kobe and Deisenhofer 1994, 1995). Domain exchange between LRR of closely related *R* genes supports their role in pathogen recognition (Ellis et al. 1999; Wulff et al. 2001). Variation among *R* genes occurs mainly in their LRR domain, typically in the solvent exposed β-strand/β-turn structure within the LRR domain. Comparison of this motif among *R* gene homologs suggests that the β-strand/β-turn structure has been under diversifying selection (Bittner-Eddy et al. 2000; Botella et al. 1998; McDowell et al. 1998; Meyers et al. 1998, 2003; Parniske et al. 1997). Evidence for this is based on the ratio of non-synonymous (*K*a) to synonymous (*K*s) nucleotide substitution at the β-strand/β-turn motif. A Ka/Ks ratio >1 indicates that diversification has occurred under positive selection pressure from the evolving pathogen. This suggests that the β-strand/β-turn motif may be involved in ligand binding as has been shown for a polygalacturonase-inhibiting protein with its polygalacturonase ligand (using site-directed mutagenesis of the β-strand/β-turn motif) (Leckie et al. 1999). A chimeric gene constructed from flax rust resistance genes *P* and *P*2 showed that amino acid changes in the β-strand/β-turn motif are sufficient to alter *P*2 to *P* specificity (Dodds et al. 2001).

The TIR domains of plant R proteins are thought to have a similar function to the homolog domains from *Drosophila* toll and human interleukin-1 receptor and act as a signaling domain (Hammond-Kosack and Jones 1997). However, analysis of recombinant alleles of *L* genes from

flax indicates that the TIR domain also may play a role in pathogen specificity (Ellis et al. 1999; Luck et al. 2000). R proteins may interact indirectly with pathogen effect or proteins, which target regulators of plant innate immunity. This idea, developed as the "guard hypothesis" (Dangl and Jones 2001), reasonably explains the interactions shown to be required for resistance mediated by RPM1 and RPS2 in *A. thaliana* (Axtell and Staskawicz 2003; Mackey et al. 2002, 2003), where RIN4 may be targeted by AvrRpm1, AvrB, or AvrRpt2. The R proteins monitor the state of RIN4 and induce the resistance response when the bacterial Avr proteins interact with RIN4. However, evidence for the guard hypothesis with respect to the TIR-NB-LRR class of *R* genes has not yet been obtained. Recognition of a pathogen by a plant initiates a rapid response localized to the infection site and manifested by changes in ion flux and production of reactive oxygen species that lead to induction of downstream signals and defense genes (Kombrink and Schmelzer 2001; Morel and Dangl 1997). Initiation of local defense also results in signals that induce systemic acquired resistance (SAR) in non-infected distal parts of the plant, resulting in broad-spectrum resistance (Dong 2001; Shah and Klessig 1999). The role of salicylic acid (SA) in plant defense and induction of SAR has been shown by treatment of plants with SA or its synthetic analogs such as 2, 6-dichloroisonicotinic acid (INA) and benzothiadiazole (Klessig et al. 1994). Furthermore, transgenic plants expressing the bacterial SA-degrading enzyme, NahG, are unable to induce SAR (Delaney et al. 1995).

Several mutants in *A. thaliana* have been identified that affect disease resistance responses associated with defense regulatory genes such as: *AtSGT*1*b* (homolog of the yeast gene *SGT*1) (Austin et al. 2002; Tör et al. 2002), enhanced disease susceptibility (*EDS*1) (Parker et al. 1996), non-race-specific disease resistance (*NDR*1) (Century et al. 1997), phytoalexin deficient (*PAD*4) (Glazebrook et al. 1997), and homolog of a barley gene required for Mla powdery mildew resistance (*RAR*1) (Muskett et al. 2002; Tornero et al. 2002). Resistance specified by the *RPS*4 gene to the bacterial pathogen *Pseudomonas syringae* expressing *AvrRps*4 and the oomycete *Peronospora parasitica* specified by *RPP*1, *RPP*2, *RPP*4, and *RPP*5, which all encode TIR-NB-LRR proteins, is abolished by *EDS*1 (Aarts et al. 1998; Parker et al. 1996; Rusterucci et al. 2001). In contrast, many CC-NB-LRR resistance genes are independent of *EDS*1 but dependent on *NDR*1 (Aarts et al. 1998; Century et al. 1997). Both *EDS*1 and *PAD*4 encode lipase-like proteins (Falk et al. 1999; Jirage et al. 1999) and function within the same defense pathways that regulate SA accumulation (Feys et al. 2001; Zhou et al. 1998). WR disease occurs on *A. thaliana* (Holub et al. 1995) and three resistance genes to *A. candida* (*RAC*) isolate Acem1 were identified (Borhan et al. 2001). Cloning was reported of the first WR resistance gene to isolate Acem1 of *A. candida* (*RAC*1) from *Ksk*-1 accession of *A. thaliana*. They also describe the effect on *RAC*-mediated resistance of standard mutations that were previously used to characterize defense signaling in DM resistance.

Resistance to *A. candida* isolate Acem1 is conferred by a single dominant gene, *RAC*1, in accession *Ksk*-1 of *A. thaliana*. This gene was isolated by positional cloning and is a member of the *Drosophila* toll and mammalian interleukin-1 receptor (TIR) nucleotide-binding site leucine-rich repeat (NB-LRR) class of plant resistance genes. Strong identity of the TIR and NB domains was observed between the predicted proteins encoded by the *Ksk*-1 allele and the allele from an Acem1-susceptible accession Columbia (Col) (99 and 98 %, respectively). However, major differences between the two predicted proteins occur within the LRR domain and mainly are confined to the β-strand/β-turn structure of the LRR. Both proteins contain 14 imperfect repeats. *RAC*1-mediated resistance was analyzed further using mutations in defense regulation, including: *PAD*4-1, *EDS*1-1, and *NahG* in the presence of the *RAC*1 allele from *Ksk*-1. WR resistance was completely abolished by *EDS*1-1 but was not affected by either *PAD*4-1 or *NahG* (Borhan et al. 2004).

Reaction of a genotype to a pathogen depends upon the genetics of both the host plant as well as the pathogen. Hence, based on the genetics of

host–pathogen relationship, the response of the host–pathogen instruction broadly can be categorized into two classes, i.e., race specific and nonspecific. In race specific, the interaction or compatibility between host genotype and pathogen determines the disease reaction to develop or not. Race nonspecific resistance, however, is quantitative in nature and is influenced by environmental conditions to a great extent in different geographical locations over the years. Hence, reaction of genotypes ranges from susceptible to resistance grading(s) under natural conditions termed as horizontal resistance and a wide range of pathogens come under this category. Understanding whether resistance to a given pathogen is race specific or nonspecific is, therefore, a prerequisite to selection of breeding strategies, and is detected by the presence or absence of crossover interaction between host genotypes and pathogen strains. For this purpose, genotype's main effects and genotype–environment interaction (GGE) biplot is the best method for visualizing important crossover interactions. Thus, understanding of *Brassica* genotypes by *A. candida* interactions is of vital importance in identifying resistant genotypes for specific adaptability. GSL-1, EC 414299, and EC 399299 showed additive gene for horizontal resistance to WR which can prove good donors in further genetic improvement programs. *B. juncea* cvs. Varuna, JMM 07-2, JMM 027-1, and JYM 10 had nonadditive gene action for pathogenicity to WR. PBC 9221, GSL 1, EC 414299, and EC 399299 were very similar in genetic makeup for disease resistance while Varuna showed maximum divergence in genetic constitution from these strains (AICRPRM 2009). The *B. napus* chromosome segment, carrying the WR-resistance gene (*Ac*2*V*1), appeared to have recombined with the *B. juncea* DNA since recombinant individuals were identified (Somers et al. 2002).

***Albugo*-specific primers:** Several workers have used markers for selective amplification of *A. candida.* Primer DC6:50-GAG-GGA-CTT-TTGGGT-AATCA-30 (Cooke et al. 2000), and LR-0: 50-GCT-TAA-GTT-CAGCG G-GT-30 reverse complementary to LR-0R (Moncalvo et al. 1995), can be used for further studies.

9.7 Mapping of Resistant Genes

A single gene (*Acr*) conferring resistance to *A. candida* was mapped on *B. juncea* RFLP map. A co-segregating RFLP marker (X140a) and two other closely linked RFLP markers (X42 and X83) were identified (Cheung et al. 1998). A locus that accounted for 18.4 % of the variation in resistance to WR was mapped to linkage group (LG) 2 near the RAPD marker Z19a. During the study, a bacterial resistance gene homologous to *Arabidopsis RPS*2 and six different *RGA*s were sequenced (Tanhuanppa 2004). A more tightly linked marker for the WR resistance gene, using AFLP in conjunction with bulk segregant analysis, and a polymerase chain reaction (PCR)-based cleaved amplified polymorphic sequence (CAPS) marker for the closely linked RAPD marker, $OPB06_{1000}$ was developed (Varshney et al. 2004). The partially resistant phenotype appeared to be controlled by a single dominant gene that has variable expression, on 7- and 13-day-old young plants and 34- and 45-day-old adult plants, did not develop hypertrophic growth or stagheads under greenhouse and field conditions (Bansal et al. 1999).

Two accessions of *A. thaliana* (*Ksk*-1 and *Ksk*-2) were used to identify and map three loci (*RAC*1, *RAC*2, and *RAC*3) of genes that confer resistance to *A. candida*. The phenotypes associated with these genes were classified as either necrotic flecks on upper surface of cotyledons and no blisters (FN) for *RAC*2 and *RAC*3 or flecks surrounded by yellowing and no blisters (FYN) for *RAC*1. Both phenotypes exhibited rapid death of host cells penetrated by the parasite (hypersensitive response), with callose deposition commonly encasing the haustorium. A fine-scale map interval and co-segregating markers for this locus, which in turn enabled mapping of a previously unnoticed source of resistance in *Ksk*-1 designated *RAC*3 that exhibits an FN phenotype hypersensitive to the FYN phenotype of *RAC*1. *RAC*3 is closely linked to the *RPP*8/*HRT* on

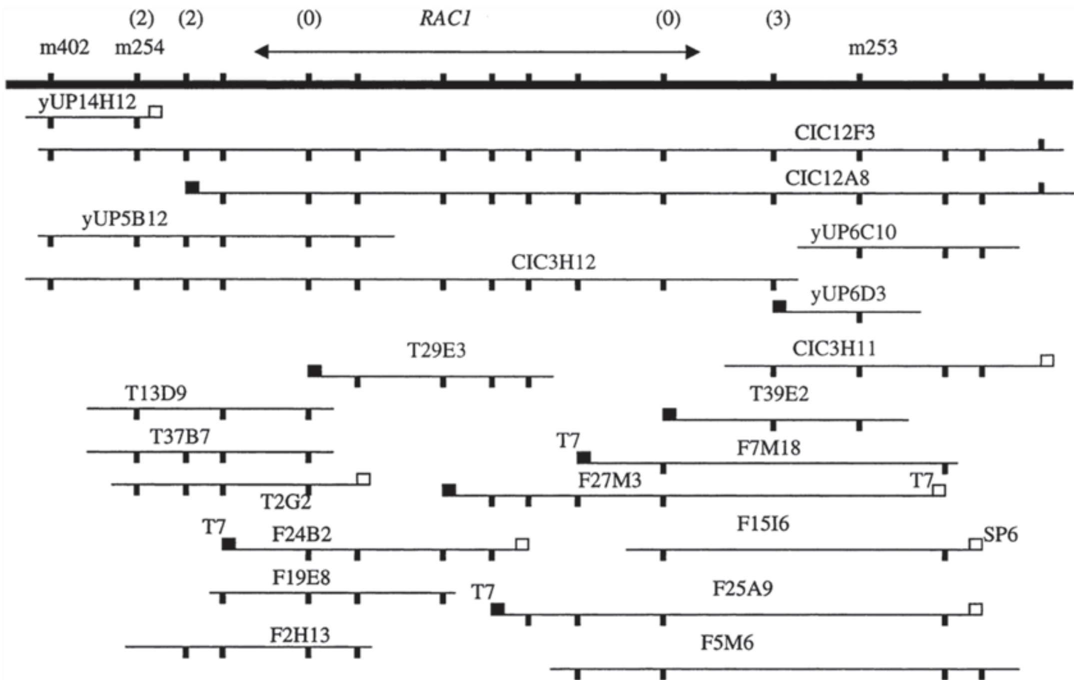

Fig. 9.4 Genetic and physical mapping of the *RAC*1 locus. Number of recombinants identified in the F_6 mapping population is shown in bracket for each molecular marker. *Solid bar* represents *RAC*1 interval on chromosome 1. *Lines* indicate YAC and BAC clones. Boxes at the end of lines are left (*solid box*) or right (*open box*) terminal used as anchoring markers. YAC contig was assembled by hybridizing to existing markers and markers developed from their terminal sequences. TAMU (T) and IGF (F) BACs were identified by screening the respective BAC libraries, TAMU (Choi and Priest 1995) and IGF (Mozo et al. 1998), with *RAC*1 flanking markers. BAC clones were ordered by cross hybridization of their terminal sequences. Based on the physical map for chromosome 1 of *Arabidopsis* (www.arabidopsis.org) the estimated distance for *RAC*1 interval, delimited by RFLP marker m254 and m253, is about 270 kb. (Borhan et al. 2001)

chromosome 5, a locus which contains specificities for resistance to DM and turnip crinkle virus. Recombinant inbred also enabled mapping of recessive resistance at *RAC*2 in *Ksk*-2 to the bottom arm of chromosome 3, in the 6 cM interval between two DM-resistance loci (*RPP*1 and *RPP*13) (Borhan et al. 2001) (Fig. 9.4; Tables 9.8 and 9.9).

In *B. rapa* the *ACA*1 locus was mapped to linkage group 4 and was flanked by RFLP marker loci (Fig. 9.5) (Kole et al. 1996). A quantitative trait loci (QTL) mapping approach using the IP scores detected the same major resistance locus for both races, plus a second minor QTL effect for AC 2 on linkage group 2. These results indicate that either a dominant allele at a single locus (*ACA*1), or two tightly linked loci, control seedling resistance to both races of WR in the biennial turnip rape cultivar Per. The map positions of WR resistance genes in *B. rapa* and *B. napus* were compared and the results indicate that additional loci that have not been mapped may be located. The alignment of these maps to the physical map of the *A. thaliana* genome identified regions to target for comparative fine mapping using this model organism (Fig. 9.6) (Kole et al. 2002).

The inheritance of avirulence and polymorphic molecular markers were studied in crosses of race 2 (AC 2), isolates MiAc2-B1 or MiAc2-B5 (metalaxyl insensitive and virulent to *B. juncea* cv. Burgonde), and race 7 (AC 7), isolate MsAc7-A1 (metalaxyl-sensitive and virulent to *B. rapa* cv. Torch). Avirulence or virulence of F_2 progeny to *B. rapa* cv. Torch gave 3:1 ratio

Table 9.8 Genetic position relative to molecular markers in *A. thaliana* of three independent loci for resistance to the *A. candida* isolate Acem1: *RAC*1 and *RAC*3 on chromosomes 1 and 5, respectively, identified in the accession *Ksk*-1; and *RAC*2 on chromosome 3 identified in *Ksk*-2. (Borhan et al. 2001; data shown for *RAC*1 was obtained from Holub et al. 1995)

Marker[a]	Position[b]	RAC1[c]		RAC2		RAC3	
		N	% R	N	% R	N	% R
m253	I-58	124	6	53	69		
m299	I-62	115	13			57	65
gl-1	III-46			53	12		
p309	III-na			53	3		
pAT389	III-na			53	3		
p3002-2	III-na			45	9		
pm249-2	III-65			54	9		
g2534	III-65			53	13		
m457	III-75	31	55			26	65
m225	V-65					34	72
m331	V-73	43	70			31	36
Cra1	V-na					38	4
nga129	V-na			53	48	36	8
m435	V-80					31	18

na not available (shown relative to physical order or markers), % *R* percentage recombination

[a] RFLP markers include: m253, m299, m331, m457, m225, m435, p3002-2, and p309. PCR-based markers include: g2534, pAT389, pm249-2, Cra1 (CAPS markers), and nga129 (SSLP markers)

[b] Chromosome and relative position (cM) on the unified genetic map of *Arabidopsis* (obtained from TAIR website at www.arabidopsis.org)

[c] *N* total number of F_6 inbred lines from *Wei*-1 × Ksk-1 for mapping *RAC*1 and *RAC*3, and from *Wei* × *Ksk*-2 for mapping *RAC*2

Table 9.9 Key recombinants that define the genetic interval of *RAC*3, a locus on chromosome 5 of *A. thaliana* for resistance to *A. candida* (isolate Acem1), relative to molecular markers that were dimorphic between the susceptible accession Wei-1 and the resistant accession *Ksk*-1. (Borhan et al. 2001)

F_6 Inbred[a]	m331	RAC3	Cra1	nga129	m435
2120, 2337	*Ksk3*[b]	*Wei*	*Wei*	*Wei*	*Wei*
2377	*Ksk*	*Wei*	*Wei*	*Wei*	*Ksk*
2098, 2145, 2273, 2338	*Wei*	*Ksk*	*Ksk*	*Ksk*	*Ksk*
2270	*Ksk*	*Ksk*	*Wei*	*Wei*	*Wei*
2073	*Ksk*	*Ksk*	*Ksk*	*Wei*	*Wei*
2160	ND	*Ksk*	*Ksk*	*W/K*	*Wei*
2087	ND	*W/K*	ND	*Ksk*	*Ksk*
2331	*Ksk*	*Ksk*	*Ksk*	*Ksk*	*Wei*
2069	ND	*Ksk*	*Ksk*	*Ksk*	*W/K*

ND not determined

[a] Recombinant inbreds from an outcross between *Wei* and *Ksk*-1

[b] *Wei* homozygous for *Wei*-1 dimorphism or uniform susceptibility at *RAC*3, *Ksk*-1 dimorphism or uniform FN resistance at *RAC3, W/K* heterozygous

in each of the three populations, supporting the hypothesis of a single dominant avirulence gene. Amplified fragment length polymorphism markers also segregated in regular Mendelian fashion among F_2 progeny derived from two F_1 hybrids (Cross-2–5 and Cross-2–7) of Cross-2. This first putative avirulence gene in *A. candida* was designated *AvrAc*1. It revealed that a single dominant

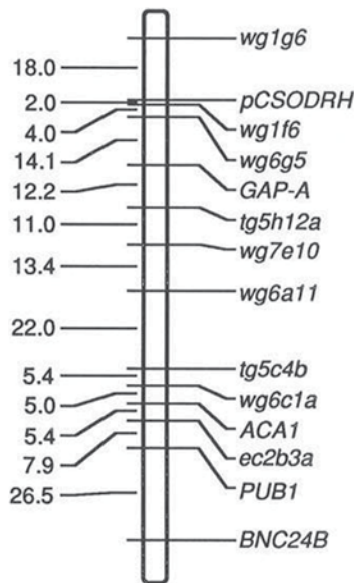

Fig. 9.5 Linkage map of *B. rapa* group 4 from analysis of F_3 families derived from cvs. Per × R-500. Locus *ACA1* controls resistance to *A. candida* race 2 and is linked to restriction fragment length polymorphism loci detected by *Brassica* genomic (*wg* and *rg*) and cDNA (*cc*) clones and heterologous probes. Locus *PUB1* controls leaf pubescence. Genetic distance to the left are in centimorgans. (Kole et al. 1996)

gene controls avirulence in race AC 2 to *B. rapa* cv. Torch and provides further evidence for the gene-for-gene relationship in the *Albugo–Brassica* pathosystem (Adhikari et al. 2003). *Diplotaxis gomezcampoi* and *Sinapis pubescens* identified WR-resistant wild species (Kumar et al. 2003).

The reaction against WR races 7a and 7v was scored in 20 seedlings from each self-pollinated F_2 individuals. The proportion of resistant plants among these F_3 families varied from 0 to 67 %. Bulked segregant analysis did not reveal any markers linked with resistance and, therefore, a linkage map with 81 markers was created (Tanhuanppa 2004). A locus that accounted for 18.4 % of the variation in resistance to WR was mapped to linkage group (LG) 2 near the RAPD marker Z19a. During the study, a bacterial resistance gene homologous to *Arabidopsis RPS*2 and six different *RGA*s were sequenced. *RPS*2 and five of the *RGAs* were mapped to linkage groups LG1, LG4, and LG9. Unfortunately, none of the *RGAs* could be shown to be associated with WR resistance (Tanhuanppa 2004).

Various generations, viz., F_1, F_1 (reciprocal), F_2, and DHs produced from the crosses were inoculated with a zoospore suspension of race 7v of *A. candida*. The partially resistant phenotype appeared to be controlled by a single recessive gene designated as *wpr* with variable expression. The simple inheritance of partial resistance has implications for disease resistance breeding against WR, as this type of resistance can be easily incorporated into elite breeding lines through conventional and DH breeding methods (Bansal et al. 2005). Transfer of resistance to white blister disease between *Brassica* species involving two genotypes each of *B. juncea* and *B. rapa* was studied in hybrids. Hybrids were identified by PCR-based inter-simple sequence repeat (ISSR) markers with both male and female species–specific bands being identified (Gupta et al. 2006).

Two accessions of *A. thaliana* (*Ksk*-1 and *Ksk*-2) were used to identify and map three loci (*RAC*1, *RAC*2, and *RAC*3) of genes that confer resistance to *A. candida* (Borhan et al. 2001). Genes for resistance to WR in oilseed *B. rapa* were mapped using a recombinant inbred (RI) population, and a genetic linkage map consisting of 144 RFLP markers, and 3 phenotypic markers. The results indicate that either a dominant allele at a single locus (*ACA*1), or two tightly linked loci control seedling resistance to both races of WR in the biennial turnip rape cultivar Per (Kole et al. 2002). Holub et al. (1995) reported incompatible interactions ranging from reduced blister formation to complete lack of asexual reproduction in the *A. thaliana* and *A. candida* system. The partially resistant phenotype appeared to be controlled by a single recessive gene designated as *wpr* with variable expression. The simple inheritance of partial resistance has implications for disease resistance breeding against WR, as this type of resistance can be easily incorporated into elite breeding lines through conventional and DH breeding methods (Bansal et al. 2005). Information on the genetics and chromosomal location of resistance to these two races of *A. candida* in the *B. rapa* genome would be useful to develop resistant varieties by marker-assisted breeding,

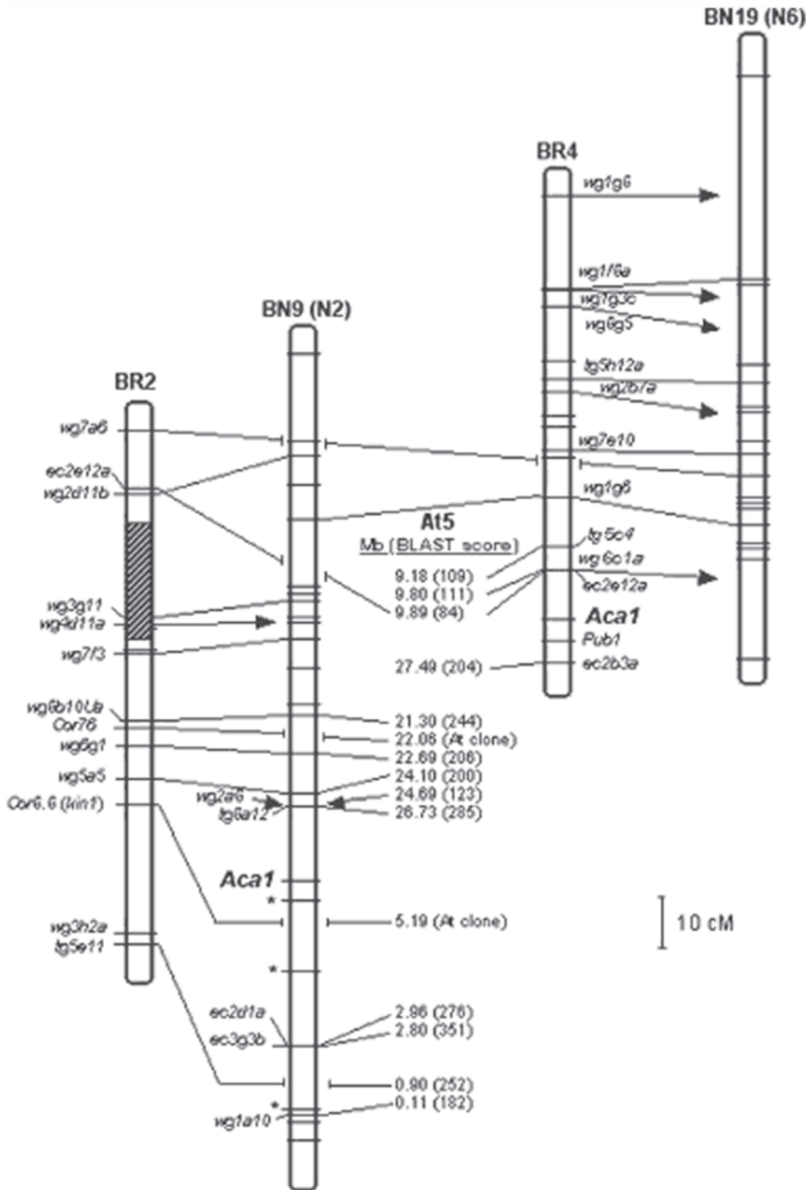

Fig. 9.6 Genetic maps of linkage groups 2 (BR2) and 4 (BR4) from *B. rapa* and linkage groups 9 (BN9), (Ferreira et al. 1994); or N2, (Butruille et al. 1999; Osborn et al. 1997; Parkin et al. 1995; Sharpe et al. 1995) and 19 (BN19), (Ferreira et al. 1994); or N6, (Butruille et al. 1999; Parkin et al. 1995; Sharpe et al. 1995) from *B. napus*. *ACA*1 on BR4 controls resistance to *A. candida* race 2 (AC 2) and race 7 (AC 7), and *ACA*1 on BN9 controls resistance to *A. candida, B. carinata* pathotype. *Pub*1 on BR4 controls leaf pubescence. *Cross-hatching* represents the one-LOD confidence interval for the QTL on BR2 controlling resistance to AC 2. RFLP loci detected by *Brassica* genomic (*wg* and *tg*) and cDNA (*cc*) clones on two or more linkage groups are shown and their positions on the different maps are connected by lines. *Lines* with *arrows* indicate RFLP loci mapped in *B. rapa* and their approximate positions on N2 and N6 in the maps of Parkin et al. (1995) and Sharpe et al. (1995). The *B. napus* linkage groups are based on "Major" × "Stellar" maps, as described by Osborn et al. 1997, although the relative position of *wg*2*a*6*b* (BN9) was estimated based on Butruille et al. (1999). The physical positions in *Arabidopsis* (Mb; chromosome 5) are shown for the DNA sequences that give the strongest matches (BLAST scores in parentheses, except for Cor6.6 and Cor78, which are *Arabidopsis* cDNA clones) to DNA sequences of the RFLP probes surrounding *ACA*1 loci. The probe detecting marker locus *wg*3*h*2*a* matched a sequence on chromosome 3 of *Arabidopsis* (BLAST score 170); loci marked with asterisks are AFLP markers and were not sequenced. (Kole et al. 2002)

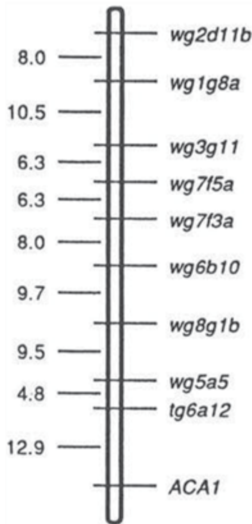

Fig. 9.7 Linkage map of *B. napus* group 9 from analysis of doubled haploid lines derived from (Major × Stella) F_1. Locus *ACA*1 controls resistance to *A. candida* isolate ACcar1 and is linked to restriction fragment length polymorphism loci detected by genomic DNA clones and designed *tg* and *wg* on *right*. Genetic distances (*left*) in centimorgans. (Borhan et al. 2008)

and also to clone the resistance genes. A single locus controlling resistance to AC 2 in *B. rapa* was mapped previously using RFLP markers and a segregating F_2 population from "Per" × "R500" (Kole et al. 1996). However, resistance to AC 7 has not been mapped. Resistance to a *B. carinata* pathotype of *A. candida* was mapped as a single locus (*ACA*1) with RFLP markers in *B. napus* (Ferreira et al. 1994). Linkage between the *ACA*1 locus and nine RFLP loci was observed by Ferreira et al. (1995) on linkage group 9 of *B. napus* RFLP linkage map (Fig. 9.7). Because *B. rapa* is one of the progenitor species of the amphidiploid species *B. napus* and maps of the two species have common RFLP loci (Osborn et al. 1997; Parkin et al. 1995), the potential homology of resistance genes from these species could be investigated by comparative mapping.

