Chittaranjan Kole *Editor*

Genomic Designing for Biotic Stress Resistant Oilseed Crops

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Dedicated to

Prof. Roger D. Kornberg
Nobel Laureate in Chemistry 2006
Professor of structural biology at Stanford University School of Medicine

With regards & gratitude for his generous appreciations of my scientific contributions and service to the academic community, and constant support and encouragement during my professional journey!

Preface

Crop production is drastically affected due to external or environmental stresses. The biotic stresses cause significant yield losses in the range of 31–42% together with 6–20% loss during the post-harvest stage. The abiotic stresses also aggravate the situation with crop damage in the range of 6–20%. Understanding the mechanisms of interaction of plants with the biotic stresses caused by insects, bacteria, fungi, viruses, oomycetes, etc., and abiotic stresses due to heat, cold, drought, flooding, submergence, salinity, acidity, etc., is critical to develop resilient crop varieties. Global warming and climate change are also causing emergence of new diseases and insects together with newer biotypes and physiological races of the causal agents on the one hand and aggravating the abiotic stress problems with additional extremes and unpredictability. Development of crop varieties resistant and/or adaptive to these stresses is highly important. The future mission of crop improvement should, therefore, lay emphasis on the development of crop varieties with optimum genome plasticity by possessing resistance or tolerance to multiple biotic and abiotic stresses simultaneously. A moderate estimation of world population by 2050 is about 9.3 billion that would necessitate an increase of crop production by about 70%. On the other hand, the additional losses due to climate change and global warming somewhere in the range of 10–15% should be minimized. Therefore, increase in the crop yield as well as minimization of its loss should be practiced simultaneously focusing on both 'adaptation' and 'mitigation.'

Traditional plant breeding practiced in the last century contributed a lot to the science of crop genetic improvement. Classical plant breeding methods including selection, hybridization, polyploidy and mutation effectively catered to the basic F^5 needs—food, feed, fiber, fuel and furniture. The advent of molecular breeding and genetic engineering in the latter part of twentieth century complimented classical breeding that addressed the increasing needs of the world. The twenty-first century came with a gift to the geneticists and plant breeders with the strategy of genome sequencing in Arabidopsis and rice followed by the tools of genomics-aided breeding. More recently, another revolutionary technique, genome or gene editing, became available for genetic correction of crop genomes! The travel from 'plant breeding' based on visual or perceivable selection to 'molecular breeding' assisted

by linked markers to 'transgenic breeding' using genetic transformation with alien genes to 'genomics-aided breeding' facilitated by known gene sequences has now arrived at the age of 'genetic rectification' employing genome or gene editing.

Knowledge on the advanced genetic and genomic crop improvement strategies including molecular breeding, transgenics, genomic-assisted breeding and the recently emerged genome editing for developing resistant, tolerant and/or adaptive crop varieties is useful to students, faculties and scientists in the public and private universities and organizations. Whole-genome sequencing of most of the major crop plants followed by genotyping-by-sequencing has facilitated identification of exactly the genes conferring resistance, tolerance or adaptability leading to gene discovery, allele mining and shuttle breeding which in turn opened up the scope for 'designing' or 'tailoring' crop genomes with resistance/tolerance to biotic and abiotic stresses.

To my mind, the mission of agriculture in this century is FHNEE security meaning food, health, nutrition, energy and environment security. Hence, genome designing of crops should focus on breeding of varieties with higher yields and improved qualities of the five basic F5 utilities; nutritional and neutraceutical compounds; and other industrially and aesthetically important products and possibility of multiple utilities. For this purpose of 'precise' breeding, employment of the genetic and genomic techniques individually or in combination as and when required will play a crucial role.

The chapters of the 12 volumes of this twin book series entitled *Genomic Designing for Biotic Stress Resistant Crops* and *Genomic Designing for Abiotic Stress Resistant Crops* will deliberate on different types of biotic and abiotic stresses and their effects on and interaction with crop plants; will enumerate the available genetic diversity with regard to biotic or abiotic stress resistance among cultivars; will illuminate on the potential gene pools for utilization in interspecific gene transfer; will brief on the classical genetics of stress resistance and traditional breeding for transferring them to their cultivated counterparts; will discuss on molecular mapping of genes and QTLs underlying stress resistance and their marker-assisted introgression into elite crop varieties; will enunciate different emerging genomics-aided techniques including genomic selection, allele mining, gene discovery and gene pyramiding for developing smart crop varieties with genetic potential to produce F^5 of higher quantity and quality; and also will elaborate the case studies on genome editing focusing on specific genes. Most of these chapters will discuss on the success stories of genetic engineering in the relevant crops specifically for generating crops with resistance and/or adaptability to diseases, insects and abiotic stresses.

There are obviously a number of reviews and books on the individual aspects of plant molecular breeding, genetic engineering and genomics-aided breeding on crops or on agro-economic traits which includes the 100-plus books edited by me. However, there is no comprehensive reviews or books available that has coverage on crop commodity groups including cereals and millets, oilseeds, pulses, fruits and nuts, vegetables and technical or industrial crops, and modern strategies in single

volumes with precise focuses on biotic and abiotic stresses. The present volumes will fill this gap with deliberations on about 120 important crops or their groups.

This volume on "*Genomic Designing for Biotic Stress Resistant Oilseed Crops*" includes eight chapters focused on Soybean, Rapeseed, Sunflower, Peanut, Rape and Mustard, Sesame, Castor Plant and Flax contributed by 67 scientists from 7 countries including Australia, Canada, China, India, Mali, Serbia and USA. I remain immensely thankful for their highly useful contributions.

I am indebted to my wife Phullara who as always has assisted me directly in editing these books and indirectly through maintaining an academic ambience to pursue my efforts for science and society pleasantly and peacefully.

New Delhi, India Chittaranjan Kole

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Chapter 1
Genomic Design for Biotic Stresses in Soybean

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Abstract Soybean is an agro-economically leading crop of the world. Soybean is rich in seed protein (about 40%) and oil (about 20%) and enriches the soil by fixing nitrogen through symbiosis with bacteria. It is widely used as food, feed, and for industrial purposes. In soybean, biotic stresses such as insects-pests and diseases have

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emerged as the major challenge for increasing production. Breeding for tolerance to biotic stresses has made excellent progress however application of novel approaches such as genomic technologies are imperative to meet the challenges. Genomic crop designing and approaches have enabled the rapid improvement of soybean than traditional approaches. Genomic designing overcomes the limitations of traditional breeding methods and accelerates the development of climate-smart soybean crops. Genomic-assisted breeding, genomic selection, genome sequencing, marker-assisted selection, genetic engineering approaches, and genomics tools have been utilized to improve tolerance to biotic stresses, yield and seed composition traits. Developing biotic stress-tolerant soybean varieties have become convenient with the availability of genome sequences of soybean and functional genomics studies. This chapter discusses the major milestones in soybean genetics, genome mapping and recent developments in comparative and functional genomics related to biotic stresses.

1.1 Introduction

1.1.1 Economic Importance of the Crop

Soybean [*Glycine max* (L.) Merr] is one of the world's major oilseed crop and an important source of protein and oil for the consumption of both the humans and animals. It is also used as a raw material for multiple human health and industrial applications. Soybean is a rich source of minerals and functional bioactives like isoflavones and tocopherols having immense nutraceuticals potential, and several health benefits. Therefore, sustainable soybean production is vital for food and nutritional security worldwide. Though the crop is cultivated globally, the United States of America, Brazil, Argentina, China, and India are the major producers. Data on world

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production of soybean indicated an increase till 2018–19 when a record area and production of 125 million ha and 358.85 million tons, respectively was obtained. During 2019–20 the respective area and production were expected to be 122.10 million ha and 341.76 million tons, indicating a considerable decline of 2.8% and 4.7% in soybean area and production, respectively compared to 2018–19.

1.1.2 Reduction in Yield and Quality Due to Stress

It has been observed that millions of acres of crop loss of soybean occur every year which could be attributed to the multiple biotic factors such as disease, insects and pests etc. Crop losses due to various biotic stresses demand robust strategies to increase soybean yield and maintain yield stability even under the constraints of biotic stresses. Therefore, genomic designing of soybean for enhancing resistance to various biotic stresses and for climate resilience is more relevant today than ever in order to ensure sustainable production with appreciable yield potential and nutritional value.

1.1.3 Growing Importance in the Face of Climate Change and Increasing Population

Considering the potential of soybean in diverse uses, it has become a highly desirable oilseed crop with a rapid growth in its demand. However, the increasing world population requires doubled food production by the year 2050, which could not be achieved at the current rate of yield improvement (Chaudhary et al. 2019). Additionally, the vagaries of monsoon and changing climatic conditions further compound the problem of yield reduction in major oil seed crops including soybean (Deshmukh et al. 2014). Biotic stresses along with extreme weather conditions negatively impact crop yield because precipitation, temperature, and solar radiations are the main drivers of crop growth and development. Therefore, the emphasis must be given toward the production of high-yielding soybeans with good nutritional value, which are environmentally sustainable and resistant/tolerant to extreme weather conditions.

1.1.4 Limitations of Traditional Breeding and Rational of Genome Designing

Conventional plant breeding has undeniably improved the soybean yield and introgressed genes to impart resistance to biotic stresses and to achieve the current level of demand. Nevertheless, the current challenges are to enhance the production potential

of soybean under the constraints induced by climate change. In addition, breeding for complex traits is a cumbersome process since these traits are governed by multiple loci or genes and are greatly affected by the environmental factors. Further, adoption of conventional breeding strategies such as single pod descent, backcrossing, pedigree breeding, and bulk population breeding which were successfully utilized in developing improved cultivars of soybean entails great deal of time. Hence, it is anticipated that a right combination of genomics science based breeding and traditional methodologies would help in developing resistant genotypes which can ensure sustainable soybean production under changing climatic scenarios.

1.2 Description of Different Biotic Stresses

1.2.1 Charcoal Rot

Charcoal rot is a serious fungal disease of soybean caused by *Macrophomina phaseolina* (Tassi) Goid in the tropical regions. In the tropical conditions, dry weather, relatively low soil moisture and nutrients conditions along with high temperature ranging from 25 to 35 °C are the major predisposing factors of the disease. Extensive reduction in yield of soybean is observed due to post emergence death and weakening of seedlings, or due to wilting and premature death of infected plants (Bowen and Schapaugh 1989). Symptomatically, the disease appears as a root rot and as a wilt. The fungus infects the root and stem base of the plant. The infected seedlings exhibit reddish brown discoloration at the point of emergence of the hypocotyl which is evident at the soil level and above. These infected seedlings become weak and die prematurely (Fig. 1.1a, b). Charcoal rot infection causes light brown discoloration of internal tissues of lower stem and upper tap root. In later stage the leaves become chlorotic and wilting ensues (Gupta and Chauhan 2005). The external lesions on the stem are generally observed during the later stages of infection. Abundant minute black sclerotia beneath the outer cortical tissues is a diagnostic feature of the disease. Following the seed germination, microsclerotia (sclerotia) in the soil or with the seed germinate on root surface and produce numerous germ tubes which penetrate host tissues through natural openings or epidermal cells. When the mycelium reaches the xylem tissues it produces microsclerotia, which plug the vessels resulting in discolouration and wilting of host tissues. Transmission of pathogen from seed to plant takes place by direct infection and from plant the infection reaches to seed by local infection through pods. The pathogen remains mostly in seed coat as mature hyphae and sclerotia. Seed borne infection can be detected by standard blotter method or by plating the pre-treated seeds on PDA containing 0.1% brassicol.

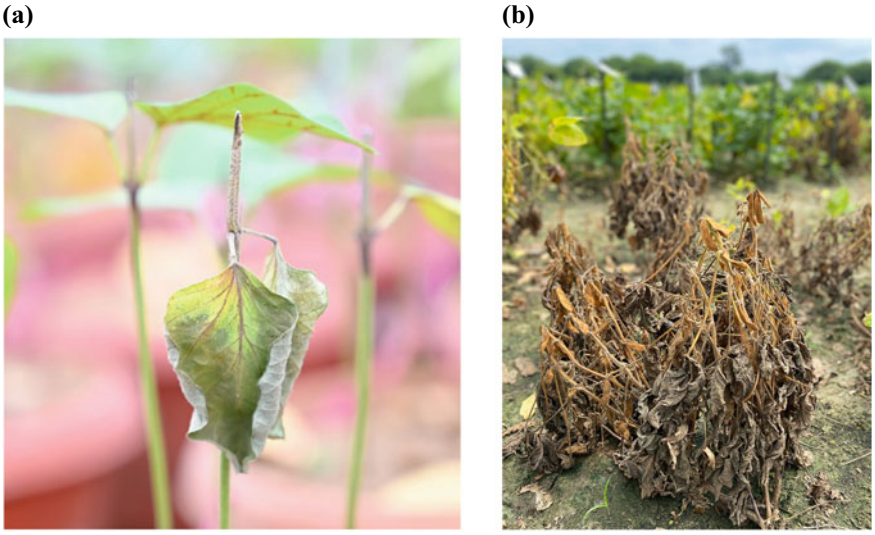

Fig. 1.1 Symptoms of Charcoal rot on soybean plant

1.2.2 Rust

Rust is caused by *Phakopsora pachyrhizi* syd. & P. Syd. and *P. meibomiae*. Infection from *Phakopsora pachyrhizi* was first reported in Japan in 1903 (Hennings 1903) and is more aggressive and predominant in Asia and Australia but now also causes rust in other countries. It is distributed throughout the soybean growing tropical and subtropical countries while *P. meibomiae* commonly occurs in south of North America, Caribbean area and South America. Rust usually appears from the middle to late in the season but infection of rust occurs in unifoliate as well as first trifoliate leaves in 3–4 weeks old seedlings (Bromfield 1984). The disease becomes severe under the conditions of moderate temperature (18–26 °C) accompanied with 80–90% relative humidity and extended leaf wetness. Temperature above 28 °C for long periods are unfavorable for rust development. Spread of rust occurs where there is high relative humidity and presence of dew over the leaf. The disease causes heavy yield reduction due to premature defoliation at pod filling stage, small sized seeds and due to the adverse effect on various yield-contributing factors. Significant losses ranging from 10 to 90% have been reported from different parts of the world. Initially, chlorotic grey brown minute spots appear on the leaves which are abundant and in groups on the lower surface which later turn tan to reddish brown angular spots. Usually, the leaf tissues around the group of spots become yellow. Slowly spots increase in size to form pustules. Leaves also turn brown causing early defoliation. Presence of loose brown powder is a characteristic symptom of soybean rust. *P. pachyrhizi* has a wide host range and can infect large number of species in the Faboidae subfamily (Bromfield 1984). Many of these may serve as collateral hosts

and may be important source of inoculum during the growing season. The life cycle of rust fungi is complex, involving different types of spores with specialization to particular hosts. Infection initiates from the germination of urediniospores originating from infected soybean or collateral hosts, in presence of free water on leaf surface and penetrate the host mainly through corticle and underlying epidermal cells. After 5 days chlorotic spots are produced and then uredinia are formed which again liberate urediniospores and help in secondary spread of the pathogen.

1.2.3 Yellow Mosaic Virus

Yellow mosaic virus (YMV) causes yield loss due to premature drying of plants, reduced number and size of pods and seeds/pod. Yield losses upto 85–100% are recorded when the plants are infected at the seedling stage (Nene 1973). Yellow mosaic infection also reduces the size and number of nodules. Symptoms of the disease appears only on leaves in the form of conspicuous yellowing along small veins of the leaves followed by severe yellow mosaic and mottling of leaves. Later, as the leaves mature rusty necrotic spots appear in the yellow areas. Mungbean yellow mosaic virus, the causative organism belongs to genus *Begomovirus* of the family *Geminiviridae*. Members of the family *Geminiviridae* have circular, ssDNA genomes encapsidated in twinned icosahedral particles (Stanley et al. 2005). Virus is spread predominantly by white flies *Bemisia tabaci* and *B. gossypiperda* and also by aphids and pollen. Comparative sequence analyses showed that the isolate from central India is a strain of Mungbean yellow mosaic India virus (MYMIV) and the southern Indian isolate is a strain of Mungbean yellow mosaic virus (MYMV) (Girish and Usha 2005). In South-East Asia the yellow mosaic disease in legumes is caused by MYMIV and MYMV. MYMIV infects several important pulse crops namely blackgram, mungbean, french bean, pigeonpea and soybean. MYMIV is a bipartite begomovirus prevalent throughout the Indian subcontinent (Haq et al. 2010; Ramesh et al. 2017a, b). Raj et al. (2006) found similarity between *Cotton leaf curl Kokhran virus* (CLCKV) with MYMIV of soybean and thus this was the first report of CLCKV as a pathogen of soybean. Genetics of YMV resistance has been reported both in cultivated *G. max* and wild *G. soja* (Bhattacharyya et al. 1999; Talukdar et al. 2013). The genes governing resistance in cultivated *G. max* and *G. soja* have been mapped by different approaches (Kumar et al. 2015; Rani et al. 2017; 2018). The molecular markers associated with YMV resistance has been utilized to introgress the resistant genes in the released cultivars through marker assisted selection. Infectious clones for screening of soybean genotypes for YMV have been found efficient in categorizing the genotypes in to different groups based on their disease reaction (Ramesh et al. 2019a). *Agrobacterium*-mediated transient expression of shRNA, targeting a conserved region of AC2 open reading frame (ORF, a VSR) of MYMIV, conferring virus resistance in soybean has showed progressive reduction of the viral titre. In addition, the newly emerging leaves exhibited symptom recovery (Ramesh et al. 2019b).

1.2.4 Soybean Mosaic

Soybean mosaic virus (SMV) is one of the major pathogen causing yield loss in soybean. Developing soybean genotypes that are tolerant or resistant to SMV is an important breeding objective for mitigating the adverse effects of the viral infection. Foliar symptoms caused by SMV include distorted and wrinkled leaves that have a mottled color pattern. Symptoms appear on young leaves, sometimes with a raised, blistered, or distorted appearance (Zhang et al. 1986). Symptoms are most obvious at cooler temperatures and often disappear when the temperatures are high. The major concern due to SMV infection is reduction of seed quality due to mottled seeds however yield is generally not affected (Goodman and Oard 1980). Nonetheless, seed mottling is associated with poor germination and may result in a grain grade reduction, when soybean is grown for food grade purposes. SMV is transmitted by more than 30 aphid species, including the soybean aphid. However, seed transmission is a prime mode of spread in the field conditions (Saghai Maroof et al. 2009).

1.2.5 Anthracnose

Anthracnose is recognized as one of the most destructive seed-borne disease of soybean, especially in warm and humid areas (Lou et al. 2009; Marmat and Ratnaparkhe 2017). Earlier two fungal species *Colletotrichum truncatum* and *Glomerella glycines* were identified, based on their cultural characteristics and pathogenicity (Manandhar et al. 1985). However, the most common pathogen associated with anthracnose is *Colletotrichum dematium* f. sp. *truncatum* or *Colletotrichum truncatum* (Schw.) Andrus & W.D. Moore. This pathogen is prevalent in almost all the soybean growing areas and extensive losses occur due to reduced seed germination, seedling blight and seed deterioration (Hartman et al. 1999). The genetic variability of the fungal isolates was further characterized using polymorphism in the internal transcribed spacer (ITS) region and by other molecular techniques (Sharma et al. 2011). When the temperature is around 35 °C along with rain, dew or fog, significant spread of anthracnose is observed. Symptoms of the disease appear on stem, petiole and on pods (Fig. 1.2). Initially, reddish to dark brown irregular spots appear which are covered by randomly arranged black fungal fruiting bodies. Infection is evident in the form of laminar vein necrosis, leaf rolling and then defoliation. When infected seeds are used for sowing, the pathogen produces dark brown sunken cankers on cotyledons and also causes seedling mortality. Infection from the mycelium of the pathogen in infected seeds or debris causes damping off of seedlings (Manandhar et al. 1987). Conidia from infected plants can also initiate the secondary infection. Transmission of pathogen from seed to plant is through systemic as well as local infection (Chen et al. 2006; Hartman et al. 1999). Several workers have identified resistant source against anthracnose. Nataraj et al. (2020) worked out genetics of anthracnose resistance in three resistant soybean genotypes, EC34372, EC457254

Fig. 1.2 Soybean pods showing anthracnose symptoms

and AKSS55 and concluded that inheritance of anthracnose resistance in each of them was through complementary fashion.

1.2.6 Soybean Cyst Nematode

Soybean cyst nematode caused by *Heterodera glycines* is one of the destructive pathogen of soybean. The race structure of the pathogen has been characterized, however the most prevalent strain is race 3, especially in the USA and China. *H. glycines* is an obligate, plant-parasitic nematode that resides in many soil types and geographical regions (Koenning and Wrather 2010). It can complete its life cycle in 3–4 week on a susceptible host, resulting in significant increase in nematode population density. Foliar symptoms associated with Soybean cyst nematode damage are often misdiagnosed as nutrient deficiencies (Niblack et al. 2006). In many cases above ground symptoms are absent altogether, even when soybean cyst nematode is causing significant yield losses. The most effective management strategy is the deployment of genetic resistance in conjunction with managed crop rotation.

1.2.7 Rhizoctonia Root Rot

Rhizoctonia solani Kühn is the causal organism of Rhizoctonia root rot (Menzies 1970). Soybean can become infected at any stage, but damage is more severe when it occurs at the seedling stage. The disease is favored by warm and wet conditions. Symptoms of this disease include reddish lesions on the hypocotyl of seedlings near the soil line. While this disease can lead to seedling death, some seedlings may survive, resulting in stunted plants. Rhizoctonia overwinters in soil and crop residue, and germinates during the spring to infect more plants. *Rhizoctonia solani* is a genetically diverse fungus, and different isolates may be more virulent on different plant hosts. The *R. solani* species complex is distinguished by its ability to undergo hyphal fusion with other strains (anastomosis), creating anastomosis groups (AG). The most common AG isolated from soybean roots is AGII-2 (Dorrance et al. 2003) which thrives well in warmer conditions.

1.2.8 Sclerotinia Stem Rot

In soybean, *Sclerotinia sclerotiorum* (Lib.) de Bary causes the destructive disease Sclerotinia stem rot, also known as white mold. The fungus survives as hard dark structures called sclerotia which are tightly packed white mycelium and covered with a dark melanized protective coat (Boland and Hall 1987). Emergence of apothecia from the sclerotia is favored by saturated soils and a full canopy. This fungus is favored by cool and moist conditions. Increasing average temperatures and more frequent rainfall increases the frequency of sclerotinia stem rot. Sclerotinia stem rot first appears as white, fuzzy mycelia on the main stem and lateral branches beginning around the R5 growth stage. The pathogen first begins its life cycle in the soil as melanized, seed-like survival structures called sclerotia. When fully mature apothecia are exposed to a slight decrease in moisture tension, commonly occurring after the morning dew dries, ascospores are forcibly ejected into the crop canopy. Apothecia exposed to humid environments continuously release ascospores. Ascospores, the primary source of inoculum, land on senescing plant parts and germinate when the temperature is between 15 and 25 °C and leaves have been wet for 2–4 h (Clarkson et al. 2003). The fungus most often infects through senescing flowers, and rarely through wounds, natural openings, and contact with neighboring plants (Grau and Hartman 2015).

1.2.9 Phytophthora Root Rot

Phythopthora root rot is caused by *Phytophthora sojae* which can infect soybeans at any growth stage. Early season symptoms include seed rot and pre- and post-

emergence damping off. A dark brown lesion on the lower stem that extends up from the taproot of the plant is an important symptom of Phytophthora root rot. The lesion often reaches as high as several nodes and girdles soybean stems, restricting flow of nutrients and water, and stunting or killing the plant. Phytophthora root rot is more severe in poorly drained soils, in no-till fields, or low-lying areas that are prone to flooding. The oomycete pathogen survives on crop residue or in the soil as oospores. When soil temperatures reach 60 °F and remains saturated, oospores germinate and produce spores, called zoospores. Warm, saturated soils after planting, are conducive to disease. Infection occurs via the roots, and from there the pathogen colonizes the roots and stems. Specific resistance genes in soybean, called resistance to *Phytophthora sojae* (Rps) genes, are responsible for resistance to various races of *P. sojae*. To date, 37 Rps genes/alleles have been identified in various soybean cultivars (Sahoo et al. 2017; Zhong et al. 2019). These Rps genes contribute to a very robust qualitative resistance against specific races of *P. sojae* (Dorrance et al. 2004). Additionally, soybeans may also contain genes that contribute to partial resistance, even though they are not classical Rps genes. These genes provide partial resistance through mechanisms such as the development of fewer lesions, smaller lesions, or allowing reduced oospore production.

1.2.10 Sudden Death Syndrome

Sudden Death Syndrome (SDS) is a root rot disease caused by a more virulent strain of the soil-borne fungal pathogen *Fusarium virguliforme*. The infection often occurs during the first 6 weeks after emergence. The disease is most often found in fields that are infested with SCN, however SDS can occur without SCN being present. The nematode after penetrating the roots, produces openings that allow the fungus responsible for SDS easy access to the internal root tissue. SDS-causing pathogens reduce yield in two-phases of disease (Roth et al. 2019). In the first phase, symptoms such as discoloration and rotting appear in the roots at the site of infection. During the second phase of the disease, infection progresses deeper into the root, the pathogen releases toxins and proteins cause foliar symptoms. Foliar symptoms appear as interveinal chlorosis that develops into necrosis, ultimately leading to premature leaf drop and pod abortion (Hartman et al. 2015). The foliar symptoms typically occur at or after flowering and are exacerbated by high soil moisture resulting from heavy rain events during flowering. Foliar symptoms can develop prior to flowering in areas with high inoculum density of *F. virguliforme* (Roth et al. 2019).

1.2.11 Bacterial Blight

Bacterial blight is a widespread soybean disease that is most common during cool, wet weather (Ashfield et al. 2012). This disease usually occurs at low threshold level

so that economic or yield loss is minimal or nil. Bacterial blight can be mistaken for Septoria brown spot. The two diseases can be distinguished by the presence of a halo around bacterial blight lesions. Bacterial blight is most common on young leaves whereas brown spot is usually seen on older, lower leaves in the plant. Bacterial blight can occur on all above ground plant parts, but is most evident on leaves in the mid to upper canopy (Sinclair and Backman 1989) Infections initially begin as small water-soaked spots which later turns yellow and then brown as the tissue dies. The spots are surrounded by yellowish-green halos. Dead patches on the leaves are observed due to merging of several small spots. These infected leaves usually remain on the plant. Infection can also occur on stems, petioles, pods, and seeds in infected pods. Infected seedlings may be stunted or killed in severe cases.

1.2.12 Bacterial Pustule

Bacterial pustule has been reported worldwide. Development of disease occurs during warm (86–91 °F) and wet weather conditions. Early symptoms include small yellowgreen spots with elevated reddishbrown centers that are visible on upper leaf surfaces (Bernard and Weiss 1973). Later, a small, slightly raised, palecolored pustule develops at the center of each lesion which is noticeable on lower leaf surfaces (Kennedy and Tachibana 1973). Leaf lesions vary from very small specks to large, irregular, mottled necrotic areas depending on the environmental conditions (Faske et al. 2021). Leaves develop a ragged appearance when the necrotic areas are torn away by stormy weather. Premature defoliation occurs during severe infection which decreases yield by reducing seed numbers and size (Weber et al. 1966). Symptoms of bacterial pustule may resemble those of bacterial blight and are commonly found to occur in the field conditions. Pustule formation and the absence of a watersoaked appearance during the early stages of lesion development distinguish bacterial pustule from bacterial blight. Bacterial pustule is caused by *Xanthomonas campestris* pv. *glycines* that resides in infested seed and soil on crop residue. The bacteria spread from diseased plants by water, rain and during cultivation when the foliage is wet (Bernard and Weiss 1973). Developing cultivars that are resistant to bacterial pustule is the commonly used strategy for controlling bacterial pustule. Cultural practices include planting disease-free seeds and adoption of tillage practices that hasten rapid decomposition of crop residue. Cultivation when foliage is wet should be avoided to reduce the spread of disease.