Borhan et al. (2008) have characterized a gene designated *WRR* 4 that encodes a cytoplasmic TIR-NB-LRR receptor-like protein in Columbia *A. thaliana* and confers broad-spectrum WR resistance to four physiological races of *A. candida* described by Pound and Williams (1963), and Liu et al. (1989). Under natural conditions, *A. thaliana* appears to be innately immune as a species to these four *A. candida* races, which instead thrive on their preferred hosts, including different *Brassica* crops and other wild crucifers such as *Capsella bursa-pastoris.* All four races belong to a predominant group of *A. candida* that is molecularly distinct from the subspecies referred to here as *A. candida* subsp. *Arabidopsis,* which is commonly found in the UK, causing WR in natural *A. thaliana* populations (Fig. 9.8) (Choi et al. 2006; Rehmany et al. 2000; Voglmayr and Riethmüller 2006). In this context, *WRR* 4 is similar to other single *R* genes that have previously been described including *RB* in the wild potato relative *Solanum bulbocastanum,* and *Bs*2 in pepper that confer broad spectrum disease resistance to late blight or bacterial speck, respectively (Tai et al. 1999; Song et al. 2003). *WRR* 4 (At1g56510) was mapped to a 40-kb region on chromosome 1 containing two additional genes that encode TIR-NB- LRR proteins (*At*1g56520 and *At*1g56540). Transgenic evidence with subclones from this region and supporting mutant analysis indicated that only one gene (*At*1*g*56510) was able to confer WR resistance. A BLAST search of the *A. thaliana* protein database identified the neighbouring genes as being the most similar to *WRR* 4 (74 and 76 % similarity). Meyers et al. (2003) previously had grouped all three genes in the same clade (TNL-H) of NB-LRR genes in *A. thaliana* Col-0. Interestingly, this clade also includes the stem canker resistance gene *RLM*1 (*At*1g64070; 54 % identity and 64 % similarity with *WRR* 4) described by Staal et al. (2006), whereas *RPP*1-*Ws* is the most closely related DM resistance gene (41 % identity and 59 % similarity). Sequence analysis of the *wrr*4-1 EMS mutant allele revealed an amino acid change of cysteine (C) to tyrosine (Y) at position 837. The point mutation lies within the β-strand/β-turn motif of the last LRR of *WRR* 4 (Jones and Jones 1997). Comparison of the solvent exposed β-strand/β-turn structure within the LRR domain of other known *R* genes shows that this motif is hypervariable and under diversifying selection (Bittner-Eddy et al. 2000; Botella et al. 1998; McDowell et al. 1998; Meyers et al. 1998).

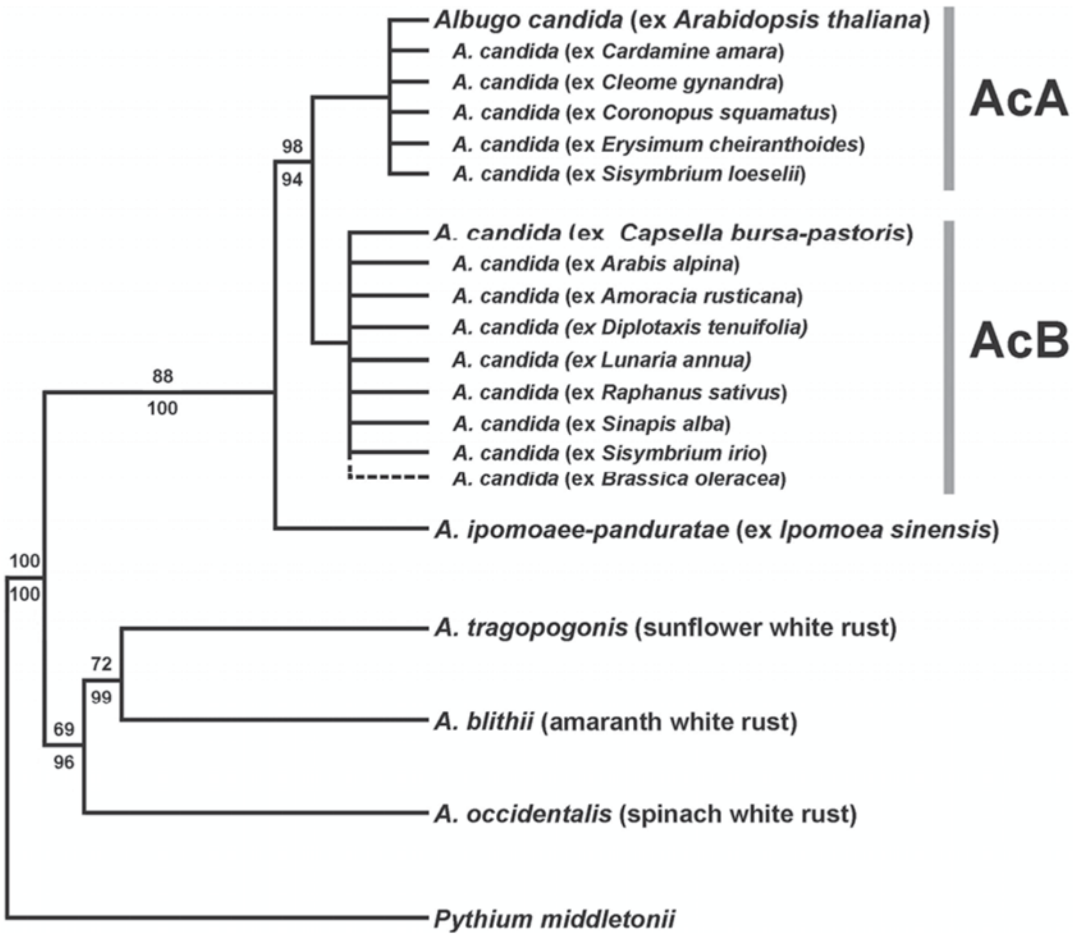

Fig. 9.8 Molecular evidence for two subgroups within *A. candida* that are labeled here as AcA (typified by isolates collected from *A. thaliana*) and AcB (typified by *A. candida* race 4 from *C. bursa-pastoris*). The phylogenetic relationship between these subgroups was described by Voglmayr and Reithmüller (2006), who compared nuclear large-subunit ribosomal DNA sequences from 60 isolates of *Albugo* spp. in a Bayesian analysis (using Markov chain Monte Carlo analysis over 5 million generations). An isolate from a *Brassica* host (*dashed line*) was not included in their study; however, additional data from AFLP and internal transcribed spacer 1 sequence comparisons indicate that *Brassica*-derived isolates (including races 2, 7, and 9 from *B. juncea, B. rapa,* and *B. oleracea,* respectively) are grouped with AcB race 4. (Choi et al. 2006; Rehmany et al. 2000)

As with these previous examples, it is plausible that the LRR of *WRR* 4 is important for recognition specificity of this novel WRR protein.

WRR 4 is structurally similar to another TIR-NB-LRR gene called *RAC*1 that previously was characterized as a gene conferring *A. candida* subsp. *Arabidopsis* resistance in *A. thaliana* (Borhan et al. 2008). As with other examples of TIR-NB-LRR disease resistance genes, *RAC*1 and *WRR* 4 each require the *EDS*1 lipase-like protein to confer resistance (Aarts et al. 1998; Feys et al. 2001; Peart et al. 2002). However, unlike the other examples, both of the *WRR* genes appear to function independently from a second lipase-like protein, PAD4. In addition, *WRR* 4 appears to function independently from FMO1 which is a positive regulator of the *EDS*1 defense pathway in *A. thaliana* and required for bacterial and DM resistance conferred by other TIR-NB-LRR proteins (Bartsch et al. 2006).

Inducible defense responses involving *EDS*1 in *A. thaliana* generally have been correlated

with SA activity (Wiermer et al. 2005). Enhanced hyphal development of *A. candida* in two mutants (*sid*1 and *sid*2) may suggest at least a partial role for constitutive levels of SA in regulating *WRR* 4-mediated resistance because these genes are involved in biosynthesis of SA. In an analogous study, Mellersh and Heath (2003) conducted mutational analyses of non-host resistance in *A. thaliana* to the basidiomycete rust pathogen from cowpea, *Uromyces vignae,* and concluded that SA plays an important role in restricting compatibility of basidiomycete rusts in *A. thaliana,* as indicated by enhanced hyphal growth and haustorial development of *U. vignae* in the *sid*2 mutant, whereas SA-dependent expression of pathogenesis-related proteins (indicative of hypersensitive defense responses) provides no significant contribution to the resistance. A role for SA in *WRR* 4-mediated resistance, however, does not appear to involve the elevated expression of the R protein itself in Columbia *A. thaliana*. Tan et al. (2007) reported that SA treatment of leaf tissue significantly elevated levels of TIR-NB-LRR proteins that are known to confer DM resistance in wild-type Columbia. However, they observed that constitutive expression of *WRR* 4 was among the lowest when compared with other known NB-LRR resistance proteins in non-elicited tissue, with the highest expression of *WRR* 4 in leaf tissue and approximately threefold lower expression in flowers and siliques; the effect of SA treatment on *WRR* 4 expression was negligible. Interestingly, the relationship between SA signals and *WRR* 4 expression may vary markedly in different genetic backgrounds because a sevenfold increase in *WRR* 4 expression was detected in the Libyan accession Mt-0 following treatment of leaf tissue with SA (Tan et al. 2007).

SA is an important molecule for signaling stomatal closure to restrict the entry of motile epiphytic bacteria into the stomatal chamber (Underwood et al. 2007). Guard cells can detect the presence of these bacteria and signal SA-mediated closure via membrane receptors such as FLS2, an LRR receptor-like kinase that detects the nonspecific elicitor protein flagellin (Gomez-Gomez and Boller 2000; Melotto et al. 2006). *A. candida* and many basidiomycete rusts such as *U. vignae* also typically attempt to invade a potential host via stomata. Thus, a report by Zipfel et al. (2004) seems particularly fascinating. They used microarray experiments for assessing the response of leaf tissue to flg22 treatment (a fragment of bacterial flagellin protein), and detected an increased expression of *WRR* 4 (approximately six fold) in wild-type *Landsberg erecta*. A similar flg22-elicited response was observed with the *WRR* 4-paralog *At*1*g*56540, whereas most other TIR-NB-LRR proteins were unaffected in expression. Some virulent bacterial pathogens release a phytotoxin coronatine that can suppress the stomatal closure process mediated by FLS2 and SA (Melotto et al. 2006).

A distinction in signaling attributes among members of the TIR-NB-LRR subfamily will be an important factor to investigate for its potential role in determining host specialization among subspecies of *A. candida.* For instance, basic compatibility of *A. candida* subsp. *Arabidopsis* in *A. thaliana* involves broad-spectrum suppression of programed cell death and innate immunity to other biotrophs (Cooper et al. 2008), including suppression of DM resistance conferred by several TIR-NB-LRR genes. However, *RAC*1 and *WRR* 4 represent important exceptions to this phenomenon as receptor-like *R* genes in *A. thaliana* that can induce defense in a manner that is non-suppressible by the corresponding isolates of *A. candida.* This suggests that they differ intrinsically from other TIR-NB-LRR genes, and particularly ones that confer DM resistance, either in timing with an ability to induce a more rapid defense response that preempts defense suppression, or perhaps in the biochemistry of the defense signaling process (e.g., PAD4-dependent defense is suppressible whereas PAD4-independent defense of *RAC*1 and *WRR* 4 may be non-suppressible by preinfection with *A. candida* subsp. *Arabidopsis*). The latter prediction could be tested by sequential inoculation of transgenic *A. thaliana* Ws-*WRR* 4, first with a virulent isolate of *A. candida* subsp. *Arabidopsis,* and subsequently with an avirulent isolate of *A. candida* (race 2, 4, or 7), similar to the experiments described by Cooper et al. (2008).

The *WRR* 4 phenotype in Col-*gl*1 does exhibit characteristics of a rapid, full immunity to at least four physiological races of *A. candida*. No symptoms were visible macroscopically and parasite development invariably was arrested in the first host cell. A rapidly induced host response was evident because an oxidative burst was detected by the accumulation of hydrogen peroxide (shown by DAB staining) following inoculation of Col-*gl*1 with the *A. candida* race 4 isolate Acem2. In contrast, the much slower, less-potent attenuation of WR development in Nd-1 did not appear to involve a rapid oxidative burst indicative of host cell death. Interestingly, colonization of Nd-1 by Acem2 was associated with a loss of turgidity, which was not evident with the other three *A. candida* isolates; and AC 9 was strictly unable to reproduce in Nd-1, whereas the other three isolates occasionally were able to produce small white blisters. This subtle phenotypic variation suggests further genetic specificity among interactions of *A. thaliana* with different *A. candida* races, potentially associated with additional receptor-like proteins. Tosa (1992) proposed that interspecific (species-level) variation in innate immunity could be explained in many cases by an accumulation of parasite-recognition-genes in natural populations of the host that collectively provide broad-spectrum, species-level disease resistance. In other words, susceptible genotypes (lacking any of the existing *R* genes) are a rare occurrence in the host species. Receptor-like genes have been proposed as important determinants of innate immunity in *A. thaliana* to higher taxa (species and subspecies) of parasites from other hosts (Holub 2007). For example, Staal et al. (2006) used transgressive segregation from a recombinant inbred mapping population to identify two TIR-NB-LRR genes that confer resistance to *Leptosphaeria maculans* (stem canker), an important *Brassica* pathogen that is rarely compatible in wild accessions of *A. thaliana*. *WRR* 4 provides the first analogous example for WR resistance, which in this case represents a single *R* gene that can effectively distinguish between different subspecies of the oomycete *A. candida*. The intercross between wild-type Columbia and Ws-3 obviously will provide a more powerful resource for further genetic dissection of non-HR or EDS-independent genes conferring WR resistance in Columbia *A. thaliana.*

Characterization of different defense-signaling attributes among these *R* genes will provide an important bridge between the much-needed exploration of *A. thaliana* in its natural habitat and the comparative biochemistry and molecular systems biology research currently being advanced in laboratory research. Investment in *Albugo* genomics, for example, will be essential to determine whether *WRR* 4 confers avirulence recognition of a highly conserved effector protein that is shared among races 2, 4, 7, and 9 of *A. candida*. Given the role of stomata as a preferred site of host entry for bacteria and rust pathogens, it will also be interesting to determine whether *A. candida* produces functional analogs of bacterial flagellin (i.e., a nonspecific elicitor of innate immunity) or coronation (i.e., a hormone-like defense suppressor) and, if so, whether this may relate directly to the effectors protein from *A. candida* that elicits *WRR* 4-mediated recognition or the sustained broad-spectrum defense suppression induced by virulent isolates of *A. candida* subsp. *Arabidopsis* (Cooper et al. 2008).

Molecular characterization of *A. candida* effector proteins should be possible because methods have been established for outcrossing *A. candida* isolates, bioinformatic identification of effector-like genes from oomycetes, and functional testing of oomycete genes via bacterial delivery (Adhikari et al. 2003; Rentel et al. 2008; Sohn et al. 2007; Whisson et al. 2007; Win et al. 2007). Parasite effectors from *A. candida* will be critical molecular determinants for investigating the relative selective pressures from *A. candida* subsp. *Arabidopsis* and races of *A. candida* derived from other hosts on the innate immunity of *A. thaliana* in natural and experimental populations (Fig. 9.9; Borhan et al. 2008).

Massand et al. (2010) have tagged two independent loci governing resistance to *A. candida* race 2V in two east European lines, Heera and Donskaja-IV. Two doubled haploid populations were used; the first population was derived from a cross between Varuna (susceptible Indian type) and Heera (partially resistant east European line),

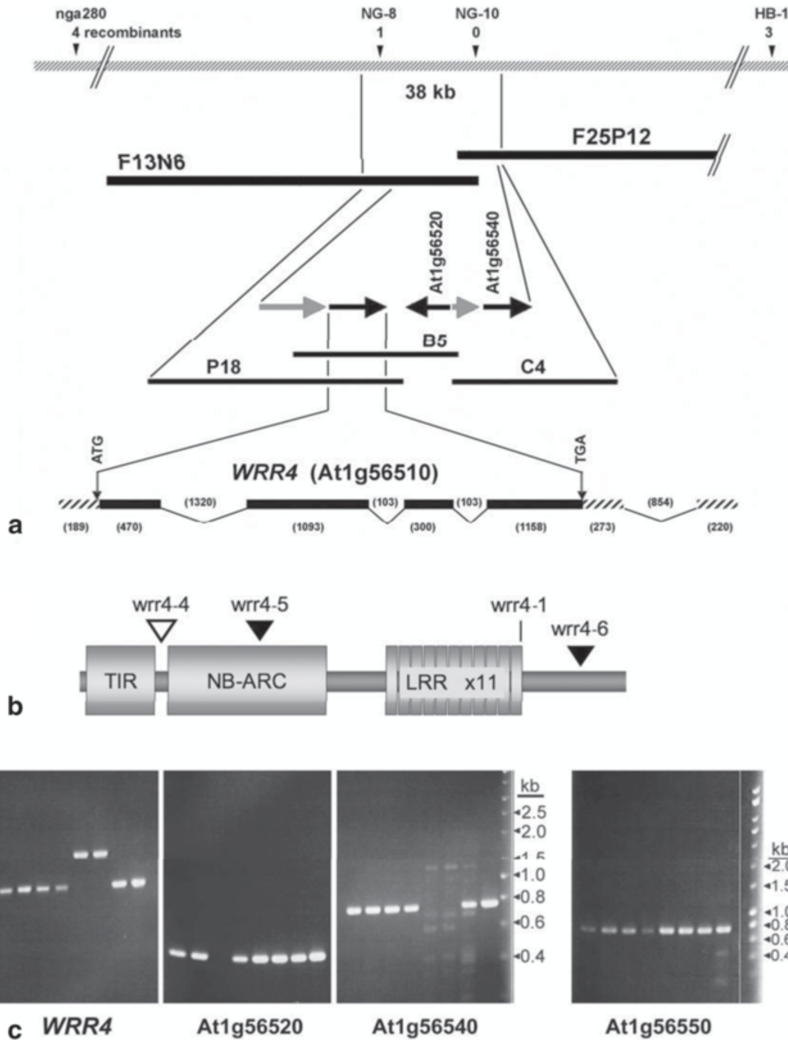

Fig. 9.9 Map-based cloning of *WRR* 4 from *A. thaliana* Columbia and supporting evidence from fast-neutron mutants. **a** Position of white rust resistance (*WRR*) gene, *WRR* 4, (*At*1g56510) on chromosome 1 (the *hatched bar*) and position of some of the markers used for mapping *WRR* 4 indicated by *arrow*. The bacterial artificial chromosomes spanning across the *WRR* 4 map contig are shown as *solid bars*. Cosmid clones spanning across this region are shown by *solid lines*. Location of exons (*solid bars*), introns (*broken lines*), and untranslated regions (*hatched bars*) are depicted in the lowest bar diagram. **b** Diagram of the *WRR* 4 including N-terminal Toll-interleukin receptor-like domain (TIR), nucleotide-binding ARC domain (NB-ARC), and 11 leucine rich-repeats (LRRs). The C-terminus is leucine rich but without a repeat structure. Mutations are indicated above, including *wrr*4-1 (EMS), which caused a single amino acid change in the last LRR; and TDNA insertion mutants in the first intron (*wrr*4-4, *open triangle*) in the NB domain (*wrr*4-5, *black triangle* indicates exon insertion) and non-LRR C-terminal region (*wrr*4-6, exon insertion). **c** Gene-specific polymerase chain reaction products were used to detect genetic rearrangements in two fast neutron mutants (*wrr*4-2 and *wrr*4-3) that affected *WRR* 4 and a paralogous gene *At*1g56540, but not middle paralog (*At*1g56520) or the next telomeric gene (*At*1g56550). The bands were generated from the following DNA sources (left to right): Col-*gl*1 (*WRR* 4), Col-0 (*WRR* 4), Ws-0 (*wrr*4-0), Col-*ndr*1 (*WRR* 4), Col-*ndr*1/*wrr*4-2, Col-*ndr*1/*wrr*4-3, Col-*wrr*4-1, and the appropriate BAC clones (F13N6 or F25P12) (Borhan et al. 2008)

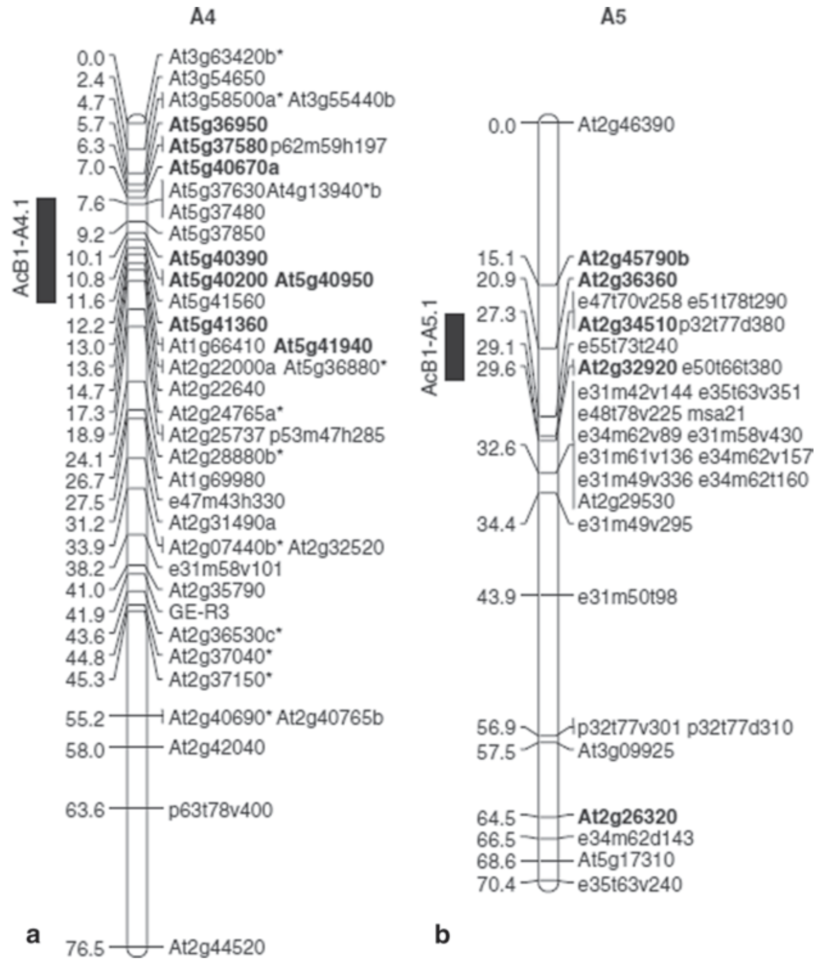

Fig. 9.10 QTL mapping of white rust resistance in two DH populations of *Brassica juncea.* **a** In the VH population one major QTL (AcB1-A4.1; *black bar*) was mapped in the linkage group A4 at a genetic interval of 7–17 cM and **b** in the TD population, a major QTL (AcB1-A5.1; *black bar*) was detected in the linkage group A5 at a genetic interval of 18–24 cM. The markers highlighted in *bold* represent the new IP markers mapped using syntenic relationship with *Arabidopsis.* (Massand et al. (2010)

and the second from a cross between TM-4 (susceptible Indian type) and Donskaja-IV (fully resistant east European line). In both the resistant lines, a single major locus was identified to confer resistance to WR. In Heera, the resistance locus *AcB*1-*A*4.1 was mapped to linkage group A4, while in Donskaja-IV, the resistant locus *AcB*1-*A*5.1 was mapped to linkage group A5. In both the cases, closely linked flanking markers were developed based on synteny between *Arabidopsis* and *B*. *juncea*. These flanking markers will assist introgression of resistance-conferring loci in the susceptible varieties (Fig. 9.10).