1.2.13 Powdery Mildew of Soybean

Powdery mildew of soybean is caused by the fungus *Microsphaera diffusa.* Powdery mildew on soybeans requires cool, cloudy weather and low relative humidity. Like most powdery mildews, the most common symptom is a white to light gray, powdery

fungal growth that covers the upper surface of leaves, although all aboveground plant parts may be affected (Lehman 1931). Infected leaves tend to be most common in the mid to lower canopy. Later symptoms may include leaf tissue yellowing and premature leaf drop. Severe infection of powdery mildew often causes premature defoliation and chlorosis of the leaves which results in considerable yield losses (Garcia et al. 1984).

1.3 Soybean Gene Pools

Soybean Gene Pool-1 consists of biological species that can be crossed to produce vigorous hybrids that exhibit normal meiotic chromosome pairing and possess total seed fertility. Gene segregation is normal and gene exchange is generally easy. Based on this definition, all soybean (*G. max*) germplasm and the wild soybean, *G. soja*, are included in GP-1. Gene Pool-2 species can hybridize with GP-1 easily and F_1 plants exhibit at least some seed fertility (Harlan and de Wet 1971). Gene Pool-3 is the third outer limit of potential genetic resources. Hybrids between GP-1 and GP-3 are lethal or completely sterile, and gene transfer is not possible or requires radical techniques (Harlan and deWet 1971). Based on this definition, GP-3 includes the 26 wild perennial species of the subgenus *Glycine* (Singh et al. 1998a). Gene Pool-4 is the extreme outer limit of potential genetic resources. Pre- and post-hybridization barriers inhibit embryo development and premature embryo abortion occurs (Singh et al. 2007). Only a few wild perennial *Glycine* species have been hybridized with soybean. Thus, majority of species belong to soybean GP-4 as they have not been hybridized with GP-1 or if hybridization did not produce viable F_1 plants (Singh et al. 1987). The wild perennial species carry resistance to several diseases (Hymowitz 2004; Ratnaparkhe et al. 2010).

1.4 Glimpses on Classical Genetics and Traditional Breeding

1.4.1 Classical Mapping Efforts

Soybean has been under the continuous scrutiny of plant breeders for improvement of the crop and to increase its productivity. The major issues are susceptibility to various biotic and abiotic stresses and improvement of seed composition traits. Improving the agronomic performance of the crop will ensure higher productivity and production, increased consumption of soybean and increase economical benefits. Traditionally, plant breeders have used crossing approaches coupled with meticulous selection methods to select better performing genotypes (Nataraj et al. 2021). Conventional plant breeding approaches led to the development of many soybean varieties.

Breeding efforts for soybean are directed towards improving the tolerance to biotic and biotic stresses and to stable yield potential of the crop. Classical genetics and traditional breeding approaches have been used to develop resistance varieties for diseases, insects, pests and other biotic stresses.

1.4.2 Breeding Objectives

Developing highly productive genotypes under biotic stress by introgressing disease resistance genes is a way forward in realizing genetic combinations supported by plant genetic resource activities. Advance phenotyping-based breeding approaches are pre-requisite and are being adopted systematically by developing early generation biparental, backcross or multi-parent intercross populations. Identified candidate for disease resistance and other biotic stresses from soybean accession has been extensively carried out and used for developing high yielding soybean varieties.

1.4.3 Limitations of Traditional Breeding

The concerns about losses due to biotic stress under climate change scenario have instilled a sense of urgency into accelerating the rates of genetic gain in molecular breeding programs. Therefore, regardless of the conventional breeding efforts, it is essential to integrate the genome designing approach to enhance production and ensuring the sustainability of the crop. To facilitate breeding advances, it is necessary to exploit molecular breeding and genetic techniques such as marker-assisted breeding, recombinant DNA technology, genome editing and "omics" to improve the soybean for disease resistance, quality and yield.

1.5 Genetic Diversity Analysis

1.5.1 Phenotype and Genotype Based Diversity Analysis

Literature on genetic diversity studies in soybean has been dominated by phenotyping-based analysis, cytogenetics and molecular studies, including isozyme variation, seed protein variation, restriction fragment length polymorphism (RFLP), random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers (Li et al. 2008; Wang et al. 2008; Shastri et al. 2019, 2021). The geographic differentiation in Chinese cultivated soybean and genetic diversity and have been studied using the coefficient of parentage (Cui et al. 2000a), morphological

traits (Dong et al. 2004), SSR markers (Li et al. 2010; Wang et al. 2015) and SNP markers (Kajiya-Kanegae et al. 2021; Saleem et al. 2021) exhibited a clear geographical effect on the genetic structure. Genetic diversity of Asian soybean landraces with North American cultivars have demonstrated that a lower level of diversity was observed in the American pools than in the Asian pools, using either phenotypic characterization (Cui et al. 2000a; Cui et al. 2001) or the coefficient of parentage (Cui et al. 2000b). Hyten et al. (2006) confirmed the reduced diversity between wild and cultivated soybeans and between Asian landraces and North American cultivars using sequence analyses. Genetic diversity studies in soybean has been covered and discussed in great detail by Carter et al. (2004). Liu et al. (2017) compared the genetic diversity between Chinese and American Soybean Accessions using High-Density SNPs. Population structure analysis, and cluster analysis indicated that the genetic basis of Chinese soybeans is distinct from that of the USA.

1.5.2 Relationship with Other Cultivated Species and Wild Relatives

Comprehensive study of biosystematic and evolutionary relationships of all species in the genus *Glycine* has been conducted. The annual (subgenus *Soja*) and perennial (subgenus *Glycine*) soybean species have diverged from a common ancestor around 5 MYA (Innes et al. 2008) and hence are significantly distantly related (Doyle et al. 2003). Initial attempts to hybridize species between the subgenus *Soja* and subgenus *Glycine* were unsuccessful. The resultant pods of interspecific hybridization eventually aborted and abscised although initiation of pod development occured (Ladizinsky et al. 1979; Hood and Allen 1980). Later, the inter-subgeneric F_1 hybrids of *G. max* × *G. clandestina*, *G. max* × *G. tomentella* and *G. max* × *G. canescens* were obtained in vitro either through embryo rescue (Newell and Hymowitz 1980; Singh and Hymowitz 1985; Singh et al. 1987) or using transplanted endosperm as a nurse layer (Broué et al. 1982). During the evolutionary process, the wild soybean (*G. soja*) has accumulated tremendously rich genetic diversity for multiple traits including morphological features like flower, pubescence, seed and hilum color, disease and insect resistance traits, physiological and biochemical traits as well as content of protein, oil and carbohydrates and their constituents (Boerma and Specht 2004).

1.6 Molecular Mapping of Resistance Genes and Quantitative Trait Loci

1.6.1 Brief History of Mapping

The first report of utilization of molecular markers in soybean is use of restriction fragment length polymorphism (RFLP) for the assessment of molecular genetic diversity of the soybean nuclear genome (Apuya et al. 1988). Subsequently RFLP markers were used extensively for genetic diversity analysis (Keim et al. 1989; Skorupska et al. 1993; Lorenzen et al. 1995) and linkage mapping (Keim et al. 1990, 1997; Diers et al. 1992; Lark et al. 1993; Akkaya et al. 1995; Shoemaker and Specht 1995; Mansur et al. 1996; Cregan et al. 1999; Ferreira et al. 2000; Yamanaka et al. 2001; Lightfoot et al. 2005) until SSR and SNP markers become popular (Hyten et al. 2010a), Lee et al. (2015), Sun et al. (2019), Ratnaparkhe et al. (2020), Kumawat et al. (2020), Ghione et al. (2021).

1.6.2 Evolution of Molecular Marker

Various molecular markers such as RFLPs, RAPDs, AFLPs, SSRs and SNPs were used for soybean diversity studies and genetic mapping. Apuya et al. (1988) analyzed 300 RFLP probes in genomic DNA of the genetically distant cultivars Minosy and Noir 1. RAPDs were also used extensively by soybean geneticists, mainly for germplasm classification because of simplicity in detection and without the prior knowledge of DNA sequence information (Thompson et al. 1998; Brown–Guedira et al. 2000; Li and Nelson 2002). A large number of AFLP markers were also utilized for linkage map construction in soybean (Keim et al. 1997; Matthews et al. 2001). The first report of SSR allelic variation and their use as marker system in plant species was from soybean (Akkaya et al. 1992; Morgante and Oliveri 1993). A high level of allelic variation in cultivated and wild soybean genotypes was observed using SSR markers (Maughan et al. 1995; Morgante et al. 1994; Rongwen et al. 1995). Akkaya et al. (1995) for the first time developed 40 SSRs and integrated them to a soybean linkage map. Later, Cregan et al. (1999) generated a large set of SSRs to develop an integrated linkage map. Song et al. (2004) developed SSRs from expressed sequence tags (ESTs), bacterial artificial chromosome (BAC)-end sequences and genomic libraries and added them to the integrated linkage map of soybean. Hisano et al. (2007) developed SSR markers using publicly available EST sequence information. Later, comprehensive sets of SSRs were developed, leading to the integration of physical map with genetic map (Shultz et al. 2007; Shoemaker et al. 2008). Utilizing the whole genome sequence, a soybean SSR database (BARCSOYSSR_1.0) containing genome position and primer sequences for SSRs was developed by Song et al. (2010).

Choi et al. (2007) identified SNPs via the resequencing of sequence-tagged sites (STSs) developed from EST sequences. These SNPs were further used for genetic

mapping studies and large number of genes were placed on the genetic map. Hyten et al. (2008) developed a multiplex assay of 384 SNPs designated as soybean oligo pool all-1 (SoyOPA-1). This custom 384-SNP GoldenGate assay was designed using SNPs discovered through resequencing efforts of diverse soybean accessions. Later, Hyten et al. (2010a) sequenced six diverse genotypes to uncover a total of 13,042 SNPs. These SNPs along with 5,551 SNPs discovered by Choi et al. (2007) were used to design GoldenGate assays designated as SoyOPA-2 and SoyOPA-3. The GoldenGate assay was designated as Universal Soy Linkage Panel 1.0 (USLP1.0). Hyten et al. (2010b) sequenced a reduced representation library of soybean to identify SNPs using high throughput approach. A total of 1,536 SNPs were selected to create an Illumina GoldenGate assay (SoyOPA-4). Chaisan et al. (2010) used publicly available ESTs derived from 18 genotypes for EST clustering and in silico SNP identification. These studies resulted in the development of large number of SNP markers in soybean which could be utilized for mapping of complex traits as well as molecular breeding applications. SNP markers in soybean which could be utilized for mapping of complex traits as well as molecular breeding applications have been developed in several investigations (Song et al. 2020). New computational approaches are also being developed for large scale analysis of soybean SNP data (Shastri et al. 2019; Jha et al. 2021).

1.6.3 Mapping Populations

Various mapping populations in soybean have been developed depending on the requirement of degree of polymorphism and target agronomic traits for analysis. F_2 populations or recombinant inbred lines (RILs) have been utilized for the construction of linkage maps in soybean. Genetic markers often show population-dependent polymorphism which greatly hinders their utility in diverse backgrounds. Several intraspecific linkage maps have also been developed, however, interspecific mapping populations contributed enormously to the saturation of the soybean linkage map. Nested association mapping (NAM) populations and multi-parent advanced generation intercross (MAGIC) population have been developed and characterized for various traits in soybean (Song et al. 2017; Diers et al. 1992; Beche et al. 2020).

1.6.4 QTL Mapping Studies

Various molecular markers have been used to map the genomic location of major genes and Quantitative trait locus (QTLs) underlying multiple traits in soybean. More than a thousand QTLs representing more than 100 agronomically important traits have been mapped in soybean (Grant et al. 2010). The updated information on all mapped QTLs in soybean is available on the USDA-ARS soybean genetic database *SoyBase* (http://soybase.org). Gene/QTLs mapping in soybean has witnessed an

impetus with the availability of whole-genome sequence (WGS) (Schmutz et al. 2010). Genome sequencing greatly aided in the development of thousands of SSRs and millions of SNP markers for genetic mapping studies. QTL analysis plays a significant role in identifying genomic regions that control over phenotypic variation, and it requires a large segregating population (biparental mapping population) such as an F_2 population or RILs. In general, QTL mapping utilizes a large number of RILs, which are established for at least several generations of selfing (typically up to F_6 or F_7). RILs are helpful for QTL detection however it estimates the influence of single QTL depending on the population size. Moreover, the outputs are highly population-specific for quantitative traits (Deshmukh et al. 2014). Plants that are homozygous for the unfavorable allele are eliminated in an F_2 population and frequencies of favorable alleles increase during inbred development. QTL mapping and marker development have progressed not only for disease resistance but also for the resistance against several insect pests and improved agronomic and physiological traits (Tripathi et al. 2021; Gupta et al. 2021).

1.6.5 QTL Mapping Software

Number of important QTL studies has been conducted to dissect various biotic stresses in last three decades. Although QTL mapping has advanced rapidly during the past few years, a large number of mapped QTLs cannot be utilized in the breeding program because of false-positive QTLs and low accuracy. However, the accuracy can be improved by adapting different QTL mapping methods and effective statistical analysis such as single marker analysis, simple interval mapping, composite interval mapping, multiple interval mapping, and Bayesian interval mapping. Also, a number of QTL mapping software have been developed such as Mapmaker/QTL, QTL Cartographer, MapQTL, MapManager, QTLMAPPER, QGene, QTLSTA, PLABQTL, PGRI, Ici Mapping, and QTL network. Utilization of QTLs for marker-assisted breeding is challenging due to the complex inheritance of unstable QTLs (Deshmukh et al. 2014). New "Meta-QTL analysis" have been proposed that compile QTL data from different reports together on the same map for identification of precise QTL region (Deshmukh et al. 2014; Sosnowski et al. 2012). The advances in sequencing technologies, statistical approaches, and software resulted in exponential intensification of research in soybean to understand plants response to various biotic stresses.

1.6.6 QTL Mapping for Disease Resistance

1.6.6.1 Root Knot Nematode

Two candidate genes *Glyma10g02150* and *Glyma10g02160* were identified in a major QTL conferring root not nematode resistance on chromosome Gm10 (Xu et al. 2013). RILs derived from a cross between Magellan and PI 438489B (resistant) was used for QTL mapping. These genes encodes a pectin methylesterase inhibitor and a pectin methylesterase inhibitor-pectin methylesterase, respectively (Xu et al. 2013). The protein encoded by *Glyma10g02160* showed homology to the *Arabidopsis* protein pectin methylesterase, PME3, which plays role in nematode parasitism (Hewezi et al. 2008). Association study for resistance to the southern root-knot nematode (*Meloidogyne incognita*) in soybean was conducted by Passianotto et al. (2017) and key SNPs were identified on chromosome 10. Recently, Vuong et al. (2021) Identified genomic loci conferring broad-spectrum resistance to multiple nematode species in exotic soybean accession PI 567305.

1.6.6.2 Soybean Cyst Nematode

The most effective way available for management of SCN is through the use of resistant varieties. A dominant resistance gene (*Rhg4*) was identified in a genotype Peking (Matson and Williams 1965). Using a positional cloning approach from soybean cultivar Forrest, Liu et al. (2012) identified a gene underlying *Rhg4* locus on soybean chromosome Gm08 and a major QTL contributing to SCN resistance. Gene *Rhg4* encodes a serine hydroxymethyl transferase enzyme and can be used to improve resistance against SCN. A recessive and codominant locus *rhg1* was also mapped (Concibido et al. 1997). Ruben et al. 2006 fine mapped the *rhg1* locus and identified *RLK* as candidate resistance gene. Srour et al. (2012) characterized the *GmRLK18-1*, and demonstrated that the dominant allele confers pleiotropic resistance to SCN and sudden death syndrome (SDS) caused by *Fusarium virguliforme*. In the absence of *Rhg4*, the *GmRLK18-1* confers partial resistance to SCN and nearly complete resistance to SDS (Srour et al. 2012). Studies indicates that LRR domain of *GmRLK18-1* binds with the CLE peptides of plants which are known to involve in tracheary element inhibition (Afzal et al. 2013).

The *rhg1-b* allele derived from the genotype PI 88788 has been used as a main resistance locus for developing several commercially cultivated SCN-resistant soybean varieties in United States (Cook et al. 2012). Fine mapping of *rhg1-b* haplotype in PI88788 identified 11 genes in soybean variety Williams 82 (Kim et al. 2010b), Cook et al. (2012) reported that resistance at *Rhg1* locus in PI 88788 is due to the copy number variation of genes *Glyma18g02580 Glyma18g02590* and *Glyma18g02610*. Further, SCN resistance is associated with multicopy *Rhg1* haplotypes that form two distinct groups (Cook et al. 2014). KASPar assays was developed by Kadam et al. (2016) using SNPs from *Rhg1* gene (*Glyma18g02590*) and *Rhg4* gene

(*Glyma08g11490*). SNP markers specific to *Rhg1* locus and *Rhg4* locus were validated using KASPar assay. A conserved region of the *Rhg1* locus was used and copy number variation at *Rhg1* locus were detected through TaqMan™ assay (Kadam et al. 2016).

Major loci conditioning resistance to SCN race 3 are *rhg1* and *Rhg4*. Gene *rhg1* is located on chromosome 18 while *Rhg4* is located on chromosome 8. Gene *Glyma.18G022500* encodes *rhg1-b* resistance which is an α-soluble N-ethylmaleimide-sensitive factor attachment protein (SNAP) which is known as *GmSNAP18*. SNP markers for *GmSNAP18* have been developed which can differentiate between susceptible and resistant cultivars. The gene *Glyma.08G108900* referred as *GmSHMT* encodes a serine hydroxymethyl transferase and is responsible for the *Rhg4* resistance. Several DNA markers have been designed based on these studies. *SCN3-11*, gene contributing to SCN race 3 resistance, lies on chromosome 11 that shows similarity with the chromosome 18 region harboring *GmSNAP18*. Patil et al. (2019) analyzed whole-genome re-sequence data of 106 soybean lines which revealed the impact of the interaction of copy number variants of the *rhg1* and *Rhg4* genes. Genetic characterization of qSCN10 from an exotic soybean accession PI 567516C revealed a novel source conferring broad-spectrum resistance to SCN (Zhou et al. 2021). Usovsky et al. (2021) further conducted fine-mapping and characterization of qSCN18 and identified a novel QTL controlling SCN resistance in PI 567516C. Recently, Kofsky et al. (2021) also developed novel resistance strategies to soybean cyst nematode (SCN) in wild soybean.

1.6.6.3 Bacterial Leaf Pustule

Bacterial leaf pustule (BPL) resistance was reported to be controlled by a single recessive gene (*rxp*) characterized in a resistant source, CNS (PI 548445) (Hartwig and Lehman 1951). QTLs analysis revealed that Satt372 and Satt486 on soybean chromosome Gm17 were strongly associated with resistance to BPL (Narvel et al. 2001; Van et al. 2004). Fine mapping in RILs derived from a cross between "Taekwangkong" (susceptible) and "Danbaekkong" (resistant) and two pair of NILs, narrowed down the BLP resistance locus to 33 Kb (Kim et al. 2010a). Two putative candidate genes, a membrane protein gene (*Glyma17g09780*) and a Zinc finger family protein gene (*Glyma17g09790*) were identified within 33 Kb sequence. The candidate genes showed high similarity with their paralogous genes, associated with bacterial leaf pustule resistance (Kim et al. 2010a).

1.6.6.4 Soybean White Mold

White mold, caused by *Sclerotinia sclerotiorum* [(Lib.) W. Phillips.] de Bary, is reported as a devastating disease of soybean and other crops (Boland and Hall 1994). Currently, only partially resistant cultivars have been used for genetic mapping due to the lack of immune type resistance genotypes. Zhao et al. (2015) identified a

major QTL on Gm13 by linkage and association mapping. Candidate genes involved in disease response and anthocyanin biosynthesis were identified at the locus near the peak SNPs (Zhao et al. 2015). These candidate genes are useful resources to perform functional validation and to utilize in soybean breeding for improving resistance to white mold. Boudhrioua et al. (2020) conducted genome-wide association mapping of *Sclerotinia sclerotiorum* resistance in soybean using whole-genome resequencing data. SNP-trait association led to discovery of a new QTL on chromosome 1. Recently, Zhang et al. (2021) identified candidate gene networks involved in resistance to *Sclerotinia sclerotiorum* in soybean. Integration of multi-method genome-wide association study (GWAS) revealed candidate genes in novel regions, which include Glyma.01g048500, Glyma.03g129100, Glyma.17g072200, and the Dishevelled (Dvl) family of proteins on chromosomes 1, 3, 17, and 20, respectively.

1.6.6.5 Sudden Death Syndrome

Sudden death syndrome (SDS) of soybean is a serious threat to soybean production (Roy et al. 1997; Wrather et al. 2010) Cianzio et al. (2014, 2019). It is caused by *Fusarium virguliforme* and once the fungus invades the root xylem tissues, the pathogen secretes toxins that cause chlorosis and necrosis in foliar tissues leading to defoliation, flower and pod drop and eventually death of plants. Sudden death syndrome can be managed with host plant resistance (Wen et al.2014). Resistance to SDS in soybean is multigenic and provides partial resistance to leaf scorch caused by fungal toxins and also provides partial resistance to root infection caused by the fungus (Njiti et al. 1998; Kazi et al. 2008) Cianzio et al. (2016) Several QTLs linked to SDS resistance have been identified through genome mapping on 12 soybean chromosomes (http://www.soybase.org/). A locus *Rfs2/Rhg1* on Gm18 provides partial resistance to root infections caused by *F. virguliforme* and SCN (Njiti et al. 1998; Triwitayakorn et al. 2005). Srour et al. (2012) characterized *Rfs2*/*Rhg1* QTL on soybean chromosome Gm18 and identified gene *GmRLK18-1* which is responsible for providing resistance to SDS and SCN. Further a genome-wide association study was conducted which identified 20 loci associated with SDS resistance (Wen et al. 2014). Several SNPs associated with SDS resistance are within the vicinity of sequences of plant disease resistance genes including SDS resistance gene *GmRLK18-1*. Another GWAS study identified a potential candidate gene (*SIK1*) on Gm02 (Zhang et al. 2015). The peak SNP locus associated with SDS resistance was present in the coding region of the *SIK1* resulting in a non-synonymous mutation. Swaminathan et al. (2019) conducted GWAS identifying novel SNP loci and candidate genes involved in soybean SDS resistance. Total eight novel genomic regions containing foliar resistance genes and five novel regions for root-rot resistance against *Fusarium virguliforme* were identified. In another study, transcriptome analysis of a susceptible soybean cultivar following *F. virguliforme* infection was conducted and key genes were identified and overexpressed. Overexpression of three genes, GmARP1, GmDR1 and GmSAMTII, enhanced SDS resistance among the transgenic soybean lines. Overexpression of GmDR1 enhanced resistance of soybean not

only against *F. virguliforme*, but also against soybean cyst nematode (SCN), spider mite and soybean aphid (Ngaki et al. 2020).

1.6.6.6 Phytophthora Root Rot

Phytophthora root rot is caused by *Phytophthora sojae* and is considered as one of the important diseases of soybean. Tolerance to *Phytophthora* root rot is multi-genic and so far 22 *Rps* loci including 26 alleles have been detected on four different chromosomes i.e., Gm03, Gm13, Gm16, and Gm18 (Li et al. 2016a). The first Rps gene was identified in the 1950s (Bernard et al. 1957). Two *Rps* genes, *RpsUN1* on chromosome 3 and *RpsUN2* on chromosome 16 were identified from a soybean landrace PI 567139B, which together confer complete resistance to 16 *P. sojae* races/isolates (Lin et al. 2013). In general, Rps genes follow the gene-for-gene hypothesis with *P. sojae* (race-specific); however, an increase in the pathotype complexity limits the utility of an Rps gene's lifespan to 8 to 15 years (Grau et al. 2004; Sugimoto et al. 2012). In the conditions of high disease pressure, cultivars with complete resistance are far effective over that with partial resistance against PRSR (Schmitthenner 1999; Dorrance et al. 2003). Contrarily, partial resistance conferred by many QDRLs is durable over complete resistance (single Rps gene) in USA where *P. sojae* races evolve at much faster rate to overcome even most effective Rps genes (Dorrance et al. 2003) suggesting the significance of both the complete and partial resistance against PRSR in different situations.

Li et al. (2016a), identified 151 kb region that harbors three disease resistance (R)-like genes, and a 36 kb region that contains four R-like genes, respectively. RNA seq analysis suggest that *Glyma.03g034600*, *Glyma.16g215200* and *Glyma.16g214900* from PI 567139B may be associated with the resistance to *P. sojae*. Later, Li et al. (2016b) identified seven candidate genes on soybean chromosome Gm13 that are probably involved in natural variations in partial resistance to *P. sojae*. These genes encode a 2OG-Fe(II) protein (*Glyma13g33900*), a PPR protein (*Glyma13g33512*), a COPI (*Glyma13g32980*), LRR domain proteins (*Glyma13g33536, Glyma13g33740*), a Zn-finger protein (*Glyma13g33260*) and a Gpi16 subunit (*Glyma13g33243*).

In the past few decades, several Rps genes have been mapped using genetic and genome mapping approaches. Around 37 Rps genes/alleles have been identified till date, and have been localized to ten different chromosomes in soybean. Most of the Rps loci have been mapped on chromosome 3 (18 genes) followed by chromosome 18 (5 genes) and chromosome 13 (5 genes). The Rps genes on these three chromosomes constitute nearly 70% of the total *Rps* genes reported. *Rps1* (with five alleles *Rps1a, Rps1b, Rps1c, Rps1d*, and *Rps1k*), *Rps7, Rps9, RpsYu25, RpsYD29, RpsYD25, RpsUN1, RpsWY, RpsQ, RpsHC18, RpsX, RpsHN, RpsGZ* and an unnamed *Rps*gene (Rps 1?) were mapped on chromosome 3 (Demirbas et al. 2001; Weng et al. 2001; Gordon et al. 2007; Sugimoto et al. 2006, 2008, 2010, 2011, 2012; Gao and Bhattacharyya 2008; Fan et al. 2009; Yu et al. 2010; Wu et al. 2011a; Zhang et al. 2013b; Lin et al. 2013; Li et al. 2016a, b; Niu et al. 2017; Niu et al. 2018; Zhong

et al. 2018b, 2019, 2020; Jiang et al. 2020). Similarly, *Rps4, Rps5, Rps6*, *Rps12* and *RpsJS* are located on soybean chromosome Gm18; *Rps2, RpsUN2*, one unknown Rps is located on chromosome Gm16; *Rps3* (three alleles *Rps3a, Rps3b* and *Rps3c*) and *RpsSN10* which was linked with *Rps8* were mapped on chromosome Gm13. Furthermore, remaining genes namely *RpsZS18, Rps11, RpsSu, Rps10*, *RpsYB30* and unnamed *Rps* are located on chromosomes 2, 7, 10, 17, 19, and 20 respectively (Sandhu et al. 2005; Gordon et al. 2006; Zhu et al. 2007; Yao et al. 2010; Yu et al. 2010, Wu et al. 2011b; Zhang et al. 2013a; Lin et al. 2013; Sun et al. 2014; Li et al. 2016a; Ping et al. 2016; Huang et al. 2016; Sahoo et al. 2017). Using bi-parent populations, several genomic regions have been repeatedly detected in different genetic mapping projects. On chromosome 3, a genomic region of ~2 Mb was found to be a hot spot involved in conferring resistance as observed in many investigations. Zhong et al. (2019) identified *RpsX* using genetic mapping with QTL-sequencing approach in soybean cultivar Xiu94-11; subsequently, it was revealed that *RpsX* was located in the 242-kb genomic region spanning the *RpsQ* locus. Zhong et al. (2020) fine mapped *RpsYD25* in $F_{3:4}$ population derived from Zaoshu18 and Yudou25 using PCR-based markers. Subsequently, 7 soybean genotypes containing *RpsYD25* were identified using five co-segregated SSR markers. Recently, Jiang et al. (2020) fine mapped *RpsGZ*to a 367.371-kb genomic region on chromosome 3 in RILs derived from a cross of the resistant cultivar Guizao1 and the susceptible cultivar BRSMG68. Sahoo et al. (2017) identified *Rps12* on chromosome 18 in a RIL population developed by crossing the *P. sojae* resistant cultivar PI399036 with susceptible AR2 line. This gene was mapped at 2.2 cM proximal to the *NBSRps4/6*-like sequence that was described to co-segregate with the *Phytophthora* resistance genes *Rps4* and *Rps6*. Genes *Rps12* and *Rps13* that confer broad-spectrum Phytophthora resistance against a large number of *P. sojae* isolates were tightly linked (Sahoo et al. 2017, 2021). Recently, a soybean gene encoding E3 ligase was identified, which guards the protein encoded by the Phytophthora resistance *Rps1-b* gene against the *P. sojae* effector proteins that are involved in disease development. The E3 ligase gene is involved in regulating the cell death pathway (Li et al. 2021).