Markers are available for the selection of *B. juncea* plants carrying the resistance gene (*AC*2*A*1) to *A. candida* race AC 2. Two markers, WR2 and WR3, linked to *A. candida* resistance, flanked the resistance locus *AC*2*A*1 and are very effective in identifying the presence or absence of the resistance gene in the doubled haploid (DH) populations (Prabhu et al. 1998). Eight AFLP markers linked to WR resistance were identified from *B. napus* (Somers et al. 2002) (Fig. 9.11). The *B. napus* chromosome segment, carrying the WR resistance gene (*AC*2*V*1) appeared to have recombined with the *B. juncea* DHA since recombinant individuals have been identified (Somers et al. 2002) (Fig. 9.11). Molecular mapping of the locus conferring resistance to *A. candida* in accession BEC-144 of *B. juncea* was accomplished by employing RAPD markers in conjunction with bulked segregant analysis of the F_7 generation RILs. The resistance locus in BEC-

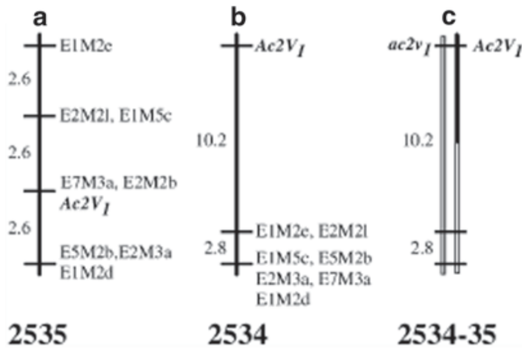

Fig. 9.11 Genetic linkage maps of AFLP markers and the *AC2V1* locus derived from *B. napus* and introgressed into *B. juncea*. **a** *B. juncea* BC3 F2 population 2535, **b** *B. juncea* BC3 F2 population 2534, **c** Schematic diagram of the *AC2V1* interval in the white rust resistant *B. juncea* plant 2534–35 showing the *AC2V1* locus to be heterozygous. *Black and white chromosome segments* represent *B. napus* and *B. juncea,* respectively. (Somers et al. 2002)

144 has been designated as AC2 (+) (Mukherjee et al. 2001).

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10 Sources of Resistance

The supply of gene(s) for disease resistance is the first concern in an ongoing resistance-breeding programme. Considerable efforts have been made to evaluate cultivar resistance to *Albugo* in oilseed *Brassica* crops. Cultivated *Brassicas* are represented by six interrelated species (Fig. 10.1), three of which are diploids: *Brassica nigra,* bb ($n=8$); *Brassica oleracea,* cc ($n=9$) and *Brassica rapa,* aa ($n=10$), and the other three are the amphidiploid derivatives of the diploid species: *Brassica carinata,* bbcc ($n=17$); *Brassica juncea,* aabb ($n=18$); *Brassica napus,* aacc ($n=19$) and *Raphanus sativus,* rr ($r=9$).

Many of the *Brassica* species consist of numerous subspecies or varieties representing a diverse range of morphotypes, and utilized for oils, condiments, vegetables and animal fodders. *Brassica* oil (rapeseed oil) ranks 5th in world commerce as a major edible and industrial oil; kales, rapes, turnips and swedes are important sheep and cattle fodder in climates too cool for maize or soya beans, whereas the cole crops and oriental brassica greens are a primary dietary vitamin source for over half of the world's population.

The cytogenetic interrelationships of the six *Brassica* spp. were first described by Morinaga (1934) and U (1935), and since then numerous studies have been made on the interspecific transfer of genes among various species of *Brassicas* (Yarnell 1956; McNaughton and Ross 1978). Intergeneric relationships between various brassicas and radish (*R. sativus*), rr ($n=9$) have demonstrated the transfer of potentially useful characters including disease resistance and high dry matter content, which have resulted in the development of the new crop genus *Raphanobrassica* (McNaughton and Ross 1978).

The three diploid species of *Brassica* are insect pollinated and strongly outbreeding with self-incompatibility controlled by a multiple allelic series of genes at the locus and under sporophytic phenotypic expression. Occasionally, genetic self-compatibility can be found and is predominant in cauliflower and sarson (yellow mustard). Selfing of incompatible plants can be accomplished by bud pollination (the placing of "self" pollen on the immature stigmas) 1–2 days prior to anthesis. Selfing in the diploid species normally results in inbreeding depression. Amphidiploid species are predominantly self-pollinating (75 % in oil seed rape) though S-alleles do exist in some populations.

Williams and Pound (1963) reported that resistance in radish cvs. China Rose Winter (CRW) and Round Black Spanish (RBS) are governed by a single dominant gene. Humaydan and Williams (1976) identified a single dominant gene, Ac-l, in radish cv. Caudatus. Bonnet (1981) reported that radish cv. Rubiso 2 contains a single dominant gene. Petrie (1985) reported that white rust (WR), *Albugo candida,* was not important due to widespread use of resistant cultivars, and later identified CRW, RBS and Burpee White (BW) as resistant to WR among 11 cultivars of radish.

Delwiche and Williams (1974) reported that all accessions of *B. napus* and most cultivars of *B. oleracea* are resistant to *A. candida.* Monteiro

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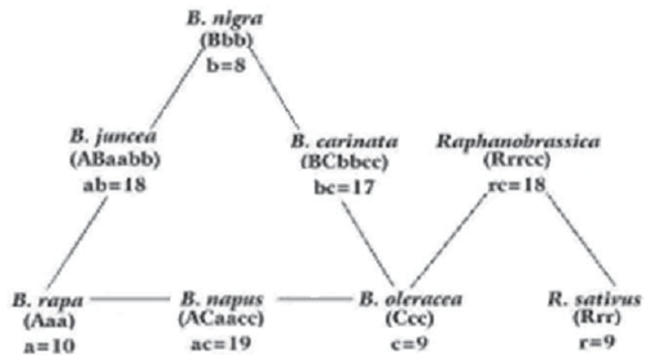

Fig. 10.1 Triangle of U is a theory about the evolution and relationship between cultivated *Brassicas* represented by six interrelated species

and Williams (1989) did not observe high resistance to *A. candida* in Portuguese cabbage and kale land races, although several accessions showed 20–30% of plants with intermediate expression of resistance. Most accessions of *B. oleracea* including Portuguese cole land races, and two Portuguese cabbage were very susceptible, and no differential reaction to the two Portuguese *A. candida* isolates were found, although some expressed resistance to both isolates; Couve Algarvia (*DI*=2.9), Couve Gloria de Portugal (*Dl*=3.4), and Couve Portuguera (*DI*=3.5) were the most resistant land races (Santos et al. 1996).

Fan et al. (1983) reported that the resistance in *B. napus* cv. Regent is governed by three genes: Ac-7-1, Ac-7-2 and Ac-7-3. In India, a number of *B. napus* genotypes resistant to *A. candida* have been reported: Gulivar (Gupta and Singh 1994; Saharan et al. 1988); GSL-l (Saharan 1996, 1997); GLS-150 1 (Gupta and Singh 1994; Saharan 1996, 1997); HNS-l (Bhardwaj and Sud 1993; Saharan et al. 1988); HNS-3, Tower (Bhardwaj and Sud 1993; Saharan 1996); HNS-4 (Jain et al. 1998; Saharan 1996, 1997); Midas (Jain et al. 1998; Saharan 1996, 1997; Saharan et al. 1988); Regent (Bhardwaj and Sud 1993; Saharan et al. 1988); GSB 7006, Norin-14 (Gupta and Singh 1994); GSL Series (10 lines), H-715, HNS-1, Norin, Tower 1,2,3,4 (Saharan et al. 1988); EC 174243, GSB 101, GLS 706, HNS-8, Tower-GO (Jain et al. 1998); ABN, Altex, EC 129126, EC 129127, EC 131625, EC 131626, EC 132121, GSA, GSB, HSN-l, Karat, Mary, Niklas, VR-OLGA, VR-WW-1313 (Bhardwaj and Sud 1993). However, all accessions of *B. napus* were reported to be resistant to *A. candida* (Bhardwaj and Sud 1993; Jain 1995; Lakra and Saharan 1989).

In India, a number of *B. juncea* genotypes resistant to existing races of *A. candida* have also been reported: CSR 142 (Kaushik and Saharan 1980; Saharan 1996); Domo-4 (Lakra and Saharan 1989; Yadav and Singh 1992); EC-126741 (Saharan et al. 1988; Yadav and Singh 1992); EC-126745 (Yadav and Singh 1992); EC 126746 (Saharan et al. 1988; Yadav and Singh 1992); EC 129126-1 (Jain 1995; Saharan 1996); KOS-l (Jat 1999); PHR-l (Jain 1995; Kolte 1987a, 1987b); RC-781 (Kaushik and Saharan 1980; Kolte 1987a, 1987b; Lakra and Saharan 1989); Scimitar (Wood and Petrie 1989); SSK-l (Gupta and Singh 1994); T-4 (Parui and Bandyopadhyaya 1973); YRT-1 (Kaushik and Saharan 1980; Kolte 1987a, 1987b; Lakra and Saharan 1989); Zem-1 (Gupta and Singh 1994; Jain 1995); Domo, Lethbridge (Kolte 1987a, 1987b; Saharan et al. 1988); Sikkim Sarson 1, YSIK 741 (Srivastava and Verma 1987); DIR 519, DIR 1507 (Jain et aI. 1998); EC-126743-2 (Lakra and Saharan 1989; Yadav and Singh 1992); DIRA-313-7, GS 7027, RN 246 (Saharan 1996); EC 126126, EC 126747, RC 1401, RC 1499 (Saharan et al. 1988); C9b, DOMO, YS-7B, Zem-2 (Jain 1995); EC 126743, EC 126743-1, EC 129121-1, RH 8541-46, RW 81-59 (Lakra and Saharan 1989); Blaze, Metapolka, Newton, Purbiraya, Stoke (Kolte 1987a, 1987b); RC-IOOl, RC-1405, RC-1408, RC-1424, RC-1425 (Kaushik and Saharan 1980; Saharan, el al. 1988); RH 8545, WRR-3-1, SV7739035 × RH-30-12-15, SV7739035 × RH-30-10-3, SV7739035 × RH-30-16-6, SV 7739035 × RH-30-2-17 (Bhatia

1994); Chamba-l, CSR 741, RC 295, RC 398, RC 1424, RC 1499 (Kaushik and Saharan 1980); MLS-7, MLS-10, MLS-11, MLS-13, MLS-16, MLS-17, MLS-18, MLS-29, MLS-30, MLS-31, MLS-32, MLS-35, MLS-39 (Velazhahan and Thiyagarajan 1994); DIRM 5, DIRM 11, Gonads 3, Gonads, IB 499-1, Kranti 43, R71-2, R75-2, RC 12, RC 43, RC 14-1, RH 861, RH 8121, RH 8176, RH 55, RLC 1015, RLM 39, RS 78, RW 15-6, RW 22, RW 33-2, RW 75-123-2, Trapal, NO 5422 (Bhardwaj and Sud 1993).

Brassica rapa cvs. Tobin (Kolte 1987a, 1987b), NDYS-2, PI-303, YSK-8502 (Jain 1995), and BSH-l; BS 15 of *B. rapa* var. Brown Sarson and Type 6, Prain YST-6 of *B. rapa* var. Yellow Sarson were resistant to WR pathogen (Kolte and Tewari 1980; Lakra and Saharan 1989); *B. rapa* var. Toria IB-586 (Kolte and Tewari 1980), KIC (Jain et al. 1998) and PI 303, PT-30 (Kolte et al. 1985) were least susceptible, immune and tolerant to *A. candida,* respectively.

In *B. carinata* HC-l (Bhardwaj and Sud 1993; Jain 1995; Saharan 1996, 1997; Saharan et al. 1988); HC-2 (Bhardwaj and Sud 1993; Jain et al. 1998; Saharan et al. 1988); HC-3, HC-5 (Bhardwaj and Sud 1993; Saharan et al. 1988); HC-4 (Saharan et al. 1988); HC-7; PC-3 (Saharan 1996, 1997); PC-5 (Gupta and Singh 1994); DIR 1510, DIR 1522, HC 9001 (Jain et al. 1998) were reported to be resistant to *A. candida.* However, all accessions of *B. carinata* were also reported to be resistant (Anand et al. 1985; Bhardwaj and Sud 1989; Lakra and Saharan 1989; Satyavir et al. 1994) to *A. candida. Eruca sativa* genotypes RTM 314, RTM 1263 and RTM 1471 were resistant to *A. candida* (Jain et al. 1998). All accessions of *Eruca vesicaria* and *Eruca pinnatifida* were resistant to race 2; all wild and two cultivated *Eruca* species were resistant to race 7 (Bansal et al. 1997).

Although, *Brassica chinensis,* cultivar Wong Bok (Sutton's) and its crosses were resistant to the foliar phase (Singh and Gangopadhyay 1976), all accessions were found to be free of staghead infection of *A. candida* (Lakra and Saharan 1989). Although all accessions of *Brassica alba* were resistant to *A. candida* (Lakra and Saharan 1989; Saharan et al. 1988), stagheads were observed in *Brassica perkinensis* (Lakra and Saharan 1989).

Brassica species and its wild allies, including *Brassica spinescens*, *Brassica tenuifolia*, *Brassica incana*, and *Diplotaxis erucoides*, *Diplotaxis siifolia*, *Dplotaxis virgata*, *Dplotaxis muralis*, *Diplotaxis vesicaria*, *Diplotaxis desnottesi* were resistant to *A. candida* (Gupta et al. 1995) (Table 10.1).

Source of multiple disease resistance (MDR) to *A. candida* has been identified (Table 10.2). Although, several genotypes are resistant to more than one disease, genotypes HC-1 and PCC-2 of *B. carinata,* and GSL-1501 of *B. napus* consistently show resistance against WR, Alternaria blight and powdery mildew diseases (Saharan and Krishnia 2001). Banga (1988) observed that some of the C genome chromosomes substitution lines of *B. juncea* and *B. napus* were practically free from WR infection.

Canadian breeders and plant pathologists in 1980 licensed *B. rapa* cv. Tobin, which was specifically bred to be highly resistant to race 7 of *A. candida*. Tobin was also found to be resistant to WR in India. In Texas, USA, some resistance has also been reported in certain varieties of spinach against *Albugo occidentalis* (Dainello et al. 1981). The *B. napus* chromosome segment, carrying the WR resistance gene (*Ac*2*V*1), appeared to have recombined with the *B. juncea* DNA since recombinant individuals were identified (Somers et al. 2002). A single dominant gene controls avirulence in race Ac2 to *B. rapa* cv. Torch and provides further evidence for the gene-for-gene relationship in the *Albugo–Brassica* pathosystem (Adhikari et al. 2003).

The resistance of *B. napus* cv. EC 151964 was transferred to *B. juncea* cv. RLM-198 through interspecific hybridization following modified pedigree method; a derivative 'NRG-49', an advance generation of interspecific cross, was found as good as *B. napus* and superior to *B. juncea* cv. RLM-198 in agronomic characters including grain yield and earliness (Pal et al. 1999). The inheritance of partial resistance to race 2 of *A. candida* was studied in *B. juncea* by crossing the partial resistant line with susceptible *B. juncea* cultivar Commercial Brown; adult plants did not develop stagheads under greenhouse and field conditions (Bansal et al. 1999). *B. alba* (=*Sinapis alba*), *B. carinata* (HC-1), *B. juncea*

Table 10.1 Sources of resistance in crucifer crops against *A. candida*. (Saharan 2010)

Crucifer host	Sources	Country	References
Raphanus sativus	China Rose Winter (CRW), Round Black Spanish (RBS)	USA	Williams and Pound 1963
	Caudatus	USA	Humaydan and Williams 1976
	Rubiso 2	USA	Bonnet 1981
	Burpee white (BW)	Canada	Petrie 1986
B. napus	Regent, Tobin	Canada	Fan et al. 1983
	GSL 1	India	Bhardwaj and Sud 1993
	S-II and S-IV from *B. napus* (single dominant gene)	India	Chauhan and Raut 2002
	Ac 2V1 (single dominant gene)		Somers et al. 2002
B. juncea	EC 399300, EC 399301, EC 399299, EC 414299, IC 443623, IC 555891	India	Saharan 2010
B. juncea	CBJ 001, CBJ 003, CBJ 004,	China	Li et al. 2008
	JR 049, JM06011	Australia	Li et al. 2007, 2009
B. rapa var. Yellow Sarson	T 6, Prain, YST 6, Tobin, NDYS 2, YSK 8502	India	Kolte and Tewari 1980; Lakra and Saharan 1989
B. rapa var. Brown Sarson	BSH 1, BS 15	India	Kolte and Tewari 1980; Lakra and Saharan 1989; Saharan et al. 1988; Saharan et al. 2005
B. rapa var. Toria	IB 586, KTC, PT 303, PT 30	India	Kolte et al. 1985
B. carinata	PBC 9221	India	
	S-III derived from *B. carinata* (single recessive gene		Chauhan and Raut 2002
Eruca sativa	RTM 314, RTM 1263, RTM 1471	India	Jain et al. 1998;
B. chinensis	All accessions	India	Gupta et al. 1995; Lakra and Saharan 1989
B. alba	All accessions	India	Saharan et al. 1988
A. thaliana	RAC-1, RAC-2, RAC-3 from *Arabidopsis thaliana*(ksk-1 and ksk-2)	Columbia	Borhan et al. 2001, 2008
B. spinescens	All accessions		
B. tenuifolia	All accessions		
B. incana	All accessions		
B. oleracea	Couve Algarvia, Couve Gloria, Couve Portuguera	Portugal	Santos et al. 1996

(DIR-1507, DIR-1522) and *B. napus* (GS-7027, Midas, Tower)) had stable and MDR (Dang et al. 2000). The segregation pattern of F_2 generation and testcrosses indicated that WR resistance in *B. juncea* cvs. Domo and Cutlass is under the control of a single dominant gene (Sachan et al. 2000). The hybrids *tourn-CMS* × BJ-38 and *oxy-CMS* × PR-1108 exhibited significantly superior specific cross combinations for disease resistance (Sheikh and Singh 2001). A new *B. rapa* cultivar AC Sungold registered in Ontario, Canada, and derived from the cross between cultivars Tobin and SV8236580, has good resistance to WR (Woods and Falk 2001).

The F_2 population of Rajat × Shiva, Pusa bahar × Domo, Varuna × EC 322092 and RH 30 × HC-1 fitted well in the ratio of 3R:1S, as well as 13R:3S, suggested that the resistance to *A. candida* was controlled by a single dominant gene, or two genes with dominant recessive epistasis. The F_2 population of cross, RH 30 × EC 322093 segregated in the ratio of 15R:1S which indicated the presence of two dominant resistant gene(s) in EC 322093 (Krishnia et al. 2000). Gen-

Table 10.2 Sources of multiple disease resistance (MDR) in rapeseed-mustard. (Saharan and Krishnia 2001)

R	R	R	R
WR and AB	WR and PM	AB and PM	WR, AB and PM
B. juncea			
EC-322090	EC-129126-1	None	None
EC-322092	EC-129126-2		
EC-322093	PR-8805		
MCB-1,BIOYSR			
EC-399296			
B. rapa			
None	Candle	None	None
	Tobin, Torch		
B. carinata			
HC-1	DHC-1	HC-1	HC-1
PCC-2	DHC-4	PCC-1	PCC-2
	DHC-9601		
	HC-1		
	PCC-2		
B. napus			
GS-7027	GSL-1501	GSL-1501	GSL-1501
GSL-1	N-20-12-2		
GSL-1501	Wester		
Gulivar			
HNS-4			
Tower sel-1			

WR white rust, *PM* powdery mildew, *AB* alternaria blight

otypes, EC-129126-1, Shiva, RC 781, ZEM-1 and PR-8805 were found resistant to the WR disease (Gupta et al. 2002). Four Chinese genotypes (CBJ 001, CBJ 002, CBJ 003, CBJ 004) and two Australian genotypes (JR 049, JM06011) were highly resistant to *A. candida* pathotypes prevailing in Australia (Li et al. 2007, 2008).

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11 Fine Structures

11.1 Haustoria

The fine structures of *Albugo candida* were studied by Berlin and Bowen (1964a, b), Davison (1968), Soylu et al. (2003), Soylu (2004) and Coffey (1975). The small stalked, capitate haustoria are connected to the much larger haustorial mother cell by a slender cylindrical neck. Haustoria contain mitochondria with tubular cristae, ribosomes and occasional cisternae of rough endoplasmic reticulum (RER). Nuclei and perinuclear dictyosomes, although present in the mother cells, but are absent in the haustoria. The fungal plasma membrane and cell wall are continuous from an intercellular hypha to the haustorium except that there is no evidence of a fungal cell wall around a portion of the haustorial stalk proximal to the haustorial head. In the host mesophyll cell, the haustorium is invariably surrounded by host plasma (HP) membrane, and/or by a thin layer of host cytoplasm. The host cell wall invaginates at the point of haustorial penetration to form a short sheath around the penetration site, but the host cell wall is absent from rest of the haustorium. A collar consisting of fibrillar material is commonly found around the proximal portion of the neck. An electron-opaque encapsulation lies between the haustorium and the HP membrane, and extends into the penetration region between the sheath and the fungal cell wall. An electron-opaque sheath surrounds the thin wall of the haustorial body but is absent from the neck region. A series of tubules is continuous with the invaginated HP membrane, which surrounds the haustorial body. These tubules contain an electron-dense core similar in appearance to, and continuous with, the sheath matrix. Host dictyosomes and their secretory vesicles are not involved in formation of the haustorial sheath. A constant feature of the haustorial apparatus is the association of flattened cisternae of host endoplasmic reticulum (ER) with the distal portion of the haustorial neck. In *A. candida*, Woods and Gay (1983) provided evidence for the presence of a neck band delimiting structural and physiological regions of the HP membrane associated with haustoria. Coffey (1983), demonstrated cytochemical specialization at the haustorial interface. Soylu et al. (2003) and Soylu (2004) observed that ultrastructural nature of the haustorium in *Arabidopsis* is clearly different from the downy mildews, white rust or the Powdery mildew (PM) fungi. Whether haustoria of *A. candida* resemble those produced by different *Albugo* spp. is unknown. Further study is needed to compare ultrastructural features of *A. candida* with other *Albugo* spp. including *Albugo occidentalis* on spinach, and *Albugo tragopogonis* and *Albugo bliti* on species of the Compositae and Amaranthaceae, respectively. Ultrastructures of *A. candida* infecting *Arabis alpine* has been observed by Baka (2008).

Transmission electron microscopy, revealed coenocytic intercellular hyphae in the intercellular spaces of host leaf tissues. The cytoplasm of intercellular hyphae contains many ribosomes, ER, lipid bodies, large and small vacuoles, mitochondria and spherical nuclei. Further growth of hyphae within intercellular spaces of host leaf

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tissue, and penetration of individual host mesophyll cell, led to the formation of haustoria. The first sign of penetration of pathogen to host cell was the formation of a penetration peg originated from the intercellular hypha. A modified thickened wall of the intercellular hyphae during penetration is observed. The haustorial body was spherical and connected to intercellular hypha by a narrow, slender cylindrical neck. Median section through penetration site shows the fungal wall to be continuous from the intercellular hypha along the entire neck of the haustorium and around the haustorial body. In the proximal region of the haustorial neck, a light staining penetration jacket, a term coined by Coffey (1975), and collar by Koch and Slusarenko (1990) is found immediately adjacent to the fungal wall.

The collar appeared to be an extension of the plant cell wall and is bounded by HP membrane. Haustoria in infected cells are surrounded by an invagination of a modified form of HP, the extrahaustorial membrane (EHM). The unit membrane structure of the HP is distinct around the penetration jacket, which encased the haustorial neck and haustorial body. The EHM around haustorial body is highly convoluted and continuous with tubular elements that extended into the host cell cytoplasm. The EHM is separated from the wall of the haustorial body by an electron-dense extrahaustorial matrix (EX). The EX is variable in width, appeared only around the haustorial body, and absent from neck region. The haustorial body is surrounded by light-stained wall and contained many mitochondria, vacuoles, ribosomes, smooth ER, lipid bodies and nucleus. Host RER is observed adjacent to haustorium. A wide foamy sheath filled with small vesicles surrounds the necrotic haustorium (Baka 2008).

11.2 Sporangia

Paramural bodies are formed in sporangia of *A. candida* solely by elaborations of the plasma membrane. Two major forms have been recognized, one consisting of invaginations of the plasma membrane projecting into the cytoplasm, and the other appearing like pockets containing vesicles and tubules. The first may be the basic form of the paramural body. In sporangia, the paramural bodies break away from the plasma membrane and undergo auto-digestion. In vegetative hyphae, the tubules and lamellae of paramural bodies break up into vesicles and are finally sequestered into the cell wall (Khan 1976a, b, 1977). The surface layer of the cell wall of the sporangia and sporangiophores of *A. candida* is composed of a series of lamellae. Evidence from freeze-fracture, freeze-etch and single-stage replicas demonstrated that the lamellae are bilayered, an organization associated with the presence of lipids. This multi-lamellate layer on the surface of the cell wall facilitates air dispersal and protects the sporangia from desiccation (Tewari et al. 1980). In *Albugo*, sporangia are produced in basipetal chains at the apices of sporangiophores and are released by the dissolution of the septa that delimit them. Hughes (1971), suggested that sporangiophores of *Albugo* produce sporangial chains by per current proliferation and that they are "apparently the morphological equivalents of annellophores (annellides)" (Hughes 1971a, b). A sporangium initially buds out from a fixed locus at the tip of the sporangiophore. After reaching a certain size, it is delimited by a basal septum and converted into a sporangium. A new initial grows out from the sporogenous locus, pushing the newly formed sporangium upwards. By repetition of this process, a basipital chain of sporangia is formed. Both layers of the sporangiophore wall grow out and take part in forming the sporangial wall. In conidium ontogeny, this mode of development is called holoblastic. During sporangial formation in *A. candida*, the sporangiophores do not increase in length; however, abnormally long sporangiophores are sometimes seen among the smaller, regular ones. There are no annellations on the sporangiophore surface and no increase in the thickness of the sporangiophore wall at its apex. Thus, none of the characteristics that have been shown to be associated with per current proliferation are present during the development of sporangia in *Albugo* (Khan 1977). In maturing sporangia, a burst of activity was observed by Khan (1976a, b). Even after formation of sporangia, the numbers of mitochon-

dria and the amounts of ER increase. Perinuclear vesicles and smooth surface cisternae differentiate into well-developed golgi apparatus, which remains secretory until complete maturation of sporangia. Maturing sporangia have autophagic vacuoles containing various cell organelles. Nuclear degeneration and mitosis proceed simultaneously. All activities decline towards the end of sporangial maturation. Golgi dictyosomes become quiescent and the numbers of mitochondria and amounts of ER decrease. There is a threefold increase in the thickness of the sporangial wall during maturation.

Scanning electron microscopy (SEM) revealed that zoosporangia of *A. candida* are produced in small to large and often found in confluent sori near the abaxial surfaces of infected leaves of *Albugo alpina,* and become exposed when the leaf epidermis ruptures. Zoosporangia are borne in chains from short club-like sporangiophores that lined the sorus base. As zoosporangia mature, they separate from one another and accumulate in the sorus cavity. Mature zoosporangia are spherical or ellipsoidal in shape and measure 12–17 to 13–18 µm. Except for a faint terminal secession scar at each end, the zoosporangium surface appears smooth when viewed with SEM (Baka 2008).

11.3 Oospores

The structure and development of oospores of *A. candida* in the stagheads of rapeseed (*Brassica rapa*) investigated by light microscopy (LM), transmission electron microscopy of ultrathin sections and SEM demonstrated that the development of an oospore was similar to that in *Pythium* (Tewari and Skoropad 1977). A reaction zone forms on the oogonial wall at the point of contact by the fertilization tube of the antheridium. The oospore has a highly differentiated, five-layered cell wall. The periplasm appears to play an active role in the deposition of the oospore cell wall. The contents of the periplasm do not disappear after maturation of the oospore; instead, they form a persistent material between it and the oogonial wall. Hence, functionally, the oospore wall complex has two additional layers, which may contribute to the longevity of the oospore (Tewari and Skoropad 1977). In a histochemical study of cytoplasmic changes during wall layer formation on the oospore of *A. candida*, Kaur et al. (1984) reported that the young multinucleate oogonium is double walled. The oospore nuclei are large and prominent and have an outer shell or sheath of proteinaceous material surrounding a central core of nucleoplasm. The first wall of the fertilized oospore is laid at the interphase of the periplasm and the ooplasm. Subsequent wall layers are formed both on the inner and outer side of the first oospore wall. The second oospore wall is formed just internal to the first one. The third wall of the oospore is formed external to the first one and appears ridged. The last wall to be formed is the innermost one, which completely surrounds the central ooplasm. This wall layer is callosic in nature. Oospore morphology is basically reticulate.

Voglmayr and Riethmuller (2006), studied the oospore wall ornamentation of *Albugo* species with LM and SEM. Enzyme digestion of the host tissue and oogonial wall with driselase works well in most cases; however, in *A. candida* and *Albugo ipomoeae-panduratae,* the oogonial wall is resistant to driselase digestion. Comparison of non-digested and digested oospores wall with both LM and SEM showed that the oogonial wall is not altered markedly during driselase treatment.

LM and SEM of the oospores showed various types of ornamentation. Oospore ornamentation was observed to be species specific. Oospores of the species are either smooth (*A. ipomoeae-panduratae*), coarsely bluntly verrucose (*A. candida*), irregularly reticulate (*A. bliti*) or more regularly reticulate (*Albugo achyranthis, Albugo amaranthi, Albugo gomphrenae, A. occidentalis, Albugo platensis, Albugo portulacae*), with a small to wide reticulum depending on the species. SEM, shows that some of these distinctly reticulate ornaments have tubercles on them (*A. achyranthis, A. amaranthi, A. occidentalis, A. portulacae*). Within *Albugo caryophyllacearum,* ornamentation can vary between irregularly verrucose with confluent verrucae to incompletely

reticulate, often even within the same oospore. *A. tragopogonis* , usually shows a combination of a very fine reticulum with fine tubercles in LM, but in SEM the reticulum is often not apparent and ornamentation appears to be distinctly finely verrucose. The reticulate oospores of *A. achyranthis* measure 50–65 mm, and the irregularly penta- to heptagonal meshes measure 4.5–7 mm diameter. SEM investigations of oospores of *A. candida* and *A. ipomoeae-panduratae* proved to be difficult due to the pronounced resistance of the oogonium wall to enzyme digestion. However, in *A. candida* it was possible to mechanically remove the oogonium wall without severe damage in a few cases. In *A. ipomoeae-panduratae*, this was not successful as the oospore wall appears to be firmly connected to the inner oogonium wall. In contrast to the other species investigated, the persistent oogonium wall of *A. ipomoeae-panduratae* was distinctly verrucose.

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12 Biochemistry of Host–Pathogen Interaction

Biochemical studies on the growth and survival of a pathogen and of the changes it induces in its host can ultimately lead to a better understanding of epidemiology, disease development, and control. With a few exceptions, such studies on white rust (WR) lag far behind those for diseases caused by other major groups of biotrophs. Ideal prerequisites for meaningful studies of the biochemistry of host–parasite interaction are (a) a clear understanding of the genetic control of virulence and avirulence in the parasite, and of susceptibility and resistance in the host; (b) precise histological and cytological descriptions of spore germination, infection, and the establishment and development of the infection; and (c) the availability of methods for growing the parasite alone and in combination with its host under controlled conditions. Unfortunately, these criteria have not been fully satisfied for any WR disease. Reduction in sugar content was proportionate to the disease severity and maximum reduction was observed in the infected leaves. Total free amino acids increased after infection in all the infected plant parts, and this increase was proportionate to the disease severity (Singh 2005).

12.1 Carbohydrate Metabolism and Respiration

A characteristic feature of the infection of plant tissues by Uredinales and Erysiphaceae is a two to four-fold increase in the rate of respiration (Heitefuss and Williams 1976; Scott 1972). Most evidence suggested that in these cases host respiration is shifted from a system that is predominantly channeled through glycolysis and the Krebs cycle to one that is dependent on the pentose phosphate pathway (Cooke 1977). Increases in the activities of metabolites produced in the Krebs cycle have also been reported, but the relative contributions of this, and the pentose phosphate pathways are not clear. A number of reports indicate that the respiration rates of tissues infected by members of the Albuginaceae also rise dramatically (Black et al. 1968a, 1968b; Williams and Pound 1964). However, the limited evidence so far suggests that such increases do not reflect any significant change in the pentose phosphate pathway. Radish cotyledons infected with *Albugo candida* increase respiration threefold, but the c1/c6 ratio, together with evidence from inhibition and feeding experiments, suggests that this is due to stimulation of existing pathways and does not involve the pentose phosphate pathway (Black et al. 1968a, 1968b; Williams and Pound, 1964). Long and Cooke (1974) suggested that host–fungus movement of carbohydrates in *Albugo–Senecio squalidus* system is maintained by hydrolysis of host sucrose and uptake of hexoses, followed by accumulation of trehalose within the mycelium and spores. Trehalose was synthesized within pustules by the fungus, but no acyclic polyols were found. Accumulation of hexoses around pustules together with increased hydrolysis of exogenous sucrose by pustular material indicated increased invertase activity within infected tissues. Accumulation of

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dark-fixed carbon compounds in WR pustules of *Senecio squalidus* infected with *Albugo tragopogonis* has been reported (Thomton and Cooke 1970).

In the leaves of *Crambe maritima* infected with *A. candida*, there is reduction in ionic content (Na^+, K^+, Ca^{2+}), pigments like chla, chlb and carotenoids, but increase in sucrose, reducing sugars and polysaccharides. In addition, increased amount of amino acids like glycine, alanine, methionine, and histidine has been observed by Aldesuquy and Baka (1992).

Eight days after inoculation of leaves, white blisters became visible on the lower surface of the leaf, although no symptoms were apparent on the upper surface. By day 14, the region of leaf invaded by fungal mycelium had become chlorotic (Chou et al. 2000). It has been hypothesized that an accumulation of soluble carbohydrates, following an increase in invertase activity, may trigger sugar signal transduction pathways leading to the repression of photosynthetic gene expression, and to the induction of defense proteins. This hypothesis was investigated by quantifying localized changes in carbohydrate and photosynthetic metabolism, and the expression of genes encoding photosynthetic and defense proteins. Quantitative imaging of chlorophyll fluorescence revealed that the rate of photosynthesis declined progressively in the invaded regions of the leaf. However, in noninfected regions of the infected leaf, the rate of photosynthesis was similar to that measured in the control leaf until later on during the infection cycle when it declined. Images of nonphotochemical fluorescence quenching (NPQ), suggested that the capacity of the Calvin cycle had been reduced in infected regions, and that there was a complex metabolic heterogeneity within the infected leaf (Chou et al. 2000).

Albugo candida also caused localized changes in the carbohydrate metabolism of the leaf; soluble carbohydrates accumulated in the infected region whereas the amount of starch declined. The reverse was seen in noninfected regions of the infected leaf; carbohydrates did not accumulate until later on during infection, and the amount of starch increased as the infection progressed. There was an increase in the activity of invertases which was confined to regions of the leaf invaded by the fungal mycelium. The increase in apoplastic invertase activity was of host origin, as mRNA levels of the ATb FRUCT1 gene (measured by semiquantitative RT-PCR) increased 40-fold in the infected region. The increase in soluble invertase activity resulted from the appearance of a new isoform in the invaded region of the leaf. Current evidence suggests that this is of fungal origin. Northern blot analysis of cab and rbcS showed that photosynthetic gene expression was repressed in the infected leaf from 6 days after inoculation compared to non-inoculated control leaves. In contrast, there was no detectable induction of defense proteins in the infected leaf which are discussed in the context of the sugar-sensing hypothesis presented above (Chou et al. 2000).

The resistant and moderately resistant cultivars contained higher amounts of chlorophyll, sugars, and total phenols than the susceptible cultivar at all growth stages. Total proteins and free amino acids, however, were higher in the susceptible cultivar at all growth stages (Singh 2000).

Higher starch contents were found in noninfected tissues, which suggest that this could be due to the higher alpha-amylase activity in diseased tissues (Debnath et al. 1998). Chlorophyll has a positive role in *A. candida* resistance in Indian mustard (Gupta et al. 1997). Thaumatin-like protein (PR-5), a protein not previously associated with the resistance of *Brassica juncea* toward *A. candida*, was detected. One protein, peptidyl-prolyl cis/trans isomerase (PPIase) isoform CYP20-3, was only detected in the susceptible variety and, its amount increased abundantly in response to the pathogen. Peptidyl-prolyl cis/trans isomerase have recently been discovered to play an important role in pathogenesis by suppressing the host cell's immune response (Kaur et al. 2011a).

12.2 RNA Content

In *lpomoea*, there was the greater reduction of RNA content in WR infected tissues than in the healthy, adjacent tissues (Misra and Padhi 1981).

12.3 Photosynthesis

Using infrared CO_2 analysis, Black et al. (1968a, 1968b) demonstrated that a decline in the photosynthetic rate in the *A. candida*-infected radish cotyledons was preceded with rise in respiration rate (Williams and Pound 1964). In another study, Harding et al. (1968) examined the pattern of pigment retention during green island development following infection of *B. juncea* cotyledons with *A. candida*, and also studied both the photosynthetic capacity and the ultrastructure of chloroplasts within the green island tissue. They found that labelled glycine-2-^{14}C was incorporated into chlorophylls a and b in both infected and non-infected tissues. Both infected and non-infected tissues fixed $^{14}CO_2$ in the light, but 4 days after infection, green islands fixed five times more $^{14}CO_2$ in the light than did noninfected tissue. Photosynthesis per mole of chlorophyll fell at the same rate in Green Island as in noninfected tissue. The maintenance of chlorophyll, and continued photosynthetic activity in green island tissue, was paralleled by delayed breakdown of chloroplasts. Electron microscopy showed that these retained their structural integrity longer in green islands than in noninfected tissue. The authors drew attention to the similarity in *B. juncea* between green islands and tissues treated with kinetin. Studies of the effects of WR and related pathogens on photosynthesis have been superficial, hence, meaningful comparisons with the rusts and powdery mildews are difficult. Extensive research has indicated that the overall activity of photosynthetic pathways declines in leaves infected by rusts and powdery mildews, and is accompanied by a decrease in chlorophyll content of the tissue (Cooke 1977; Daly 1976). Around the sites of infection, however, green islands of chlorophyll are retained, and within them, photosynthesis continues. Thus, the green islands are regions within which synthetic processes are maintained, and the movement of metabolites from host to parasite may continue even after the rest of the leaf has become nonfunctional. Enhanced fixation of CO_2 in the dark also occurs in green islands, but this appears to be due to the ability of the fungus itself to fix CO_2 in the dark. The similarity between green islands and the sites of exogenous application of cytokinins to plant tissues has been widely noted, although there is no clear evidence that these or other plant growth substances, are actually responsible for the green island phenomenon.

12.4 Accumulation of Metabolites

Long et al. (1975) demonstrated that infection of *S. squalidus* by *A. tragopogonis* results in an increase in the activity of acid invertase at the infection site. They concluded that sucrose from the host is first hydrolyzed to glucose and fructose, and then absorbed by the parasite. They also suggested that invertase may play a key role in the provision of substrate for the accumulation of starch at infection sites; where there is a surplus of soluble carbohydrate, particularly sucrose, hydrolysis by invertase might provide hexose for starch synthesis within chloroplasts. Invertase may thus mediate a system by which the excess soluble carbohydrate at infection sites is converted to osmotically inactive polysaccharides. In contrast, Whipps and Cooke (1978a) suggested that starch does not accumulate in *S. squalidus* infected by *A. tragopogonis*. Further research is required to resolve this conflict. Dhingra et al. (1982) found decreased amounts of free protein, total protein and total phenolic compounds in floral parts and floral axes of *Brassica rapa* infected with *A. candida*. Dhawan et al (1981) and Pruthi et al. (2001) correlated resistance of *Brassica juncea* cv. RC-781 with higher concentrations of phenols compared with the susceptible cultivars Prakash, Varuna and RH-30, where greater amounts of sugars were present.

Components of disease reaction, including incubation period, pustule types, inoculum production and disease index (DI), and contents of protein, phenols, soluble, reducing and nonreducing sugars were investigated in cotyledonary and true leaves of *A. candida*-infected *B. juncea* cvs. Varuna, Kranti, EC-399296, EC-399299, EC-399313 and EC-399301. Disease indices (DIs) at the cotyledonary leaf stage in the above six genotypes were 67, 65, 32, 31, 31, and 38%, respectively;

DIs 21, 28, 12, 17, 9, and 4% respectively at 14 days after inoculation, and 35, 45, 17, 19, 20, and 6%, respectively at 21 days after inoculation Protein contents were highest in the genotypes with the highest DIs (Varuna at the cotyledonary leaf stage and Kranti at the true-leaf stage), and lowest in the genotypes with the lowest DIs, (EC-399299 at the cotyledonary stage and EC-399301 at the true-leaf stage). Total phenols, and total reducing and nonreducing sugars were generally negatively correlated with DI, but were not always consistent, particularly when differences in DI were small. The results indicated that factors conditioning the response of host genotypes to *A. candida* infection may differ or operate in different ways at different growth stages (Mishra et al. 2009).

In WR-infected leaves of *Achyranthes aspera*, Purohit et al. (1980) observed gradual increases in peroxidase and IAA-oxidase activities with increased severity of WR infection. A decrease in total and o-dihydroxy phenols with increased poly-phenol-oxidase activity was observed in infected leaves compared in healthy tissue. A gradual increase in enzymatic activity with loss of phenolics shows a state of high catabolism induced by pathogenesis. Singh et al. (1980) demonstrated that cellulase, endo-PMG and endo-PG were produced in *B. juncea* leaves infected with *A. candida*. According to Maheshwari and Chaturvedi (1983, 1985a), the swelling and disruption of subcellular particles rich in lysomal acid hydrolases was produced by acid phosphatase activity centered primarily in the infected tissues of *B. juncea*. Acid phosphatase activity in antheridia, oogonia, and oospores of *A. candida* indicates that the enzyme plays a very important role in the synthesis of fungal organs.

12.5 Growth Substances

Infection of host plants with *Albugo* causes hyperplasia and hypertrophy of leaf, stem, and floral parts. Kiermayer (1958) found that these symptoms are produced in plants infected with *A. candida* due to the production of indole acetic acid (IAA). Hirata (1954, 1956) found that infection with *A. candida* causes an initial increase in diffusible auxin in diseased stems and leaf sections, followed by a decrease before maximum development of the galls. The auxins in healthy and *Abugo*-infected inflorescences of *Brassica napus* have now been identified and estimated quantitatively by Srivastava et al. (1962). Malformed *B. napus* inflorescences produce IAA, indole-3-acetonitrile (IAN), accelerator L, and an ether-insoluble growth substance designated as A.

Kumari et al. (1970) and Lal et al. (1980) studied the quantitative and qualitative changes in the amino acid contents of diseased (hypertrophied) and healthy tissues of mustard and radish, and suggested that the amino acid contents are greatly changed because of disruption in the metabolism of plant organs under the influence of a pathogen (Kumari et al. 1970). The infection causes the breakdown of plant proteins, releasing small quantities of tryptophan, which reacts with endogenic phenolic acid to produce IAA which is responsible for hypertrophied growth.

12.6 Fatty Acid Composition

Fatty acids (FAs) play an important role as the major energy storage in oomycetes. Some of them may as well be an important factor for normal growth and development of these organisms (Herman and Herman 1985). The composition of fatty acid patterns in three WR species, *Aposphaeria amaranthi, A. candida, and A. tragopogonis*, was determined by Spring et al. (2005). The fatty acid composition of these biotrophic WR species was identified by means of gas chromatography (GC) and GC/mass sprectometry (MS) analyses. Besides the common saturated fatty acids of C14–C22 chain length, several unusual monounsaturated and polyunsaturated fatty acids were present in significant amounts in the lipid profiles of the three species; each taxon revealed its own characteristic pattern which was uniform among populations of different geographic origin, and independent from the host species. Fatty acids were obtained from minute amounts of sporangia collected from the surface of desiccated host plants. Comparison of herbarium specimens

Table 12.1 The fatty acid composition (identified by GC/MS analyses; ratio to total peak) of *A. amarathi* (Aa568), *A. candida* (Ac505), and *A. tragopogonis* (At567). (Spring et al. 2005)

Fatty acid	*Amaranthi*	*Candida*	*Tragopogonis*
14:0	0.4	5.3	2.6
16:0	21.5	9.1	41.7
16:1n-9c[a]	–	3.0	–
16:1n-7	1.3	1.2	7.4
16:2[b]	0.4	0.1	0.6
18:0	21.3	26.3	11.5
18:1n-9	4.5	16.8	3.3
18:2n-6	26.6	10.3	13.9
18:3n-6	7.3	6.3	2.0
18:3n-3	4.3	1.7	3.6
18:4n-3	0.9	1.7	1.7
20:0	4.3	2.7	2.6
20:3n-3	–	0.7	0.7
20:4n-6	–	3.3	7.0
20:5n-3	–	1.3	0.5
22:0	1.6	2.1	1.1
24:0	–	tr	0.3

tr trace amounts (<0.1), – not detected
[a] No standard available, position of the double bond tentatively assigned
[b] Position of the double bond not determined

revealed a decrease of polyunsaturated compounds after storage for 30 months at room temperature, but still allowed unquestionable species differentiation (Table 12.1).

12.7 Phytoalexins and Polar Metabolites

In general, the metabolic responses of plants vary according to the type of stress. These responses can be rather specific, since the metabolic pool of plant defenses is composed of a variety of constitutive and induced metabolites. Phytoalexins are induced antimicrobial metabolites produced de novo by plants in response to biotic or abiotic stress, whereas phytoanticipins are constitutive metabolites with a defensive role (Pedras et al. 2007b). *B. rapa* canola is reported to produce several phytoalexins after $CuCl_2$ sprays (Rouxel et al. 1991). The metabolite profiles of phenylpropanoids in turnip leaves after insect infestation (Widarto et al. 2006), and methyl jasmonate sprays (Liang et al. 2006a, 2006b) are reported.

Obligate biotrophs are capable of penetrating and colonizing plant host tissues in ways that prevent immediate recognition by the host. It has been suggested that a biotroph may induce production or suppression of different proteins in the host, and suppress induced resistance mechanisms such as phytoalexin production and hypersensitive host cell death (Mendgen and Hahn 2002; O'Connell and Panstruga 2006). Recently, a mutant of *Arabidopsis thaliana* that accumulated significantly higher levels of the phytoalexin camalexin was reported to be more resistant than wild-type plants to virulent strains of the biotrophic pathogen *Hyaloperonospora parasitica* (Veronese et al. 2004). Nonetheless, little is known about the chemical defense pathways of plants, including phytoalexin elicitation and accumulation during biotrophic infections (Mendgen and Hahn 2002; O'Connell and Panstruga 2006).

Phytoalexins, phytoanticipins, and polar metabolites from leaves of *B. rapa* cvs. Torch and Reward with four races of *A. candida* provide a model to analyze metabolic responses in compatible and incompatible interactions between a crucifer and a biotroph. This work established consistent phytoalexin production in response to

inoculation with a biotroph (*A. candida* races 2V, 2A, 7V, and 7A). Although, the accumulation of spirobrassinin, cyclobrassinin, and rutalexin in leaves inoculated with *A. candida* races 7V, 7A, 2A, or 2V, was similar during the first 4 days, their concentrations decreased, and eventually undetectable, in incompatible interactions; in the compatible interactions the concentrations of all three phytoalexins increased, with the highest concentration at 7–10-days postinoculation. These results suggest that during the initial stage of the interaction, leaves of *B. rapa* have a similar response to avirulent and virulent races of *A. candida* with respect to the accumulation of chemical defenses. After this stage, despite the higher phytoalexin concentration, the "compatible" races could overcome the plant defense system for further infection, but growth of the "incompatible" races was inhibited. The induction of invertase activity and defense proteins occurred very rapidly in leaves of *A. thaliana* after the initial challenge with *A. candida* in the incompatible interaction. By contrast, in compatible interactions (*A. candida–A. thaliana*) invertase activity, accumulation of sugars, and the repression of photosynthetic gene expression occurred several days after infection (Chou et al. 2000; Pedras et al. 2008).