1.6.6.7 Charcoal Rot

For identification of charcoal rot resistance genotype, a core set of 100 diverse soybean genotypes were subjected to screening for resistance (Talukdar et al. 2009). Details of the work done in finding the resistance source against charcoal rot is presented in Table 1.1. None of the genotypes were immune but seven genotypes (viz. DS 9712, DS9814, JS 335, PK 564, EC 439618, EC 439619 and DS61) were identified (Talukdar et al. 2009) as resistant. Expression of the disease reaction is continuous, that is, a wide variety was observed starting from highly susceptible through moderately resistant to highly resistant suggesting the involvement of multiple genetic locus in controlling the resistance of the disease. Advancements have been made to map QTL for charcoal rot resistance in soybean and identification of linked molecular markers (Talukdar et al. 2009). Coser et al. (2017) identified a set

Table 1.1 Screening of soybean genotypes against Charcoal rot using different methods

S. No.	Genotypes with resistant reaction		Screening method	References
1	Resistant	DS9712, DS9814, JS335, PK564, EC439618, EC439619, and DS61	Paper towel method	Talukdar et al. (2009)
	Moderately Resistant	BR11, DS-201-A, NRC67, NRC37 AND NRC7		
2	Resistant	DS9712, DS9814, PK564, EC439619, JS335, EC439618 and EC44303	Field screening	Gowda et al. (2014)
	Resistant	JS335, NRC7, NRC37, PK564, EC44303, EC439618, C439619, DS61, DS9712 and DS9814	Pot inoculation	
	Resistant	DS9712, DS9814 and JS335	Blotter paper	
	Resistant	DS9814 and JS335	In-vitro screening	
3	Moderately Resistant	DT97-4290	Field screening	Paris et al. (2006)
4	Moderately resistant	DG3905, Manokin, DT99-16864, DT99-17483, DT98-7553 and DT99-17554	Field screening	Mengistu et al. (2011)
5	Partially resistant	PI 548302 and PI 548414	Cut-stem inoculation method	Pawlowski et al. (2015)
6	–	DT97-4290, DT98-7553, DT99-17554 and DT99-16864	Cut-stem inoculation method	Twizeyimana et al. (2012)
7	Absolute resistance (AR)	JS 20-69, RVS 2001-4 and MACS 1336	Field screening at Amravati	Personal commuications

Table 1.2 Genes/QTL identified against charcoal rot

S. No.	Gene/QTL	Chromosome	Phenotyping	Population	Reference
1	Glyma.04g053100	4	Field screening	A collection of 459 PI lines spanning MG I to MG III from USDA	Coseret al. (2017)
	Glyma.14g002000	14			
	Glyma.18g248100	18			
	Glyma.18g228600	18			
	Glyma.06g176100/ Glyma.06g176200	6	Cut stem inoculation method		
	Glyma.08g306800/ Glyma.08g306900	8			
	Glyma.08g315900/ Glyma.08g316500	8			
	Glyma.09g230300	9			
	Glyma.12g216200	12			
	Glyma.12g006300	12			
	Glyma.18g262800	18			
	Glyma.20g197000	20			

of candidate genes governing charcoal rot resistance in a collection of 459 PI lines through association studies (Table 1.2). QTL mapping of charcoal rot resistance in PI 567562A soybean accession was done by da Silva et al. (2019) and genomic regions governing resistance to charcoal rot in soybean were identified on chromosome 16. Later, da Silva MP (2020) conducted bulked segregant analysis using next-generation sequencing for identification of genetic loci for charcoal rot resistance in soybean. Three genomic regions on chromosomes 5, 8 and 14 were identified associated with charcoal rot resistance in soybean.

1.6.6.8 Rust

The development of durable genetic resistance to soybean rust (SBR) depends greatly on understanding of the genetic and molecular basis of the resistance response. Several genes for ASR resistance have already been identified including *Rpp1*—PI 200492 (McLean and Byth 1976), *Rpp2*—PI 230970 (Bromfield and Hartwig 1980), *Rpp3*—PI 462312 (Bromfield and Hartwig 1980; Hartwig and Bromfield 1983), *Rpp4*—PI 459025 (Hartwig 1986) and *Rpp5* (Garcia et al. 2008; Morceli et al. 2008), *Rpp6* and *rpp7 (*King et al. 2015; Li et al. 2012; Liu et al. 2016; Childs et al. 2018; Table 1.3). Other soybean genetic sources harbouring genes for resistance include PI 239871A, PI 239987B, PI 230971, PI 459024, TK 5, TN 4 and a wild *Glycine* spp. Although resistance controlled by single gene is relatively easy to work with in a backcrossing program, as desirable traits can be moved into elite breeding stock in a relatively short time through marker assisted backcross breeding (MABB) approach. However, *P. pachyrhizi* might easily overcome any single-gene resistance.

Table 1.3 List of identified rust resistance genes and their genomic positions

Gene locus	Source	Chromosome	Physical position Glyma.Wm82.a2[a]	References
Rpp1/Rpp1-b	PI 200,492 PI 594538A PI 587,886 PI 587880A PI 594760B PI 561,356 PI 594767A PI 587,905 PI 594,177 PI 587,855	18	56,182,523 – 56,797,174	Chakraborty et al. (2009); Garcia et al. (2011); Hossain et al. (2014); Hyten et al. (2007); Kim et al. (2012); McLean and Byth (1976); Ray et al. (2009); Rocha et al. (2015); Morishita et al. (2015); Yamanaka et al. (2016)
Rpp2/rpp2	PI 230,970 PI 224,270 PI 417,125	16	27,937,049 – 30,478,472	Bromfield and Hartwig (1980); Calvo et al. (2008); Garcia et al. (2008); Hartwig and Bromfield (1983); Nogueira et al. (2008); Silva et al. (2008); Yu et al. (2015)
Rpp3/Rpp? (Hyuuga)	PI 462,312 PI 506764[b] PI 628,932 PI 567099A PI 416,764	6	44,049,891 – 45,995,029	Brogin (2005); Hartwig and Bromfield (1983); Hossain et al. (2014); Hyten et al. (2009); Kendrick et al. (2011); Monteros et al. (2007); Ray et al. (2011)
Rpp4/Rpp4-b	PI 459025B PI 423,972	18	51,397,064 – 51,584,617	Garcia et al. (2008); Hartwig (1986); King et al. (2017); Meyer et al. (2009); Silva et al. (2008)
Rpp5/rpp5	PI 200,526 PI 200,456 PI 200,487 PI 471,904 PI 506764[b]	3	29,862,641 – 32,670,690	Calvo et al. (2008); Garcia et al. (2008); Kendrick et al. (2011)
Rpp6	PI 567102B PI 567068A PI 567104B	18	5,953,237 – 6,898,528	King et al. (2015); Li et al. (2012); Liu et al. (2016)

(continued)

Table 1.3 (continued)

Gene locus	Source	Chromosome	Physical position Glyma.Wm82.a2[a]	References
Rpp7	PI 605,823	19	39,462,291 – 39,616,643	Childs et al. (2018)

Right combination of single genes will be useful to develop rust resistance in soybean and in a resistance management program. Partial resistance may also contribute to the control of soybean rust by decreasing the buildup of rust spores. Fewer spores produced over time could effectively reduce the need for multiple fungicide applications. Soybean genotypes with resistance to all known races of *P. pachyrhizi* are not yet available, and more basic research is also needed on the pathogen itself. Crucial elements in the infection process of the fungus and novel plant protection strategies need to be identified. One important step forward in this direction is the assessment of fungal gene expression during distinct phases of the plant–pathogen interaction. The application of molecular technologies will help in developing resistant cultivars and the threat of Asian soybean rust can successfully be averted in the major areas of soybean cultivation. Ratnaparkhe et al. (2020) conducted whole genome re-sequencing of soybean accession EC 241780 and identified candidate *Rpp1* rust resistant gene. The SNPs and InDels identified within the candidate genes can be used for the marker assisted breeding of *Rpp1* rust resistant gene.

1.7 Marker-Assisted Breeding for Disease Resistance Traits

Identification of molecular marker(s) linked to the disease resistance gene would greatly facilitate screening of breeding materials and thus accelerate the development of new resistant cultivars. Continuous efforts are required for the identification of new disease resistant genes and for the development of tolerant cultivars. During last two decades tremendous progress has been made on the marker assisted breeding for disease resistance.

1.8 Map-Based Cloning of Resistance Genes

1.8.1 Strategies Landing and Walking

Availability of genomic clone libraries with large DNA inserts is one of the essential requirements for plant genome analysis, primarily for physical mapping, gene isolation, and gene structure and function analysis. The BAC vectors have been used widely for generating genomic DNA libraries in economically important crop plants including soybean. The development of BAC libraries is considered a critical step towards physical mapping and positional cloning of important genes.

1.8.2 Libraries: BAC/YAC Libraries

In soybean, several BAC libraries have been developed from different genotypes for genomic research as well as for cloning of stress tolerance loci. These libraries are useful resources for positional cloning of agronomically and biologically important genes. Yeast artificial chromosomes (YAC) were initially developed with a view to utilize the resource for chromosome walking and in situ hybridization (Zhu et al. 1996). BAC libraries covering the whole soybean genome were generated by early genomic researchers (Marek and Shoemaker 1997; Danesh et al. 1998; Tomkins et al. 1999; Salimath and Bhattacharyya 1999). BAC libraries encompassing a variety of genotypes in combination with diverse enzymes have led to the development of early physical contigs (Marek and Shoemaker 1997). Efforts were made to develop a physical map of soybean genome using BAC-based libraries (Wu et al. 2004). A physical map of soybean cultivar Williams 82 was generated from BAC clones (http://soybeanphysicalmap.org/). Furthermore, SSR markers derived from BAC ends sequence (BES) were mapped and integrated into the physical map (Shoemaker et al. 2008). Six dimensional BAC clones pools were employed to demonstrate the anchoring of genetic markers to the soybean BAC clones (Wu et al. 2008). The the physical framework was further accomplished by associating the contigs to the molecular markers (Song et al. 2004; Choi et al. 2007; Katayose et al. 2012). The soybean physical map was made available public under Soybean Breeders Toolbox (SBT) set up in soybase website (http://www.soybase.org) for the greater benefit of the research community. Later, physical maps of soybean and related wild species were used for comparative and functional genomics studies (Innes et al. 2008; Ashfield et al. 2012). BAC libraries have also been constructed for several wild species of soybean: *G. soja, G. syndetika, G. canescens, G. stenophita, G. cyrtoloba, G. tomentella, G. falcata*, and the polyploid, *G. dolichocarpa.*

1.9 Genomics-Aided Breeding

1.9.1 Details of Genome Sequencing

Soybean genome sequencing project was accomplished by the US Department Of Energy-Joint Genome Initiative (DOE-JGI)-Community Sequencing Program (CSP). The genome sequence assembly was termed as Glyma-1.0. The protein-coding regions were predicted to be 66,153, of which over 46,000 genes were predicted with a high confidence level (Schmutz et al. 2010). New sequencing technologies have the potential to rapidly change the molecular research landscape in soybean (Lam et al. 2010; Libault et al. 2010; Li et al. 2013; Li et al. 2014, Chung et al. 2014; Chu et al. 2021).

1.9.2 Application of Structural and Functional Genomics in Genomics-Assisted Breeding

Several research projects include genome re-sequencing, gene expression, and whole transcript profiling have provided large scale datasets for comparative and functional genomics studies (Kim et al. 2019; Kajiya-Kanegae et al. 2021). Structural variations play important roles in driving genome evolution and gene structure variation which in turn contribute to agronomic trait variations. Valliyodan et al. (2019) reported reference-quality genome assemblies and annotations for two accessions of soybean and one accession of *Glycine soja*. Liu et al. (2020) selected 26 accessions and performed de novo genome assembly for soybean accession. Through a comparative genome analysis, a total of 14,604,953 SNPs and 12,716,823 InDels, 27,531 copy number variations and 723,862 present and absent variations were identified.

Gene expression studies are an imperative constituent of any crop improvement programme. The global gene expression pattern analysis forms an integral part of soybean functional genomics. The gene expression patterns are being investigated using the techniques like high-density expression arrays, microarray systems, serial analysis of gene expression (SAGE) meant for both quantitative and qualitative gene expression analysis and through transcriptome sequencing. Microarray-based expression investigation on soybean was initiated using cDNAs arrayed on a filter in high-density expression arrays format (Vodkin et al. 2004). Later on the usage of microarray on soybean gene expression studies were very sparse like the instances of comparison of gene expression between root and shoot (Maguire et al. 2002), comparing transcript expression pattern during somatic embryogenesis (Thibaud-Nissen et al. 2003). Structural and functional genomics studies have also been carried out on MicroRNAs. MicroRNAs (miRNAs) are key regulators of gene expression and play important roles in many aspects of biotic stress tolerance and in plant development. Turner et al. (2012) identified number of novel miRNAs and previously unknown family members for conserved miRNAs in the recently released soybean genome sequence. They classified all known soybean miRNAs based on their phylogenetic conservation (conserved, legume- and soybean-specific miRNAs) and examined their genome organization, family characteristics and target diversity. Comparative and functional genomics of soybean has been covered in great detail by Ma et al. (2010), Livingstone et al. (2010), Ratnaparkhe et al. (2013), Kavishwar et al. (2021). Comparative and functional genomics studies have been largely benefitted by the development of several soybean genome databases (Table 1.4). SoyBase provides the genetic and genomics data of soybean and USDA soybean germplasm information. The loci information of more then 100 traits for QTLs mapping and GWAS studies are available on SoyBase (Grant et al. 2010). The SoyKB is a web-based database that provides data of genomics, transcriptomics, metabolomics, and molecular breeding (Joshi et al. 2017). The comparative genomic analysis also provides evolutionary information, polyploidization, copy number variation, and

Table 1.4 List of online databases for soybean comparative and functional genomics

Databases	Features	Tools	Website
SoyBase and the Soybean Breeder's Toolbox	Genetic and physical maps, QTL, Genome sequence, Transposable elements, Annotations, Graphical chromosome visualizer	BLAST search, ESTs search, SoyChip Annotation Search, Potential Haplotype (pHap) and Contig Search, Soybean Metabolic Pathways, Fast Neutron Mutants Search, RNA-Seq Atlas	http://soybase.org/
SGMD-The soybean genomics and microarray database	Integrated view genomic, ESTand microarray data	Analytical tools allowing correlation ofsoybean ESTs with their geneexpression profiles	http://bioinformatics.towson.edu/SGMD/
SoyKB-Soybean knowledge base	Multi-omics datasets, Genes/proteins, miRNAs/sRNAsMetabolite profiling, Molecular markers, information aboutplant introduction lines and traits,	Germplasm browser, QTL and Trait browser, Fast neutron mutantdata, Differential expression analysis, Phosphorylation data, Phylogeny	http://soykb.org/
SoyDB-Soybean transcription factorsdatabase	Protein sequences, Predicted tertiary structures, Putative DNAbinding sites, Protein Data Bank (PDB), Protein familyclassifications	PSI-BLAST, Browse database, Family prediction by HMM, FTP dataretrieve	http://casp.rnet.missouri.edu/soydb/
SoyMetDB-The soybean metabolomedatabase	Soybean metabolomic data	Pathway viewer	http://soymetdb.org
SoyTEdb-Soybean transposableelements database	Williams 82 transposable element database	Browse for repetitive elements, transposable element and mapposition, data retrieval tools	www.soybase.org/soytedb/

(continued)

Table 1.4 (continued)

Databases	Features	Tools	Website
SoyProDB-Soybean proteins database	Several 2D Gel images showing isolated soybean seed proteins	Search tool for 2D spots, Navigation tools for protein data	http://bioinformatics.towson.edu
DaizuBase-An integrated soybeangenome database including BAC-basedphysical maps	BAC-based physical map, Linkage map and DNA markers, BACend, BAC contigs, ESTs, full-length cDNAs	Gbrowse, unified map, gene viewer, BLAST	http://daizu.dna.affrc.go.jp/
Soybean network (SoyNet)	Database for co-functional networks for soybean	Network based algorithms	www.inetbio.org/soynet
A knowledge database of soybean functional networks(SoyFN)	Database of soybean functional gene networks	Functional gene network, microRNA functionalnetwork, gene annotation, genome browser	http://nclab.hit.edu.cn/SoyFN
SoyXpress-Soybean transcriptomedatabase	Soybean ESTs, Metabolic pathways, Gene Ontology terms, Swiss-prot Identifiers and Affymetrix gene expression data	BLAST search, microarray experiments, pathway search etc.	http://soyxpress2.agrenv.mcgill.ca
SoyGD-The Soybean GBrowseDatabase, Southern Illinois	Physical map and genetic map, Bacterial artificial chromosome(BAC) fingerprint database, Associated genomic data	Sequence data retrieval tools, Navigation tool for sequenceinformation of different builds	http://soybeangenome.siu.edu/
Deltasoy-an internet-based soybean database for official variety trials	Official variety trial (OVT) information in soybean, MississippiOVT data, including yield, location, and disease information	Comparison tools for variety trail data, phenotypic data and diseaserelated data	http://msucares.com/deltasoy/testlocationmap.htm
Soybean cyst nematode proteins database (SCNProDB)	Soybean Cyst nematode protein database	SCN protein identification, 2D gel images data	http://bioinformatics.towson.edu/

(continued)

Table 1.4 (continued)

Databases	Features	Tools	Website
Soybean functional genomics database (SFGD)	Functional Genomics database	Gbrowse, microarray expression profiling,transcriptome data, gene co-expressionregulatory network, acyl-lipid metabolismpathways, cis-element significance analysis	http://bioinformatics.cau.edu.cn/SFGD/
Soybean proteome database	Soybean Proteome database	Proteome, metabolome, transcriptomedatasets, 2D-PAGE and proteomicsinformation, comparative proteomics underflooding, drought and salt stress	http://proteome.dc.affrc.go.jp/Soybean/
Soybean-VCF2Genomes	To map single sample variant call format (VCF) file against known soybean germplasm collection for identification of the closest soybean accession	WebApp to identify nearest cultivar	http://pgl.gnu.ac.kr/soy_vcf2genome/

presence-absent variations (PAV). Ha et al. (2019) developed a database Soybean-VCF2Genomes to identify the closest accession in soybean germplasm collection. A recently developed SoyTD integrated database (http://artemis.cyverse.org/soykb_dev/SoyTD/) of WGRS and transcriptomics gives the information of natural variations and expression of soybean transporter genes (Deshmukh et al. 2020) Lai et al. (2021) developed a comprehensive framework consisting of bioinformatics big data mining, meta-analysis, and a gene prioritization algorithm. Comparative and functional genomics have been applied extensively in soybean for identification of genes associated with key agronomic and physiological traits and for understanding the genome structure (Ma et al. (2010), Livingstone et al. (2010), Kim et al. (2010b), Shastri et al. (2019), Paganon et al. (2020), Liu et al. (2020), Valliyodan et al. (2021), Kumar et al. (2021).

1.9.3 Comprehension of Biotic Stress Resistance in Soybean Utilizing Transcriptomic Approaches

Biotic stress tolerance in soybean has been dissected through the application of RNA-sequencing approach in general and transcriptome sequencing coupled with metabolomic and proteomic techniques too to decipher the molecular basis of biotic stress in few instances (Table 1.5). However, the large quantum of data generated from the RNA-seq data requires sorting and analysis to arrive at a meaningful outcome so that it could lead to identification of suitable biomarkers associated with resistance. In many instances the miRNA target transcripts are not characterized warranting further validation and alternate approaches (Chen et al. 2016). Interestingly the integration of transcriptome and metabolome data and use of model plants and their mutant sources has revealed a robust lead in analyzing the Rhizoctonia foliar blight (RFB) disease (Copley et al. 2017). However, it is clear that combination of approaches transcriptomics, metabolomics and proteomic-based techniques are indispensable to dissect the molecular basis of stress tolerance. Alternatively plants are considered to function as holobionts associated with the microbiota in its vicinity. Hence, sudden death syndrome of soybean caused by *Fusarium virguliforme* was analyzed in the mycorrhizal and non-mycorrhizal soybean plantlets (Marquez et al. 2019). This study revealed that AMF-colonized plants showed upregulation of genes involved in defence and disease resistance concomitant with the down regulation of genes involved in cell wall modification and peroxidases shedding light on tolerance of mycorrhizal plants to sudden death syndrome (Marquez et al. 2019).

Table 1.5 Application of transcriptomic approaches in delineating biotic stresses in soybean

Sl. No.	Disease	Genotypes	NGS platform	Inferences	References
1	Bacterial leaf pustule (BLP) caused by *Xanthomonas axonopodis pv. glycines (Xag)*	BLP-resistant NILs and BLP-susceptible line, Taekwangkong	Illumina GA II and sequencing-by-synthesis technology	A list of 2415 differentially expressed genes in BLP-resistant and BLP-susceptible NILs following Xag inoculation	Kim et al (2011)
2	Asian soybean rust caused by *Phakopsora pachyrhizi* Sydow & Sydow	Embrapa 48 (susceptible) and PI561356 (resistant)	Illumina sequencing	Identified 29 novel miRNAs from water deficit and rust infections of Glycine max	Kulcheski et al. (2011)
3	Asian soybean rust caused by *Phakopsora pachyrhizi* Sydow & Sydow	PI459025B (having Rpp4-gene resistant genotype) and Williams 82 (susceptible)	Gene ChipSoybean Genome Array (Affymetrix)	Identified 14 transcription factors common to the resistant and susceptible responses along with fourteen transcription factors unique to R-gene-mediated resistance response	Morales et al. (2013)
4	Root knot nematode (*M. javanica*)	Resistant PI 595099 (PI) and susceptible BRSMG 250 'Nobreza' genotypes	454 GLX titanium sequencer	A model involving the phytohormones such as auxin, GA along with the participation of DELLA proteins and ROS signaling was proposed to account for the resistance trait	Beneventi et al. (2013)

(continued)

Table 1.5 (continued)

Sl. No.	Disease	Genotypes	NGS platform	Inferences	References
5	Phytophthora root and stem rot (PRR) caused by *Phytophthora sojae*	Williams 82 (Susceptible), 10 near isogenic lines each having unique Rps gene/allele	Illumina HiScanSQ	4330 DEGs in Williams versus 2014 to 5499 DEGs in individual NILs following *P. sojae* inoculation Putative ethylene, jasmonic acid, ROS, and MAPK regulatory networks underlying the defense responses	Lin et al. (2014)
6	Soybean mosaic virus isolates, L (G2 strain), LRB (G2 strain) and G7 (G7 strain).	Williams 82 (susceptible)	small RNA (sRNA)-seq, degradome-seq and whole transcriptome sequencing	A complex miRNA-mRNA regulatory networks identified that the majority of the targets are involved in protein synthesis and modification	Chen et al. (2016)
7	Rhizoctonia foliar blight (RFB) caused by *Rhizoctonia solani* AG1-IA	Williams 82	Combined RNA-seq and metabolomics (^{1}H NMR) analysis	A comprehensive metabolite and transcript pathway network analysis of host responses to the pathogen	Copley et al. (2017)
		Arabidopsis thaliana (ecotype Bensheim, Be-0) and the ADH mutant (ADH-R002)	Role of ethanol in RFB resistance	Alcohol dehydrogenase activity and alcohol may have a role in RFB resistance	

(continued)

Table 1.5 (continued)

Sl. No.	Disease	Genotypes	NGS platform	Inferences	References
8	Mungbean yellow mosaic India virus (MYMIV) and Mungbean yellow mosaic virus (MYMV)	Field grown soybean	–	Stress-responsive proteins and proteins involved in metabolism such as proteasome subunit alpha type 5, Nudix hydrolase 16, glutathione S-transferase, annexin, oxygen evolving enhancer 2, carbonic anhydrase are found to be upregulated	Kumar et al. (2017)
9	Mungbean yellow mosaic India virus (MYMIV)	UPSM 534 (resistant) and JS-335 (susceptible)	–	miRNA mediated argonaute homeostasis is involved in MYMIV resistance	Ramesh et al. (2017a)
10	Floral bud distortion syndrome (FBD), (or) witches' broom syndrome	JS- 335	Illumina HiSeq 2000 platform	Divulged 17,454 DEGs, 5561 TFs, 139 pathways and 176,029 The indispensable roles of positive modulator ofDNA gyrase (PmbA), Zn-dependent protease, phytoplasma virulence effector protein (SAP) family and auxin responsive system in disease progression revealed	Jaiswal et al. (2019)

(continued)

Table 1.5 (continued)

Sl. No.	Disease	Genotypes	NGS platform	Inferences	References
11	Soybean downy mildew (SDM) caused by Peronospora manshurica	Jilinxiaoli1 (highly resistant) and Kefeng1 (highly susceptible)	Illumina HiSeqTM 2000/MiSeq	GmWRKY31 binds to cis-element of GmSAGT1 gene encoding serine/alanine glyoxylate aminotransferase enzyme. GmSAGT1 was activated by fungal infection and salicylic acid Proposed that GmWRKY31 might regulate GmSAGT1 through SA pathway	Dong et al. (2019)
12	Soybean aphid (*Aphis glycines* Matsumura)	Aphid-susceptible (Dongnong47), the antibiotic genotype (P746), and the antixenotic line (P203)	Illumina HiSeq 4000 platform	The expression of TFs in Susceptible and antixenotic genotypes was similar following the aphid infestation. JA behavior and callose deposition. In antibiotic genotype, SA suppressed the JA pathway, and callose deposition happened more rapidly and efficiently	Yao et al. (2020)

1.10 Genetic Engineering for Resistance Traits

Genetic engineering for imparting biotic stress tolerance or resistance is a viable approach because of its efficiency and specificity against target pests and pathogens. Compilation of genetically modified soybean at ISAAA website (https://www.isaaa.org/gmapprovaldatabase/default.asp) that has been approved for field or commercial cultivation reveals that 38 transgenic events have been approved. However none of the approved events are catering to disease resistance trait. Six transgenic events have been approved with respect to insect or pest resistance (https://www.isaaa.org/gmapprovaldatabase/commercialtrait/default.asp?TraitTypeID=2&Trait=Insect%20Resistance). Almost all the approved transgenic events in soybean pertaining to biotic stress tolerance are aimed at achieving lepidopteran insect resistance through the expression *Bacillus thuringiensis* derived *cry1Ac, cry2Ab2, cry1A.105and cry1F* either alone or in combination with herbicide tolerance trait. Nevertheless, there were promising studies to achieve various other biotic stress tolerance in soybean. Soybean plants resistant to *M. incognita* were developed utilizing RNA interference technique by silencing tyrosinase phosphatase gene and another gene encoding mitochondrial stress-70 protein precursor (MSP) (Ibrahim et al. 2011). The genetically altered plants exhibited >90% reduction in gall formation exemplifying the potential of RNAi in soybean. Search for resistant genes in related or distant legume species have also provided resistance to devastating disease like Asian soybean rust (Kawashima et al. 2016). Heterologous expression of *P. pachyrhizi* resistance gene *CcRpp*1 (*Cajanus cajan* resistance against *P. pachyrhizi*) from pigeonpea confers resistance to the pathogen attack in soybean (Kawashima et al. 2016). Similarly, expression of wheat gf-2.8-(germin) gene conferred resistant against the fungal pathogen *Sclerotina sclerotiorum* infecting soybean (Donaldson et al. 2001). When the host factor a soybean transmembrane protein 199 (GmVma12), vacuolar-ATPase (V-ATPase), was found to interact with soybean mosaic virus (SMV) encoded protein, RNAi based silencing of *GmVma12* conferred SMV resistance (Luan et al. 2020).

1.11 Recent Concepts and Strategies Developed

Application of genome editing (GE) technologies utilizing CRISPR/Cas (clustered regularly interspaced short palindromic repeat/CRISPR-associated) proteins have opened up novel avenues for the development of commercially important crops either through transgenic or non-transgenic approaches. The combination of CRISPR/Cas and developments in the field of plant regeneration has offered opportunity to introduce the commercially important trait in the cultivated genotypes. Though soybean has undergone CRISPR/Cas-based targeted genome edits for imparting altered seed protein or oil composition and herbicide tolerance it is anticipated that the technology

would be explored for defining biotic stress tolerance (Bao et al. 2021). Besides developing various products of interests, genome editing could be used in the functional genomics studies of soybean wherein unequivocal assignment of gene-function relationship was possible (Xu et al. 2020). Though GE technologies are powerful it may not replace the genetic modification technologies that are in vogue due to various challenges including its regulatory regimen.

1.12 Prospects and Limitations of Genomic Designing for Soybean

Availability of high quality genomic resources and use of conventional and molecular breeding techniques have helped greatly in designing soybean crop. Soybean being a commercial crop of importance has intensively utilized the platforms such as genomics science and other technologies such as genetic modification to incorporate phenotypic traits of commercial importance. It is also anticipated that modern tools like Genome editing would once again assist in developing designer soybean crops. With the advent of omics technologies application of genomics approaches have gained momentum in the identification and characterization of rare alleles. Though much advancement have been made in the application of novel technologies to develop soybean resistant to biotic stresses, much remains less understood in the field of soybean-pathogen, soybean-pest molecular interactions, and in combining the multiple or dual resistance conferring genes in a single genotype. A combination of multiple omics approaches involving proteomic, transcriptomic, metabolomic and ionomic along with genomic science is required to comprehensively uncover the soybean-pathogen (pest) interactions. Also the role of regulatory set up requires a comprehensive policy revisit in embracing technologies such as genome editing or *cis*genics in the context of soybean being widely used for food and oil.

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Chapter 2
Genomic Designing for Biotic Stress Resistance in Rapeseed

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Abstract *Brassica napus*, rapeseed or oilseed rape, is the major oilseed Brassica crop originated from multiple hybridization events among *Brassica rapa* and *Brassica oleracea*. However, its production has been reduced drastically by various biotic factors. These biotic stresses affect growth and development of the plants resulting in huge reduction in oilseed production at global level. Moreover, the control measures are considerably broader in case of diseases than in insect-pest, resulting in more confrontation from the latter. The development of resistant varieties that can survive under stress conditions is of utmost importance. Various management tools like crop rotation and use of resistant varieties etc. are unsuccessful in most of the cases, as insect control measure mostly depends on use of chemicals/insecticides. The information regarding different molecular and cellular mechanisms is essential to understand the biotic stress tolerance in rapeseed. Thus, the use of genetic or genomics information and microarray tools is vital to speed up the productivity potential in genetic improvement programs. The advancement in genomic techniques as well as availability of genome sequences offer opportunities to produce new plant genotype for any particular character. The identification of various genes and/or effectors against various diseases/pests in rapeseed through omics has opened up the way for more studies to depict the host–pathogen interactions and to characterize the gene function and expression. In this context, this chapter provides up-to-date information on the various biotic stresses faced by the crop across the globe, progress made through conventional plant breeding techniques, genomics, bioinformatics, transgenics and genome editing approaches with a particular focus on the trait mapping and molecular marker assisted breeding approaches. The chapter also offers an overview of the latest genomic findings and tools, such as omics that have been widely employed to unravel the genomic and molecular intricacies against various biotic stresses and its potential applications for further rapeseed improvement. So, the combination of classical genetics, genome editing and integrated omics can accelerate rapeseed production globally.

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2.1 Introduction

Among various oilseed crops, Brassicas are economically more important since they are cultivated for various uses including as oil, vegetables, fodder, condiments, etc. Brassicas include different species viz., *Brassica napus* L. (rapeseed), *Brassica juncea* L. (raya or Indian or oriental mustard), *Brassica oleracea* L. (cabbage and cauliflower) and *Brassica rapa* L. (turnip rape, simply rape or field mustard) (McVetty et al. 2016). Among all the *Brassica* species, *Brassica napus* also known as rapeseed is placed at 2nd position for its production after soybean as oilseed field crop. The family of rapeseed is Brassicaceae having 338 genera and approximately 3709 species generally cultivated annually, biennially or perennially particularly in moderate/mild temperature and hilly regions (Warwick et al. 2010). This crop is supposed to be originated from turnip rape (AA genome, $2n = 20$) and cabbage (CC genome, $2n = 18$) after natural hybridization and its genome is $2n = 38$ (AACC) (Koh et al. 2017). It is one of the major oilseed crops grown worldwide over an area of 36 million hectares with 73 million tons production (www.fao.org/faostat/). The main leading production countries are European countries, Canada, China, India and Australia with a production of 25.5, 20.3, 13.3, 8.4, and 3.9 million metric tons, respectively (FAO Database in 2018–19). In this crop, the oil content is about 31–48.5% and oil profile has vital fatty acids such as oleic acid (56.80–65%), palmitic acid (4.19–5%) and linoleic acid (17.13–21%). The amount of α-tocopherol is approximately 13–40% of the total oil contents (Matthaus et al. 2016).

As oilseed crop (Brassica) attained considerable expansion, however, the yield potentials are very less because of various biotic stresses. Moreover, oilseeds have an imperative part in the diversification in cropping system as long as making available the quality food by fulfilling the fat requirement. Rapeseed is often subjected to various biotic stresses that have major effect on the biochemical, physiological and molecular functions of the crop plant leading to reduction in vigor as well as production causing several losses (Raza 2020). The fungal pathogens like *Alternaria* spp., *Fusarium oxysporum, Albugo candida* and *Leptosphaeria maculans* reduce the oilseed brassicas production the most (Fitt et al. 2006). There are several diseases and pests, supposed to exist in *Brassica napus* growing regions as mentioned in Table 2.1. The biotic stresses mainly have an effect on leaves and stems, while only few insect pests and diseases have an effect on pods and seeds. In addition to this, painted bug, bihar hairy caterpillar and southern blight were only reported in mustard crop from India. Globally, clubroot and sclerotinia stem rot are the major biotic stresses in *B. napus* as well as flea beetles signify main stress from insect-pest. *Phyllotreta* spp. are main flea beetles in spring oilseed crop growing regions, such as Canada, whereas in *rabi* season, *Psylliodes* spp. are existing (Zheng et al. 2020). Undoubtedly, biotic factors are major obstacle in increasing the rapeseed productivity.

Table 2.1 Diseases and insect pests on rapeseed worldwide

Diseases	Causative agent	Root	Seedling	Leaf	Stem	Buds/flowers	Pods/seeds
Alternaria spots	*Alternaria* spp.		x	x	x		x
Clubroot	*Plasmodiophora brassicae*	x					
Downy mildew	*Peronospora parasitica*			x			
Fusarium wilt	*Fusarium oxysporum* f.sp. *conglutinans*				x		
Grey mould	*Botrytis cinerea*			x			
Light leaf spot	*Pyrenopeziza brassicae*			x			
Powdery mildew	*Erysiphe cruciferarum*			x	x		x
Sclerotinia stem rot	*Sclerotinia sclerotiorum*				x	x	
Seedling disease complex	*Rhizoctonia, Fusarium, Pythium* spp.	x	x				
Stem canker and blackleg	*Leptosphaeria* spp.			x	x		
Southern blight[a]	*Sclerotium rolfsii*	x	x		x		
Verticillium stem striping	*Verticillium longisporum*	x			x		
Virus	*Turnip yellows virus Turnip mosaic virus*			x			
White rust	*Albugo candida*			x		x	
Insect pests							
Army cutworms	*Euxoa auxiliaris*			x			
Baris coerulescens	*Baris coerulescens*	x			x		
Bertha armyworm	*Mamestra configurata*			x			x
Bihar hairy caterpillar[a]	*Spilosoma obliqua*			x			
Brassica leaf beetle	*Phaedon brassicae*			x			
Brassica pod midge	*Dasineura brassicae*						x

(continued)

Table 2.1 (continued)

Diseases	Causative agent	Root	Seedling	Leaf	Stem	Buds/flowers	Pods/seeds
Budworm native	*Helicoverpa* spp.			x		x	x
Cabbage aphid	*Brevicoryne brassicae*			x	x	x	x
Cabbage root fly	*Delia radicum*	x					
Cabbage seedpod weevil	*Ceutorhynchus obstrictus (syn.assimilis)*						x
Cabbage stem flea beetle	*Psylliodes chrysocephala*			x	x		x
Cabbage stem weevil	*C. pallidactylus*			x	x		
Cabbage webworms	*Hellula rogatalis*			x		x	
Cabbage white	*Pieris rapae*			x			
Cabbage/striped flea beetles	*Phyllotreta cruciferae, P. striolata*, *Phyllotreta* spp.		x	x			x
Diamondback moth	*Plutella xylostella*			x	x		x
Green peach aphid	*Myzus persicae*			x	x		
Mustard sawfly	*Athalia lugens proxima*			x			
Painted bug[a]	*Bagrada hilaris*			x			
Pollen beetle	*Brassicogethes aeneus*					x	
Rape stem weevil	*Ceutorhynchus napi*				x		
Rape winter stem weevil	*C. picitarsis*				x		
Rape-leaf nitidulid	*Strongyllodes variegatus*			x			
Rapeseed stem weevil	*Ceuthorrhynchus asper*				x		
Redlegged earth mite	*Halotydeus destructor*		x				
Turnip aphid	*Lipaphis erysimi*			x		x	
Turnip moth	*Agrotis segetum*	x			x		

(continued)

Table 2.1 (continued)

Diseases	Causative agent	Root	Seedling	Leaf	Stem	Buds/flowers	Pods/seeds
turnip sawfly	*Athalia rosae*			x			
Nematodes		x					
Slugs	*Deroceras* spp.		x				

[a] Diseases and insect-pest reported from India on mustard
Source https://doi.org/10.3389/fagro.2020.590908

The long-lasting aim of crop improvement against biotic stresses in plants is a main purpose of breeders. Improved biotic stress tolerance against various biotic stresses and losses caused from them is very important globally to enhance oilseeds production as well as productivity of rapeseed. The conventional plant breeding approaches have not given complete defense against biotic stresses so far. The various breeding techniques used in the past to develop resistance against biotic stresses have failed as new pathogens are competent to overcome that resistance by modifying their metabolic cycles. Therefore, there is necessity to reduce the unfavorable effects of biotic stresses in order to increase the production as well as productivity to meet out the oil requirement. To overcome these problems there is an imperative requirement to produce disease and insect-pest resistant crop varieties by means of innovative technologies by knowing metabolic and biochemical pathways of host plants and pathogens.

In order to avoid these biotic stresses various techniques such as use of microbes that can tolerate stress, various enzymes and genes are extremely crucial, whereas numerous techniques such as deployment of genetic markers for indirect selection of improved genotypes accelerate the selection procedure by reducing laborious techniques as well as direct screening under screen house and field conditions, identification of quantitative trait loci (QTLs) can provide additional help to improve resistance against biotic stresses in rapeseed. Genetic and genomic tools that help to recognize DNA regions that are tightly linked to stress resistance in rapeseed should be used. Therefore, the use of one particular method or the combination of different approaches has a prospective to enhance the resistance against various biotic stresses in rapeseed.

2.2 Description of Biotic Stresses in Rapeseed

It is estimated that biotic stresses are responsible for reducing crop production up to 50–60% in Brassica crop and results in considerable economic losses (Kim et al. 2000; Shukla 2005; Sotelo et al. 2015).

2.2.1 Diseases in Rapeseed

In all the rapeseed growing regions Sclerotinia stem rot (or white mold), blackleg and clubroot are predominant diseases which are commonly found (Zheng et al. 2020). It is found that rapeseed grown during spring season has less incidence of fungal diseases as compared to winter and semi-winter crop. In tropical and subtropical regions, white rust is a chief destructive disease (Asif et al. 2017). From the studies it was observed that various major diseases on rapeseed are because of soil borne pathogens (*Plasmodiophora brassicae, Verticillium longisporum*) or by pathogens that have an effect on the stem (*Sclerotinia sclerotiorum, Leptosphaeria maculans*, *L.*

biglobosa, Alternaria spp., *Pseudocercosporella capsellae*, and *Pyrenopeziza brassicae*). In all rapeseed growing regions, Sclerotinia stem rot disease is observed as the main disease-causing agent leading to substantial loss in yield ranging from 0.19 to 1.4%. (Kirkegaard et al. 2006; Del Rio et al. 2007). The pathogen *Sclerotinia sclerotiorum*, is a destructive organism affecting a large number of plant species (Mizubuti 2019). At seedling stage, it attacks cotyledon and leaves and at later stages stem and leaves resulting in water-soaked lesions, necrotic tissues with fluffy white mycelium and sclerotia inside of stems (Khan et al. 2020). The sclerotia of this pathogen can live in the soil for more than four years, that may affect crop rotation. In the cool and moist weather condition during anthesis, ascospores are formed and blowout within the canopy to lower parts of stems and dispersed through insects or wind to leaves and silique as well as adjoining plants (Link and Johnson 2007).

Blackleg disease produced by ascomycete fungus *Leptosphaeria maculans* is a main constraint in rapeseed production. This disease has been reported in all rapeseed cultivating areas except China causing yield reduction up to 5 to 50% (Zhou et al. 1999; Aubertot et al. 2004; Fitt et al. 2006; Hwang et al. 2016). At seedling stage, the pathogen results in necrotic cotyledon and leaf lesions, whereas blackening of stem and cankering occur at maturity, confining the nourishment of the plant and in harsh conditions results in complete mortality of plants (Van de Wouw and Howlett 2020). The air-borne ascospores are responsible to initiate the disease (Gladders and Musa 1980), these may be spread by wind over longer distances (Piliponyte-Dzikiene et al. 2014).

Clubroot produced by *Plasmodiophora brassicae* is a main risk to rapeseed production worldwide since the last two decades. The pathogen may cause loss in productivity up to 0.04 ton per hactare over each 1% increase in disease severity (McGrann et al. 2016), whereas the whole losses in yield may range up to 100% (Ren et al. 2014; Strehlow et al. 2015). Earlier researches confirmed that half-life of resting spores is more than three years (Wallenhammar 1996). Moreover, the spores of this pathogen in the absence of host plants remain in soil for more than four years. This disease is primarily dispersed through field apparatus or by water erosion (Ricarova et al. 2016).

Stem striping disease is produced by *Verticillium longisporum* which is a soil-borne pathogen. It is a vascular disease resulting in 10 to 50% injury in the crop as well as comparatively moderate losses in yield because symptoms of the disease arise at the time of maturity (Dunker et al. 2008; Depotter et al. 2019; Zheng et al. 2019b). In addition, the vascular spread in upward direction in the stem can be enhanced by higher soil temperature (Zheng et al. 2019b). The resting spores, microsclerotia, continue in plant debris and disperse in the soil after harvesting. Besides, current research showed that this pathogen might be spread by seeds of spring season rapeseed rather than winter grown rapeseed (Zheng et al. 2019a). The white rust disease in rapeseed is caused by the oomycete pathogens, *Albugo candida* which is a biotrophic pathogen. The zoospores invade its host plant via stomata, where they grow and start colonization of mesophyll cells. After this, the oomycete produces zoosporangia that look like as white pustules rupturing the epidermis, henceforth establishing noticeable disease symptoms (Cevik et al. 2019). In addition to all these diseases, there

is another disease called as downy mildew produced by the oomycete *Hyaloperonospora brassicae* (earlier known as *Peronospora parasitica*) that affect the aerial portion of the plant. At seedling stage, the causal agent generally presents on cotyledons and on leaf portion as pale green, yellowish growth on leaf bases, whereas in mature plants, it produces uneven angular yellow blotches that have dark speckling (Thines and Choi 2016; Lee and Lee 2019).

2.2.2 Insect-Pest in Rapeseed

Insect-pests play larger role among biotic stresses in rapeseed production worldwide than diseases and result in yield losses and quality (Table 2.1). Globally, the average annual loss in yield due to insect-pest infestation is 13% as they play significant role in yield reduction in rapeseed growing areas (Cramer 1967; Milovac et al. 2017). Most of the insect-pests are not confined to the damaged field but can spread and travel over longer distances because of their high mobility nature, thus not only affecting nearby areas but most probably spread on a landscape level.

In most of the rapeseed growing regions, the prevailing insect-pests are aphids, flea beetles, diamondback moth, brassica pod midge, cabbage root fly and red-legged earth mites. Many current findings showed that flea beetle, brassica pod midge and cabbage root fly can affect rapeseed production on larger scale while their comparative status differs with respect to country and years (Williams 2010; Reddy 2017). In all rapeseed growing regions, where mostly *rabi* season rapeseed is cultivated, aphids are one of the major insect-pest to cause maximum yield losses. In addition to this, diamondback moth is the only insect species that is present worldwide. So far, flea beetles and the cabbage root fly have been reported in the Northern hemisphere.

During winter and semi-winter season, the rapeseed production is largely affected by Flea beetles (*Psylliodes* spp.). The beetles affect leaf area from 25 to 50% within hours during hot weather conditions and result in loss of entire produce if treatment delayed for 1–2 days (Sekulic and Rempel 2016). In case of cabbage stem flea beetle, crop is affected from cotyledons and leaves by adult feeding and thus reduction in vigor within petioles and stems occur. In severe cases, farmers had to resow the field with any substitute crop resulting in 21% reduction of the rapeseed cultivated areas by this cabbage stem flea beetle (Wynn et al. 2017). The flea beetles may remain alive for 6–7 months even in the absence of host and can migrate up to 4 km distance for host plants (Bonnemaison 1965; Finch and Collier 2000). The adults of flea beetles result in reduction in growth and vigor, low silique development and lodging of crop (Juran et al. 2011).

2.3 Management Tools for Biotic Stresses in Rapeseed

The biotic stresses are arising at an alarming rate, in spite of the use of improved protection measures in rapeseed crop in the recent decades indicating that the recent management practices are not sustainable. Recent studies and reports signify that the risk from insect-pests is the major threat to rapeseed production at global level which threatens the overall production as well as productivity of the crop (Menzler-Hokkanen et al. 2006; Arthey 2020). This may be due to the lack of resistance source in case of insect-pests as well as the partial and declining capability in insect control than variety of choices of disease control that are accessible. Thus, management of insect-pests is essential for enhancing the productivity potential of rapeseed in hot spot regions. There are various management practices available for controlling diseases and insect-pests in rapeseed crop, but the accessibility of these practices differs for these two biotic stresses. Generally, stresses from biotic factors can be managed in two possible ways in rapeseed.

2.3.1 Crop Management

On the farm level, extensive choice of methods for diseases control in rapeseed is available. There are numerous control measures available for controlling disease incidence such as use of resistant cultivars, biological control agents, crop rotation, soil tillage and use of chemicals, while in case of insect-pests use of insecticides is preferred. Introduction of exotic lines having gene of interest can assist in the development of plants with vigor, growth and high yield potential thus providing biotic stress resistant plants (Clair et al. 2016). Resistance source for clubroot and stem canker are present. In addition, breeding for resistance to Verticillium is underway. Four biological control agents have been registered for disease control namely *Coniothyrium minitans* CON/M/91–08 against *Sclerotinia sclerotiorum*, *Phythium oligandrum* M1 against *S. sclerotiorum* and *Leptosphaeria maculans*, *Bacillus amyloliquefaciens* MBI 600 against *L. maculans* and *B. amyloliquefaciens* QST 713 against *Sclerotinia* spp. during 2020 (Zheng et al. 2020). The rapeseed production is also enhanced by crop rotation practices as the risk of soil borne and seed borne diseases such as Sclerotinia, clubroot, Verticillium can be reduced as these pathogens leave their fungal inoculum in the particular fields (Sieling et al. 1997; Sieling and Christen 2015). Another management option for reducing disease incidence is soil tillage as destruction of plant debris having fungal infection/ inoculum is most important to control a particular disease.

Moreover, the management practices for insect-pest control are considerably less as compared to diseases. Because the resistant source as well as biocontrol agents are not available in case of insect-pest and the effect of soil tillage and crop rotation is also scarcer. So, the main means of insect control in rapeseed is the use of insecticides (Zheng et al. 2020). Overall, in order to introgress single gene for disease or insect

resistance in the susceptible high yielding varieties, backcross method is the generally preferred technique.

2.3.2 Biotechnological Approaches

During plant microbial interaction, candidate genes are involved to lower the virulence traits of pathogens e.g., pathogen cell wall degrading enzymes and toxins that result in disease resistance. When these genes are incorporated into the crop plants the formation of plant defense proteins like pathogenesis related (PR)-proteins, phytoalexin, reactive oxygen species (ROS), saponins, antimicrobial peptides, etc. is accelerated. The plant defense proteins give resistance against pathogens by affecting their disease-causing factor (Tian et al. 2016). The resistance against various biotic stresses can be achieved by inserting such genes in the plant genome. Gene stacking or gene pyramiding techniques can be used in order to provide broad spectrum resistance against the diseases and insect-pest by combing two or more genes.

2.4 Classical Genetics and Breeding for Biotic Stresses

2.4.1 Alternaria Blight

Identifying resistance mechanisms at the genetic and genomic level has been a prime concern for the researchers over the recent years. Various sources suggest that the resistance against *Alternaria* is polygenic (Tripathi et al. 1980; Zhang et al. 1996; Krishnia et al. 2000; Meena et al. 2016). On the contrary, other findings confirmed that disease resistance is mainly controlled by only additive genes or dominant nuclear genes (Tripathi et al. 1980; Zhang et al. 1996; Krishnia et al. 2000; Panja and De 2005; Meena et al. 2016). However, Kumar et al. (2020) evidenced that inheritance of *Alternaria* blight resistance is governed by more than one gene and fixable and non-fixable gene effects are vital in the genetic control of *Alternaria* blight resistance. In *Arabidopsis*, six quantitative trait loci (QTLs) governing *Alternaria* blight resistance were identified. Among these QTLs, five were population specific and one was universal among all mapping populations. Presence of both universal and population specific QTLs indicates that resistance against *Alternaria* blight is quantitative and more than one gene potentially governs the resistance (Rajarammohan et al. 2017).

2.4.2 Clubroot

In rapeseed, some varieties are recognized to carry the dominant resistance against clubroot disease. Ayers and Lelacheur (1972) in their study identified that the clubroot resistance was present in rutabaga cultivars and this resistance was controlled by single dominant gene. Williams (1966) also identified two rutabaga accessions that show clubroot resistance and these accessions were reported to carry resistance in several other studies also (Walker 1939; Ayers and Lelacheur 1972; Hasan et al. 2012). Vigier et al. (1989) found several Swedish accessions which were showing clubroot resistance. In one study, clubroot resistance was transferred from rutabaga into cabbage through interspecific hybridization. The resulting F1 hybrids were also resistant to clubroot disease (Chiang et al. 1977).

2.4.3 Blackleg

Blackleg resistance genes were mapped on linkage groups N2 (*LepR1*), N7 (*Rlm1, Rlm3, Rlm4, Rlm7* and *Rlm9*) and N10 (*Rlm2, LepR2* and *LepR3*) of rapeseed were of the dominant resistance type (Delourme et al. 2004; Yu et al. 2005, 2008). Yu et al. (2012) reported that *LepR4a* and *LepR4b* were recessive genes which show resistance to blackleg in rapeseed. Saal et al. (2004) identified recessive gene *rjlm2* from rapeseed genotypes carrying B-genome chromosome additions derived from raya.

2.4.4 Aphid

Resistance to aphid may be controlled by single dominant or recessive gene or polygenes. To study the inheritance of aphid resistance, interspecific hybridization was carried out between *Brassica napus* and *B. oleracea* and hybrid plants were produced by embryo culture (Quazi 1988). F2 segregation ratios indicated that resistance to aphid was controlled by a single dominant gene. One resistant line was also isolated from a cross between the F1 and forage rape (Quazi 1988). Two dominant, aphid resistance genes (*Vat* and *Mi-1*) have been isolated and both of these genes encode nucleotide binding site- leucine rich repeat (NBS-LRR) proteins (Dogimont et al. 2010).

2.5 Molecular Mapping and Cloning for the Biotic Stresses

2.5.1 Clubroot

Clubroot causing pathogen *Plasmodiophora brassicae* has a broad choice of host counting both cruciferous as well as non-cruciferous crops (Ren et al. 2016). Yield loss from the clubroot disease ranged up to 21–30% in China (Chai et al. 2014) and 50% in Canada (Strelkov and Tewari 2005). In rapeseed, some resistance loci have been characterized for various isolates. In one study, two QTLs controlling clubroot resistance to race 2 were identified. These QTLs accounted for 58% and 15% of the observed phenotypic variation (Landry et al. 1992). Manzanares-Dauleux et al. (2000) also identified one major gene, *Pb-Bn1*. Werner et al. (2008) detected 19 QTLs showing resistance to seven dissimilar isolates of the pathogen. Piao et al. (2009) identified 16 QTLs on eight different chromosomes (N02, N03, N08, N09, N13, N15, N16 and N19) of rapeseed. Fredua-Agyeman and Rahman (2016) mapped clubroot resistance to a DNA segment that composed 12 markers linked to the *CRa* locus. Hasan and Rahman (2016) mapped resistance to a genomic segment on chromosome A8 used rutabaga-derived populations. This region was identified to confer resistance to the five tested pathotypes. This resistance locus was flanked by the simple sequence repeat (SSR) markers sS1702 and A08_5024. Li et al. (2016) carried out a genome-wide association study (GWAS) to identify the loci conferring clubroot resistance. They identified a total of nine loci conferring resistance to clubroot. Zhang et al. (2016) also identified a marker GC1680, linked to the clubroot resistance gene *CRa*. Hejna et al. (2019) used an associative transcriptomics approach and identified two major loci and seven minor loci controlling resistance. Two major loci were present on chromosomes A2 and A3 rapeseed. In another study, 45 single nucleotide polymorphisms (SNPs) and four PCR-based markers were recognized (Fredua-Agyeman et al. 2020). They were strongly associated with resistance to thirteen pathotypes (2F, 3H, 5I, 6 M, 8 N, 2B, 3A, 3O, 5C, 5G, 5 K, 5L and 8P). These markers were located on the top and bottom segments of chromosome A03 and the middle segment of chromosome A08 of rutabaga. These genomic regions are the hotspots of resistance to the various *P. brassicae* pathotypes, where almost all CR genes (*CRk, Crr3, CRd, CRa, CRbKato, Rcr1, CRb*, and *Crr1*) on the A-genome are located (Fredua-Agyeman et al. 2020).