Lower amounts of cyclobrassinin and higher amounts of rutalexin in infected leaves, and absence of rutalexin in $CuCl_2$-sprayed plants, suggest that these metabolites (and/or their biosynthetic precursors) play an important role in the plant–pathogen interaction. Results of bioassays showed that cyclobrassinin and brassilexin were stronger inhibitors of *A. candida* than rutalexin; this apparent redirection of the phytoalexin pathway toward increased amounts of rutalexin, and decreased level of cyclobrassinin and brassilexin accumulation might be caused by the pathogen. This exchange would, therefore, favor the pathogen which consistently lacks brassilexin production in infected plants, since, brassilexin is produced only in $CuCl_2$-sprayed leaves. This hypothesis appears reasonable, since, cyclobrassinin is a biosynthetic precursor of both rutalexin and brassilexin, and the biosynthesis of rutalexin from cyclobrassinin is unlikely to involve more than three enzymatic steps (oxidation, hydrolysis, and methylation) (Pedras et al. 2007b). Alternatively, *A. candida* might be able to detoxify both cyclobrassinin and brassilexin, similar to necrotrophic fungi (Pedras and Ahiahonu 2005). Although indolyl-3-acetonitrile, arvelexin, and caulilexin C were previously reported as phytoalexins of a few plant species (Pedras et al. 2007b), these metabolites are phytoanticipins but not phytoalexins in cv. Torch and Reward, because they were detected in control leaves also, and their production increased upon elicitation. In a study using 43 accessions of *Brassica* species, it was found that a *B. rapa* line 29 produced the phytoalexins cyclobrassinin, brassilexin, and cyclobrassinin sulfoxide 48 h after spraying with $CuCl_2$ (Rouxel et al. 1991).

Different types of polar metabolites, including esters and glucosides of hydroxycinnamic acid and their malic derivatives, flavonoids, ionone glucosides, amino acids, and nucleotides were isolated from leaves of cv. Torch infected with *A. candida* (Pedras et al. 2008). Among this large pool, quantitative differences were detected in leaf extracts by HPLC for indole glucosinolates and tryptophan. In fact, the production of 4-methoxyglucobrassicin (13b) was substantially higher in sprayed than in control leaves (Pedras et al. 2008). These results are consistent with the accumulation of higher amounts of arvelexin (10, tR = 14.5 min) in sprayed-leaves, as glucosinolates are known to give nitriles, isothiocyanates, etc., upon enzymatic degradation (Bones and Rossiter 2006). Also, since crucifer phytoalexins are biosynthesized from tryptophan, an increase of its concentration was expected; the highest concentration of tryptophan was detected 8 days after elicitation. Nonetheless, since tryptophan is a biosynthetic precursor of many different metabolites, its concentration increase could be related with other biosynthetic pathways. Previously, it was reported that polar metabolites from cruciferous plants, including glucosinolates (Fahey et al. 2001), polar indole metabolites (Hahlbrock et al. 2003), soluble compounds (Hagemeier et al. 2001), and wall-bound phenolics (Tan et al. 2004), were induced and might play an important role in crucifer defense. Glucosyl

indole-3-carboxylic acids were identified from *A. thaliana* roots infected with *Pythium sylvaticum* (Bednarek et al. 2005). Also, monolignol glucosides, e.g., coniferin, were characterized from *A. thaliana* roots infected with *P. sylvaticum,* but their biological functions were not established (Whetten and Sederoff 1995).

Naturally-occurring phenylpropanoids such as hydroxycinnamic acid, caffeic acid, ferulic acid, and sinapic acid can be present in plants as glucose esters and glucosides, or as esters of organic acids, such as quinic acid, malic acid, and tartaric acid. The six new methyl malates 14e–j were isolated from canola leaves and their structures were established using spectroscopic data and comparison with the corresponding free acid derivatives. The four hydroxycinnamoyl malic acids 14a–d as well as 5-hydroxyferuloyl malic acid were detected and characterized in extracts of turnip leaves by NMR analysis (Liang et al. 2006a, 2006b). The hydroxycinnamoyl malic acids 14a–d is also isolated from radish (Brandl et al. 1984). These phenolic derivatives appeared to be related with UV resistance, but not with pathogen defense (Li et al. 1993; Hagemeier et al. 2001). No substantial quantitative differences in the accumulation of these metabolites were detected in biotically or abiotically-stressed canola leaves (Pedras et al. 2008).

The correlations observed between phytoalexin production in infected leaves of canola and rapeseed (biotrophic elicitation), and the outcome of the plant-pathogen interaction, suggest that *A. candida* is able to elude the plant defense mechanisms by redirecting the phytoalexin biosynthetic pathway. Considering both the nonpolar and polar metabolite profiles, it is clear that canola metabolic responses to the biotroph *A. candida* are distinct from responses to abiotic stress.

12.8 Phytoalexins and Metabolites from Zoosporangia

The main chemical components of zoosporangia of *A. candida* races 2V and 7V were determined to ensure that induced metabolites (detected or isolated) were produced by canola leaves and not by sporangia. Zoosporangia contained the phytoalexin spirobrassinin (1, 7 nmoles/g of zoosporangia), rapalexins A and B (5, <2 nmoles/g of zoosporangia), and trehalose (37 mg/g of zoosporangia) (Pedras et al. 2008). The presence of spirobrassinin was not surprising, since this is a phytoalexin produced in leaves of many brassicas including brown mustard (*B. juncea*), canola and rapeseed (*B. rapa*) from where zoosporangia of races 2V and 7V were isolated (spirobrassinin may adhere to zoosporangia surface). On the other hand, rapalexins A and B are much less abundant than spirobrassinin, and therefore, their presence was less expected. Nonetheless, because these compounds are induced by $CuCl_2$ as well, there is no doubt that they are produced by the plant. Trehalose is a storage carbohydrate in microorganisms accumulated mainly during starvation, and has been connected with stress protection (Rolland et al. 2006), as well as, thermo- and cryo-protector and protein stabilizer (Voit 2003). Trehalose is one of the most important storage carbohydrates as it is present in almost all living organisms except mammals (Benaroudj et al. 2001).

It is worthy to note that the phytoalexins cyclobrassinin, rapalexin A, and brassilexin displayed substantially more potent inhibitory activity against zoospore release at much lower concentrations (ca. 2 mg/l) than the commercial fungicides benomyl and ridomil MZ (ca. 50 mg/l). As ridomil MZ was found to be one of the most active fungicides for treatment of *A. candida* infections (Godika and Pathak 2005), rapalexin A could be a good lead structure to develop natural protection agents. It would be of great interest to analyze lines of *Brassica* species to determine, if the production of cyclobrassinin, rapalexin A, and brassilexin correlates positively with resistance to *A. candida* (Pedras et al. 2008).

12.9 Enzyme Activity Changes

The quantitative analysis of peroxidase, α-amylase, invertase, IAA-oxidase, and ascorbic acid oxidase activity was observed in healthy and diseased *B. juncea* leaves and inflorescence

Table 12.2 Enzymatic activity from healthy and infected parts of *Brassica juncea* (Singh et al. 2011a)

Plant parts	Alfa-amylase (µg/g)	Invertase (µg/g)	Peroxidase (µg/g)	IAA-oxidase (µg/g)	Ascorbic acid oxidase (µg/g)
Healthy leaf	0.023	0.36	0.055	4.30	0.57
Infected leaf	0.033	0.13	0.076	2.75	0.32
Healthy inflorescence	0.031	0.52	0.36	2.55	0.67
Infected inflorescence	0.040	0.76	0.56	1.75	0.12

(Table 12.2). Maximum peroxidase activity was observed in infected leaves and disease inflorescence as compared to healthy leaves and infected inflorescence; α-amylase was maximum in healthy leaves and minimum in infected inflorescence. Maximum invertase activity was found in infected leaves of *B. juncea* and minimum in healthy leaves of *B. juncea*. IAA-oxidase activity was higher in infected leaves and inflorescence as compared to healthy leaves and inflorescence. The activity of ascorbic acid oxidase decrease with the increase in infection in plants (Singh et al. 2011b).

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13 Disease Management

The information on the management of white rust (WR) of cultivated crops have been generated on the aspects of fungicidal control, cultural control, host resistance, biological control and their use in an integrated ways as follows.

13.1 Fungicidal Control

Early work on the chemical control of WR diseases was focussed on the use of copper-based fungicides to control the leaf phase (LP) of the disease. Frickhinger (1932) and Neumann (1955) reported that *Brassica* diseases can be controlled by frequent sprayings with copper fungicides. Vasudeva (1958) recommended the use of bordeaux mixture or perenox for the control of WR of *Brassicas* and other diseases. With the progress in the development of dithiocarbamate, control of WR was attained with multiple applications of protectant fungicides. However, these fungicides provided little protection from the staghead phase (SP) of the disease. Acylalanines, which are specifically active against Peronosporales, have now been proven useful to control both the LP and SP of WR, either with a seed dressing or soil drenching, and with one or two foliar applications. Three sprays of 0.2 % polyram M at 15-day intervals were most effective in controlling WR of *Brassica rapa* var. Sarson in Pakistan (Perwaiz et al. 1969). Many fungicides have been evaluated for efficacy against WR of *Brassica* crops in India. Benlate 0.1 %, calixin 0.1 %, difolatan 0.2 %, dithane Z-78 0.2 %, miltox 0.3 %, thiovit 0.3 %, mancozeb 0.2 %, mancozeb + metalaxyl (ridomil-MZ) 0.05 % and ridomil-MZ 0.2 % are effective in controlling both leaf (LP) and SP of the disease and in increasing crop yield (Tables 13.1 and 13.2; Bains and Jhooty 1979; Gaur and Ahmed 1980, 1985; Gupta and Sharma 1978; Gupta et al. 1977; Kolte 1982, 1985a, b, 1987a; Lakra and Saharan 1988d; Saharan 1980, 1984; Saharan et al. 1982, 1984, 1990; Sharma and Kolte 1985; Singh et al. 1980, 2002).

For maximum disease control and high seed yield, treatment of seed with metalaxyl (6 g Apron SD-35/kg of seed) followed by three sprays of dithane M-45 or metalaxyl was recommended (Kolte 1985a, b; Saharan 1984; Saharan et al. 1984, 1990; Sharma 1983). Seed treatment with apron SD-35 protects the crop from *Albugo candida* infection for at least 60 days (Saharan et al. 1984, 1990). To control the SP, foliar sprays of mancozeb, metalaxyl or a mixture of mancozeb + metalaxyl are required (Kolte 1987a). Proper foliage cover and one spray coinciding with flower initiation are very important to arrest secondary spread of the disease and flower bud infection (Lakra and Saharan 1988a; Saharan et al. 1984, 1990) (Tables 13.3 and 13.4). Three foliar applications of fungicide at 15-day intervals after the appearance of the disease, or at 40–45 days of crop growth, control the disease effectively and economically (Table 13.5) (Saharan et al. 1984, 1990). Seed treatment with mercurial fungicides may be effective to control WR of rape (*B. rapa*) in Canada, since these compounds inhibit oospore germination or kill the zoospores on

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Table 13.1 Efficacy of different fungicides in controlling *Albugo candida* in mustard during 1985–1986 and 1986–1987. (Lakra and Saharan 1988)

Treatments	Disease index of leaf phase					Rate of disease development/month (r)	Disease index of systemic infection (110-day-old crop)	Yield (g/plot)
	Before first spray (60 days)	Before second spray (75 days)	Before third spray (90 days)	After third spray (105 days)	Final disease index (110 days)			
	1985–1986/1986–1987	1985–1986/1986–1987	1985–1986/1986–1987	1985–1986/1986–1987	1985–1986/1986–1987	1985–1986/1986–1987	1985–1986/1986–1987	1985–1986/1986–1987
Ziride at 0.2%	6.6/0.0	28.2/17.0	44.7/37.0	49.0/47.0	49.6/47.5	2.0/2.0	19.5/17.7	580.0/565.0
Galixin at 0.2/0.05 %	6.7/0.0	26.6/20.0	39.0/39.5	43.0/48.5	3.1/50.5	1.9/2.0	17.0/15.3	200.0[a]/601.2
Carbendazim at 0.1%	6.7/0.0	23.4/10.0	35.0/24.8	39.2/33.5	39.2/35.3	1.9/1.8	16.8/15.6	622.5/602.5
Difolatan at 0.2%	6.6/0.0	19.8/8.5	29.5/20.5	33.5/29.5	33.3/31.5	1.8/1.8	11.6/9.5	665.0/650.0
Mancozeb at 0.2%	6.7/0.0	16.7/6.7	26.7/17.5	31.5/26.0	31.5/26.4	1.8/1.7	10.0/8.0	747.5/732.5
Metalaxyl 25 WP at 0.2%	6.7/0.0	11.5/3.5	17.0/9.5	20.3/19.5	20.7/16.9	1.6/1.5	1.5/0.9	805.0/797.5
Control	6.7/0.0	40.0/28.5	66.5/60.0	70.0/68.0	71.5/70.0	2.2/2.2	27.1/26.9	482.5/475.0
Critical difference (CD) at 5%	NS/NS	1.29/0.96	1.34/2.39	1.59/2.07	3.54/3.36	0.1/0.1	3.85/2.39	50.37/36.74

Days in parenthesis = age of crop

NS non-significant

[a] Exteremly poor yield was due to the phytotoxicity of chemical and concentration, reduced from 0.2 to 0.05%

Table 13.2 Efficacy of chemicals on the rate of white rust development (leaf phase) in mustard during 1985–1986 and 1986–1987. (Lakra and Saharan 1988a)

Treatments	Initial amount of disease (x_0) in 52-day-old crops		Final amount of the disease (x) in 110-day-old crops		$t=t_2-t_1$ months	r=rate of disease development/month	
	1985–1986	1986–1987	1985–1986	1986–1987		1985–1986	1986–1987
Ziride at 0.2%	1.0	1.0	49.6	47.5	1.93	2.01	1.98
Galixin at 0.2% (1985–1986) and at 0.05% (1986–1987)	1.0	1.0	43.1	50.5	1.93	1.94	2.02
Bavistin at 0.1%	1.0	1.0	39.2	35.3	1.93	1.89	1.83
Difolatan at 0.2%	1.0	1.0	33.3	31.5	1.93	1.81	1.77
Dithane M-45 at 0.2%	1.0	1.0	31.5	26.4	1.93	1.77	1.68
Metalaxyl 25 WP at 0.2%	1.0	1.0	20.7	16.9	1.93	1.56	1.45
Control	1.0	1.0	71.5	70.0	1.93	2.20	2.19

t time in month between x and x_0, t_2 time when x (final amount of disease) was noted (110 days), t_1 time when x_0 (initial amount of disease) was noted (52 days)

Table 13.3 Efficacy of apron SD-35 and dithane M-45 against white rust of Indian mustard. (Saharan et al. 1990)

Treatment	Concentration (%)	Disease intensity (%)	Yield (q/ha)
Apron SD-35 ST	0.2	13.1 (21.2)	19.33
Dithane M-45 ST	0.2	25.1 (30.1)	17.31
Apron SD-35 ST + Dithane M-45 SP	0.1 + 0.1	20.1 (26.6)	18.94
Apron SD-35 ST + Dithane M-45 SP	0.2 + 0.2	10.1 (18.5)	20.71
Dithane M-45 ST + Dithane M-45 SP	0.2 + 0.2	20.3 (26.8)	19.29
Apron SD-35 ST + Dithane M-45 ST + Dithane M-45 SP	0.1 + 0.1 + 0.2	16.0 (23.5)	20.74
Dithane M-45 SP	0.2	22.8 (28.6)	19.51
Control	–	35.5 (36.4)	17.63
CD at 5%		11.69	0.093

ST seed treatment, *SP* spraying, *CD* critical difference

Table 13.4 Efficacy of different fungicides against white rust of mustard. (Saharan et al. 1990)

Treatment	Concentration (%)	Disease intensity (%)	Yield (q/ha)
Dithane M-45	0.2	24.2 (29.5)	20.3
Difolatan	0.15	13.3 (21.3)	20.8
Ziram	0.15	23.2 (28.8)	19.4
Ready-mix	0.5	16.8 (24.2)	19.0
Apron SD-35 ST + Ready-mix SP	0.2 + 0.5	12.6 (20.7)	19.9
Control		29.8 (33.1)	18.3
CD at 5%		03.31	0.12

ST seed treatment, *SP* spraying, *CD* critical difference

emergence (Table 13.6; Verma and Petrie 1979). Two applications of protectant fungicides like Bravo, manzate 200 and DPX 164 reduced foliar infection in turnip rape (Dueck and Stone 1979; Verma and Petrie 1979). Three sprays of acylalanine (metalaxyl) fungicides, CGA 2912 and CGA 48988, were the most effective in reducing staghead infections in rape (Berkenkamp 1980; Dueck and Stone 1979; Stone 1977; Stone and Dueck 1977). Stone et al. (1987a, b) advocated

Table 13.5 Effect of number of sprays and stage of plant growth on the control of white rust of mustard. (Saharan et al. 1990)

Number of sprays/days after sowing	Disease intensity (%)	Disease control (%)	Yield (q/ha)
30, 45, 60, 75	14.4 (22.3)	56.5	20.1
45, 60, 75	17.8 (24.9)	46.2	20.6
60, 75	25.2 (30.1)	23.9	18.5
30	30.3 (33.4)	8.5	17.3
45	31.2 (33.9)	5.7	17.3
60	29.8 (33.0)	10.0	17.7
75	32.4 (34.6)	2.1	17.4
Control	33.1 (35.1)	–	16.9
Critical difference at 5 %	10.457	–	0.14

successful control of *A. candida* race 7 in *B. rapa* cv. Torch through foliar, seed and soil applications of metalaxyl. Foliar applications at 2.0 kg a.i./ha reduced systemic or staghead infection when applied at growth stages 3.2 or 4.1. To control staghead infection, a fungicidal spray at the flowering stage is essential. Seed dressing alone with metalaxyl at 5.0 g a.i./kg, or soil drench applications, reduced primary infections from germinating oospores. This subsequently reduced sporangial inoculum on the foliage during the growing season, as metalaxyl uptake in rape is efficient through root absorption when applied to the soil. Using bioassays and chemical and gas chromatographic analyses of rapeseed plants grown from metalaxyl-treated seeds, and in metalaxyl-drenched soil, Stone et al. (1987b) showed that the fungicide was readily taken up by plants from the soil solution or from seeds. The greatest accumulation was in the lower leaves, with concentrations decreasing in the leaves farthest from the roots. Only small concentrations were detected in the stem and inflorescence. These results confirmed the observations of Sharom and Edgington (1982), indicating that root absorption is a major factor in metalaxyl uptake. The adsorption, mobility and persistence of metalaxyl in soil and aqueous systems are greatly influenced by soil type, amount of rain and other environmental factors (Sharom and Edgington 1982). Control of WR of radish was most effective when four sprays of difolatan 0.3 %, daconil 0.1 %, dithane M-45 0.2 %, ridomil 0.1 % or aliette 0.1 % were applied at 8–10-day intervals (Glaeser 1973; Holtzhausen 1978; Sharma and Sohi 1982). Dithane M-45 also increased yield significantly (Sharma 1983). In the past, brestan, captan and bordeaux mixture and zeneb were used to control WR of horseradish (Boning 1936; Frickhinger 1932; Hammarlund 1954; Kalchschmid and Krause 1976). However, later studies showed that mancozeb and metalaxyl were more effective in controlling the disease and increasing the yield of horseradish (Szith and FurIan 1979). Foliar sprays of captan and cyprex have been used to control WR of spinach (Chambers et al. 1974). Jones and Dainello (1983) suggest that effective control can be achieved with four sprays of metalaxyl 0.28 kg a.i./ha, chlorothalonil 1.17 kg a.i./ha or ethylene bisdithiocarbamate ethylene bisdithiocarbamate (EBDC) 3.59 kg a.i./ha. Tank mixing of only half the recommended rates of both metalaxyl and EBDC (0.15 + 0.58 kg a.i./ha) was as effective as the full rates of the individual compounds. Dainello and Jones (1983, 1984) studied the influence of continuous leaf wetness as a parameter for scheduling fungicide applications, and achieved the same level of control as the fixed schedule with metalaxyl and chlorothalonil. In Brazil, copper fungicides are recommended to control WR of *Ipomoea horsfalliae* (Goncalves 1955). Doepel (1965) suggested weekly sprays of zeneb or maneb 1 oz in 4 gallons of water to control WR of Gerberas.

Efficacy and economics of fungicidal management of WR and downy mildew (DM) complex in mustard was studied by Mehta et al. (1996). Three sprays of ridomil MZ-72 (metalaxyl + mancozeb) at 0.25 % at 20-day intervals starting 40 days after sowing gave maximum

Table 13.6 Percentage inhibition of germination of *Albugo candida* oospores by chemicals at a concentration of 500 ppm active ingredients. (Verma and Petrie 1979)

Product name[a]	Active ingredients' percentage and formulation	Source	Total percentage inhibition adjusted[b]
Bayleton	Triadimefon (L-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazole-1-yl)-2-butanone) 50 %, WP	Chemagro	24.1
Benlate	Benomyl (methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate) 50 %, WP	Du Pont	24.5
Bravo	Chlorothalonil (tetrachloroisophthalonitrile) 54 %, Fwble	Diamond-shamrock	21.2
Bromosan	Thiophanate-methyl (dimethyl-4.4–0-phenylenebis-(3-thioallophanate)) 16.67 % + thiram (tetramethylthiuram disulfide) 50 %, WP	Cleary	46.2
Calixin	Tridemorph (2, 6-dimethyl-4-tridecylmorpholine) 75 % Soln.	BASF	27.9
Chlorophenate	Chlorophenate mixture 18 %, WP	Cleary	56.7
Cyprex	Dodine (N-dodecylguanidine acetate) 65 %, WP	Cyanamid	24.9
Dexon-PCNB	p-dimethylaminobenzenediazo sodium sulfonate 35 % + pentachloronitrobenzene 35 %, WP	Chemagro	21.0
Dowco-269	Pyroxychlor (2-chloro-6-methoxy-4-(trichloromethyl) pyridine) 97 %, Soln.	DOW	46.5
DPX 3217	2-Cyano-N-(ethylaminocarbonyl)-2-(methoxyimino) acetamide 50 %, WP	Du Pont	55.9
Duter	Fentin hydroxide (triphenyltin hydroxide) 19 %, WP	Ciba Geigy	27.9
Kocide-101	Copper hydroxide 83 %, WP	Kennecott	13.2
Manzate-200	Mancozeb (zinc and manganese ethylene-bisdithiocarbamate) 80 %, WP	Du Pont	59.8
Mersil	Mercury chloride ($HgCI_2$) 14 % + mercurous chloride (Hg_2CI_2) 28 % + mercury equivalent 34 %, WP	May and Baker	75.7
N.F. 48	Thiophamine (2-(3-methoxycarbonyl-thioureido)-analine) 80 %, WP	Nippon Soda	22.5
N.F. 65	Thiophamine 40 % + bis-(dimethylthiocarbomoyl disulfide) 40 %, 80 %, WP	Nippon Soda	25.5
Panogen	Methylmercury dicyandiamide 0.9 %, WP	Morton	74.5
PMA-10	Phenyl mercuric acetate 10 %, Soln.	Later	75.7
Polyram	Metiram ((ammoniates of ethylene-bis-dithiocarbamate zinc) 83.9 % + (ethylenebis-dithiocarbamic acid)) 16.9 %, WP	Niagara	37.5
Sicarol	Pyracarbolid (2-methyl-5. 6-dihydro-4H-pyran-3-carboxanilide) 50 %, WP	Hoechst	19.8
Tersan SP	Chloroneb (1,4-dichloro-2, 5-dimethoxybenzene) 65 %, WP	Du Pont	22.0
Terraclor	Quintozene (pentachloronitrobenzene) 75 %, WP	Olin	20.4
Terrazole	Ethazole (5-ethoxy-3-(trichloromethyl)-1, 2, 4-thiadiazole) 35 %, P	Olin	59.8
Topsin M	Thiophanate methyl (dimethyl 4,4-0-phenylenebis-(3-thioallophanate)) 70 %, WP	Pennwalt	14.8
Vitavax	Carboxin (5, 6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide) 75 %, WP	UniRoyal	28. 8
R-28921	O,O-diethyl-2-((3-methoxycarbonyl) thioureido 1 phenyl phosphoramidothioate) 50 %, WP	Stauffer	21.2

Table 13.6 (continued)

Product name[a]	Active ingredients' percentage and formulation	Source	Total percentage inhibition adjusted[b]
LFA 2043	Iprodione	May and Baker	21.5
Control[b]			

WP wettable powder, *P* powder, *Soln* solution, *Fwble* flowable

[a] The use of trade names in this publication does not imply endorsement by Agriculture Canada of the products named or criticism of similar ones not mentioned

[b] At least 400 spores counted per sample. Percentages of non-germinated oospores in the control (18) were subtracted from those in the treatments to obtain percentage inhibition due to fungicide

disease control (82 %), followed by seed treatment with apron SD-35 (metalaxyl) at 2 g a.i./kg seed along with two foliar applications of ridomil MZ-72 at 30-day intervals; both treatments were also effective in reducing the staghead incidence. When non-systemic fungicides mancozeb and chlorothalonil were sprayed thrice following seed treatment with apron SD-35, disease control was 47 and 49 %, respectively. But four sprays with mancozeb alone controlled the disease up to 42 %. However, the maximum cost–benefit ratio was either with four sprays of mancozeb or seed treatment with apron SD-35, followed by three sprays of mancozeb (Tables 13.7–13.9).

Absorption and degradation of metalaxyl were studied in mustard plants after its application as a seed dresser, a foliar spray and a combination of both, under subtropical conditions in India (Mehta et al. 1996). Absorption of metalaxyl increased up to 30 days when applied as a seed dresser; thereafter, it started declining and was not detectable after 60 days of sowing. The maximum residues (average, 9.03 ppm) of metalaxyl were detected 1 day after spraying. The dissipation of metalaxyl after initial deposits on mustard plants was almost complete 15 days after spraying. The safe waiting period of metalaxyl was calculated to be 62 and 8 days for seed dressing and foliar application, respectively. The seeds produced from metalaxyl-treated plants were completely free from any detectable amount of metalaxyl residues (Mehta et al. 1996).

Seed treatment with metalaxyl (apron at 6 g/kg seed) followed by two sprays of metalaxyl + mancozeb (ridomil MZ-72 at 0.25 % at 50 and 70 days after sowing (DAS)), or three sprays of mancozeb (dithane M-45 at 0.2 %) at 45, 60 and 75 DAS, were the most effective schedule in reducing percentage disease index (PDI) on leaves, percent staghead formation and increasing seed yield by 3–5 q/ha with 1:2 and 1:3 cost–benefit ratios, respectively (Bartaria et al. 1998).

Khangura and Sokhi (2000) reported that the two sprays of ridomil-MZ at 450 g a.i./ha, followed by another spray of mancozeb at 600 g a.i./ha or blitox-50 at 375 g a.i./ha, at 15–20-day intervals to a 70-day-old crop, significantly reduced both foliage and floral infection. These treatments significantly increased the seed yield over control and other treatment combinations, without having an adverse effect on the oil content and fatty acid composition of the oil; same fungicide regime was also effective when applied to a 50- or 60-day-old crop under delayed-sown conditions. The seeds harvested from the plots treated twice with ridomil- MZ exhibited no detectable residue of metalaxyl and mancozeb (Meena and Jain 2002). Application timing of fungicides may be critical in the control of WR, particularly in the late-sown crop (Khangura and Sokhi 2000). Meena et al. (2005) found that metalaxyl + mancozeb (ridomil-MZ) sprayed on leaves did not show any disease development (Table 13.10).

13.2 Cultural Methods of Control

To control WR of cauliflower and horseradish, Savulescu (1960) and Glaeser (1973) suggested the collection and burning of diseased plants in order to prevent the formation of oospores. Furthermore, these crops should be isolated from other crucifers. Reductions of relative humidity

Table 13.7 Efficacy and spray schedule of fungicides against white rust in mustard during 1991–1992 and 1992–1993 crop seasons. (Mehta et al. 1996)

Fungicide	Concentration (%)	Percent disease index[a]							Percent disease control		
		Number of sprays	1991–1992		1992–1993		Average		1991–1992	1992–1993	Average
			60 DAS	90 DAS	60 DAS	90 DAS	60 DAS	90 DAS			
Mancozeb	0.2	4	2.7	26.2 (30.5)[b]	11.3	30.7 (33.5)	7.0	28.4 (32.0)	39.7	45.2	42.4
Kavach	0.2	4	3.5	28.2 (32.1)	–	–	3.5	28.2 (32.1)	35.0	–	35.0
Difolatan	0.2	4	2.7	22.4 (29.3)	–	–	2.7	22.4 (29.3)	48.2	–	48.2
Ridomil MZ-72	0.25	3	0.2	7.8 (15.9)	04.7	10.3 (18.7)	4.0	9.1 (17.3)	82.0	81.6	81.8
Apron SD-35[c] + Ridomil MZ-72	0.25	2	0.0	12.9 (21.0)	12.3	18.7 (25.4)	6.1	15.8 (23.2)	70.3	66.6	68.5
Apron SD-35[c] + mancozeb	0.2	3	0.4	22.9 (28.5)	–	–	0.4	22.9 (28.5)	47.4	–	47.4
Apron SD-35[c] + Kavach	0.2	3	1.2	21.9 (27.8)	–	–	1.2	21.9 (27.8)	49.6	–	49.6
Apron SD-35[c] + difolatan	0.2	3	1.8	20.9 (27.2)	–	–	1.8	20.9 (27.2)	51.8	–	51.8
Apron SD-35[c]	–	–	2.2	27.3 (31.5)	15.3	40.0 (39.0)	8.7	33.6 (35.2)	36.9	28.6	32.7
Unsprayed (control)	–	–	4.8	43.4 (41.2)	23.7	56.0 (48.4)	14.2	49.7 (44.8)	–	–	–
LSD ($P<0.05$)					2.51		5.75		4.10		

DAS days after sowing
[a] Average of four replicates
[b] Angular transformed values
[c] Seed treatment at 2 g a.i./kg seed

Table 13.8 Efficacy of fungicides against staghead of Indian mustard. (Mehta et al. 1996)

Fungicides	Number of spray	Staghead incidence[a] (%)	Staghead length[a] (cm)	Staghead score[a]
Mancozeb	4	8.8 (16.2)[b]	7.9	2.1
Kavach	4	2.4 (8.9)	8.9	1.4
Ridomil MZ-72	3	1.6 (6.4)	5.4	1.5
Apron SD-35[c] + ridomil MZ-72	2	5.2 (12.0)	8.1	2.0
Apron SD-35[c] + mancozeb	3	2.6 (9.2)	1.9	1.6
Apron SD-35[c] + kavach	3	2.9 (9.9)	3.7	1.7
Apron SD-35[c]	–	13.9 (19.0)	10.4	2.3
Unsprayed (control)	–	25.8 (30.4)	14.5	3.4

[a] Average of four replicates based on 2-year mean
[b] Angular transformed values
[c] Seed treatment at the rate of 2 g a.i./kg seed

Table 13.9 Comparative yield increase and cost–benefit ratio of fungicides against white rust of Indian mustard. (Mehta et al. 1996)

Fungicides	Yield (kg/plot)[a]	Increase in yield over control (%)	Cost–benefit ratio[b] (Rs)
Mancozeb	1.164	28.5	2.2
Kavach	1.116	23.3	1.6
Ridomil MZ-72	1.352	49.3	1.2
Apron SD-35[c] + Ridomil MZ-72	1.216	34.2	1.1
Apron SD-35[c] + Mancozeb	1.115	22.1	2.1
Apron SD-35[c] + Kavach	1.090	20.4	1.3
Apron SD-35[c]	1.050	15.9	20.6
Unsprayed (control)	0.950	–	–

[a] Plot size = 3.0×2.1 m^2
[b] Based on prevalent market price in 1992 = Raya = Rs 800/q; mancozeb = 152/kg; ridomil MZ-72 = 950/kg; kavach = 333/kg; labour-5 labour/spray/ha at the rate of Rs 40/per labour
[c] Seed treatment at the rate of 2 g a.i./kg seed

Table 13.10 Effect of fungicides on development of WR on detached leaves of Indian mustard. (Meena et al. 2005)

Fungicide	PDI[a]	Percent disease control
Metalaxyl + mancozeb (ridomil)	4.0 (0.0)	100.0
Mancozeb	22.0 (27.9)	56.5
Baynate	34.7 (36.1)	31.5
Blitox-50	29.3 (32.8)	42.1
Sulfex	40.0 (39.2)	21.0
Antracol	45.3 (42.3)	10.5
Aliette	39.3(30.0)	22.4
Control	50.7 (45.4)	–
Critical difference (P < 0.005)	4.0	

[a] Average of three replications (three leaves per replication) and figures in parentheses are actual percent white rust severity and others arc sine transformed values

around the plants by adequate aeration, and avoiding dense sowing and growth of weeds, also help to reduce disease. The application of organic manures, avoidance of excess nitrogen and addition of phosphorus and potassium increase resistance in plants. Time of planting is very important for escaping staghead infection in *Brassica* crops. In India, early (September)

Table 13.11 Effect of planting dates on the severity of WR and DM of Indian mustard cv. Varuna. (Saharan 1992b)

Sowing date	Percent WR and DM disease intensity		
	Hisar	Kanpur	Pantnagar
06.10.1978	10.0	–	–
21.10.1978	8.6	–	–
28.10.1978	18.6	–	–
06.11.1978	55.4	–	–
18.11.1978	68.5	–	–
02.12.1978	72.8	–	–
01.10.1979	–	24.16 (29.45)	–
10.10.1979	04.6	28.30 (32.14)	–
20.10.1979	10.0	34.34 (35.86)	–
30.10.1979	22.5	36.18 (36.97)	–
09.11.1979	46.8	40.91 (39.77)	–
19.11.1979	57.5	46.15 (42.76)	–
03.10.1980	–	–	15.04
23.10.1980	–	–	19.85
13.11.1980	–	–	32.85

"–" means no staghead recorded

and timely (mid-October) sown crops escape staghead infection and suffer less disease on the leaves (Table 13.11) (Kolte 1987a, b; Lakra and Saharan 1988d, 1990; Saharan 1984, 1992a, b). In Western Australia, crops of rape sown in early June generally have a higher incidence of stagheads, whereas crops sown in mid-July escape infection (Barbetti 1981). This suggests that the incidence of stagheads in a crop is at least partially determined by environmental conditions.

An increase in crop density by reducing inter-row and intra-row planting distances of *Brassica juncea* cv. Pusa Bold, although reduced per plant yield and increased disease intensity of *A. candida*, increased the total per plot yield in field experiments (Verma and Bhowmik 1996).

Cultural management involved early sowing (1 October) and removal of 50 % lower leaves at 60 days of crop age. Early sowing resulted in disease escape from WR and DM (*Hyaloperonospora parasitica*) staghead formation. There was a significant interaction between date of sowing and removal of lower leaves in relation to the severity of Alternaria blight (AB), WR and DM diseases. However both cultural management practices had independent effects on seed yield (Koul and Singh 1999).

13.3 Host Resistance

Control of WR through host resistance has been attempted by identifying sources of resistance (Edwards and Williams 1982, 1987; Fan et al. 1983; Petrie 1988; Pidskalny and Rimmer 1985; Pound and Williams 1963; Saharan et al. 1988; Thukral and Singh 1986a, b; Tiwari et al. 1988a, b, c; Williams and Pound 1963). All sources of resistance identified so far are race specific and governed by major genes. Zero erucic acid and zero glucosinolate *B. rapa* cv. Tobin (Canola), highly resistant to *A. candida* race 7, was developed by the Agriculture Canada Research Station, Saskatoon, Saskatchewan, Canada, and has been licensed for commercial use in Canada since 1980 (Klossen, Verma, Downey, unpublished data). Recently, Petrie (G.A. Petrie, personal communication), discovered occurrence of a race 7a to which cultivar Tobin was found susceptible.

13.4 Biological Control

The bacterium, *Pseudomonas syringae*, caused a soft rot disease preferentially on the green developing stagheads, of field-grown Canola (*B. rapa*)

Table 13.12 Effect of plant products on white rust development on detached leaves of Indian mustard. (Meena et al. 2005)

Plant product	PDI[a]	Percent disease control
Azadirachta indica leaf extract	4.0 (0.0)	100.0
Ocimum sanctum leaf extract	18.7 (25.6)	62.7
Datura stramonium leaf extract	30.0 (33.2)	40.0
Allium sativum bulb extract	3.4.0 (35.7)	32.0
Ovis	38.0 (38.1)	24.0
Zetron	38.7 (38.5)	22.7
Control	50.0 (45.41)	–
Critical difference ($P<0.05$)	3.2	

[a] Average of three replications (three leaves per replication) and figures in parantheses are actual percent white rust severity and others are arc sine transformed values

in Alberta, Canada, and on *B. juncea* in India, leading to abortion of the developing oospores. This natural biological control of *A. candida* may regulate its overwintering population in the field, and may have implications towards developing planned biological control strategies for this pathogen.

13.5 Plant Extracts

Crude extracts of *Rhus coriaria, Anagallis arvensis* and *Mesphilus germanica* inhibits zoospore release from sporangia of *A. candida* at 50 ppm (Omranpour et al. 2011). Meena et al. (2005) found leaf extract of *Azadirachta indica* very effective in controlling WR under field conditions (Table 13.12).

13.6 Integrated Disease Management

No single method or approach can be viable, stable, effective, environmentally safe and economically feasible in dealing with any biological system, particularly the disease management system. Therefore, all means of control measures like cultural, biological, chemical and host resistance, including genetic engineering, should be used to manage rapeseed-mustard diseases in an integrated way (Mukerji et al. 1999; Saharan and Mehta 2002). The following general practices should be followed to manage the diseases.

13.7 Cultural Practices

Cultural control of diseases of cruciferous crops is largely a matter of sanitation, and of manipulating the environment to the advantage of the host and to the detriment of the pathogen. Various cultural practices useful in reducing diseases are as follows:

- Because *A. candida* survives in the form of thick-walled oospores, diseased debris containing these resting structures should be burnt or destroyed after harvesting the crop.
- Deep ploughing should be done during hot summer months, to expose and probably destroy (May-June) the resting propagules of the pathogens.
- Crop rotation for a period of at least 3 years should be followed with non-cruciferous crops after epidemics.
- Proper plant-to-plant and row-to-row spacing should be maintained to make microenvironments less conducive to disease.
- High relative humidity around the plant canopy should be avoided to have proper aeration.
- The cruciferous and other weed host plants should be removed.
- Avoidance of continuous cropping of rapeseed-mustard, particularly the same variety,

helps in reducing disease severity and build up of oosporic inoculums (primary source of infection).

- Optimum doses of fertilizers should be used.
- Time of sowing of the crop should be adjusted depending upon weather conditions conducive to disease development in particular area.
- Early sowing (up to 20 October) of the crop reduces incidence of the major diseases, including WR and AB.
- Planting of cultivars with high degree of tolerance.
- Plant healthy and treated seeds.
- Use foliar fungicidal sprays, if necessary.

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14 Techniques

14.1 Mycelium

The nonseptate and intercellular mycelium of *Albugo* species feeds by means of globose or knob-shaped intracellular haustoria, one to several in each host cells. The detail of haustorial formation and development has been given by Berlin and Bowen (1964a, b); Coffey (1975, 1983); Davison (1968); Fraymouth (1956), and Wager (1896).

14.2 Sporangiophore

The sporangiophores are short, hyaline, clavate, thick walled, especially toward the base, 30–45 × 15–18 µm^2 diameter, basally branched, club shaped and give rise to simple chains of sporangia. The number of sporangia produced is indefinite. They are formed in basipetal succession; that is, the sporangiophore forms a cross wall or septum, cutting off that portion which is to become a sporangium. The sporangiophore increases in length, a second sporangium is cut off, and the process continues, resulting in the simple chains of multinucleate sporangia.

14.3 Sporangia

The number of sporangia produced is indefinite in basipetal succession; that is, the sporangiophore forms a cross wall or septum, cutting off that portion which is to become a sporangium; sporangia are globose to oval, hyaline with uniform thin wall, and 12–18 µm diameter. As sporangial production continues, the older, terminal portions of the chain breaks, releasing the individual sporangia. The sporangia germinate by the formation of zoospores and, on rare occasions, by means of a germ tube (Heald 1926; Wager 1896; Walker 1957; Zalewski 1883).

14.4 Gametogenesis, Fertilization, and Oospore Formation

One or more antheridia come to occupy a position close to an oogonium. There are two types of egg organization within an oogonium. In *Albugo candida*, the protoplast becomes differentiated into a peripheral or external zone, the periplasm, which contains many nuclei, and a central mass, the egg cell or ooplasm, which contains a single nucleus. The antheridium, which is a multinucleate cell, produces a short tube-like outgrowth, the fertilization tube which penetrates the periplasm and comes in contact with the egg cell or ooplasm. The antheridial or male nuclei are discharged through this tube into the egg cell. In the uninucleate egg, the female nucleus fuses with a single male nucleus, where as in the multinucleate egg, female and male nuclei fuse in pairs. This nuclear union constitutes the process of fertilization (Heald 1926; Walker 1957). Following fertilization, the egg is gradually transformed into a thick-walled oospore. The periplasm is absorbed, the oospore wall darkens and thickens, and develops a characteristic external ridges, reticulations or knobs,

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while the interior of the oospore becomes filled with an abundance of reserve food in the form of oily or fatty globules. The fully developed oospore lies within the old empty oogonial cell. The oospores are released only by weathering and decaying of the host tissues (Heald 1926). The characteristics of oospores are useful criteria for distinguishing species of *Albugo*; the epispore of the oospore is tuberculate or ridged. Zalewski (1883) and Stevens (1901a, b, c) confirmed that, this is the more specialized group, where there is complete development of the epispore with cytological phenomena.

14.5 *In vitro* Callus Culture

Preliminary dual *in vitro* culture of *Albugo ipomoeae-panduratae* and species of *Ipomoea* (Singh 1966), *A. candida* race 2 and *Brassica juncea* (Lahri and Bhowmik 1993, Nath et al. 2000), and unidentified race of *A. candida* and *B. juncea* (Goyal et al. 1995) have been established. Although the authors report presence of zoosporangia and oospores in callus tissues derived from hypertrophied stems (Singh 1966; Goyal et al. 1995), or hypertrophied peduncles, or thickened terminal leaves (Lahiri and Bhowmik 1993), the origin of both sexual and asexual spores is questionable, because the hypertrophied tissues used as explants in their studies are known to almost entirely composed of thick-walled oospores (Verma and Petrie 1975a, 1979; Saharan and Verma 1992; Verma and Bhowmik 1988).

Using explants from freshly-inoculated leaves, Goyal et al. (1996) very successfully established dual *in vitro* callus cultures of *A. candida* race 7, and *Brassica rapa* cv. Torch on Murashige and Skoog (MS) medium (Murashige and Skoog 1962) supplemented with 1.0 mg L^{-1} Y-naphthalene acetic acid and 1.0 mg L^{-1} benzene amino purine (Table 14.1; Fig. 14.1a–14.2h). These authors provided evidence for: (a) production of zoosporangia, oospores, and parthenogenetic–like oospores; (b) establishment of haustorial connections with host callus cells; (c) origin and development of both antheridia and oogonia; and (d) the pathogenicity of the zoospores derived from *in vitro* produced zoosporangia and oospores (Fig. 14.1a-h, 14.2a-h).

The authors (Goyal et al. 1996b) reported that:

a. Callogenesis was observed within 7–8 days of incubation, and the proportion of callused explants was significantly affected by the type and concentration of growth regulators.
b. Under both light and dark conditions, the length of incubation period significantly affected the presence and development of haustoria, zoosporangia, oogonia, antheridia, and oospores.
c. The callus tissues incubated in the light were hard, nodular, and green, compared to soft, watery, and beige-yellow in the dark (Goyal et al. 1996b; Table 14.1).

Zoosporangia were observed in the largest numbers of calli at 8-day incubation, and after this their numbers declined consistently; zoosporangiophores without zoosporangia grew out of the callus cells after day 18 of incubation. In callus cells, the zoosporangiophores were long, knotted, branched, and indeterminate, compared to short, club shaped, unbranched, and determinate in infected leaves (Goyal et al. 1996b; Table 14.1).

By subculturing the calli every 2 weeks, for 18 weeks, the *A. candida*–*B. rapa* dual cultures were maintained. After 18 days of incubation, and until the end of the observation period, haustoria, similar to those reported in infected leaf tissues (Verma et al. 1975), were observed in the cytoplasm of callus cells, or between cell wall, and the cell membrane (Goyal et al. 1996b).

The development of antheridia and oogonia among the callus cells were observed after 13 days of incubation, and until the end of the observation period. Two types of oospores, mature oospores with characteristic features such as wall layers and a coenocentrum, or two coenocentra, and parthenogenetic-like oospores were observed after 18 days of incubation. The parthenogenetic–like oospores were oval, devoid of wavely layers like typical mature oospores, often germinated by a germ tube, and were associated with haustoria inside the callus cells (Goyal et al. 1996b; Table 14.1; Fig. 14.1a-h, 14.2a-h). Pathogenicity tests on seedlings of *B. rapa* cv. Torch using zoospores derived from *in vitro* produced

Table 14.1 Maximum likelihood estimates (±S.E.) of the proportion of callus tissues with various stages of *Albugo candida* over time under dark and light conditions. (Goyal et al. 1996b)

Time (days)	Days after subculture	Incubation condition	Proportion of calluses			
			Zoosporagia	Haustoria	Oogonia	Oospores
8		Light	0.42±0.06	0	0	0
		Dark	0.45±0.06	0	0	0
13		Light	0.39±0.06	0.56±0.06	0.69±0.06	0
		Dark	0.41±0.06	0	0.56±0.06	0
18	3 Days after subculture II	Light	0.10±0.04	0.75±0.06	0.61±0.06	0.48±0.06
		Dark	0.03±0.02	0.66±0.06	0.59±0.06	0.46±0.06
23	8 Days after subculture II	Light	0	0.58±0.06	0.60±0.06	0.63±0.06
		Dark	0	0.63±0.06	0.57±0.06	0.55±0.06
28	13 Days after subculture II	Light	0	0.72±0.06	0.67±0.06	0.47±0.06
		Dark	0	0.75±0.06	0.70±0.06	0.48±0.06
33	3 Days after subculture III	Light	0	0.63±0.06	0.58±0.06	0.63±0.06
		Dark	0	0.70±0.06	0.63±0.06	0.47±0.06

zoosporangia and geminating oospores confirmed the viability and the virulence of *A. candida* in dual callus cultures (Goyal et al. 1996b). The *A. candida–B. rapa* dual culture system reported by Goyal et al. (1996b) has potential for sexual studies of the fungus, because it was possible to trace the development of antheridia and oogonia from the mycelium, which confirm homothallic nature of the *A. candida* race 7V isolates (Fig. 14.1, 14.2).

14.6 Sporangial Viability Test

A fluorescent microscopy technique to test sporangial viability was suggested by Vyalykh and Lanetskii (1974). This method provides information on the number of viable sporangia of *Albugo* in a population within minutes. Sporangia are stained with a 0.01% aqueous solution of acridine orange for 5–10 min, and viewed under a fluorescence microscope. The dead sporangia are fluorescent green.

14.7 Sporangial Preservation

Freezing (Zalewski 1883) has been used to maintain the white rust (WR) fungus on living plants. In 1958, O'Brien and Webb (1958) suggested that *Albugo occdentalis* can be preserved for 5–6 months by storing infected spinach leaves at −10°C. According to Lakra and Saharan (1989d), sporangia of *A. candida* from *B. juncea* survive for 4–5 days at 15°C on detached infected leaves, but lose their viability in 18 h if stored as a dry powdered mass. However, sporangia can be stored as a dry powdered mass for 105 days at −40°C.

14.8 Inoculation Applicator

A micro ULVA-controlled droplet applicator was developed as a tool for inoculating *A. candida* (race 2) in the field and greenhouse by Perry and Williams (1984). The severity of the disease is greater in the field or greenhouse when large spray droplets (60–70 µl) are used instead of small droplets (30–40 µl). Since relatively small volumes of inoculum are required, this inoculator is potentially a valuable tool for field use.