2.5.2 Sclerotinia Stem Rot

Sclerotinia stem rot is caused by the pathogen *Sclerotinia sclerotiorum* which has a wide host range (Zhao et al. 2004). Yield losses by *S. sclerotiorum* can reach up to 80% in China (Mei et al. 2011) and cause a major loss of AU$ 23 million in Western Australia (Dafwa 2015). Zhao and Meng (2003) first identified three QTLs conferring leaf resistance. They also identified three other QTLs for stem resistance in

the seedling and adult stages. Zhao et al. (2006) also identified eight QTLs which were explaining 7–23% of the observed phenotypic variance. Yin et al. (2010) used three inoculation procedures, and detected ten, one, and ten QTLs in single doubled haploid (DH) population. They also identified two common QTLs in three procedures. Wu et al. (2013) identified two major QTLs, LRA9 on LG A9 and SRC6 on LG C6, and a candidate resistance gene, *BnaC.IGMT5*, was identified. Fomeju et al. (2014) first reported 64 genomic regions involved in stem rot by using genome-wide association study (GWAS). Wei et al. (2016) identified 17 significant regions on the A8 and C6 chromosomes of rapeseed using combined GWAS and SNP array analyses. In another similar study, Wu et al. (2016) identified three loci, *DSRC4*, *DSRC6* and *DSRC8* for stem rot resistance. Gyawali et al. (2016) carried out GWAS study and found that 34 loci were significantly associated with stem rot resistance. Some distant hybridization combined with marker assisted selection (MAS) experiments were also carried out due to lack of resistance sources in rapeseed. Mei et al. (2011, 2013, 2015) successfully introgressed resistance from wild *B. incana* into *B. napus* through hexaploidy hybridization and marker assisted selection (MAS). Majority of the QTLs were recognized in the C genome (Li et al. 2015), representing that cabbage has source of resistance genes for stem rot. Wei et al. (2016) identified 17 SNPs for stem resistance on chromosomes A8 and C6. Behla et al. (2017) identified QTLs for stem rot resistance on linkage group A7, A9 and C6 of rapeseed. Qasim et al. (2020) identified 17 QTLs for resistance against Sclerotinia Stem Rot using SNP markers during three seasons. During 2016, they have identified seven QTLs viz., SRA2a (Chr A2), SRA9a (Chr A9), SRA9c (Chr A9), SRC3a (Chr C3), SRC3b (Chr C3), SRC3c (Chr C3) and SRC3d (Chr C3), during 2017, five QTLs viz., SRA2b (Chr A2), SRA2c (Chr A2), SRC2a (Chr C2a), SRC3a (Chr C3), SRC4 (Chr C4) and during 2018, five QTLs like SRA9b (Chr A9), SRA9a (Chr A9), SRC2a (Chr C2a), SRC2b (Chr C2b) and SRC6 (Chr C6) were identified. Evidences from previous studies and this study regarding the QTLs positions having genes showing resistance against Sclerotinia Stem Rot is shown in Fig. 2.1.

2.5.3 Downy Mildew

Hyaloperonospora parasitica is the causal organism of the downy mildew disease. Earlier five R genes conferring resistance to *H. parasitica* that encode both TIR- and CC-NBS-LRR have been cloned in Arabidopsis. *RPP2* (Sinapidou et al. 2004) and *RPP5* (Parker et al. 1997) were detected on chromosome 4, while *RPP8* (McDowell et al. 1998), RPP1 (Botella et al. 1998) and *RPP13* (Bittner-Eddy et al. 2000) were detected on chromosome 3 (Botella et al. 1998) of Arabidopsis. Lucas et al. (1988) identified that rapeseed cultivar Cresor was resistant to 14 isolates of *Peronospora parasitica.* This resistance was studied to be controlled by a single gene. McDowell et al. (2005) identified a major gene *RPP31* for adult plant resistance on chromosome 5 in *A. thaliana.* Another major locus *Pp523* conferring resistance at the adult stage was identified on chromosome C8 in *B. oleracea* (Farinho et al. 2004; Carlier et al.

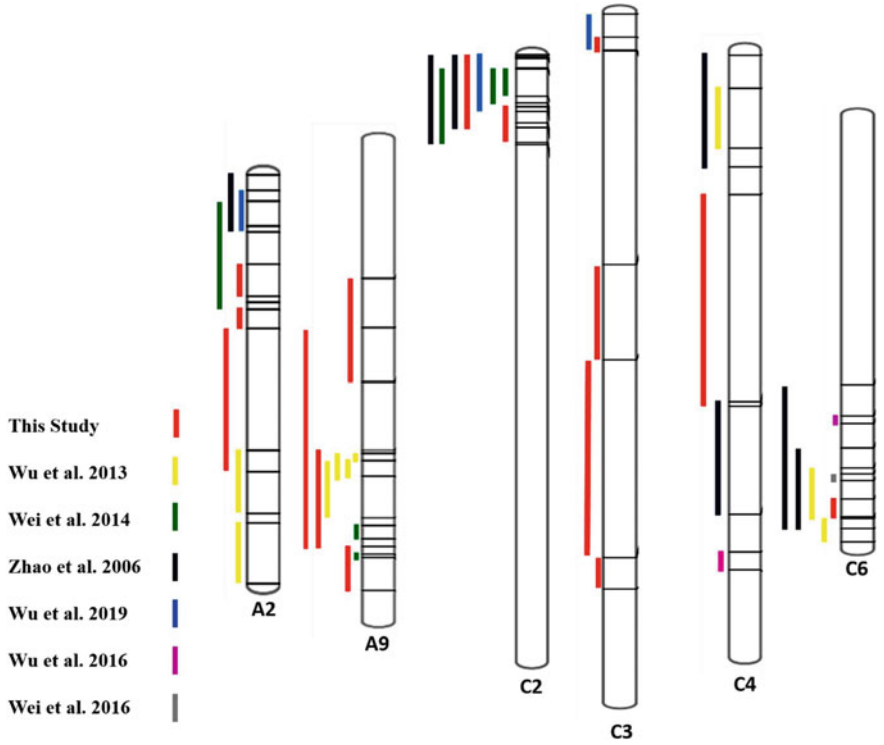

Fig. 2.1 QTLs identified for resistance against sclerotinia stem rot from different studies. (Zhao et al. 2006; Wu et al. 2013, 2016, 2019; Wei et al. 2014, 2016) (Adapted from Qasim et al. 2020)

2012). This region was syntenic to a locus on chromosome 1 in *A. thaliana* (Farinho et al. 2007). Singh et al. (2012) mapped a single dominant R gene *Ppa3*, in *B. oleracea* using molecular markers. Since many resistance genes have been mapped in Arabidopsis, the orthologous genes in rapeseed can be studied through comparative analysis of genomes (Yu et al. 2014) and pan-genome analysis (Golicz et al. 2016).

2.5.4 Blackleg

Most of the blackleg resistance genes/QTLs are reported to be originated from the A genome of *B. napus*. *Rlm1*, was the first R gene identified to be involved in the gene for gene type of resistance interaction against the blackleg pathogen in rapeseed (Ansan-Melayah et al. 1998). Ferreira et al. (1995a) first identified a major locus *LEM1* on N7 chromosome of rapeseed. In other studies, major genes *LmFr1* (Dion et al. 1995) and *LmR1* (Mayerhofer et al.1997) were detected. Delourme et al. (2004) mapped resistance loci as a cluster consisting of five R genes. Work of fine mapping

of the resistance conferring loci was carried out extensively after 2010. Yu et al. (2005, 2008) mapped blackleg resistance *LepR1, LepR2* and *LepR3*. Long et al. (2011) identified two major genes, *BLMR1* and *BLMR2* conferring resistance to blackleg. Fine mapping of the locus *BLMR1* was also carried out which resulted in the identification of the closest marker at 0.13 cM distance. Jestin et al. (2011) identified five novel alleles for blackleg resistance using an association mapping approach. Raman et al. (2012) identified a new major locus, *Rlm4*. Blackleg resistance genes were also transferred from wild relatives of *B. rapa* and *B. oleracea* to *B. napus* (Yu et al. 2012). Larkan et al. (2013, 2014) used map-based cloning method to first clone the blackleg disease resistance gene *LepR3*. They also detected the *Rlm2* gene on chromosome A10 of the rapeseed cultivar 'Glacier'. More recently, another blackleg resistance gene, *Rlm9*, was cloned. This gene encodes a wall-associated kinase-like protein, which is a newly discovered class of race specific plant RLK resistance genes (Larkan et al. 2020). In addition to the major locus, some QTLs were detected, which are stable under different environmental conditions (Haung et al. 2016; Larkan et al. 2016). In another study, Fu et al. (2019) identified the *Rlm1* gene in rapeseed cultivar 'Quinta' which was located on chromosome A07, between 13.07 to 22.11 Mb. Further fine mapping of *Rlm1* gene was carried out into a 100 kb region from 19.92 to 20.03 Mb. *BnA07G27460D*, a potential resistance gene was identified in this *Rlm1* region. Raman et al. (2020) identified two race-specific resistance R genes, *Rlm3* and *Rlm4* and 21 marker associations.

2.5.5 White Rust

Albugo candida race 2 is primarily linked with the white rust disease of *B. napus/B. juncea*. The white rust resistance is found to be governed mostly by a single dominant gene (Delwiche and Williams 1974; Tiwari et al. 1988; Kole et al. 1996). Verma and Bhowmik (1989) reported that it is controlled by two dominant genes while minor genes control was reported by Kole et al. (2002). Ferreira et al. (1995b) identified the *ACA1* locus on the linkage group 9 of rapeseed. This *ACA1* locus was linked with the nine restriction fragment length polymorphism (RFLP) loci. The genetic distances were given in centimorgans. Kole et al. (2002) also identified the loci conferring resistance to white rust disease. QTL mapping using the IP scores detected the same major resistance locus for both races (race 2 {AC2} and race 7 {AC7}). In addition, a second minor QTL effect for *AC2* was detected on linkage group 2.

2.6 Addressing Biotic Stresses in Rapeseed in the Post-genomics Era

The Brassica crops are susceptible to various biotic factors including fungal and bacterial diseases viz. Alternaria leaf blight (*Alternaria brassicae* and *A. brassicicola)*, Sclerotinia stem rot (*Sclerotinia sclerotiorum*), blackleg (*Leptosphaeria maculans*), powdery mildew (*Erisiphe spp.*), white rust (*Albugo candida*), downy mildew (*Hyaloperonospora parisitica*), various viruses like Cauliflower mosaic virus, Turnip mosaic virus (TuMV), Turnip yellow virus (TuYV), and aphids (*Brevicoryne brassicae* (L.); *Lipaphis erysimi* and *Myzus persicae)* which, poses a serious threat to Brassica production worldwide (Kumar 2012; Kumar et al. 2017). Effective management of biotic stresses for oilseed Brassicas remains challenging due to poor efficacy of chemical and cultural control measures. Therefore, new feasible biotechnological methods must be exploited to protect the Brassicas against varied biotic stresses.

2.6.1 Genomics and Bioinformatics

The recent advances in sequencing of five *Brassica* genomes of "U triangle" excluding *B. carinata* has led to novel insights into the evolutionary genomics, development of molecular markers and identification of novel genes and/or gene discoveries. Literature cites many examples in *Brassica* species where sequencing, whole genome re-sequencing, genotyping by sequencing using latest next generation sequencing (NGS) platforms has tremendously advanced the field of Brassica genomics. Among six species, *B. rapa* was the first *Brassica* species sequenced by a Chinese group (Wang et al. 2011). Also, several Brassica reference genome assemblies for example *B. rapa* (Chiifu *B. rapa* ssp. *trilocularis* Z1), *B. oleracea, B. nigra, B. napus* and *B. juncea* are presently available in public domain (Chalhoub et al. 2014; Liu et al. 2014; Parkin et al. 2014; Yang et al. 2016; Bayer et al. 2017; Sun et al. 2017; Belser et al. 2018; Zhang et al. 2018; Paritosh et al. 2020; Perumal et al. 2020).

Recently, omics technologies have been used extensively to unravel the genomic and molecular intricacies against various biotic stresses. For instance, RNA sequencing, comparative transcriptomic analysis, associative transcriptomics, functional genomics, proteomics, metabolomics, and phenomics have emerged in an efficient way to evaluate host signaling pathways, gene networks including up-regulated and down-regulated gene(s), gene expression profiling, metabolic profiling against disease and/or insect pests. Ding et al. (2019, 2020) demonstrated the important role of calcium signaling in production of ROS during *S. sclerotiorum* infection through transcriptomic analysis in *B. oleracea.* Also, several other transcriptomic studies in *B. napus* and *B. oleracea* emphasized salicylic acid (SA) and jasmonic acid (JA) involvement against *S. sclerotiorum* resistance (Wang et al. 2019, 2020). Qasim et al. (2020) revealed the crucial role of genes in *S. sclerotiorum* resistance including

TIR–NBS–LRR, and in the synthesis of hormone and secondary metabolites in *B. napus*. Likewise, in *B. napus,* comparative RNA sequencing analysis revealed up–regulation of plant hormones viz. ethylene, JA, SA, abscisic acid (ABA), auxins, and cytokinin against clubroot (Shah et al. 2019). Another transcriptomic study in *B. rapa* exhibited the involvement of effector triggered immunity (ETI) pathways including pathogenesis–related (PR) genes, WRKY transcription factors, calcium–binding proteins and chitinases against *Plasmodiophora brassicae* infection (Chen et al. 2013; Ji et al. 2018, 2020). In addition to differential expression analysis RNA-Seq data is also used for identifying exonic SNPs that can be converted to functional markers. Hejna et al. (2019) exploited associative transcriptomic approach to identify candidate SNPs associations with the differential gene expression data against clubroot resistance.

2.6.2 Proteomics

The proteomics studies in *Brassica* species has also facilitated the identification of various proteins that are expressed during the host–pathogen/pest interaction. For instance, Sharma et al. (2008) demonstrated that various enzymes required for CO_2 fixation, H_2O_2 scavenging, and redox metabolism were up-regulated during *B. napus–L. maculans* interaction. Using comparative proteomics in *B. rapa* several researchers revealed the involvement of ubiquitin–related proteins, lignin biosynthesis, proteins associated with tryptophan and glutathione biosynthesis, ROS activation, thioredoxin association with oxidative stress, and MAPK signaling pathway proteins against *P. brassicae* (Song et al. 2016; Lan et al. 2019; Moon et al. 2020).

2.6.2.1 Metabolomics and Phenomics

Recently several new strategies like metabolomics and phenomics have been widely used in crop plants. Metabolomics deals with the study of detection of primary and secondary metabolites and metabolic pathways whereas phenomics or high–throughput phenotyping (HTP) deals with the study of phenotype of the crop plants by using various high throughput imaging techniques (Walter et al. 2015; Razzaq et al. 2019; Raza 2020). For example, Raza (2020) did metabolic profiling of 52 compounds in different rapeseed varieties using mass spectrometry. In case of HTP, phenotyping centres have been developed across the world. Some of them are Phenome and National Plant Phenomics Center (NPPC) in UK, High resolution Plant Phenomics center (HRPPC) in Australia, and European Plant Phenotyping Network (EPPN) in Europe.

2.6.3 Bioinformatics Approaches

Several bioinformatics tools nowadays have been used for various genome annotations, databases, SNPs discovery, gene prediction and many more studies. With the advancement of sequencing, numerous completed sequences have been deposited in public data bases such as BRAD (Cheng et al. 2011), Brassica genome (Stein et al. 2002), Phytozome (Goodstein et al. 2012; Nordberg et al. 2014), Ensembl Plants (Bolsers et al. 2018), and TAGdb (Marshall et al. 2010). For domain and motif analysis various software's such as Hidden Markov Model (HMM) (Yoon, 2009), SMART (http://smart.embl–heidelberg.de/) (Schultz et al. 2000), Prosite (http://prosite.expasy.org/), pfam (http://pfam.xfam.org/), and InterProScan5 (http://www.ebi.ac.uk/Tools/pfa/iprscan5/) were used. Several others software's viz. GenBank (Pruitt et al. 2009a, b), PRG db (Sanseverino et al. 2010), Cutadapt 1.7.1 (Martin 2011), Sickle 1.33 (Joshi and Fass 2011), SOAPdenovo 2.04 (Luo et al. 2012), Velvet 1.2.10 (Zerbino and Briney 2008), and Patho Plant (Bolívar et al. 2014) have tremendous use in transcriptomic analysis, de novo assembling, plant–pathogen interaction studies. Also, tools like DEGseq (R–package, Wang and Wang 2020), DAVID (https://david.ncifcrf.gov/), PlantGDB (http://www.plantgdb.org/BrGDB/), agriGO (http://bioinfo.cau.edu.cn/agriGO/), and MEGA 6.0 (Tamura et al. 2013) were used to identify and annotate the differentially expressed genes/resistance/pathogenicity associated genes.

2.7 Transgenics and Genome Editing

The identification of various genes and/or effectors against various diseases/pests in Brassicas through omics has opened the way for more studies to define the host–pathogen interactions and to characterize the gene function and expression. Several transgenics in *B. na*pus have been developed by incorporating various gene(s) from other species. The list of transgenic Brassicas developed against various biotic stresses was provided in Table 2.2. Although the functional validation of selected candidate and/or effector genes was carried out either by (1) over–expression of the gene (Knock in) and (2) Dysfunction of genes in the host species (Knock out). Strategies using RNA interference (RNAi), host induced gene silencing (HIGS), virus induced gene silencing (VIGs), spray induced gene silencing (SIGS) and by genome editing using clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated proteins (CRISPR/Cas) system (Yin and Hulbert 2015; Majumdar et al. 2017; Li et al. 2018; Ma et al. 2019; Wu et al. 2020).

Using sRNA induced gene silencing we can improve host resistance and expand disease resistant resources. Several researchers deciphered the role of miRNA and/or siRNA in mediating RNAi against various biotic stresses (Nowara et al. 2010; Pitino et al. 2011; Cao et al. 2016a, b; Mamta et al. 2016; Fan et al. 2017). Using high throughput deep sequencing Cao et al. (2016b) identified 280 miRNAs in inoculated

Table 2.2 The list of successfully developed transgenic rapeseed against various biotic stresses

S. No.	Gene(s)	Stress resistance	References
1	*DRR206*	*Leptosphaeria maculans*	Wang and Brian (2001)
2	*Oryza cystatin I*	*Myzus persicae*, *Aphis gossypii* *Acyrthosiphon pisum*	Rahbe et al. (2003)
3	*gf-2.8*	*Sclerotinia sclerotiorum*	Dong et al. (2008)
4	*WRR4*	*Albugo candida*	Borhan et al. (2010)
5	*PmAMP1*	*Alternaria brassicae*, *Leptosphaeria maculans* *Sclerotinia sclerotiorum*	Verma et al. (2012)
6	*Thaumatin like tlp* *GDSL1*	*Sclerotinia sclerotiorum*	Zamani et al. (2012) Ding et al. (2019)

and un–inoculated *B. napus* leaves *S. sclerotiorum* out of which 53 are novel and 227 are variants of miRNA gene families. And also reported that these miRNAs have some role in defense mechanism like R genes, and reactive oxygen species (ROS) related genes. HIGS induces the production of small interfering RNA by expressing the double stranded RNA fragment of the pathogen gene and then silencing the target gene of the pathogen, interfering the growth and development of the pathogen, formation of infection structure, and thus reducing the virulence of the pathogen (Weiberg et al. 2013). In recent years, HIGS, VIGS and SIGS technologies has been successfully applied to verify the role of pathogenic factors of *B. cinearea* and *S. sclerotiorum* in *B. napus* and turnip yellow mosaic virus in *B. rapa* (Yu et al. 2018).

The gene editing tool namely clustered regularly interspaced short palindromic repeat (CRISPR) or CRISPR/Cas system has been exploited for functional validation analysis as well as incorporating targeted genome modification in Brassica improvement in recent years. The CRISPR/Cas system is categorized into three types viz. I, II and III out of which type II was the most commonly used (Harrison et al. 2014; Barakate and Stephens 2016; Sun et al. 2018). Literature cites few reports in *B. napus*, *B. campestris* and *B. oleracea* where gene editing was carried out by using CRISPR/Cas9 against various biotic stresses (Sun et al. 2018; Yang et al. 2018; Xiong et al. 2019). Recently this system has been widely used by many researchers in targeted genome editing of quality/yield traits in rapeseed like *BnFAD2* (Okuzaki et al. 2018), *SPL3* gene (Li et al. 2018) and *JAGGED* gene (Zaman et al. 2019). In case of biotic stresses for instance, Sun et al 2018 deciphered the role of WRKY transcription factors *BnWRKY11*, and *BnWRKY70* using the CRISPR/Cas9 approach against *S. sclerotiorum.*

2.8 Future Perspectives

Biotic stresses play significant role in decreasing the rapeseed productivity to fulfill the increasing demand of oil, as diseases and insect-pest effects oilseed production globally. There is an utmost prerequisite to diminish the unfavorable effects of these stresses. The fungicides and pesticides have helped to control such stresses to some extent, though their use is detrimental. Therefore, plants own defense mechanisms for biotic stress control is imperative for enhancing productivity. The traditional techniques of plant breeding have not given complete defense from these biotic factors. So, for the development of high yielding resistant varieties, the fundamental study about the inherent capacity of disease and insect-pest resistance and knowledge about host–pathogen interactions is of utmost importance. Likewise, identification of earlier unidentified resistance mechanisms is possible through genetic mapping of plant mutations. Molecular markers accelerate the selection process by reducing time consuming approaches. In addition to this, high-throughput DNA sequencing and microarray analysis tools are used for mapping and cloning of resistant gene against various biotic stresses. The genetic information regarding host plant as well as pathogens/insect-pest speed up the process of investigating these stresses. Thus, genomic tools are required to recognize DNA regions that are tightly linked to particular trait of interest in rapeseed as they provide ease for the functional annotation of genes associated with resistance and susceptibility reaction. In rapeseed, several transgenics have been developed by incorporating various gene(s) from other species. The CRISPR or CRISPR/Cas technology has been used for functional validation analysis as well as incorporating targeted genome modification in rapeseed improvement.

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Chapter 3
Designing Sunflower for Biotic Stress Resilience: Everlasting Challenge

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Abstract Sunflower, a relevant crop for oil production in temperature regions, is subjected to various biotic stresses. Significance of a particular stress agent, both spatially and temporally, is determined by the environmental limitations and the pest population variability. This chapter provides a review of the major sunflower diseases and pests, with an emphasis on their distribution and description of the damage they may cause. Besides, we discuss different strategies used in sunflower breeding for biotic stress resistance, strategy that is reliable, durable, cost effective and with low negative impact on environment, for pest and disease control. During a long history of sunflower cultivation, several major breakthroughs in breeding for resistance to diseases and pests were made. Recent breakthrough in sunflower genomics and availability of genome data of both sunflower and its pathogens opens up the new possibilities for introduction of biotic stress resistance into cultivated sunflower. In the light of changes made over the history and the recent findings we discuss new tools available for designing sunflower crop resilient to biotic stresses.

Keywords *Helianthus annuus* · Breeding for resistance · Genomics · Diseases · Pests · Broomrape

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3.1 Introduction

Breeding for resistance to biotic stress is a central focus of plant breeding programs. For a variety to be accepted for production in particular area complete package of agronomic and quality traits such as high yield, disease resistance, agronomic performance, seed quality, oil content needs to be attained (Poland and Rutkoski 2016). This especially stands for sunflower (*Helianthus annuus* L.), the fourth most important oilseed crop in the world, since it hosts large number of pathogenic microorganisms, most of them fungi, which could lead to significant reduction of its yield and seed quality. The increase in sunflower production on global scale has been singnificant, from 10 million tons cropped on area of 9.6 million ha in 1975 to 52 million tons cropped on area of 27 million ha in 2018: meaning that increase in yeald was twice as much compared to production area, pointing at the same time on changes in market position which is connected to significant technical progress (Pilorge 2020). However, the increase of acreage and stability in present area of sunflower production could be indangered, among other factors, by the presence of pathogens causing disease outbreaks in every region where it is grown.

In this chapter we made a review of the most important sunflower pests and diseases of and available genetic resources and tools for tackling this everlasting challenge in sunflower breeding and production and designing pest- and disease-resilient sunflower.

3.2 Major Pathogens of Sunflower

Sunflower is a plant that is suitable as a host for a large number of pathogenic microorganisms. Disease incidence and severity depends on the host susceptibility and environmental conditions. Some sunflower pathogens are widely distributed and considered a major constraint in sunflower production while others are of regional or minor importance. Changing weather patterns can favor one disease over another making sunflower crop under pressure of different pathogens with possibility of predicting changes in pathogen incidence with the use of prediction models (Debaeke et al. 2017).

3.2.1 Oomycete

Pathogenic oomycetes are causing several sunflower diseases. During periods of prolonged soil wetness before and after emergence, soilborne pathogenic *Phytium* spp. and *Phytophtora* spp. could cause dumping off and collar rot of young sunflower plants. These diseases are less important than other two diseases also caused by pathogenic oomycetes—downy mildew and white rust.

Downy mildew of sunflower, caused by the specialized biotrophic oomycete *Plasmopara halstedii* ((Farl.) Berl. and De Toni), has a potential to severely damage sunflower crop. Pathogen presence on sunflower has been proved in the majority of countries growing sunflower, but not in sunflower regions of Australia and New Zealand (Spring 2019). Its impact on yield on global scale, using current control measures, is estimated at 3.5% of commercial seed production (Gascuel et al. 2014). Yield loss is directly connected with the incidence, severity and distribution of diseased plants. Disease appearance early in season, combined with the scattered distribution pattern and low incidence, does not result in yield loss due to sunflower good compensation ability. Cumulative damage increases with the increase in disease incidence and grouping of diseased plants in patches. Characteristic disease symptoms include plant stunting and leaf chlorosis with white cover of pathogen sporangia (Fig. 3.1a). Soilborne inoculum penetrating into the root and subsequent development of pathogen in host is referred as systemic infection. Airborne inoculum from sunflower leaves is resulting in appearance of angular leaf spots that are considered not damaging to the host plant. This local infection can potentially become systemic and could lead to seed contamination (Spring 2009). Number and arrangement of severely affected plants in a field determine the level of damage. Discovery of resistance genes made possible description of pathogen virulence chategorised as races. Beginning of race determination, when low number of races was described, was charaterised by marking of races with numbers or capital letters depending on particular research group. Increase in number of races led to adoption of code system based on susceptibility of groups of three differential lines (Tourvieille de Labrouche et al. 2000). Number of races is rising as a result of pathogen adaption to the host. This increasing diversity of virulent pathotypes makes downy mildew a persistent and highly dangerous treat for sunflower production.