14.9 Components of Partial Resistance

Partial resistance may be the result of low infection frequency, low spore production, and a long incubation period, and/or a short infectious period. Fox and Williams (1984) stated that spore production by *A. candida* on *B. rapa* is highly

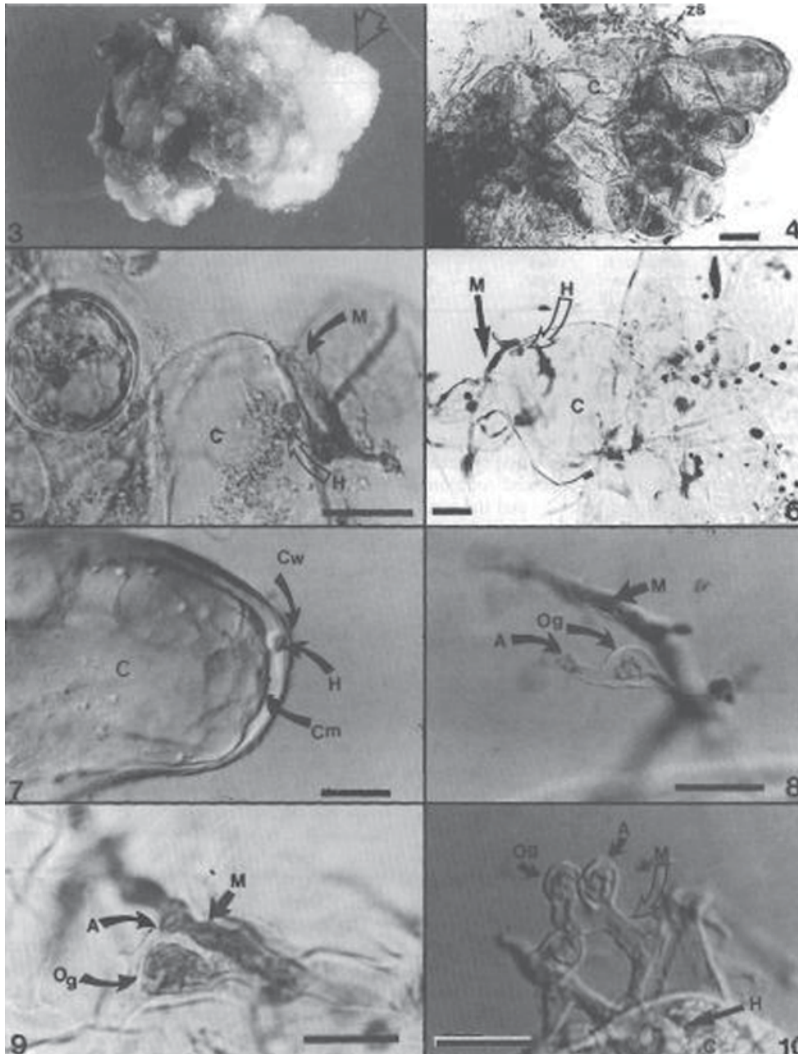

Fig. 14.1 **a** *B. rapa* cv. Torch leaf callus cells in association with *Albugo candida* race 7V (dual culture) on MS medium supplemented with 1.0 mg L^{-1} naphthaleneacetic acid (NAA) and 1.0 mg L^{-1} 6-benzyloaminopurine (BAP). Light or phase contrast microphotographs of hand-cut sections of callus tissues infected with *A. candida* (bar scale = 50 μM). Abbreviations: *antheridium (A), callus cells (C), coenocentrum (Co), cell membrane (Cm), cell wall (Cw), haustorium (H), mycelium (M), oospore (O), oogonium (Og), parthenogenetic-like oospore (pO), zoosporangiophore (z), zoosporangia (zs)*. **b** Zoosporangiophores with zoosporangia in association with callus cells after the 8th day of callus initiation. **c** and **d** A mycelium and a haustorium inside the cytoplasm of callus cell, and **e** in between the cell wall and the cell membrane of a callus cell. **f** Early development of antheridium and oogonium on the mycelium. **g** Late development of antheridium and oogonium on the same mycelium as in **f.** **h** Antheridium and oogonium originating from common mycelium (Goyal et al. 1996b)

correlated ($r = 0.93$) with a visual WR interaction phenotype (IP) rating scale. Plants are rated from 0—9 according to the amount of leaf necrosis or area covered by WR pustules. Means of spore production on plants rated as 1, 3, 5, and 7 are significantly different ($p = 0.05$) from each other. These results support the use of a visual scale for selection of components of partial resistance to the WR disease. Black and Dainello (1986) made comparisons of percent leaf area

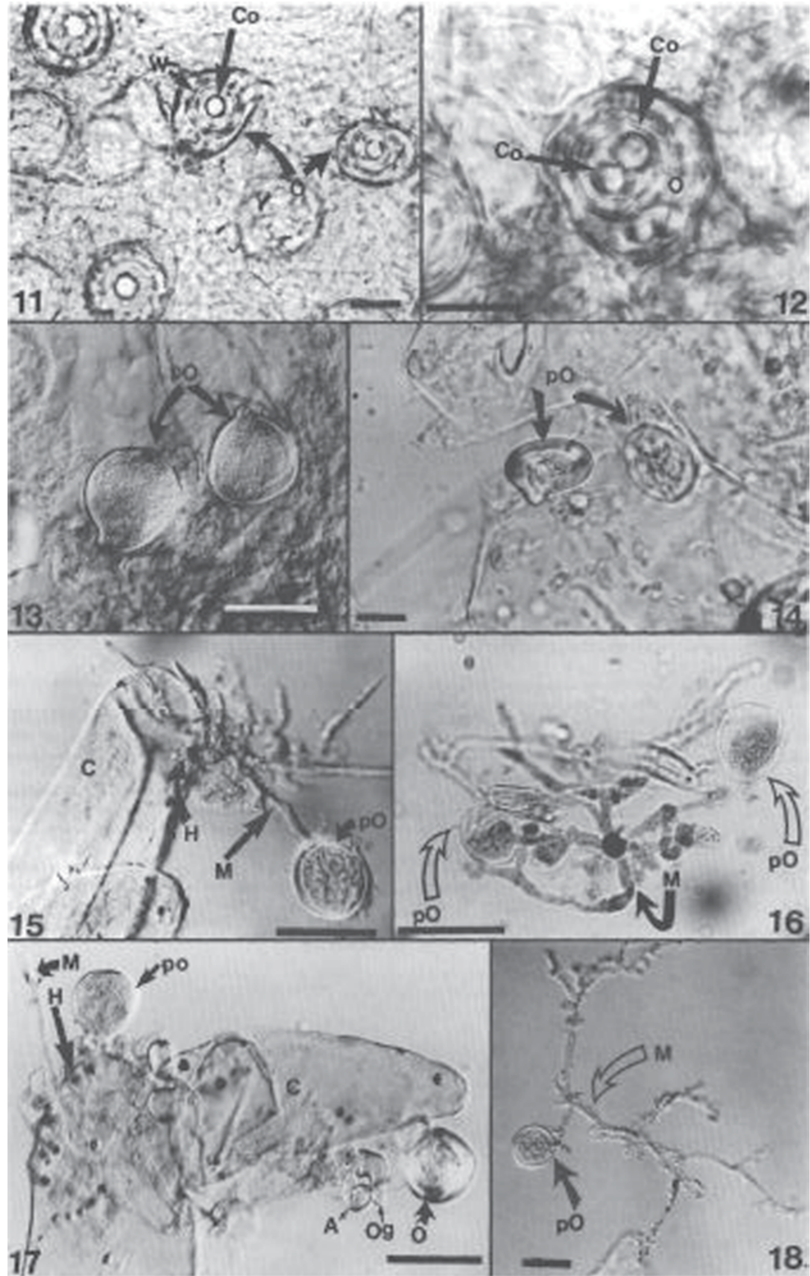

Fig. 14.2 **a** Mature oospores with one coenocentrum. **b** With two coenocentra. **c** Parthenogenetic-like oospores among callus cells. **d** Thick walled parthenogenetic-like oospores without the wall layers and coenocentrum characteristic of mature oospores. **e** Parthenogenetic-like oospores with attached mycelium and haustorium inside a callus cell. **f** Germinating parthenogenetic-like oospores with attached mycelium. **g** Callus cells with mycelium, haustoria, antheridia, oogonia, mature oospore, and parthenogenetic-like oospore. **h** Parthenogenetic-like oospore with branched mycelium (Goyal et al. 1996)

with WR lesions, and used two other methods for evaluating partial resistance to *Albugo occidentalis* in spinach. Lakra and Saharan (1988b), using length of incubation period for determining level of resistance in several *B. juncea* cultivars, found that the decision between compatible (susceptible) and incompatible (resistant) reaction of *Albugo–B. juncea* system is made within 80 h of inoculation. Verma et al. (1975) also reported that this decision is made within 72 h of inoculation, when the single haustorium formed in the resistant host is encapsulated by a marked hallo. In a histopathological study, Liu et al. (1989) and Verma et al. (1975) showed that the earliest event

distinguishing a compatible from an incompatible interaction occurs after formation of the first haustorium, and that resistance is not manifested until the host mesophyll cell had come in contact with the first haustorium.

14.10 White Rust as a Weed-Control Tool

Disease induced by *A. candida* has a significant effect on the survival and reproduction of *Capsella bursa--pastoris* (shepherd's purse), a weed plant. The timing of infection is very important in determining the ultimate effect of the disease. Systemic (primary) infection of seedlings causes a high degree of mortality (88 %) prior to reproductive maturity. Localized or systemic infection does not affect survival (Alexander and Burdon 1984). In North America, Hartmann and Watson (1980) suggested use of WR fungus *Albugo tragopogonis* to control common ragweed (*Ambrosia artemisiifolia*). When the plants are inoculated at the two-leaf stage, pollen production is reduced by 99 % and seed production by 98 %, if the infection is systemic.

14.11 Germination of Oospore

Germination of oospores of *A. candida* in the laboratory was first reported by DeBary in 1866. Petrie and Verma (1974); Petrie and Verma (1975b); Verma (2012) developed a reliable technique by which 70 % oospore germination was achieved. Verma and Petrie (1975b) refined this technique (Petrie and Verma 1974) and developed two more techniques to get even better results. In all three techniques, hypertrophied tissues were finely ground with a mortar and pestle, and the grindings screened through a 60-mesh sieve to give a brown powder consisting largely of oospores. The oospore were germinated by incubating them on moist filter paper for 21 days at 10–15 °C, by slowly leaching them for 15 days or more on sintered glass filters, or by washing them for a few days on a rotary shaker followed by a day of still culture. Oospores germination (> 80 %) was obtained by agitating them for 24 h in sterile distilled water (SDW) containing a 1–2 mixture of β-glucorinidase and arylsulfatase followed by 3 days of washing on a rotary shaker at room temperature, and chilling for 15 h at 13 °C (Liu and Rimmer 1993; Meena and Sharma 2012). The importance of oospore as a source of primary inoculum was also explored in a field experiment conducted under irrigated and dry land conditions (Verma and Petrie 1980). The treated plots were seeded with seeds of susceptible *B. rapa* cv. Torch mixed with an equal weight of oospore powder. The control plots did not receive any oospore powder. Both number of pustules per infected leaf, and the percentage of plants with stagheads, were significantly higher in oospore-infested than those in the noninfested plots. These results convincingly suggest that oospores overwintered in plant debris in soil, or carried on the seed, are most likely the primary source of infection. Verma and Bhowmik (1988) suggested that pretreatment of oospores with $KMn0_4$ gives better germination.

Cotyledons containing oospores, and hypertrophied inflorescence (stagheads) produced on artificially-inoculated plants were stored at room temperature for at least 2 weeks, ground to fine powder with a mortar and pestle, and four collections of oospores representing different stages of maturity were examined: oospores from (1) senescent, dried cotyledons, (2) light-brown stagheads collected at plant maturity (growth stage (GS) 5.5), (3) dark-brown stagheads collected 2 week after maturity, and (4) stagheads from naturally-infected plants of *B. rapa* stored at − 15 °C for over 10 years. Oospore suspensions are prepared by suspending small amounts of oospore powder in 50-ml Erlenmeyer flasks with 20 ml SDW containing 0, 0.5, 1.0, 1.5, or 2.0 % mixture of β-glucuronidase and arylsulfatase. The oospore suspensions are kept on a rotary shaker (200 rpm) at room temperature for up to 24 h, washed three times with SDW by pelleting in a bench centrifuge, resuspended in 20 mL SDW, and after returning back on to the rotary shaker for 72 h, 10 ml aliquots of each spore type were chilled at 13 °C for about 20 h.

Germination of oospores is recognized by the disappearance of the central reserve globule and granular contents and formation of sessile vesicles. Counts of germinated oospores are made using light microscope at 400× magnifications between 0 and 34 h of chilling at 4 h intervals (Liu and Rimmer 1993; Meena and Sharma 2012).

14.12 Disease Scoring Scale

Pound and Williams (1963) reported a procedure to assess symptoms of WR on mature cruciferous plants. This procedure has since been modified by different workers. Mayee and Datar (1986) suggested the use of 0–9 scale based on growth stages of the crop for leaf as well as staghead infections. To assess both leaf (LP) and staghead phase (SP) infections separately, and/or in combination, a 0–5 scoring scale was proposed by Lakra and Saharan (1990). A descriptive and pictorial WR assessment scale for sunflower was proposed by Siddiqui et al. (1975). The scale is based on the percentage of total leaf area infected, and on the morphological development of the plant. Details of WR assessment scales used by different researchers are given in Chap. 2 at 2.5.1.

14.13 Induction of Stagheads Flower Bud Inoculation

A number of plant pathologists believe that the hypertrophies or stagheads are produced as a result of early infection of young seedlings and systemic development of the fungus in the plant. This theory was rejected when Verma and Petrie (1980) and Goyal et al. (1996b) routinely obtained stagheads by artificially inoculating flower buds of plants grown under growth chamber and greenhouse conditions. The results of several field experiments (Verma and Petrie 1979, 1980) conclusively proved that a large percentage of stagheads in the field are produced as a result of secondary infection of flower buds rather than a systemic development of the fungus in the plant. The flower bud inoculation technique at GS 3.1 is routinely used for screening advanced breeding lines (Goyal et. al. 1996b). Results of these studies have also proved useful in determining actual time of application of both protectant and systemic fungicides. Verma and Petrie (1980) suggested two possible mechanisms for staghead development:

- Early infection of young cotyledons and leaves with oospore-infested seeds, and infection progressing systemically throughout the development of the plant, and
- Infection of young flower buds by zoospores arising from wind- or water-borne sporangia.

According to Verma and Petrie (1980) and Verma (2012), oospores of *A. candida* race 7 mixed with seeds of turnip rape prior to sowing results in high levels of leaf and staghead infections. Over 55 % staghead infections were induced by flower bud inoculation. Lakra and Saharan(1989) although failed to induce stagheads in artificially inoculated *B. juncea* plants, they were able to induce > 66 % stagheads in plants grown in soil infested with 5 g/pot of oosporic inoculum at sowing time, and with subsequent sporangial spray inoculation at the seedling, branching, and flowering stages. Systemic infection was found to be directly correlated with the host age.

14.14 Germplasm Screening

Many procedures have been described for evaluating host germplasm for resistance to WR (Fox and Williams 1984; Pound and Williams 1963; Verma et al. 1975; Verma and Petrie 1980).

14.14.1 Preparation for Sowing of Seeds

Seeds are sown into steam-treated potting mix (composted pine bark:cocoa peat:river sand, 2:1:1 by vol.) in 8-cell plastic trays, and plants grown at 13/18 °C night/day temperatures with a 16 h photoperiod for screening against pathogen under artificially controlled conditions.

Untreated seeds are sown 5 mm deep in 5 × 5 cm^2 Jiffy pots containing soil less peat-

based compost mix and placed inside a propagator ($57 \times 29 \times 21$ mm^3). Approximately 5 mm diameter hole was made in the base of the Jiffy pots to allow water drainage. Five to six Jiffy pots are used for each line. Each propagator contained 11–12 lines including susceptible controls. Five or more seeds are sown in each jiffy pot, but later thinned to five prior to inoculation to maintain a uniform number of seedlings per pot at the same growth stage. Some lines only had a few seeds, so a lesser number of seeds are sown. After sowing, the propagators are immersed in water filled trays for 1 h to allow absorption of water through the base. After water absorption, holes in the base of each propagator are cleared of debris and perforations made between the junctions of jiffy pots, so that air would pass through them when they were placed on the airflow bench to provide the plants with a spore-free environment in the glasshouse. The air is exhausted through the two ventilators on the top of the propagator lids and through the junction between the lids and the propagator base. After 4–5 days, each propagator is watered again, if needed in the same manner for 30 min to maintain proper soil moisture prior to inoculation. The propagators are then returned to the airflow bench. The seedlings are grown under the same conditions and inoculated using the same method as used for isolate maintenance. Approximately 20 and 40 ml of spore suspension is used to spray inoculate the seedlings and five to seven leaf stage plants, respectively, in each propagator (Meena 2007).

14.14.2 Maintenance of *A. candida* Isolates and Inoculum Preparation

A. candida isolates used in the present investigation were maintained separately on 1-week-old seedlings of the susceptible host raised from untreated seeds sown in Jiffy pots (Manufactured by Jiffy A/S Denmark and supplied by Nursery trades (Lee Valley) Ltd., Cheshunt, U.K.) filled with moist sterilized soilless compost placed in propagators ($16 \times 20 \times 5$ cm^3). The propagators were placed in an air-flow-bench under spore-free conditions in the glasshouse at 18 °C ± 2 °C (Jenkyn et al. 1973) with supplementary light to maintain a 16 h light/8 h dark; day/night cycle. The seedlings were sprayed with SDW to clean the surface of cotyledons 24 h prior to inoculation.

Sporangial suspension was prepared by adding 1–2 ml SDW to glass vial containing the excised sporulating cotyledons from the previous stock (kept in the freezer or excised from freshly sporulating material). The vial was then closed and shaken vigorously on a vortex to rupture the pustules and to facilitate the release of the sporangia from the sporangiophores. The concentration of the suspension was then adjusted to 2.5×10^4 sporangia/ml with the aid of a hemocytometer (particle counting chamber).

Each cotyledon was inoculated with two 5 µl droplets of sporangial suspension using a micropipette. Alternatively, the propagator was sprayed to run-off with the spore suspension using an atomiser. After inoculation, the propagators were covered with clear plastic lids and sealed with insulation tape to allow relative humidity (RH) to increase approximately 100 %. They were then placed in a growth chamber for 12 days at 16 °C with 8 h darkness initially, followed by 16 h photoperiod with 70–120 µmol/m^2/s irradiance. After this period, peak sporulation was expected to occur.

Infected cotyledons were excised and placed into 5 ml glass vials, used immediately for the preparation of spore suspensions, or stored in the freezer for future use. All the work pertaining to preparation of inoculum and inoculation was carried out in a sterile environment inside a Class II laminar flow cabinet.

14.14.3 Pathogen Culture and Inoculation Method

Sporangial suspension of *A. candida* is prepared by adding 1–2 ml SDW to glass vial containing the excised, sporulating cotyledons from the previous stock (kept in the freezer or excised from freshly sporulating material). The vial was then closed, and shaken vigorously on a vortex to rupture the pustules and to facilitate the release

	A	B	C	D	E
1	Dilution =	ml of spore suspension required x spores required per 9 large squares			
2		number of spores in 1 large square x 9			
3					
4	number of spores in 1 large square:	103	ml of spore susp. Re	12	(20ml per tray at seedling stage; 40ml per tray for reinoculation)
5	spores required per 9 large squares:	15	dilution:	0.19	
6	(9 for 1st inoculation at seedling stage; 27 for reinoculation after scoring)				
7					
8	Concentration (spores per ml) =	number of spores in 9 large squares x 10000			
9			9		
10					
11	number of spores in 9 large squares:				
12	spores per ml	0.00E+00	spores per ml	0.00E+00	
13	zoospores per ml	0.00E+00	zoospores per ml	0.00E+00	

Fig. 14.3 Calculation formulae in Microsoft Excel for dilution and spore concentration

of the sporangia from the sporangiophores. The concentration of the suspension was then adjusted to 2.5×10^4 sporangia/ml with the aid of a hemocytometer (particle counting chamber) (Fig. 14.3).

Prior to inoculation, sporangial concentration is adjusted to 1×10^4 sporangia/ml as opposed to 2.5×10^4 sporangia/ml used for maintenance, because fresh sporangia are used rather than frozen sporangia. The sporangia burst to release eight zoospores, so the concentration of zoospores is, therefore, 8×10^4 per ml.

Seedlings are inoculated 7 days after sowing by spraying to runoff with sporangial suspension using an atomiser (spray inoculation) or by placing 10 µl drops of zoosporangial suspension onto the adaxial surface of each of the two lobes of each cotyledon (Fig. 14.4). When preparing a sporangial suspension containing more than one isolate, the concentration is adjusted using a formula: (number of isolates/2) × original sporangial concentration. For example, if six isolates are combined in one suspension: $(6/2) \times 1 \times 10^4 = 3 \times 10^4$ sporangia/ml giving a zoospore concentration of 2.4×10^5 zoospores/ml. After inoculation, the pots are covered with clear plastic lids and the ventilators and margins are sealed with insulation tape to allow the RH to increase to almost 100 %. The seedlings are then placed in a growth chamber at 16 °C, 8 h dark period, followed by 16 h light/8 h dark; day/night cycle, at 70–120 µmol/m^2/s irradiations for 12 days to reach peak sporulation. The WR pustules are collected, 10 days

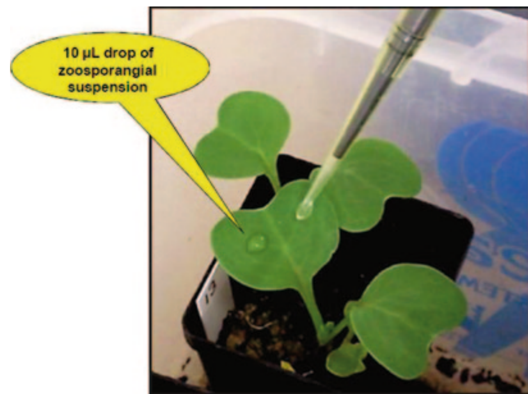

Fig. 14.4 Seedlings are inoculated by placing 10 µl drops of zoosporangial suspension onto the adaxial surface of each of the two lobes of each cotyledon

after inoculation, and immediately frozen in liquid nitrogen, and stored at −80 °C for further analysis (Meena 2007). Based on the percentage of cotyledon area covered with pustules, disease is assessed on 0 (resistant) to 9 (highly susceptible) scale (Williams 1985). Leckie et al. (1996) developed a disease scoring system to assess the host plant response as well as the growth of the pathogen, 14 days after inoculation. Accordingly, seedlings were categorized into eight interaction phenotype classes.

In a screen house tests using oospore-infested-seeds, Kolte et al. (1998) observed, both in *B. juncea cv.* Varuna and *B. rapa* var. Toria cv. PT 303, very distinctive WR pustules in 20 days after seeding. The pustules on cv. Varuna were more conspicuous than cv PT 303. The cotyledonary-infected plants were 79.5 % in cv. Varuna compared to only 4–12 % in cv. PT 303. Two to fifteen percentage of infected plants of cv. Varuna produced stagheads compared to none in cv. PT 303. Results of these preliminary experiments suggest that in India two different biological races of *A. candida* exist on *B. juncea* and *B. rapa*; more comprehensive studies are needed to confirm these findings.

14.15 Detached Leaf Culture

Verma and Petrie (1978), and Verma (2012) developed a very reliable and reproducible detached-leaf inoculation technique, where 12–14-days-old detached leaves of *B. rapa* are placed, (lower surface down on medium) within 15 min of detachment, into Petri dishes containing 20–25 ml of autoclaved medium containing 0.5 ppm benzyladenine and 0.8 % agar, drop inoculated with a zoospore suspension (75,000–100,000 zoospore/ml) derived from sporangia of *A. candida* race 7, and incubated under 100 % RH for 72 h with day–night temperature regime of 21 and 16 °C, respectively. Observations for WR pustules are recorded 10–17 days after inoculation.

The detached-leaf culture technique was used to study the influence of temperature on the temporal progression of WR, the development of disease on leaves of different ages, and the development of disease on leaves detached at the end of light and dark periods (Verma et al. 1983; Verma 2012). This information was necessary for using detached-leaf culture technique for screening of rapeseed cultivars for resistance against *A. candida.*

Temperature, leaf age, time of leaf detachment, and the interaction of these factors had a significant effect on the temporal development of *A. candida* race 7 on detached leaves. Of the temperatures tested (3–32 °C), 21 °C gave the best disease development, with 18.5 °C being the calculated optimum. The disease did not develop at 3, 29, and 32 °C and was slow to develop at 9, 12, and 27 °C. There was a highly significant ($p < 0.01$) interaction between length of incubation period and temperature. Unlike intact plants, detached leaves developed pustules on both surfaces. Infection occurred on leaves of all ages, but medium-aged leaves supported the maximum number of pustules, followed by the younger leaves. Leaves detached at the end of a dark period developed more pustules than those detached at the end of the light period. When using the detached leaf culture technique for screening germplasm for resistance to WR, we advise an adaxial surface inoculation of cotyledons, medium-aged-leaves, and an incubation temperature of 18–22 °C.

The detached-leaf inoculation technique is ideal to screen breeding lines for resistance to *A. candida*, for establishment and maintenance of single zoospore cultures, and different biological races. The technique results in greater uniformity of experimental units, more economic use of growth chamber space, greater chance of manipulation, and environmental control. From the plant breeder's point of view the program efficiency is increased, since the breeder can select resistant material for intercrossing from among vigorously growing plant population rather than a weak group of resistant plants that have survived the unfavorable environment necessary to obtain differential infection on potted plants.

14.16 Growth Chamber and Greenhouse Screening

Verma and Petrie (1978, 1979, 1980), Verma et al. (1975, 1983, 1999), Singh and Singh (1983, 1999), and Goyal et al. (1995, 1996a, b) in their several comprehensive studies described growth chamber or growth room inoculation technique, with some variation between studies, as follows: Seeds of host plants are sown 2 cm deep in a modified Cornell soilless mix (Stringam 1971) in 16 cm diameter plastic pots and placed in 7 cm deep trays in a growth chamber with an 18 h photoperiod (312 μEM^2S^{-1}), and day–night temperatures of 18° and 14 °C, respectively. The plants are watered by flooding and draining the trays. Two weeks after seeding, plants are inoculated by spraying zoospore (100,000–150,000 per ml) suspension to runoff. Control plants are sprayed with distilled/tap water. Following inoculation, plants are exposed to continuous misting for 72 h with day–night temperature of 18° and 14 °C, respectively. Except for an initial 24 h dark period, an 18 h day was maintained for the duration of the experiment. Two weeks after inoculation the number of diseased plants per pot and disease severity ratings on the most heavily infected leaf on each diseased plant are recorded using 0–4 disease severity scale (Verma et al. 1999). Sporangial spray inoculation technique has also been reported by several other researchers (Fan et al. 1983; Fox and Williams 1984; Pound and Williams 1963; Singh and Singh 1983; Stone et al. 1987; Verma and Bhowmik 1986). This technique has also been used successfully for producing hypertrophies of inflorescence (stagheads) in artificially-inoculated plants by Verma and Petrie (1980), and Goyal et al. (1996a).

14.17 Field Screening

Verma and Petrie (1980), and Verma (2012) were first to demonstrate the potential importance of using oospore-infested seeds for initiating WR infection. In their 2 years of field experiments, seeding oospores of *A. candida* race 7 with seeds of turnip rape (*B. rapa*) cv. Torch resulted in a significant increase over the control in both foliar- and systemically-infected (stagheads) plants. Saharan et al. (1988) and Lakra and Saharan (1988c) also established a WR disease nursery by infesting soil with a staghead powder continuously for 3–5 years by broadcasting, by seeding oospores-infested plant material, and also by sowing oospores-infested seeds. Frequent irrigations are given to maintain high humidity. Sporangial spray inoculations were also made at the seedling, branching, and flowering stages of the crop. Genotypes were usually grown in paired rows between highly susceptible "infector" rows of plants. Flowers were inoculated by plucking the main shoot about 6 inches" from the tip of the test row genotype. This allowed the sporangial inoculum of the taller infector plants to fall on to the test plants. Rapid identification of resistant genotypes can be reliably identified under suitable controlled environment conditions at the cotyledonary, seedling, or flowering stages (Li et al. 2007).

14.17.1 GGE Biplot Analysis of Brassica Genotypes for WR Disease Severity Under Aided Epiphytotic Conditions

It involves analysis of variance which represents precentage of the total sums of squares accounted for by genotype (G), environment (E), and genotype–environment (GE) interactions over the location under testing, and mean performance and stability of genotypes which includes, the interrelationship among genotypes and locations; mean performance of genotypes at different locations; stability of genotypes across the locations; comparision among the genotypes; and "which-won-where" pattern to identify the best genotypes in each environment for genotypes of *Brassica* under test.

Analysis of variance: The percentage of the total sums of squares accounted for by G, E, and GE interactions are used as an indicator of variation attributed seed yield (Table 14.2). Variation due to G or GE interaction is a measure of how cultivars respond across environments/locations.

Table 14.2 Degree of freedom, sum of squares, significance levels, and total percentage of total variation of G, E, and GE interaction. (Meena et al. 2011)

Seed yield per hectare	Source	DF	MS	F	P	SS
	Environment (E)	12	1361.3	20.1	0.00001	60.6
	Genotypes (G)	14	447.3	6.6	0.00001	19.9
	GE	168	436.7	6.5	0.00001	19.5

DF = degree of freedom, *F* = Fisher's value, *P* = *p*-value, *SS* = sum of squares

Fig.14.5 Genotypes by locations relationship with respect to white rust disease index. (Meena et al. 2011)

The environmental component (years and locations) represents how the genotypes are different across the locations in spatial stability. The total sums of squares are 18.4 % for environment, 48.1 % for genotype, and 33.5 % for the interaction for disease severity. As genotype by environment interaction accounted for 33.5 % of the total variation for disease index indicates the presence of crossover interactions due to nonspecific response of genotypes for disease intensity at different locations.

14.17.1.1 Mean Performance and Stability of the Genotypes Across the Locations:

(a) Interrelationship Among Genotypes and Locations Figure 14.5 provides the summary of the interrelationship among the environments (locations over years) for different genotypes, disease severity response at different locations during the years. The lines that connect the biplot origin and the markers for the environments are called environment vectors. The angle between the vectors of two environments is related to the correlation cofficient between them. The cosine of the angle between the vectors of two environments approximates the correlation cofficient between them. Based on the cosine of angles of location vectors, Morena and Pantnagar locations and different agroclimatic conditions for WR development comapred to rest of the locations. Among the years, 2008 had similar climatic conditions at all locations, while 2009 and 2010 had maximum dissimilarity in climatic

parameters. The presence of wide obtuse angles, i.e., strong negative correlations among the locations, is indication of strong crossover genotype by environment interactions. The vector length of location(s) meausres discriminating power of the location for screening genotypes sensitive to WR disease severity as the ideal environment should have both power of discriminating and representativeness for selecting wide adaptive genotypes. Hence, genotypes like EC 414324, RM 604, and EC 414109 found to be the most susceptible, while GSL 1, PBC 9221, and EC 414299 resistant to WR disease (Meena et al. 2011).

(b) Stability of Genotypes Across the Locations The ideal genotype should have both high mean performance coupled with high stability to give wide adaptability in the target region. But in this case low mean value is desirable because high value means more susceptibility to disease. The single-arrowed line, called average-environment coordination abscissa (or AEA), points to higher mean disease index across the locations. So far, stability of resistance is concerned, GSL 1, EC 414299, and EC 399299 showed consistent performance at different locations over the years showing wider adaptability. On the other hand, Varuna and JMM 07-2 showed inconsistent behavior with respect to disease reaction over the locations and years. Similarly, GSL and PBC 9221 exhibited maximum resistance to WR at all the locations. However, some of the genotypes, viz., JMM 07-2, JYM 10, and EC 399299 exhibited specific adaptability for resistance to WR at different locations. Genotypes like EC 414324, RM 604, Varuna, and EC 414322 exhibited the susceptible reaction at all locations over the years except Varuna at Morena during 2008 (Meena et al. 2011).

(c) Ranking of Genotypes Based on Performance at Specific Location and Across the Locations Suppose we want to see the differential response of genotypes Varuna at different locations across years, for this, a line will be drawn which passes through the biplot origin and Varuna cultivar. Varuna found to be susceptible at most locations over years except, where this showed resistant reaction. Similarly only two genotype, viz., GSL 1 and EC 414299 were free of disease at locations, while rest of the genotypes exhibited mixed reaction with different locations. Similarly, we can visualize response of particular locations for disease score among the genotypes. In present investigation, different years viz-a-viz locations gave different ranking of disease score for WR disease indicating the clear-cut presence of crossover interactions (COI), which necessitates exploitation of genotype–environment interaction (GEI). It is very clear from reversal sensitivity of genotypes to disease index indicating that natural selection had played its role in restructuring genetic makeup of host–genotypic interaction for specific geographical regions. This means that specific adaptability of genotypes resistance for these locations is entirely different and GEI can be exploited while selecting genotypes for cultivation rather than ignoring (Meena et al. 2011).

(d) Comparison Among the Genotypes The distance between two genotypes approximates the Euclidean distance between them, thus is a measure of dissimilarity among the genotypes. Therefore Varuna and PHR 2, RM 604 and EC 414324 are quite different in their genetic makeup from JYM 10, JMM 07-1, to JMM 07-2. Similarly, GSL 1, PBC 9226, and EC 414299 from JGM 901 to EC 414322. The biplot origin also represent a "virtual" genotype that assumes the grand mean values and zero contribution additive effect of G and multiplicative interactions (GE). The vector length of a genotype of the origin of biplot is due to the contribution of G or GE, or both. Genotypes those are located near to the origin have little contribution, viz., EC 399299, PHR 2, and JGM 901 to either G or GE, and genotypes having longer vectors indicate the contribution of G or GE, or both. Therefore genotypes with the longest vectors are either best (PBC 9226 and GSL 1), or the poorest (EC 414324), or most unstable (Varuna). The GSL 1 can be considered as best genotype as it is very close to ideal (Meena et al. 2011).

14.18 Detection of White Rust Pathogen

A polymerase chain reaction (PCR) assay has been developed to detect *A. candida* internal transcribed spacer region 1 (ITS 1) of the ribosomal DNA (rDNA). Field observations suggest that the *Albugo* can exist as latent, asymptomatic, systemic infections. The wild crucifer species *Lepidium campestre* (L.) R.Br., *Arabidopsis lyrata* L., and *Erysimum menziesii* ssp. *eurekense* R.A. Price were chosen as models to test this hypothesis. Detection of the parasite in asymptomatic host tissue was based on PCR amplification of rDNA ITS 1 of *A. candida* using primers designed to discriminate between plant and parasite DNA template. *A. candida* was detected in high proportions of asymptomatic field plants (vegetative and reproductive), greenhouse grown vegetative rosettes, and laboratory germinated seedlings of each species. For example, *A. candida* appeared to be systemic in all 20 field-grown plants and 10 inoculated rosettes of *L. campestre. A. candida* was present in non-inoculated rosettes and seedlings (up to 98 %) suggesting seed borne transmission of the pathogen. Although, the parameters controlling symptom development are still unclear, presence of the pathogen appeared not to be sufficient for symptom expression. Persistent, asymptomatic, systemic infections that are vertically transmitted through seed suggest that *A. candida* may possibly form endophytic relationships with at least some of its crucifer hosts (Jacobson et al. 1998).

A PCR-based assay for the detection of ITS 1 of the rDNA of *A. candida* has been developed. Similar PCR-based approaches have become widely used to detect *Albugo* spp. (Jacobson et al. 1998). Primers were designed based on sequence differences in the ITS 1 sequences between *A. candida* and *Lepidium oleraceum*. The primers preferentially amplified the *A. candida* ITS 1 from tissue samples where the plant ITS 1 template predominated. *A. candida* ITS 1 DNA was detected in a number of asymptomatic plants of *L. oleraceum*, as well as in surface-sterilised seeds collected from plants grown at Auckland Regional Botanic Gardens (ARBG). These findings suggest that in *L. oleraceum, A. candida* can be transmitted vertically through seed and can also exist as a latent asymptomatic infection. With further development, this technique could have the ability to detect *Albugo* in wild and *ex situ* populations, and could assist in efforts to ensure that wild stocks are not placed at risk by contamination from restoration plantings (Armstrong 2007).

14.19 Production of Oospores

Zoospore suspensions are prepared according to the method described by Williams (1985). Zoosporangia are placed in Erlenmeyer flask containing 20 ml of double distilled water, the flasks are covered with Parafilm, and shaken gently for even distribution of zoosporangia. The resulting suspensions are incubated at 12–16 °C for about 3 h to induce zoosporangenesis. Six days after seeding, cotyledons of *Brassica* plants are inoculated with a zoospore suspension ($1–2 \times 10^5$ zoospore ml^{-1}). A 10-µl droplet of inoculum is placed on the adaxial surface of each half cotyledon using a pipette. The inoculated seedlings are incubated for 24 h in a mist chamber at 20 °C with the initial 12 h in dark. Afterward, they were transferred to a growth chamber and grown under 18-h photoperiod at day/night temperature of 22/17 °C.

Two methods are used to produce oospores in inoculated cotyledons. With the first method (Method I), infected cotyledons are detached 5–7 days after inoculation, rinsed with distilled water to remove asexual spores (zoosporangia), and then placed on moist filter paper in 9-cm petri dishes. The petri dishes are incubated in the growth chamber. Detached cotyledons are moistened by adding distilled water regularly with a pipette. After 10–14 days of culture, cotyledons are fixed, cleared, and examined for the presence of sexual spores (oospores) using light microscopy. With the second method (Method II), the infected cotyledons are allowed to senesce naturally on seedlings grown under the same conditions as described above. Brownish cotyledons are collected at the time of abscission and stored in envelopes at room temperature. Segments of dried cotyledons are crushed in a drop of distilled water on a microscope slide and examined for the presence of oospores by light microscopy. The experiments are repeated. Analyses of vari-

ance are computed to test the difference between the two isolates in oospore production with either Method I or Method II. Data from the experiments using the two different methods are also pooled and analyzed as a split-plot design to test the effects of methods on production of oospores by two isolates (Liu and Rimmer 1993.

14.20 Induction of Stagheads

Plants of *B. rapa cv*. Torch are inoculated with a zoospore suspension at the seedling, two-leaf, six-leaf, or early-bud stage. Three different inoculation methods are tested: (1) drop inoculation of cotyledons or leaves, (2) spray inoculation of whole plants, and (3) injection of stems and flower buds. Hypertrophic stems and floral parts are induced through inoculation of plants at all four growth stages, but the highest percentage of plants with stagheads are obtained in plants inoculated at GS 3.1. The three inoculation methods are equally effective in inducing staghead formation. To hasten staghead formation, development and maturity, the rapid-cycling lines of *B. juncea* CrGC4-2 and *B. rapa* CrGC1-19 (Williams and Hill 1986) are used as hosts. Plants are grown in 15-cm-diameter plastic pots filled with Metro-Mix, liquid fertilizer (20–20–20, N–P–K) applied at 7–10-days intervals during the growth period, and leaves and flower buds of CrGC4-2 and CrGC1-19 are spray-inoculated with a zoospore suspension when plants are at GS 3.1. The inoculated plants are incubated in a mist chamber for 72 h and transferred to the growth chamber under continuous daylight at 20±2 °C for 4 weeks. Plants with stagheads or hypertrophied stems are then moved to the greenhouse and grown to maturity (Liu and Rimmer 1993).

14.21 Biotic and Abiotic Elicitation of Rapeseed and Preparation of Extracts

B. rapa cvs. Torch (rapeseed) and Reward (canola) were sown in individual pots in peat-lite at day/night temperature 20/16 °C, and 16 h photoperiod (250 µmol s^{-1} m^{-2}). Seven-day-old fully expanded cotyledons and 14-day-old leaves were used for inoculation. For time course analysis by high-performance liquid chromatography (HPLC), three individual plants in separate pots were used as replicates for each cultivar, with three leaves per plant collected and extracted as reported below (Pedras et al. 2008).

A. candida races 2A, 2V, 7A, and 7V were used for biotic elicitation. Two-week-old leaves of cvs. Torch and Reward were inoculated with zoospore suspensions of 10 µl droplets per leaf, and inoculated plants were incubated in a growth chamber in the dark for 24 h at 16 °C at 100 % RH. After 24 h, the chamber environment was changed to the growth conditions described above. Abiotic elicitation studied in $CuCl_2$-sprayed plants. After elicitation, the plants were incubated in a growth chamber under conditions described above. Three leaves per plant samples were collected every 24 h up to 10 days, 500–800 mg leaves were frozen and ground in liquid N_2, and extracted with MeOH (6–8 ml) on a shaker at 120 rpm for 3 h. The leaf solids were removed by filtering, and the solvent evaporated to give a concentrated sample. The resulting oily residue was rinsed with CH_2Cl_2 (5 ml) and the remaining solid residue (polar extract) dissolved in MeOH–H_2O (1:1, 200 ll) for HPLC analysis. The CH_2Cl_2 extract (nonpolar fraction) was concentrated under reduced pressure and dissolved in CH_3CN (60 µl) for HPLC analysis (Pedras et al. 2008).

14.22 Inhibition of Zoospore Release from Sporangia

Sporangia of *A. candida* race 2V and 7V (ca. 10 mg) were added to a 250 ml Erlenmeyer flask containing 100 ml of 10 % MeOH in SDW (v/v), and the flask swirled to suspend zoosporangia. This zoosporangium suspension (10 ml) was dispensed into 20 ml vials and 10 µl of the test solution (phytoalexin or compound in DMSO) was added to vials to make final concentrations of 5, 10, and 50 µM. Stock solutions of fungicides benomyl and ridomil-MZ were made in 10 %

aqueous MeOH, which were then added to assay vials to make final concentrations of 5, 10, and 50 µg/ml. Dimethyl sulfoxide (DMSO) (10 µl) was added to control assay vials. All vials were incubated at 16 °C, in complete darkness for 3 h. The experiments were repeated three times with six replications. Mobile zoospores were counted using a hemocytometer under a phase contrast microscope (40 ×), and the percentage inhibition relative to controls was calculated according to the following equation: Percentage inhibition = ((number of zoospores in control solution – number of zoospores in test solution)/number of zoospores in control solution)× 100 (Pedras et al. 2008).

14.23 Inhibition of Cyst Germination on Cellulose Dialysis Membrane

A cellulose dialysis membrane is cut into ca. 1 cm squares and autoclaved dialysis membrane pieces are placed on 2 % H_2O agar to ensure even hydration (Francis et al. 1996). After 24 h, the membrane pieces are dipped separately in 5, 10, and 50 µM concentration of phytoalexin or 5, 10, and 50 µg/ml of fungicide in 10 % aqueous MeOH and each piece placed back on agar; solutions are allowed to evaporate in laminar flow hood for 15 min. For controls, cellulose dialysis membranes are dipped in 10 % aqueous MeOH and solutions are allowed to evaporate in laminar flow hood for 15 min. *A. candida* zoospore suspension (10 µl) is added to each membrane piece and plates are incubated at 16 °C in complete darkness for 24 h. Cyst germination is assessed under a phase contrast microscope (40 ×), and the percentage inhibition of cyst germination is calculated according to the above equation (Pedras et al. 2008).

14.24 Extraction of Zoosporangia

Zoosporangia of *A. candida* races 2V and 7V are obtained from leaves of 21-day-old *B. juncea* cv. Commercial Brown and *B. rapa* cv. Torch, respectively (Rimmer et al. 2000). Zoosporangia (300 mg) are suspended in EtOAc (2 ml), sonicated for 20 min, the solvent is separated from zoosporangia by filtration, and concentrated under reduced pressure using a rotary evaporator. The extracted zoosporangia are resuspended in MeOH (2 ml) and sonicated for further 20 min. The MeOH extract is separated by filtration and concentrated; zoosporangia are autoclaved and discarded. Both organic extract residues are combined (19 mg), concentrates rinsed with dichloromethane (2 ml) to yield a dichloromethane extract (7.6 mg), and dissolved in MeOH (7 mg) or H2O (4.8 mg). Each extract is subjected to HPLC-diode array detector (HPLC-DAD), HPLC-mass selective detector (HPLC-MSD), and nuclear magnetic resonance (NMR) analysis to determine the chemical components (Pedras et al. 2008).

14.25 Measurement of Spores

Quantitative microscopy is a very useful tool to resolve morphological factors of a pathogen. It requires observations of shape, size, and other ornamental features using stage and ocular micrometry as follows:

The measurement of length/width using the light microscope requires the following:

- Stage micrometer, positive 5 = 100/100 y, D = 0.17 mm as the object.
- Eyepiece cross line micrometer 10:100, d = 26 mm in the eyepiece.

Before length measurement using the microscope can be performed, the micrometer of scale value of the objective/eyepiece reticule combination must be determined. This scale value is exactly that distance in the specimen which complies with one interval of the used cross line micrometer.

For calibration, align the scales of the stage micrometer and the cross line micrometer parallel to each other by turning the eyepiece, and make the zero line of both scales exactly congruent. If, for example, 99 increments (10 µm each) of the stage micrometer correspond to exactly 100 increments of the cross line micrometer, the resulting scale value K’ for the used objective/

eyepiece reticule combination (A-Plan 10x/0.25 and cross line micrometer 10:100) is

$$K' = 99/100\text{x } \mu\text{m} = 9.9 \ \mu\text{m}$$

The distance to be measured should be ≥5 mm in the eyepiece field to keep the influence of random measuring deviations as low as possible. Other measuring errors can occur if the eyepiece has not been inserted into the tube until stop.

14.26 DNA extraction by CTAB Method

For DNA extraction methodology described by Lee and Taylor (1990) is with the steps as follows:

- Mycelium is harvested from liquid cultures by filtration through Whatman No. 3 filter paper and damp dried.
- Grind freeze dry mycelium into a fine powder in liquid nitrogen and place 50 mg in eppendorf centrifuge tubes.
- The homogenized material is transferred to pre-warmed 2× CTAB DNA extraction buffer (100 mM Tris (pH-8.0), 20 mM ethylenediaminetetraacetic acid (EDTA) (pH-8.0), 1.4 M NaCl, 2% cetyl trimethylammonium bromide (CTAB), and 2 µl/ml β-merceptoethenol).
- Incubate for 1 h in a 60 °C water bath, occasionally mixing gently.
- After removing from water bath, equal volume of chloroform–isoamylalchohol (24:1) is added, and mixed by inversion for 15 min.
- Spin at 15,000 rpm (10,000 g) for 15 min at 4 °C
- The aqueous phase is taken and transferred to another tube.
- Prechilled equal volume of absolute alcohol or six volume of isopropanol is added to precipitate the DNA.
- DNA–CTAB complex is precipitated as a fibrous network, lifted by a pasture pipette, and washed in solution.
- Centrifugation at 15,000 rpm for 15 min at 4 °C.
- Pellet is washed with 70% alcohol, micro centrifuge (10,000 rpm) three times, each for 10 min at 4 °C, and dried overnight.
- The dried DNA pellet is redissolved in tris-EDTA (TE) buffer (pH-8.0) by keeping overnight at room temperature.

14.27 Purification of DNA

RNA is removed by treating the sample with DNase free RNase. Protein including RNase is removed by treating with Chloroform:Isoamyl alcohol (24:1). The purification is carried out by following the steps of method given by White et al. (1990):

- 2.5 µl of RNase is added to 0.5 ml of crude DNA (2.5 µ of RNase =25 µg of RNase, so treatment is 50 µg/ml of DNA preparation).
- Mix gently and incubate at 37 °C for 1 h.
- After 1 h, a mixture of 0.3–0.4 ml of chloroform:isoamyl alcohol (24:1) is added, and mixed thoroughly for 15 min till an emulsion is formed.
- Centrifuge for 15 min at 15,000 rpm.
- Supernatant is taken avoiding whitish layer at interface.
- The DNA is reprecipitated by adding double the quantity of absolute alcohol.
- To pellet DNA, the tube is centrifuged for 5 min at 5,000–10,000 rpm.
- The pellet is washed with 70% alcohol and dried overnight.
- The DNA is redissolved in TE buffer.

14.28 Gel Analysis by Agarose Gel Electrophoresis

The integrity of DNA is judged through gel analysis by the method of Hudspeth et al. (2000) in following steps:

- Cast 150 ml of 0.8% agarose gel in 1× tris-borate-EDTA (TBE) buffer containing ethidium bromide (0.5 µg/ml).
- Load 2 µl of DNA with 6× loading dye (bromophenol blue).

- Load a known amount of uncut Lambda phage DNA as control in the adjacent well.
- Run the gel at 50 V for 1 h.
- Visualize gel under UV light.
- Presence of single compact band at the corresponding position to phage DNA indicates high molecular weight of isolated DNA.

14.29 Quantification of DNA

The quantification of DNA is done by observing it at 260 and 280 nm wavelength by using a spectrophotometer (UV visible) method of Hudspeth et al. (2000):

- Take 3 ml TE buffer in a cuvette and calibrate the spectrophotometer at 260 nm as well as at 280 nm.
- Add 3 µl of DNA, mix properly and take reading of optical density (OD) at both 260 and 280 nm.
- Estimate the DNA concentration employing the following formula: Amount of DNA (µg/ml) = (OD) 260 × 50 × dilution factor/1000.
- Check the quality of DNA from the ratio of OD value recorded at 260 and 280 nm.

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15 Future Research Priorities

During the past century, mycologists/plant pathologists/researchers in the world have endured enormously to excel in the comprehension of white rust disease of crops. However, some priority areas of research pointed below may give quantum jump in the production and productivity of crops suffering from white blister rust. Formation of inter- and intra-institutional working groups at national and international level may give boost to get reliable and repeatable research results:

- Generation of enough data on epidemiological elements of white rust to develop disease forecasting system.
- Recommendation of a package for integrated disease management.
- Determination of morphological and histological basis of host resistance along with induced resistance mechanism.
- Identification of genotypes with few numbers of stomata and narrow stomatal aperture to ensure mechanical host barrier.
- Information regarding presence/production of oospores inside the seeds, and their possible importance in the survival of the pathogen is lacking.
- Role of simple or branched germ tube from germinating oospores need to be studied.
- Single zoospore cultures from germinating sporangia and oospores must be prepared, and their pathogenicity compared.
- After screening lines for resistance against foliar infections, some select advanced lines must also be screened for production of stagheads using flower-bud inoculation technique.
- Mycologists and taxonomists may consider the division of *Albugo candida* complex into different species depending on host specificity.
- There is a need to standardize the host differentials in each crucifer species internationally, and more so in the form of isogenic lines to get true pictures of *A. candida* races/pathotypes.
- Nomenclature of the *A. candida* races should be standardized internationally, viz., AC jun 1, 2 for *Brassica juncea* isolates, AC rap 1, 2 for *Brassica rapa* isolates, AC nig 1, 2 for *Brassica nigra* isolates, AC ol 1, 2, etc. for *Brassica oleracea* isolates, respectively.
- Identification of sources of resistance should be based on broad spectrum effectiveness of a genotype against specific races.
- Efforts should be made to identify resistance loci in the genotypes along with alleles for resistance in each locus.
- Genotypes exhibiting attributes of slow white rusting, disease tolerance, and partial resistance may be categorized.
- Studies on inheritance of virulence may be undertaken along with virulence spectrum.
- Mapping, cloning, characterization, and identification of marker genes for resistance and virulence at molecular level may be strengthened.

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- Genetics of *Albugo–Hyaloperonospora* association may be determined both at phenotypic and genotypic levels.
- Strong and weak genes for resistance in the host with their suitable combinations for durable resistance may be looked into.
- Sources of multiple disease resistance should be explored.
- Morphological, histological, and biochemical basis of host resistance should be identified.
- Rapid identification of latent infection under field conditions using modern techniques of biotechnology is needed.
- Using PCR technique, presence or temporal progression (vertical growth) of *A. candida* mycelium from infected cotyledons or primary leaves should be determine importance of systemic infection in the production of stagheads.

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