White rust in sunflower was for a long time considered restricted to several countries in South hemisphere and to be the disease of hot and dry climate (Kolte 1981). However, there are reports on recent spreading of the disease to other areas (Gulya et al. 2002a; b; Thines et al. 2006). Causal agent has been described as *Pustula helianthicola* (Rost and Thines 2012). Seed-borne and soil-borne oospores are source of primary infections (Vijoen et al. 1999; Lava et al. 2013). It creates on host leaves characteristic chlorotic blisters and white layer of sporangia on adjacent side of the leaf. Numerous symptoms in early stages of plant development can cause premature leaf senescence (Kolte 1981). Logging of plants due to stem breakage is described as particularly damaging (van Wyk et al. 1995). It is important constraint in production of ornamental sunflower that loses economic value after symptoms appear (Lava et al. 2013).

3.2.2 Fungi

Majority of the most damaging sunflower diseases is a result of parasitism of fungal pathogens. They are causing damage to sunflower roots and above-ground parts.

Fig. 3.1 Sunflower plant with **a** symptoms of reduced growth and chlorosis characteristic for downy mildew, **b** symptoms of interveinal chlorosis and necrosis as a result of *Verticillium dahliae* parasitism, **c** symptoms of wilting as a result of *Sclerotinia sclerotiorum* parasitism and **d** emerged flowering broomrape plants

Symptoms are visible in various forms depending on the pathogen. Some pathogens can be identified by the disease symptoms and structures present on the plant surface or inside of the diseased tissue. In favorable environment and in the presence of susceptible host, high incidence of pathogen inoculum can result in significant yield losses.

***Sclerotinia* spp**. Several *Sclerotinia* spp. have been identified as causal agents of sunflower diseases. They differ in economic significance and adaptation to the environmental conditions. *Sclerotinia minor* Jagger and *Sclerotium rolfsii* Sacc. are root parasites, causing root rot and plant wilting. In addition to the symptoms of wilting, fungus *Sclerotinia sclerotiorum* Lib. de Bary is causing airborne rot of all above-ground parts of sunflower plant.

White mould is a disease caused by *S. sclerotiorum.* Fungus is affecting number of plant hosts with several important crops among them. Sunflower is more affected in temperate regions particularly with high amount of rain (Smolinska and Kowalska 2018). Yield loss as a result of plant wilting is significant if infection occurs at any time after flowering to near maturity and similar to the effect of drought and defoliation (Saharan and Mehta 2008). Disease is both soilborne and airborne and can appear in every stage of sunflower development. Sclerotia, fungus surviving structures, are able to penetrate sunflower roots upon myceliogenic germination in soil. As a consequence of pathogen extensive colonization leading to host cells collapse (Davar et al. 2012), symptoms of disease can be seen as sunken pale lesions on the stem base with or without white puffy mycelium (Fig. 3.1c). In time, lesion becomes large enough to result in wilting of plant. Disease is more damaging if wilting occurs during and closes after flowering (Maširević and Gulya 1992). Plant suffering from disease is easily visible among healthy plants, scattered in the field or forming small patches. Fungus can be identified by formation of black sclerotia inside or on the surface of diseased plants. Sclerotia scattered near the soil surface can produce cuplike fruiting bodies dispersing numerous ascospores. This type of inoculum is responsible for the occurrence of leaf spots and rot of leaf petioles, stem logging and soft rot of capitula (Fig. 3.2d). Disease on capitula can be highly damaging and may cause average yield reduction of 10–20% (Fusari et al. 2012). Time frame in the disease epidemiology for sunflower is flowering period when ascospores germinate and invade sunflower tissue mainly through anthers (Says-Lesage and Tourvieille de Labrouhe 1988; Rajender et al. 1996). The process of apothecia formation is susceptible to soil moisture, temperature and light intensity (Sun and Yang 2000). If the conditions are not conductive and for apothecia formation or their formation is not synchronized with flowering stage disease will be absent.

Collar rot is a disease caused by pathogen *S. rolfsii* occurring in regions of Asia, Africa, South and North America and Europe with warmer climate where it can be considered as one of the most important sunflower diseases (Gandhi et al. 2019). Symptoms of disease are mainly visible in a form of water-soaked stem base lesions that enlarge and encircle the stem. Damage is in a form of rotting of host tissues results in plant wilting. Inside and on the surface of roots and stem, large number of small brown sclerotia form. Affected plant wilts and has smaller heads with light seeds (Gulya et al. 1998).

Verticillium wilt is a vascular disease caused by ubiquitous pathogenic fungus *Verticillium dahliae* Kleb. Based on differential reaction of sunflower genotypes, several physiological races of *V. dahliae* were described, with the reports of increasing virulence (Garcia-Ruiz et al. 2014; Clemente et al. 2017). Ultrastructural changes as a consequence of parasitism in sunflower plant are degradation of xylem vessel walls, inhibition of necrotic band, degradation of remote mesophyll tissue and degradation of phloem (Kolte 1981). Disease symptoms are visible as interveinal chlorotic and necrotic areas on leaves. Infected plants have reduced photosynthesis, leaf expansion and seasonal duration of plant leaf area (Sadras et al. 2000) (Fig. 3.1b). In general, plant growth is impeded which ultimately results in reduced size of capitula and premature ripening of plant.

Fig. 3.2 Sunflower plant with symptoms of **a** Septoria leaf spot **b** Phomopsis stem canker, start of disease on leaf **c** Alternaria on leaves and stem and **d** head rot caused by *Sclerotinia sclerotiorum*

Charcoal rot, disease caused by pathogen *Macrophomina phaseolina* (Tassi.) Goild., a multi-host pathogen adapted to warm and dry environmental conditions. Symptoms of disease can appear any time during sunflower vegetation, but usually they are visible after flowering period as grey areas on lower stem parts. Stem pith is filled with pathogen sclerotia which can be additional sign for identification. Infection rate and low soil moisture is synergistic to disease incidence and decrease in yield (Jordaan et al. 2019). Prematurely ripen plants as results of the disease are directly related with yield decrease. This disease also reduces seed germination, mean seed weight and seedling vigour index (Ijaz et al. 2013).

Fusarium rot is characterized with symptoms such as wilt and root rot and is caused by *Fusarium* spp. This disease was reported as significant and highly damaging in Russia (Gontcharov 2014). It has been detected in the most of the sunflower producing areas and pathogen is often found associated with other

root pathogens. *Fusarium* pathogens can induce seedling decay and rot of young sunflower plants grown on moist and cold soils. Infection of roots and stem base can appear as black in color. Plants with these symptoms are commonly found in the patches in field. Pathogen disintegrates stem pith that may be pink in color which can be used for identification of causal agent. After flowering, symptoms of wilt appear. Reduction of capitula and seed size is cause of yield reduction.

Stem canker is a disease with high damage potential caused by *Diaporthe helianthi* Munt.-Cvet., although other species belong to same genus were detected in sunflower diseases tissues with similar symptomatology (Mathew et al. 2015). Upon the first outbreak of the disease, in former Yugoslavia causal agent was identified as *Phomopsis* spp. (Muntañola-Cvetković et al. 1981; Mihaljčević et al. 1985). Susceptible genotypes in conditions conductive for disease development can be greatly affected due to plant logging, as a result of damage to stem tissues. Yield decrease is related to the percentage of logged plants. The disease is spreading by air-borne ascospores, which after germination can penetrate into leaf tissues. This is clearly visible as a characteristic V-shape necrotic area surrounded by chlorosis on leaf (Maširević and Gulya 1992) (Fig. 3.2b). Depending on weather patterns, progress of the disease can be slowed or even stopped particularly at temperatures above 30 °C. Upon reaching the stem, symptoms in form of elongated brownish or greyish lesions appear. The pathogen can be present other parts of plant such as cotyledon leaves, stem base or capitula but symptoms are not easily distinguished compared to stem lesions.

Phoma black spot is caused by *Phoma macdonaldii* Boerema (teleomorph *Plenodomus lindquistii* (Frezzi) Gruyter, Aveskamp and Verkley, with sunflower as the primary hosting plant. It is a widely occurring disease that can affect every aboveground plant part. The pathogen is highly adaptable, and the disease is absent only in dry and warm weather conditions. It penetrates host tissues directly without formation of appressoria (Al Fadil et al. 2011). Necrosis on leaves, midvein and leaf petioles are the first visible symptoms. Characteristic symptoms are visible on the stem in form of dark black lesions around 5 cm in length. High disease severity can cause numerous stem lesions and lead to plant defoliation. *Phoma macdonaldii* can cause premature ripening of sunflower if stem base is affected. Incidence of prematurely ripen plants increases with the increase in plant density and increased level of nitrogen (Sessau et al. 2012). Plant with this type of symptoms have decreased leaf area, lower dry matter and consequently reduced grain yield (Quiroz et al. 2014).

Rust is caused by *Puccinia helianthi* Schw., a macrocyclic and autoecious pathogen. Damage caused by rust can cause yield losses that can reach 80%. Teliospores of pathogen overwinter on sunflower debris which in spring produce basidiospores. Once formed uredinia with uredinospores which are disseminated by wind, have potential for fast spreading. Favorable conditions are warm weather combined with periods of dew formation. For germination of uredinospores, 6–10 h of leaf wetness is required. Under optimal conditions of 15–25 °C, it takes 8–10 days from inoculation to pustule formation (Shtienberg and Vintal 1995). The disease incidence and severity are increased if sunflower field is in proximity of the infected field from previous year. Sunflower genotypes differ in reaction to the disease, making

possible the description of pathogen virulence through physiological races similar to that of *P. halstedii*.

Alternaria blight and leaf spot. Several *Alternaria* spp. are connected to the occurrence of symptoms on sunflower above-ground parts. Depending on the host genotype and pathogen species, symptoms may vary, but they are commonly in a form of necrotic leaf spots with more or less visible surrounding chlorotic area. In time, necrotic area can coalesce leading to the leaf collapse (Fig. 3.2c). On the stem and leaf petioles, symptoms appear as small spots that are commonly found on the old senescing leaves. The damage on the plant is occurring in the case of disease spread on the most of leaf area, when yield losses can reach 60% (Carson 1985). The disease has negative effect on the number of parameters that are crucial for high yield (Gopalakrishnan et al. 2010). In the case of high pathogen presence and highly susceptible host, sunflower plants may not reach flowering stage.

Septoria blight is a widely-spread sunflower leaf disease caused by *Septoria helianthi* Ell. et Kell.. It has been recorded in new areas with the increase in sunflower production (Maldaner et al. 2009). It causes appearance of small size spots scattered on the leaves that usually have yellow halo (Fig. 3.2a). In time, black pycnidia, visible as black dots, are produced. Infection is starting on lower leaves and spreads to upper leaves. Necrotic areas can become angular and a leaf withers and dries as a result of lesion coalesce. Moist and warm weather is conductive for this disease, especially in a high-density crop. Severity of the disease is positively corelated with the duration of leaf wetness (Brand et al. 2018) and may result in yield reduction of up to 10–15% (Carson 1985).

Powdery mildew is a disease common in a number of countries with sunflower production. Sunflower powdery mildew is usually caused by *Golovinomyces cichoracearum* (DC.) V. P. Heluta. However, from sunflower leaves were also isolated other fungi that cause powdery mildew like *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff (Chen et al. 2008), *Golovinomyces orontii* (Castagne) V. P. Heluta and *Leveillula taurica* (Lév.) G. Arnaud (Mulpuri et al. 2016). Generally, it is not considered to be of a high economic importance, but has been a serious constraint for sunflower in some regions of India (Kulnakarni et al. 2015). Symptoms appear in the form of white mildew areas on leaves, stem and bracts. It appears on older leaves and gradually advances. Affected leaves can became chlorotic and brown, and senesce.

Grey mold is a disease caused by ubiquitous, seed transmitting facultative pathogen *Botrytis cinerea* Pers. Symptoms of rot on plant parts can be observed during vegetation in moderately warm and humid conditions (Kanyion and Friedt 1993). The most damaging is disease development on sunflower capitula. Early symptoms appear as sunken watery lesions much like those caused by *S. sclerotiorum.* High humidity induces sporulation of fungus which can be observed as grey layer covering lesion or area between seeds. Inside capitula tissues are slimy and sticky appearance. This can be potentially highly disruptive during sunflower harvest (Ladsous et al 1991).

Rhyzopus head rot is a disease caused by several species of *Rhyzopus* that can occur in a complex or solely. The fungi are abundantly present in environment but

infection of sunflower can be successful in a case of the mechanical injury by insects, hail or birds. The symptoms of disease are visible in the form of soft watery spots that turn dark in time. Symptoms may be localized on surface tissues of a head. Disease progress inside of the sunflower head is easily noticed by the presence of mycelial strands and numerous black sporangia. Damage to sunflower varies significantly depending on the region and the genotype susceptibility (Gontcharov 2014).

3.2.3 Bacteria

Sunflower bacterial pathogens belong to genus *Pseudomonas* and *Xanthomonas*. *Pseudomonas syringae* pv. *tagetis* Hellmer and *P. syringae* pv. *helianthi* Kawamura, are commonly found and other bacteria are restricted (Gulya et al. 1998). Damage resulting from foliar bacterial diseases of sunflower is low. More impact on sunflower may come from rot caused by ubiquitous *Pectobacterium carotovorum* (Jones) Waldee emend. Prtier et al.

Apical chlorosis is caused by *Pseudomonas syringae* pv. *tagetis* commonly found in younger plants. In the field it is easily observed by appearance of chlorosis of the youngest leaves leaf blade. The chlorotic and bleached appearance is result of tagetitoxin that inhibits RNA synthesis and disrupts chloroplast thylakoid membranes (Gulya et al. 1997).

Bacterial stalk and head rot of sunflower is caused by bacterial species *P. carotovorum* subsp. *carotovorum* and *P. atrosepticum* (van Hall) Gardan et al. The disease on the stem starts on petiole axils and is favoured by presence of water and mechanical injury leading gradually to dark brown or black appearance of stem with the characteristic rotting potatoes odour (Harveson et al. 2018). Symptoms on capitula resemble that of fungal pathogens at first. Water soaked lesions enlarge gradually and on the surface foam-like exudate appears which is of diagnostic importance.

3.2.4 Viruses

Sunflower virus diseases are considered not to be of high economic importance in the most of sunflower regions. Because of varying and not distinctive symptoms they are usually overlooked of misidentified for other diseases or disorders. This is combined with high-cost and laborious identification process. Sunflower is, however, severely affected by some viruses such as *Tobacco Streak Virus* that can result in substantial loss in the yield (Sharman et al. 2009).

Tobacco Streak Virus strain has been connected to sunflower necrosis disease (Ravi et al. 2001). The disease was described as highly dangerous for sunflower production in India (Bhat and Reddy 2013). The disease has spread and the virus was detected in other sunflower regions (Sharman et al. 2008; Hosseini et al. 2012; Cabrera Mederos et al. 2019). Symptoms are visible as leaf and stem necrosis

resulting in plant stunting and dieback (Ravi et al. 2001). Disease outbreaks were recorded with substantial losses in yield (Shirshikar 2010). Several other viral diseases of sunflower are described as minor threat to crop or with potential to affect sunflower if disease outbreak occurs (Gulya et al. 1997; Lenardon et al. 2001; Gulya et al. 2002a, b; Salamon 2003).

3.2.5 Broomrape

Broomrape (*Orobanche cumana* Wallr.), a sunflower root parasite, source of severe damage to sunflower crop where present (Seiler 2019; Shi and Zhao 2020). This broomrape species probably adapted to sunflower from host plants of wild Asteraceae species (Pineda-Martos et al. 2014). It connects to sunflower vascular system, depleting host form water and nutrients (Krupp et al. 2019). Broomrape form aboveground stem with rudimentary leaves and numerous flowers (Fig. 3.1d). Each plant is capable of producing abundant durable minute seeds, thus increasing probability of successful contact with the host. Host plant could be completely destroyed or more commonly parasitism is resulting in the decrease in size of all plant parts. Sunflower genotypes react differentially to broomrape and up to date races are named, using Latin letters, from A to H (Molinero-Ruiz et al. 2015; Cvejić et al. 2020).

3.3 Major Pests of Sunflower

Cultivated sunflower (*H. annuus*) is native to North America and the insects associated with this species have coevolved with these plants for centuries. When sunflowers were altered from a wild to a cultivated form, the changes also affected the insects associated with the crop. For example, stem-boring beetle, *Dectes texanus*, oviposits (lay eggs) more frequently in cultivated than in wild sunflower, since cultivated sunflower has the softer petioles and lower amount (Michaud and Grant 2009). According to Knodel et al. (2015) a number of insect species transited from wild to cultivated plant form, and now some of these species have become economically important pests.

In the major sunflower producing areas, about 15 insect species can cause significant plant damages and economic losses. However, during growing seasons, only a few species occur in abundance that requires control measures. Sunflower is attacked by a number of insect pests at different developmental stages but most of them are not specific to sunflower and originate from other crops, weeds or plant remains. Based on the plant part they attack sunflower pests can be divided in several groups: soil-dwelling pests, stem borers, foliage feeders and head and seed feeders (Knodel et al. 2015).

3.3.1 Soil-Dwelling Pests

Wireworms (Coleoptera: Elateridae) *Agriotes* spp., *Melanotus* spp., *Limonius* spp. etc., are soil-dwelling larvae of click beetles that damage roots of sunflower seedlings. As a result of feeding activity plants wilt and die. When a heavy infestation occurs, bare soil spots appear in the field which requires reseeding (Fig. 3.3b). Adults and larvae of **False wireworms** (Tenebrionidae, Coleoptera) (Striate false wireworm—*Pterohelaeus alternates* Pascoe, Eastern false wireworm—*Pterohelaeus darlingensis* Carter and Southern false wireworm—*Gonocephalum macleayi* Blackburn) also damage sunflower plants at the beginning of the vegetation which results in patchy stands as well. Larvae feed on decaying plant material in the soil, on germinating seeds and on vegetative growth points on seedlings. Damages by both larvae and adults may impose a need for reseeding (Knodel et al. 2015).

Fig. 3.3 Sunflower pests **a** Thistle caterpillar on sunflower head, **b** wireworm and a damaged sunflower seedling and **c** Head borer moth on sunflower leaves

Sunflower root weevil *Baris strenua* LeConte (Coleoptera: Curculionidae) adults cause non significant mechanical injuries to leaves of sunflower plants, but the main injury is caused on roots by larval feeding (Ziaee 2010). If the infestation is severe, plants wilt and lodge, but the damage is usually localized.

Cutworms (Lepidoptera: Noctuidae), Darksided cutworm—*Euxoa messoria* Harris, Redbacked cutworm—*Euxoa ochrogaster* Guenee, Dingy cutworm—*Feltia jaculifera* Guenee, Black cutworm—*Agrotis ipsilon* Hufnagel. Larvae of these species can damage stems of young seedlings at or near soil level causing plant lodging. Young leaves also may be chewed by cutworms and sometimes young plant is partially dragged into the soil. Similar to the wireworms and false wireworms, the presence of cutworms can be visible by patchy areas in the field as well as wilted and/or dead plants (Floate 2017).

Black scarab beetles/black sunflower scarab (*Pseudoheteronyx* sp.) (Coleoptera: Scarabeidae) larvae cause wilting and death of seedlings when feedin on taproots while beetles can defoliate plants (Charlestone 2013).

3.3.2 Stem Feeders

Stem borer *Dectes texanus* LeConte (Coleoptera: Cerambycidae) is first mentioned as a potential pest of sunflower at the early 1970s, causing significant damage in south-central Texas. Larvae are the most destructive stage and due to their feeding stalks lodge at a girdle point, about 7 to 9 cm above the ground. Damages caused by the adult feeding are neglectible because they do not penetrate the cortex nor encircle the stalk. A female chews a hole through the epidermis and oviposits a single egg inside the pith. The larvae stay within the petiole feeding on the pith for 1 to 2 weeks prior to boring into the main stem. Once in the main stem, the larvae tunnel up and down the plant feeding on pith and eventually, larvae tunnel down to the base of the plant, girdle the inside of the stem about 5 cm above the ground surface and plug the tunnel with frass (Rystorm 2015). Knodel et al. (2015) mention that perennial sunflower species are resistant to this pest, making breeding of resistant cultivars possible. Also, lowering plant populations, with plants that have thicker stems, less prone to logging, may reduce negative impact of pest.

Sunflower stem weevil *Cylindrocopturus adspersus* LeConte (Coleoptera: Curculionidae) larvae damage stalks that may become weakened by tunnelling and break, causing a head loss before the harvest. The most severe stalk lodging, as a result of stem weevil attack, is during drought periods or under strong winds because plants are drying prior to harvest. The sunflower stem weevil also has been involved in the Phoma black stem (*Phoma macdonaldii*) epidemiology and Charlet et al. (2002) mention that it may predispose plants to the infection by Charcoal stem rot (*Macrophomina phaseolina*).

Black sunflower stem weevil *Apion occidentale* Fall (Coleoptera: Curculionidae) has been associated with the transmission of *Phoma macdonaldii*, but otherwise it does not have economic importance.

Sunflower maggot *Strauzia longipennis* Wiedemann (Diptera: Tephritidae) is the only tephritid species recorded to attack cultivated sunflower. Larvae can be commonly found in sunflower stalks, up to now, economic losses have not been documented for this species. Damaged stalks are not weakened and seed yield is not affected (Knodel et al. 2015).

3.3.3 Foliage Feeders

The most comprehensive review of sunflower foliage pests was published by Knodel et al. (2015), naming the following species as the most devastating for cultivated sunflower.

Palestriped flea beetle *Systena blanda* Melsheimer (Coleoptera: Chrysomelidae) injures plants from seedling emergence until plants develop four leaves. Under heavy attack, significant stand losses may occur.

Sunflower beetle *Zygogramma exclamationis* F. (Coleoptera: Chrysomelidae). Adults and larvae feed on both cultivated sunflower and Helianthus species causing plant defoliation. Adult beetles damage plants as they emerge from overwintering. Damage to cotyledons is generally low, but the first true leaves may be completely consumed. Crop can be severilz affected if beetles are hign in abundance.

Thistle caterpillar *Vanessa cardui* L. (Lepidoptera: Nymphalidae) larvae consume leaves which can result in plants defoliation. The effect of defoliation on sunflower yield depends on the severity of defoliation (Fig. 3.3a). Larvae produce silk webbing during feeding.

Tobacco caterpillar *Spodoptera litura* F. (Lepidoptera: Noctuidae) host range covers at least 120 species including wild and cultivated sunflowers. On majority of crops, damage are a result of intensive larval feeding, leading to complete stripping of the plants.

Jassids, *Amrasca biguttula* Ishida (Homoptera: Cicadellidae). Heavy infestations by this pest make the leaves turn yellow, curl up and fall off. The insects also secrete honeydew that is suitable surface for the development of sooty mould that restricts the amount of light reaching the plants and reduces the yield.

Thrips (Thripidae: Thysanoptera), Onion thrips—*Thrips tabaci* Linderman, Tomato thrips—*Frankliniella schultzei* Trybom, Plague thrips—*Thrips imaginis* Bagnall, Western flower thrips—*Frankliniella occidentalis* Pergande. The highest thrips abundance can be expected during a hot and dry springs following a mild and dry winter. Adults and nymphs cause leaf damages as a result of feeding activity, where they rasp surface plant tissue and suck the exuded juices. Although damages are ussualy not significant, at high abundance, thrips can cause distortion and browning of the cotyledons and leaves. Additionaly, thrips are the most important vectors of tobacco streak virus (TSV) (Knodel et al., 2015).

Loopers (Lepidoptera: Noctuidae), Tobacco looper—*Chrysodeixis argentifera* Guenee, Vegetable looper—*Chrysodeixis eriosome* Doubleday, Soybean looper—*Thysanoplusia orichalcea* F., Cabbage semilooper—*Trichoplusia ni* Hübner and Bihar hairy caterpillar—*Spilosoma obliqua* Walker (Lepidoptera: Arctiidae). Loopers are occasional pests of sunflower and can be distinguished from Helicoverpa species by their 'looping' action when moving. They have two pairs of hind legs, while Helicoverpa caterpillars have four. Larvae feed on leaves and the damages increase as the loopers become older. According to Charlestone (2013), 80% of defoliation is done by medium- large larvae, but it only on rare occasions. However, if severe defoliation (>50% total leaf area) occurs during budding and flowering, it will result in loss of yield and oil content.

3.3.4 Head and Seed Feeders

Tarnished plant bug *Lygus lineolaris* Palisot de Beauvois and other *Lygus* species (Hemiptera: Miridae). *Lygus* bugs cause kernel brown spot on confectionary or non-oilseed sunflowers, which results in necrosis around the feeding site due to the injection of enzymes. Tissue destruction and brown spots on the kernel, results in a bitter taste of the seeds, and only 0.5% damage is tolerated for the final product. Oilseed type of sunflower is not at high risk *Lygus* damages.

Weevils. Several weevil species represent the most devastating pests of cultivated sunflower. The most comprehensive report on these species was presented by Charlet and Brewer (1997) and Knodel et al. (2015).

Sunflower headclipping weevil *Haplorhynchites aeneus* Boheman (Coleoptera: Attelabidae) causes very distinctive damage—clipped heads of sunflower plants. Damages occur frequently along field margins and although the percent of clipped heads is ussually very low (1 to 3%), losses up to 25% have been reported in individual fields.

Red sunflower seed weevil *Smicronyx fulvus* LeConte (Coleoptera: Curculionidae) is not a pest of economic importance. Larvae consume the kernel, most often partially, and oil loss from hull damage is insignificant. Some seeds may be completely consumed. Adults feed on the bracts but it does not cause significant damages or losses.

Grey sunflower seed weevil *Smicronyx sordidus* LeConte (Coleoptera: Curculionidae) larvae are internal seed feeders and usually are found near the bottom of the developing seed. As a result of low population levels and low fecundity, this pest does not cause economic damage, especially in oil sunflower fields.

Banded sunflower moth *Cochylis hospes* Walsingham (Lepidoptera: Tortricidae) was well described by McLeod (2002). Larvae (early instars) primarily feed on the bracts, and later (3rd and later instars) feed on pollen, disk florets and seeds, causing most of the economic damage by consuming a part or entire seed content (Jyoti and Brewer 1999a, b; Knodel et al 2015). Sunflower plants are susceptible to moth's

infestation only during the flowering period. Small areas of silken webbing on mature sunflower are indicators of larvae presence.

Sunflower bud moth *Suleima helianthana* Riley (Lepidoptera: Tortricidae). Yield loss is noticeable only when larvae burrow into unopened buds, preventing the head development. The larvae keep an open tunnel and continually push frass in to the hole. This pest does not cause economically significant yield losses, although larval injury causes malformations in heads and stalks.

Sunflower and European sunflower moth *Homoeosoma electellum* Hulst and *H. nebulella* Denis and Schiffermüller (Lepidoptera: Pyralidae). First-instars feed primarily on pollen. Larvae may tunnel through the corolla to feed on pollen inside disk florets. Silk webbing over the face of the sunflower head is indicator of pest. The accumulated debris in the larval webbing and tissue damage may predispose the head to *Rhizopus* infection (McLeod 2002; Knodel et al. 2015; Sikora 2017).

Sunflower midge *Contarinia schulzi* Gagne (Diptera: Cecidomyiidae) larvae cause brown scar tissue and necrosis at base of bracts as well as head distortion and development of heads with little or no seeds. Early symptom of midge presence is the loss of ray flowers, because larvae firstly feed on bracts before moving into the head and feeding at the base of developing seeds. Early losses from sunflower midge range from 5–20%. At some regions and some vegetative seasons, midge infestation may cause losses high enough to discourage sunflower growers. Heavily damaged heads are knotty and hollow, often with a hole or depression in the centre. If available, growers should consider using tolerant or resistant hybrids (Glogoza et al. 1997).

Sunflower seed maggot *Neotephritis finalis* Loew (Diptera: Tephritidae) newly emerged larvae tunnel into the corolla of young blooms and cause seed sterility. Ona larva can tunnel through 12 ovaries while mature larvae feed on older sunflower heads can destroy only one to three seeds. The severity of sunflower seed damages depends largely on the stage of larval and seed development (Knodel et al. 2015).

Sunflower head fly, *Melanagromyza minimoides* Spencer (Agromyzidae) is a significant pest of sunflower in South America, with reported damages up to 20–30% in Argentina and Uruguay (Zerbino 1991; Ves Losada and Figueruelo 2006). This pest is the most damaging when late sowing of sunflower.

Head borers, *Helicoverpa* sp. (Lepidoptera: Noctuidae) includes two pests significant for sunflower production—the native budworm *Helicoverpa punctigera* and the corn earworm *H. armigera.* These species usually occur from late budding until late seed fill. Although the damage from this pest is obvious and can be severe, they are not considered pests of major economic importance in sunflowers since the plant is able to tolerate large infestations and still produce satisfactory yield. The larvae feed on various plant parts, causing damage to reproductive organs, and reducing yields by lowering seed values (Krinski and Godoy 2015). On sunflower plants, the larvae feed on leaves, buds and petals or on the small green bracts surrounding the head. Feeding on the back of the head predisposes the plant to secondary infections by casual agents of head rots. Larvae can cause the deformation of sunflower heads, and sometimes head loss due to larval chewing of its connection with the stem (Fig. 3.3c).

3.4 Genetic Resources for Resilience Improvement

As in other crop species, in sunflower as well genetic resources are a very important link as genetic diversity is the prerequisite in breeding for future challenges (Anđelković et al. 2020). The amount and distribution of genetic material in cultivated plants, contained in germplasm collections, is a product of evolutionary processes during their domestication phase from wild to modern cultivars and with domestication history of around 4000 years ago sunflower became one of the most important sources of edible oil. Sunflower genetic resources consists of cultivated sunflower germplasm, open-pollinated varieties and local populations and wild sunflower species. A special place in the breeding of sunflower is occupied by wild species because their diversity and sources of resistance genes have enabled the survival of sunflower in the economic sense and its use as a source of edible oil.

3.4.1 Inbred Line Gene Pool

As outlined in Filippi et al. (2020), the major sunflower research centers have been developed in Western Europe, United States of America, Canada and Argentina. Public research institutions such as United States Department of Agriculture (USDA), the University of British Columbia (UBC) in Canada, L'Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement (INRAE) in France, and Instituto Nacional de Tecnología Agropecuaria (INTA) in Argentina, made invaluable efforts to the development of the initial breeding material that served as the breeding basis for many current seed companies. Public Institutes in South-east Europe (Balkan region) have also done significant contribution in development of outstanding genetic diversity in sunflower. It should be noted that one of the larger and more significant sunflower collections, originating from different genetic sources, is developed and maintained by the Institute of Field and Vegetable Crops (IFVCNS) from Novi Sad, (Atlagić and Terzić 2014; Terzić et al. 2020). Also, important and well-known research institutes are Trakya University from Edirne in Turkey, National Agricultural Research and Development Institute from Fundulea, Romania (NARDI Fundulea) and Dobroudja Agricultural Institute from General Toshevo, Bulgaria. Although not publicly available, except under certain agreements, the existing genetic stocks of these public research institutions are an important source of genetic diversity in sunflower, especially bearing in mind that these gene pools are developed in different environments and therefore are characterized by different traits. There is also a momentous sunflower collection at the Vavilov research Institute in Saint Petersburg with over 400 open-pollinated varieties (Gavrilova et al. 2014).

USDA-ARS collection was established in Texas in late 1970s. This collection contains numerous CMS, B and Rf sunflower lines, with desirable genes that were utilized as an initial breeding material for creation of different synthetics or in the recurrent selection for introduction of many valuable genes, including biotic stress

resistance (Seiler and Jan 2010; Terzić et al. 2020). Some of the inbred lines developed in the USDA are used around the globe as a source for resistance to different diseases, notably RHA 443, RHA 478, RHA 479, RHA 480, HA 444 and HA 481 (Miller et al. 2006a, b; Koehler et al. 2019).

Inbred line gene pool of IFVCNS contains more than 7000 lines with different genetic background (Atlagić and Terzić 2014). What makes this research center stand out is the development of the first source of resistance to *Phompsis* from interspecific cross with *H. tuberosus* (Škorić 2016; Anđelković et al. 2020). In the field test, Škorić (1985) discovered that several sunflower lines are resistant to *Phomopsis*, designated as Ha-22, Ha-74, Ha-BCPL and the restorer line SNRF-6. Using these lines, the same author developed the first sunflower hybrids resistant to *Phomopsis*. One of the latest achievements of this breeding centre is development of lines resistant to newer races of broomrape (Cvejić et al. 2020).

3.4.2 Open-Pollinated Varieties

Although sunflower originates from the North America, its evolution as an industrial plant began in Russia. By selecting plants from local populations based on their phenotypic appearance Russian peasants created a large number of cultivars of which some had improved agronomic traits and resistance to broomrape and sunflower moth (Seiler and Jan 2010). The significance of so-called local populations is their adaptability to specific soil and climate conditions, as well as resistance and tolerance to prevalent constraints. Open-pollinated varieties created in Russia are valuable source of important traits such as high seed yield, quality and disease resistance. Using local open-pollinated varieties, V. S. Pustovoit managed to create the genotypes that combine several traits such as resistance to broomrape (race B) and high oil content (>50%) (Jocić et al. 2015). Peredovik is the most famous variety created by Pustovoit. This variety served as a source for the development a great number of inbred lines with good agronomic characteristics and resistance to rust, *Verticillium*, broomrape and European sunflower moth. Another group of important open-pollinated varieties are created by academician Zhdanov, designated as Zhdanovsky 6432, Zhdanovsky 8281 and Stepnyak. These varieties are developed at Saratov experimental station because the production of sunflower became endangered by the appearance of a new broomrape race (Gorbachenko et al. 2011). Open-pollinated varieties created in Russia have also been used as a base for breeding programs around the world and with the introduction of hybrids in production served as the source of a large number of lines. Mennonite immigrants from Russia introduced sunflower and Russian open-pollinated varieties into Canada, where seed production on large scale started in 1943 (Putt and Sackston 1956).

3.4.3 Wild Relatives

In sunflower, the wild relatives are invaluable source for many important genes, especially for resistance to biotic constraints. Genus *Helianthus* include 53 species with different level of ploidy with 14 annuals and 39 perennials (Stebbins et al. 2013; Seiler et al. 2017). Their usefulness largely depends on the possibility of crossing with cultivated sunflower and the success to transfer genes of interest. Bearing in mind that *Helianthus* genus contains species of various levels of ploidy, obtaining interspecies hybrids is usually associated with implementation of specialized techniques such as embryo rescue or tissue culture (Terzić et al. 2020). Cytogenetic studies have increased the success in obtaining interspecies hybrids and enhanced introduction of genes for many important traits as differences in the ploidy level cause cross incompatibility resulting in embryo abortion, sterility or reduced fertility in progeny, as well as low dormancy in hybrids (Seiler et al. 2017).

Russian scientists were the first who realized importance of wild species and work on interspecific hybridization was continued with Galina Pustovoit (Škorić 1988). The first use of wild relatives in sunflower breeding dates from the first half of twentieth century and attempt to transfer rust resistance from interspecific cross with *H. argophyllus* (Cockerell 1929). Resistance genes for many sunflower fungal diseases such as rust, downy mildew, *Verticillium* wilt, *Alternaria* leaf spot, powdery mildew, *Sclerotinia* wilt/rot as well as for parasitic weed broomrape have been introduced from wild *Helianthus* species (Seiler et al. 2017). The introduction of these genes into cultivated sunflower enabled sustenance of sunflower production through years. The most notable example is introduction of *Phomopsis* resistance. The occurrence of *Phomopsis* epidemic in 1981, IFVCNS collection was extensively screened in the pursuit for tolerant genotypes. Four lines were evaluated as high tolerant with two lines originating from interspecific crosses with *H. tuberosus*, and one was derived from the cross *H. argophyllus* x Armavirski 9345 (Miladinović et al. 2019). As a result, the first sunflower hybrids tolerant to *Phomopsis* were created, designated as NS-H-43, NS-H-44 and NS-H-45. Another example comes from Canada where as a result of intensive cultivation, without crop rotation, rust disease caused by *Puccinia helianthi* appeared. Sunflower rust was causing severe damages in production and aggravating circumstance was that there were no resistant varieties available from Europe or South America. Derived from a single resistant plant, from a natural cross with wild sunflowers, rust resistant variety "Beacon" was released for commercial production in 1954 (Putt and Sackston 1956).

3.4.4 Induced Mutations

Mutation-based breeding in plants has been used for more than eight decades to generate new genetic variability based on random genetic variations, in order to create desirable traits (Cvejić et al. 2014). Mutagens have potential to induce hereditary

alterations in plant genome and thereby enhance the frequency to obtain desired individuals. Induced mutation breeding is based on the induction of mutations by various reagents and their detection using different methods. Mutagenic agents have been used in the breeding by many authors but generally have been restricted to obtaining dominant traits while recessive ones have largely been lost during selection (Barkley and Wang 2008). However, with the introduction of targeting induced local lesion in genome (TILLING), mutation breeding has become a powerful tool for identification of key genes, but also for creating and discovering new traits and detection of very rare recessive mutations (Colbert et al. 2001; Kumar et al. 2013).

Use of induced mutations in sunflower has not given significant results in terms of increasing resistance to diseases. Positive results of induced mutations for increasing disease resistance in sunflower are scares. Only achieved results in resistance were obtained for sunflower rust, Alternaria leaf spot, broomrape, downy mildew. Lofgren and Ramaraje Urs (1982) obtained plants with early maturity and plants resistant to sunflower rust. Darvishzadeh et al. (2007) identified mutants with partial resistance to phoma black stem. Encheva and Shindrova (2011) used induced mutation and embryo culture to develop resistance to downy mildew race 330. With the application of ultrasound treatment on immature zygotic embryo Encheva et al. (2012) developed a mutant line R 12,003 having high oil content and resistance to broomrape.

3.4.5 Diversity Analyses

Several great sunflower gene banks in which a large number of cultivated and wild sunflower accessions are maintained (Terzić et al. 2020). These large collections of sunflower germplasm are very demanding as it takes a lot of effort, time and finances for maintenance. However, this is an investment in sunflower sustainable production of tomorrow. Sunflower is an important oil crop of the future bearing in mind the predicted climate changes, as it is a resilient crop with good adaptive potential. Molecular analysis and characterization of the different sunflower collections are a good basis for accelerating their implementation in sunflower improvement.

Some of the collections from different gene banks were used to create association mapping populations for mining of different agronomically important traits. Mandel et al. (2011) examined genetic diversity by use of expressed sequence tag-simple sequence repeat (EST-SSRs) of the sunflower primary gene pool consisting of 433 accessions of cultivated sunflower from North America and Europe and 24 wild populations sampled from the United States, Canada and Mexico. Within the population different genetic constitutions are represented: oil and non-oil maintainers (HA) and restorers (RHA), landraces, open-pollinated varieties (OPV), introgressed material. The authors defined a core set consisting of 288 genotypes that capture almost 90% of allelic diversity of the primary gene pool. This USA-SAM1 population which is distributed by Germplasm Resources Information Network (GRIN -https://www.ars-grin.gov/) consists of accessions from USDA-NPGS and INRA, France and has been employed in several association studies (Mandel et al. 2011; Nambeesan et al.

2015; Horn et al. 2019). Moreover, Mandel et al. (2011) identified a minimal core set capturing almost 50% of allelic diversity constituting of 12 different accessions (maintainers, restorers, oil and non-oil) which may be a good basis for creating a MAGIC (Multi-parent advanced generation inter-cross) population (Dimitrijevic and Horn 2018). Filippi et al. (2015) exploited SSR and single nucleotide polymorphism (SNP) markers for evaluation of an association mapping population consisting of 137 inbred lines of INTA, 12 open-pollinated varieties and 20 composite populations maintained at AGB-IM and reported that both types of markers are efficient in proper evaluation of population structure and genetic diversity. Pérez-Vich et al. (2018) reported great variability in several traits (100 seed weight, plant height head diameter and days to flowering) in population consisting of 196 confectionary accessions of CRF-INIA, Spain, while SSR analysis showed moderate variability and existence of two separate genetic pools.

3.5 Genetic Basis of Biotic Stress Resistance

3.5.1 Genetic Resistance

Genetic control of pathogens in sunflower is considered the most effective and sustainable way to eliminate or reduce many biotic constraints which cause yield losses. The genetic basis of sunflower resistance to various pathogens has been studied since sunflower started to be bred, and many resistance genes have been identified so far. In genetic terms, resistance is generally defined by the number of genes, their effect, and the mode of inheritance. There is a significant difference between the resistance of controlled by oligo-genes (one or more genes with a major effect) and polygenes (multiple genes with a minor single phenotypic effect) (Balconi et al. 2012). Moreover, terms aggressiveness and virulence are often used to express the impact of pathogens on sunflower. Aggressiveness indicates the quantitative component of pathogenicity expressed horizontally, while virulence denotes the qualitative component of pathogenicity expressed vertically (Sakr 2011). In sunflower, both qualitative and quantitative resistances have been reported against different pathogens. Qualitative resistance has monogenic control, while quantitative resistance is controlled by several genes or quantitative trait loci (QTLs).

3.5.1.1 Qualitative Resistance

Qualitative resistance is simply referred to as race-specific or gene-for-gene resistance. Based on previous researches, resistance genes are mostly dominant in action; such as, genetic resistance to downy mildew, rust, wilt, and powdery mildew (Miladinović et al. 2019). In these cases, a single dominant gene provides complete resistance to progeny for a particular pathogen when crossed resistant and susceptible plants.

Besides dominance, there are few reports on recessive resistance in sunflower, e.g. gene for broomrape resistance to race F and higher (Akhtouch et al. 2016).

Qualitative resistance is highly efficient in parasite inhibition and has become preferable among plant breeders as it is easily introduced and maintained in the breeding material (Boyd et al. 2013). As sunflower production can be severely jeopardized by presence of different pathogens, introduction of pathogen-resistance genes into commercial sunflower lines and hybrids, as the most effective manner of controlling pathogens, is imperative for obtaining resilient, high-yielding hybrids. The development of molecular markers in sunflower enabled introduction of marker-assisted selection (MAS) into the breeding process. Over the years, MAS become a powerful tool for acceleration of the introduction of qualitative resistance into cultivated sunflower through marker-assisted backcross breeding for gene introgression. It is especially useful in introduction of recessive resistance and gene pyramiding, that is used to enhance the durability and degree of pest and disease resistance (Kaya et al. 2012; Qi et al. 2019).

3.5.1.2 Quantitative Resistance and QTLs

Quantitative resistance is also known as a partial resistance, a field or horizontal resistance and is usually not race-specific. It is usually controlled by multiple genetic factors (quantitative trait loci or QTL) and its effect does not comply with simple Mendelian inheritance. Talukdar et al. (2009) reported a continuous distribution of the partially inherited disease reaction, ranging from highly susceptible through moderately resistant, to highly resistant. Quantitative resistance is usually more effective when environmental or plant tissue conditions are favorable to disease. So with sunflower, the incidence of diseases such as Phomopsis helianthi Munt.-Cvet., Phoma macdonaldii Boerema, Sclerotinia sclerotiorum (Lib.) de Bary, Macrophomina phaseolina, Botrytis cinerea Pers. is highly dependent on climatic conditions (Tourvieille de Labrouhe et al. 2008) and the use of quantitative resistances in widely grown hybrids does not appear to affect pathogen populations (Miladinović et al. 2019). The introduction of quantitative resistance using classical breeding is a complicated and long-term process since phenotypic selection is not as efficient as in qualitative, monogenic resistance. In recent years, association mapping studies are a valuable alternative to classical and marker-assisted breeding for quantitatively inherited traits (Dimitrijević and Horn 2018). Another option is a genomic selection that effectively uses genome-wide molecular data to select QTL (Bernardo 2008).

3.5.1.3 Durable Resistance

Durable resistance applies to the resistance that remains effective during prolonged use in favorable environments to the pathogen or disease distribution (Kou and Wang 2010). A very efficient strategy to obtain durable resistance is to combine

resistance genes from different sources can be a very efficient strategy to obtain durable resistance, especially in complex traits with multiple gene effects. Strategies proposed for sustainable, durable reduction of disease and discovery and use of resistant genes implemented in selected resistant genotypes. Furthermore, the key to developing genotypes with long-term resistance is a diversity strategy depending on the number of genes and the mechanism of action of these genes (Rubiales 2018). It is widely acknowledged that quantitative resistance is likely to be more durable than qualitative resistance. This strategy can be achieved by pyramiding of different resistance genes or by combining monogenic and polygenic resistance against different pathogens. Since resistance genes for these particular traits are largely unknown, it is essential to examine the unspecific defense mechanisms of plants for quantitative resistance and identify unspecific genes involved in the mechanism. In sunflower, durable resistance in the single genotype is possible by combining two types of resistance (quantitative with monogenic) (Vear et al. 2008) or introducing genes from different sources (Jocić et al. 2010). Durable resistance for *Plasmopara halstedii* has been achieved by combining minor genes providing partial resistance with major resistance genes (Tourvieille de Labrouhe et al. 2008). Škorić (2016) reported that Phomopsis resistance is positively correlated with Macrophomina and Phoma resistance and drought tolerance. Current biotechnological approaches can offer various opportunities to use quantitative resistance in crop improvement efficiently. In this area, research in epigenetics and epigenomics can provide new tools and new techniques to breeding for quantitative and durable resistance (Tirnaz and Bartley 2019; Varotto et al. 2020).

3.5.2 Traditional Breeding Methods

Traditional sunflower breeding methods have played an essential role in the development of biotic stress-resistant sunflower genotypes. In the sunflower breeding program, the main objective is the development of high-yielding hybrids with high oil content. However, the limiting factors are different pathogens, pests, and parasitic weed—broomrape that attack sunflower plants and cause yield reduction. Another major problem is that process of appearance of new races of the pathogens is continuous therefore it is necessary to constantly search for new sources (genes) of resistance. The narrow genetic base of cultivated sunflower affects deficiency in genes for resistance to causal agents of biotic stress factors. Sources of resistance or improved levels of tolerance for most diseases and broomrape have been primarily found in wild species of Helianthus genus (Škorić et al. 2006; Seiler et al. 2017). Nevertheless, sunflower breeders accomplish great success by identifying genes for resistance or high tolerance to major pathogens and transferring them from wild species into the cultivated sunflower.

In order to develop high-yielding and healthy sunflower hybrids, breeders have to follow certain steps (Fig. 3.4). First, due to the narrow genetic variability of cultivated sunflower, it is essential to have sources of resistance/tolerance to main

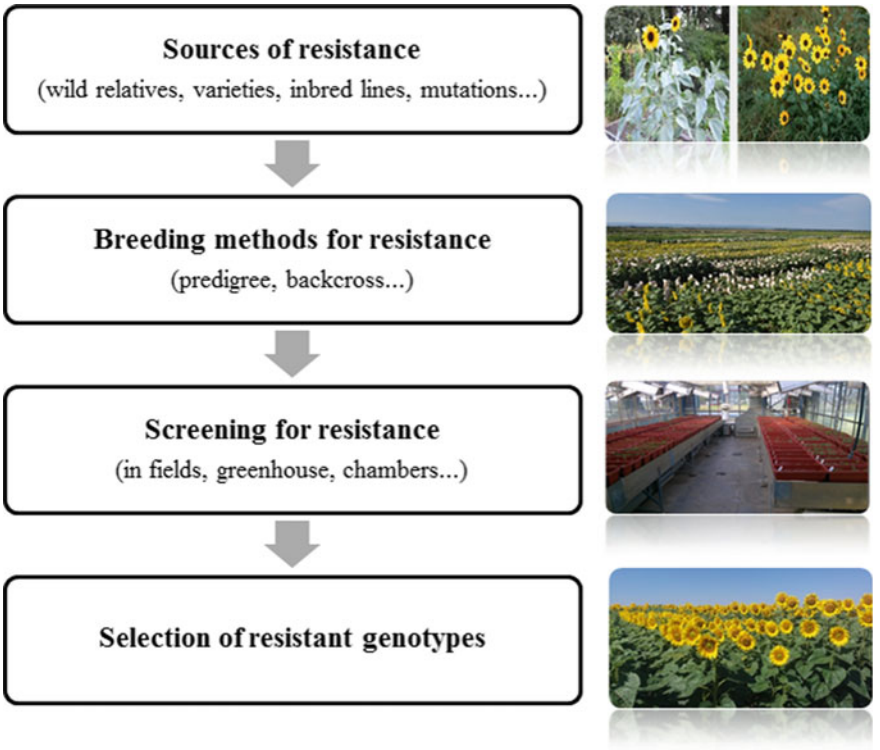

Fig. 3.4 Milestones in breeding for resistance

biotic constraints. These sources are used as starting material for breeding and are usually found in the existing genetic collections. The collections mainly consist of wild sunflower species, gene bank of cultural inbred lines, genotypes created by induced mutations, varietal and local populations, as well as synthetic populations obtained by different selection methods. A second request for successful breeding is the use of adequate methods of breeding for resistance. In the past fifty years, sunflower breeding programs have been focused on the development of sunflower hybrids by using the phenomenon of heterosis. From the genetic point of view, heterosis is the result of intra-allelic interaction (domination and super domination) and inter-allelic interaction (epistasis). This is, in fact, the state of maximum heterozygosity, most successfully achieved by crossing genetically divergent self-pollinated homozygous inbred lines (Jocić et al. 2015). The main advantage of hybrids over open-pollinated varieties is higher production, crop uniformity, easier gene introduction, especially incorporation of resistance genes. Thus, method of creating heterotic hybrids includes the creation of inbred lines and testing the combining ability of heterotic hybrids. Furthermore, which method for resistance breeding will be used, depend on to whether resistance is qualitative (downy mildew, broomrape, rust, Verticillium) or follows quantitative inheritance (white rot, Phomopsis, Alternaria, grey

rot) (Vear 2010). Various methods of selection were used for in the development of biotic stress resistant sunflower and are discussed below.

3.5.2.1 Creation of Inbred Lines

The proper selection of initial material for inbred line creation is essential for success in sunflower breeding. As initial material for inbred line creation, most often used are: local populations, new or commercial varieties, intervarietal, interline, and interspecific hybrids, populations obtained by planned crossing, and populations improved by recurrent selection.

An inbred line is the progeny of one self-pollinating homozygous plant (Borojević 1992). The most common process for the creation of inbred lines is self-pollination through six or more generations. Individual plants are selected from the initial population which contains high genetic variability. Plants are selected based on the yield, oil content, and other desirable traits, such as disease resistance. Seeds or plants are screened to particular pathogen using standardized methods and resistant plants are selected. Resistant plants continue to be sown according to the pedigree method of selection. The screening and selection of the resistant plants from the best progenies is continuing carried out in next generations. Uniform resistant lines for most characteristics appeared after six to eight generations of self-pollination.

The most commonly used method for sunflower breeding to biotic stress resistance is pedigree selection. The method is based on individual plant selection in segregating generations and evaluating selected plants for resistance to particular disease until the creation of inbred lines. During the vegetation, test plants are preferably subjected to artificial inoculation methods and perform phenotypic observations. The chosen plants selfed by isolation with paper bags immediately before flowering. It is essential to exclude extremely self-incompatible genotypes in the first year of selfing, as it is the trait that hampers creation of sunflower inbred lines.

During the development of sunflower inbred lines, two types of resistance can be discerned: single genes following a Mendelian pattern of inheritance, and quantitative resistance, which are explained by QTLs with complex patterns of inheritance. Generally, resistance followed by a single gene is more easily introduced into non-resistant material than quantitative resistance. In the past, transferring resistance genes in a developmental line are often based on the phenotypic evaluation, but lately, MAS has been performed at a larger scale. These include marker-assisted backcrossing (MAB), combining MAS with phenotypic screening in the cases of traits with low heritability, when a large number of QTL are of interest, as marker-assisted gene pyramiding. As quantitative resistance becomes more commonly used, the focus should be evermore on identifying and applying QTL.

3.5.2.2 Backcross Breeding

Backcross breeding is one of the most commonly used methods to introduce single disease resistance genes into a susceptible high yielding inbred line (Hussain 2015). Sunflower breeders have used this method to develop disease-resistant genotypes in most cases (Jocić et al. 2015). The inbred line with good performances is the recipient, and the resistant line is the donor, and they are crossed during the first year. After one year, the progeny will have 50% genetic content of both lines and is called first backcross (BC1). After six backcrosses, the line will recover good performances together with the gene from the donor line. In each generation of backcrosses, it is important to test for the resistance in order to choose resistant progenies. Besides introduction resistant gene(s), backcrosses are widely used for the fixation of respective genes in the good standard inbred lines (Jocić et al. 2010).

3.5.2.3 Hybrid Development

Heterosis in sunflower is exploited chiefly by creating two-way (single-cross) hybrids by crossing two genetically diverse inbred lines. The main advantage of hybrids over the varieties is 25–30% increased seed yields (Kaya et al. 2012). Besides the higher genetic potential for seed yield, it is easier to insert genes for resistant sunflower diseases, rendering hybrids more resistant than varieties. In addition, hybrid breeding allows a combination of resistance genes from different inbred lines, which gives durable resistance to a particular pathogen (Jocić et al. 2015).

3.6 Breeding for Biotic Stress Resistance

Breeding for biotic stress resistance is regarded as the most cost-efficient method of pest and disease control. Therefore, sunflower breeders must be thoroughly familiar with the general principles of resistance breeding, major approaches in resistance gene management, the stability of sunflower resistance to specific pathogens, monitoring interactions between host (sunflower), pathogen and environment, and resistance type (vertical and horizontal) (Škorić 2016).

The main problem in modern sunflower production is many diseases that cause a significant reduction in yield. Over 40 pathogens attack the sunflower, but not all of them cause economically considerable damage, and not all are present in all areas of sunflower cultivation. There is several pathogens that are frequently associated with significant yield decrease, Sclerotinia head rot and stalk rot, stem canker, rust and downy mildew. Several sunflower diseases although of high impact on yield are of regional importance, Verticillium wilt in Argentina or white rust in South Africa. Sunflower pathogens are either with narrow host range (*P. helianthi, A. helianthi,* and *P. halstedii*) or with broad host range (*S. sclerotiorum*) (Seiler and Gulya 2016). Another major obstacle resulting in continuous breeding process

is permanent adaptation of pathogens in a manner of new pathotypes emergence. Hence, breeding programs aiming to achieve durable disease resistance should rely on strategy of using diverse resistance genes. This strategy, if successful, should present a significant constrain for pathogen to overcome resistance trough development of number of effectors (Zhang and Powles 2006).

Increasing resistance to the predominant pests and diseases is one of the main tasks in sunflower breeding, preferably to achieve long-term tolerance or resistance to a particular pathogen (Jocić et al. 2012). Current results in sunflower breeding for disease resistance could be classified into four groups: genetic resistance to diseases such as *P. halstedii*, *P. helianthi*, *Verticilium spp*, and *G. cichoracearum*; high level of tolerance to diseases such as *Phomopsis, Macrophomina, Albugo,* and *Alternaria* spp.; acceptable tolerance of diseases such as *P. macdonaldii* and *S. sclerotiorum*; and partial disease tolerance against *Rhizopus* spp., *B. cinerea*, and other pathogenic fungi (Kaya 2016).

3.6.1 Downy Mildew

Resistance to downy mildew is controlled by dominant genes, designated as Pl genes, which provide resistance to one or more downy mildew races (Jocić et al. 2012). So far, twenty-two Pl genes (Pl1 to Pl21, PlArg, PlPMI3) have been identified and genetically mapped (Pecrix et al. 2018), and they confer resistance to at least one race of P. halstedii. Races are determined by set of differential lines with particular level of resistance (Tourvieille de Labrouhe et al. 2000). Pl genes are race-specific (Miller and Gulya 1988; Vear et al. 2008), and that often means complete but relatively not durable disease control. Although not lasting, this is highly efficient control method for downy mildew of sunflower the most efficient methods of controlling downy mildew (Miladinović et al. 2019). Emergence of new pathotype and resistance break is usually solved with introduction of new resistance genes trough breeding process.

Sunflower and *P. halstedii* have a "gene-for-gene" relationship. Sunflower genotype with effective resistance gene that will counteract the pathogen's virulence gene, will stop disease development. This reaction is easily observable trough presence or absence of pathogen sporulation on cotyledon and true leaves, a trait that is widely used in biotests. Depending on resistance gene resistant a reaction named "cotyledon limited infection", describing a resistant reaction visible as scarce sporulation of pathogen was also described (Gulya et al. 1991). Resistance is based on hypersensitive reaction (HR) ending in cell death of infected hypocotyls tissues (Mouzeyar et al. 1993, 1995). Glutationperoxidase gene (Herbette et al. 2003) and hsr230J-like gene (Radwan et al. 2004) are linked with this type of resistance reaction, leading to a cascade of processes constraining pathogen growth.

As the majority of downy mildew resistance genes have been reported to be monogenic dominant, this is an excellent example of exploitation of molecular markers in MAS. Pl_1 gene was mapped by use of random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) markers on chromosome *8*

by Mouzeyar et al. (1995). This gene is clustered together with Pl_2, Pl_6 and Pl_7. Out the four genes of the cluster, Pl_6 proved to be very efficient in controlling downy mildew for a long time, as it was resistant to all present races at the time, except for race 304. Bouzidi et al. (2002) developed sequence-tagged sites (STS) markers which belong to the TIR-NBSLRR class of RGA (resistance-gene analogue), while Panković et al. (2007) developed cleaved amplified polymorphic sequence (CAPS) markers for detection of Pl_6, which have been used in MAS for creating numerous downy milder resistance lines (Dimitrijević et al. 2010; Jocic et al. 2010; Miladinović et al. 2014). However, with the appearance of new downy mildew races, Pl_6 lost in its significance, and other genes were in the focus of molecular studies, among them and Pl_8 and Pl_{Arg}. Both genes originate from wild *H. argophyllus* and are positioned on chromosome *1* and *13*, respectively. Pl_5/Pl_8 cluster was examined by Radwan et al. (2005) who developed STS markers for the cluster. Moreover, this region was enriched by developed single-strand conformational polymorphism (SSCP) markers, RGC15/16 and RGC251, which were closely linked to Pl_8 (Bachlava et al. 2011). As, SSCP markers are labor intensive, they are not best suited to be used in MAS. Recent study, showed that two SNP markers, NSA_002220 and NSA_000423, could be very useful in MAS, as they were identified as good diagnostic markers in crosses in which RHA 340 was adonor of Pl_8 (Qi et al. 2017).

Pl_8 gene is losing in its significance, as it does not confer resistance to all present races (Gilleyet al. 2016). On the other hand, Pl_{Arg}, together with Pl_{17}, Pl_{18} and Pl_{33} confer resistance to all downy mildew races present in the USA (Qi et al. 2019). Pl_{Arg} was mapped, originally, by use of SSR (Dußle et al. 2004) markers, and the region was further explored by SSRs, SNPs and RGCs (Wieckhorst et al. 2010; Imerovski et al. 2014) with the ORS716 marker being identified as the most effestive one in MAS. Recently, Solodenko (2018) performed validation of reported SSR closely linked not only to Pl_{Arg}, but to Pl_8 and Pl_6 in diverse plant material. Concerning Pl_{Arg}, the author reported that five SSRs (ORS509, ORS605, ORS610, ORS1182 and ORS1039) reliably identified donor line of Pl_{Arg}, RHA 419. Qi et al. (2017) examined the cross HA 89 × RHA 464 (donor of Pl_{Arg} gene) and narrowed down the area with Pl_{Arg} locus to an interval of 2.83 Mb with the two nearest SNP markers being NSA_007595 and NSA_001835. Furthermore, the authors identified nine SNPs with a solid diagnostic potential for MAS of Pl_{Arg}.

Concerning the remaining complete resistant *Pl* genes known so far, Qi et al. (2015) identified ORS963 and SFW04052 as the closest flanking markers to Pl_{17} on chromosome *4*. The region surrounding the Pl_{17} gene had high recombination frequency of 0.59 Mb/cM. Later on, Ma et al. (2018) identified two flanking SNP marker to Pl_{17}, C4_5711524 and SPB0001, in an interval of 15-kb and six SNPs with good diagnostic potential in MAS. The authors also identified a potential candidate gene for Pl_{17}, HanXRQChr04g0095641, encoding a typical TNL (Toll/interleukin-1-receptor, nucleotide-binding site, and leucine-rich repeat) resistance gene protein. Pl_{18} gene was the first *Pl* gene mapped to chromosome *2* flanked by two SSRs (CRT214 and ORS203) and ten SNPs. Pl_{33} gene was recently mapped on chromosome *4* in F_2 and F_2-derived F_3 populations of HA 434 × TX16R (donor of Pl_{33}) (Liu et al 2019). The gene was co-segregating with SSR markers ORS963 and ORS644

and SNP markers SFW04052 and SFW04901, while two SNPs (NSA_008496 and NSA_006089) were mapped 0.2 cM proximal to the gene. Liu et al. (2019) further on exploited the region on chromosome *4* and found that SNP NSA_003564 is a common marker in maps of Pl_{17}, Pl_{19} and Pl_{33}, with Pl_{19} being downstream comparing to Pl_{17} and Pl_{33}. Pl_{19} was originally mapped by Zhang et al. (2016) on chromosome *4* with two flanking SNPs: NSA_003564 and NSA_006089, while later on Ma et al. (2018) found that the only gene present in the region in the reference genome is HanXRQChr04g0095951, with predicted function of coding an RNA methyltransferase family protein.

Recent molecular studies placed eight *Pl* genes on chromosome *1*, with Pl_{13}, Pl_{14}, Pl_{16} and Pl_{25} forming a cluster on the lower end of the chromosome, and Pl_{Arg}, Pl_{23}, Pl_{24} and Pl_{35} forming the second cluster far from the first one (Qi et al. 2017; Pecrix et al. 2018). Pl_{13} and *Pl16* are linked to two SSRs, HT636 and ORS1008 as they are closely positioned (Liu et al. 2012). Bachlava et al. (2011) developed RGS markers that are tightly linked to Pl_{14}. Qi et al. (2019) identified four co-segregating SNPs with Pl_{35} derived from wild *H. argophyllus*. Genes $Pl_{23–25}$ were mapped on chromosome *1* by Pecrix et al. (2018) in a comprehensive study in which the authors identified 10 probably new *Pl* genes. Apart from the *Pl* genes reported on chromosome *1*, these authors identified seven more *Pl* genes: one on chromosome *2* (Pl_{26}), three on chromosome *4* ($Pl_{27–29}$) closely positioned with Pl_{17}, one on chromosome *11* (Pl_{30}) and two on chromosome *13* (Pl_{31} and Pl_{32} closely positioned to Pl_8). Pl_{30} was the first *Pl* resistance gene identified on chromosome *11*. The 10 identified resistance genes conferred resistance to 15 French *P. halstedii* pathotypes and pathotype 330 from Spain. Chromosomes *8* and *13* harbor large clusters of resistance genes. Besides already mentioned, Pl_1, Pl_2, Pl_6, Pl_7 and Pl_{15} on chromosome *8*, recent study conducted by Ma et al. (2018) showed presence of a new gene, Pl_{20}, on this chromosome with four SNP markers (SFW09076, SFW02745, S8_11272046 and S8_11272025) co-segregating with it. Chromosome *13* harbors already mentioned, Pl_5 and Pl_8, and a new contingency of *Pl* genes. Pl_{21} was positioned in close vicinity to previously mapped Pl_5 (Vincourt et al. 2012), while Pl_{22} was the first *Pl* gene that was physically mapped to a region of 1.7 Mb on the chromosome *13* (Pecrix et al. 2018). Hypersensitive response effector PhRXLR-C01 and the Pl_{22} are proposed as an avirulence/resistance gene.

As it can be seen, there is a variety of genes to choose from, some conferring resistance to certain *P. halstedii* pathotypes, while some conferring complete resistance to all present races. The diagnostic markers reported can be used in MAS and pyramiding of *Pl* genes for breeding purposes and achieving sustainable resistance toward downy mildew.

3.6.2 Rust

Several genes conferring rust resistance in sunflower have been reported (Bachlava et al. 2011; Qi et al. 2012). Dynamism of resistance genes and pathogen races

have similarities with pathosystem of sunflower and *P.* halstedii. One example of successful control is the gene *R11* that provides rust races 336 and 777, pathotypes spread in the Northern Great Plains of USA (Gulya and Markell 2009; Qi et al. 2011a, 2012). High pathogen pressure is making many R-genes obsolete. One way to tackle this problem is by pyramiding multiple resistance genes into a single host genotype could reduce the possibility of resistance ineffectiveness resulting from pathotype evolution.

Extensive molecular studies were conducted for detecting diagnostic markers for rust resistance. As *Pl* genes, the majority of rust resistance genes or *R* genes are monogenic dominant. First markers developed for *R* gene resistance were RAPD and sequence characterized amplified region (SCAR) for R_1 and R_{adv} genes (Lawson et al. 1996, 1998). R_2 gene was recently mapped on chromosome *14* with SFW01272 and NSA_002316 flanking the gene (Qi et al. 2015) which should be combined together in MAS of the gene. As previously mentioned, chromosome *13* possess *R* gene rich regions in which the genes are clustered, usually as a combination of *Pl* and *R* genes. R_4, R_{u6}, R_{11}, R_{adv}, R_{13a} (R_{HAR6}), and R_{13b} are located on chromosome *13* (Bachlava et al. 2011; Qi et al. 2011b, 2012b; Gong et al. 2013; Bulos et al. 2014). R_4 gene is flanked by ORS581 and ZVG61 (Qi et al. 2011b) and linked to R_{13a} (R_{HAR6}) and R_{13b} on the lower end of the chromosome *13* (Bulos et al. 2013; Gong et al. 2013; Qi et al. 2015), while Lawson et al. (1998) and Bachlava et al. (2011) reported co-segregating SCARs and tightly linked RGCs and SSRs to R_{adv}. Further studies showed that P_{u6} and R_4 genes are in vicinity, mapping 6.25 cM from each other Bulos et al. (2014), however no diagnostic markers can be identified for MAS of P_{u6} gene since all mapped marker were found to be distant from the gene. R_{11} gene, also found at the lower end of chromosome *13* was mapped 0.3 cM proximal from ORS728, a common marker for Rf_5 and R_{11}. R_{16} is the newest *R* gene discovered in wild *H. annuus* accession TX16 (Jan and Gulya 2006) mapped on the lower end of chromosome *13* and flanked by two SNP markers: SFW04317 and SFW08875 (Liu et al. 2019). This lower end of chromosome *13* is the second largest cluster of NBS-LRR encoding genes, both *Pl* and *R*. By analyzing this region with SSRs and RGCs, Gong et al. (2013) suggested that this big cluster may be divided into two sub-clusters, with R_{adv} and R_{11} forming sub-cluster I, and R_4, $R_{13a,}$ R_{13b}, R_{16}, Pl_5, Pl_8, Pl_{21} subcluster II (Liu et al. 2019). Of the genes, R_{13a}, R_{13b} and R_{16} confer resistance to all present races of *P. helianthi* in North America (Liu et al. 2019).

So far, the only molecularly characterized rust resistance gene on chromosome *2* is R_5 flanked by two SSRs, ORS653a and ORS1197-2, and two SNPs NSA_000267 and SFW03654, that is the closest marker to the gene 0.6 cM distant to it (Qi et al. 2012, 2015). New resistance gene derived from HA-R8, R_{15}, was also, recently characterized on chromosome *8* (Ma et al. 2018). While SSR markers were inefficient in detecting linkage with R_{15}, SNPs were more efficient. By use of genotyping-by-sequencing (GBS) the authors located the gene and mapped co-segregating SNPs: SFW01920, SFW05824, SFW00128 and NSA_008457. R_{12} and R_{14} have been positioned in the middle of chromosome *11* between ORS1227 and ZVG53, but originating from two different wild sunflower accessions (Gong et al. 2013; Zhang et al. 2016). Fine mapping of R_{12} genes provided breeders with markers that are closely

associated with the gene, NSA_003426 and NSA_004155 (Talukder 2014), while NSA_000064 was mapped 0.7 cM from R_{14} and showed no polymorphism between RHA 464 (R_{12}) and PH3 (R_{14}) (Zhang et al. 2016). Two SNPs, NSA_001392 and NSA_001570, mapped bellow R_{12}, and showed polymorphism between two resistant lines. Out of the examined SSRs, ORS542 was linked 3.5 cM proximally of R_{14}.

As already mentioned, the markers identified for detection of different *R* genes can be used in gene pyramiding. However, a proper choice of genes to be included in the pyramiding process needs to be made. A very nice example of pyramiding *R* genes was provided by Qi et al. (2015) who saturated the big sub-cluster R_4, R_5, R_{13a}, and R_{13b} with different SNP markers, thus identifying the closest ones to the genes of interest (under 1 cM distant) and exploited the identified SNP markers together with SSRs in identifying homozygous "double-resistant" plants, harboring R_5 and R_{13a}. The "double-resistant" progeny plants were more resistance to races 336 and 777 compared to the ones with one *R* gene. More recent study exploited several markers with the aim of combining different rust resistance genes with *Pl* genes ($R_4/R_{12}/Pl_{Arg}$, $R_5/R_{12}/Pl_{Arg}$, $R_{13b}/R_{12}/Pl_{Arg}$, R_{15}/R_{12}, *and* R_{13b}/R_{15}) to obtain sunflower lines resistant to all present races both pathogens in a single genotype (Qi and Ma 2020).

3.6.3 Stem Canker

Genetic control of *Diaporthe /Phomopsis helianthi* remains unclear concerning the number of gene(s), their expression, and modes of inheritance (Miladinović et al. 2019). The first report on the genetics of sunflower resistance to Phomopsis came from Vranceanu et al. (1983), who noted that several genes with partial dominance controlled the resistance and positively correlated with the stay-green trait. Similar findings of intermediate or partial dominance as a mode of inheritance and resistance for this disease controlled by complementary genes were reported by Škorić (1985). Research of Tourvieille et al. (1988) indicated recessive resistance or resistance dependent on interactions between genes and the polygenic nature of resistance. Therefore, it could be concluded that combinations of inbred lines with the best levels of resistance give the best hybrids and that it should be possible to obtain increased resistance by selecting combinations from different sources.

Hybrids with a high level of tolerance to Phomopsis have been available since Škorić (1985) obtained highly tolerant inbred lines. By that time, many commercial hybrid tolerant to Phomopsis have been developed. Literary data have lately been mainly related to its occurrence in countries where there has been no strong attack in the previous period, such as Argentine and Uruguay (Huguet 2006), Australia (Thompson et al. 2011) and the United States (Methew et al. 2012).

D. helianthi Munt.-Cvet. et al. resistance in sunflower has been the least examined on the molecular level. Phomopsis stem canker reduces oil content, seed weight and yield in sunflower, at any stage of seed development (Diaz and Ortegon 1997). As resistance is achieved by presence of minor genes, (Bert et al. 2002) initially mapped

15 resistance QTLs on seven sunflower chromosomes: *3*, *4*, *8*, *10*, *14*, *11* and *17* in a cross XRQ × PSC8. Individually, detected QTLs explained between 7.2 and 24.7% of the phenotypic variation. Opposite, to this research Langar et al. (2004) examined RILs population of HA 89 × LR 4 and detected resistance QTLs on at least eight chromosome regions. A major QTL for final expansion rate of lesions on leaf blades was mapped on chromosome *6* flanked by markers ACH9AA-ACH7TC, explaining 24% of the phenotypic variation. Another major QTL associated to frequency of attack at flowering with seminatural infections was mapped on chromosome *15* and was flanked by markers CCL6TA-CCH5AC Since two types of inoculations were tested, artificial infection of leaves and seminatural infection, the resistance QTLs detected differed between these two inoculation types. This confirmed the hypothesis of Langar et al. (2004) that different molecular mechanisms are responsible for resistance on leaf blades, petioles and stems.

3.6.4 White Rot

Resistance to S. sclerotiorum is under polygenic control (Bert et al. 2004). Since sources of resistance have not been found, degrees of tolerance were determined (Škorić et al. 2006). The high degree of tolerance was achieved in more lines and hybrids to a stem form. Satisfactory tolerance is achieved and the forms of root and head diseases in selected inbred lines (Vasić et al. 1999).

As resistance to Sclerotinia is a quantitative trait, several authors mapped resistant QTLs in attempt to identify the major ones applicable for accelerating development of resistant sunflower genotypes. Mestries et al. (1998) used two methods (analysis of variance and interval mapping) for detection of QTLs associated to *Sclerotinia* resistance and mapped several RFLP markers across sunflower genome linked to the detected QTLs. The QTLs detected differed between generations for leaf while the same QTLs in all generations were detected for capitulum Sclerotinia resistance (on LGs A and M). Gentzbittel et al. (1998) mapped a major QTL explaining 50% of the phenotypic variance and identified a protein-kinase gene co-segregating with the QTL in three different crosses. Later on, Bert et al. (2002) identified 15 QTLs associated to the resistance parameters (mycelium on leaves and capitulum, percentage attack and latency index) to white rot in a cross XRQ × PSC8, with the individual QTLs explaining between 9 and 41.2% of the phenotypic variation, while Bert et al. (2004) mapped 14 QTLs associated to resistance parameters (attacks on terminal buds, attacks on capitulum and latency index) in a cross FU x PAZ2. In both studies, RFLP and AFLP markers were used for QTL mapping. Mićić et al. (2004, 2005a; b), conducted a comprehensive study for analysis of Sclerotinia resistance in sunflower. In their research, the authors analyzed F_3 and RIL populations of NDBLOS × CM625 and F_3 population of TUB-5–3234 × CM625 for validation of resistance QTLs. The authors identified two genomic regions that carried resistance QTLs consistent across generations (on chromosomes *8* and *16*) (Mićić et al. 2004, 2005a). When comparing

detected QTLs between two mapping populations NDBLOS × CM625 and TUB-5–3234 × CM625, there were some similarities and some differences. For example, for stem lesion, one QTL, detected on chromosome *4*, out of four QTLs was common in both crosses (Mićić et al. 2005b). This QTL originated from a common parent in both crosses. Later on, seven and nine QTLs for disease severity and disease incidence respectively were identified in a different cross, HA 441 × RHA 439 (Yue et al. 2008). Individually, detected QTLs explained between 8.4 and 34.5% of the phenotypic variation. Chromosome *10* was identified as very promising in MAS for Sclerotinia resistance, as in numerous studies it was shown that it carried resistance QTLs to stalk and head rot (Mestries et al. 1998; Bert et al. 2002; Mićić et al. 2005b; Ronicke et al. 2005). Recently, Amoozadeh et al. (2015) employed SSR and SNP markers for mapping 14 QTLs that conferred partial resistance to two *S. sclerotiorum* isolates. QTL mapped on chromosome *1* was linked to the glutathione S-transferase gene and was identified as potentially useful in MAS. Development of new molecular techniques enabled Livaja et al. (2016) to develop and perform a 25 K SNP genotyping array based on Illumina® Infinium assay for combining phenotypic data from Mićić et al. (2005a) with newly obtained molecular data and mapped six resistance QTLs for the following traits: stem lesion length, speed of fungal growth and leaf length with petiole. Individually, detected QTLs explained between 8.1 and 35.2% of the phenotypic variability, with the chromosome *8* harboring QTLs explaining the largest portion of the variability. Zubrzycki et al. (2017) used Illumina Oligo Pool Assay in examining RIL population obtained from PAC2 x RHA266 and detected 36 main effect QTLs and 13 epistatic QTLs on 14 chromosomes. The authors highlighted importance of chromosomes *1*, *10* and *15* in detection of candidate genes for Sclerotinia head rot.

Najafzadeh et al. (2018) were the first to use retrotransposon-based markers for association analysis of sunflower resistance to three isolates of each, *S. sclerotiourm* and *S. minor*. The authors examined 100 sunflower oilseed lines and identified 15 and 14 loci associated with resistant traits by use of general and mixed linear model, respectively. Individual QTLs explained 1 to 23% of the phenotypic variation. Four markers were associated to resistance traits to more than one isolate: UF1, LTR1064-A13, LTR1061-UBC818 and LTR1064–65.

3.6.5 Phoma Black Stem

Development of sunflower hybrids with partial resistance to Phoma black stem is an effective way to control the disease (Roustaee et al. 2000). Genetic variability for this trait in sunflower genepool has been reported (Peres et al. 1994; Bert et al. 2004). Roustaee et al. (2000) wrote that variation of reaction to the seedling test among genotypes studied is due to the additive and dominant effects of genes controlling black stem partial resistance. In addition, partial resistance reduces epidemic severity and is more durable (Mundt 2014). Therefore, developing methods that

enable improved partial resistance to Phoma black stem may improve sunflower sustainability (Schwanck et al. 2016).

Study of Mirleau-Thebaud et al. (2011) showed that *P. macdonaldii*-induced premature ripening impacts plant nutrition via its effect on sunflower plant organs (roots, stems, leaves), yield and yield components, while the disease influences oil quality and the balance oleic-linoleic fatty acids. As resistance to *P. macdonaldii* is a quantitative trait, Bert et al. (2004) mapped four and two resistance QTLs by composite interval mapping (CIM) and simple interval mapping (SIM), respectively in a cross FU × PAZ2. QTLs mapped explained 20% of variability in total. Rachid Al-Chaarani et al. (2002) identified seven resistance QTLs on seven sunflower chromosomes: *3*, *6*, *8*, *9*, *11*, *15* and *17*, explaining 92% of the phenotypic variation in RIL population of PAC 2 × RHA 266, while a Abou Alfadil et al. (2007) analyzed RIL population of the same cross and mapped 27 resistance QTLs to four basal stem and root necrosis isolates from France. Out of the 27, 13 QTLs were isolate-specific, while the rest were isolate-non-specific. These QTLs were mapped on chromosomes: *5*, *6*, *8*, *12*, *13* and *15* and had a major effect for resistance to each isolate. Moreover, Darvishzadeh et al. (2007) used RILs of the same cross (PAC 2 × RHA 266) and detected ten resistance QTLs, of which four were isolate-non-specific, and the rest isolate-specific, individually explaining between 6 and 20% of the phenotypic variation. Even though both authors used RILs of the same cross they used different fungi isolates which lead to detection of different QTLs. Consequently, molecular markers recommended for MAS differed between the studies. While, Al Fadil et al. (2007) recommended use of markers E33M48_26, HA3555 and E33M48_20, located on chromosomes *6*, *12* and *13*, respectively, Darvishzadeh et al. (2007) recommended use of HA3700, ORS523_1, SSU25 and ORS1097, located on chromosomes *5*, *15* and *17*.

Finally, Bordat et al. (2017) examined resistance to *P. macdonaldii* in the field conditions using controlled inoculation for the first time. The authors examined two RIL mapping populations of crosses XRQ × PSC8 and FU × PAZ2 at different stages, mapping different QTLs in both examined populations. Two most significant QTLs were located on chromosome *7* and *10* for FU × PAZ2 and XRQ × PSC8, respectively. These QTLs were detected 49 days after infection. The authors concluded that diverse genetic factors are included in the development of the disease, depending on the stage of disease development as well as infection process leading to premature ripening or black stem.

3.6.6 Alternaria Leaf Spot

Although genetic resistance is the most economical means of plant disease control, the non-availability of resistance sources to Alternaria helianthi, leaf spot disease, has been a significant constraint in sunflower breeding programs. However, a few sources of different levels of resistance to A. helianthi have been reported and quantitative differences among genotypes have been developed but still limited under epidemic

conditions (Morris et al. 1983; Carson 1985; Lipps and Herr 1986; Das et al. 1998). In Argentina, the most endangered area by Alternaria leaf spot, both qualitative genes and quantitative resistances have been proposed (Bertero de Romano and Vazquez 1982; Kong et al. 2004).

Not much work has been done on study of Alternaria resistance on molecular level. There is only report dealing with this topic, in which Murthy et al. (2005) attempted to develop mutant lines of sunflower resistant to Alternaria and markers that could be useful for marker-assisted selection in breeding. In their work, single marker and stepwise regression analysis carried out in relation to percent disease index indicated that the RAPD markers OPC5-B, K, J, OPA12-D and OPA15-A are strongly associated with *Alternaria* resistance.

3.6.7 Powdery Mildew

Powdery mildew is a widely spread disease in countries with sunflower crop production (Aćimović 1998). Cultivated and wild sunflower genotypes have been inexhaustible sources of resistance (Dedić 2012). Genetic bases of resistance to powdery mildew in sunflower have been studied. Two genes controlling inheritance were proposed by Rojas-Barros et al. (2006). Christov (2008) reported the existence of two modes of inheritance in wild Helianthus species. In one, the resistance was controlled by a single dominant gene, whereas in the other, it was polygenic. Similarly, the existence of both single and polygenic inheritance was reported by Naggayya (2013).

Kallamadi and Mulpuri (2020) studied the inheritance of powdery mildew resistance and mapped QTLs for resistance to powdery mildew in a sunflower multiple disease resistance line, TX16R (PI 642,072). The authors identified three genomic regions for resistance to this disease, two of which were mapped on chromosome *10* and one on chromosome *5*. This work is the first report on mapping of powdery mildew resistance in sunflower.

3.6.8 Charcoal Rot

In the light of global change of climatic conditions, sunflower breeding for charcoal rot, caused by *M. phaseolina,* resistance is gaining importance. Although *M. phaseolina* is monotypic and no physiological races have been reported, it has high genetic variability. The most effective method is the use of resistant/tolerant sunflower genotypes. Resistance to *M. phaseolina* is horizontal and controlled by polygenes (Kaya 2016). Resistant genes against *M. phaseolina* do not exist or are unknown (Khan 2007). Generally, all commercial cultivars are susceptible to the disease, and only a moderate level of resistance was found in cultivated sunflower germplasm (Ijaz et al. 2013; Jalil et al. 2014) and in wild relatives (Seiler et al. 2010; Warburton et al.

2017; Shehbaz et al. 2018). Khan (2007) found that the resistance in some sunflower genotypes has a dominant character and presence of two complimentary genes, *MP 1* and *MP 2*, is essential in resistant cultivars.

3.6.9 Breeding for Broomrape Resistance

Breeding for broomrape (O. cumana) resistance includes discovering resistance gene(s) and incorporation into resistant sunflower genotype. Since broomrape is a devastating constrain in many regions, a lot of effort is given to developing resistant hybrids. First, five races of broomrape have been distinguished (named A-E) by Vranceanu et al. (1981). They identified five single dominant genes (Or1-Or5) for resistance to these five races and established the set of differential lines. Many inbred lines and hybrids resistant to race E were successfully developed, thus improving sunflower production worldwide. Perez-Vich et al. 2013 reported that resistance to race E is qualitative or race-specific or gene-for-gene resistance and dominantly inherited.

Later on, resistance was overcome by the appearance of race F. The resistance remained race-specific, controlled by a single dominant gene Or_6 (Pacureanu-Joita et al. 1998; Pérez-Vich et al. 2004a, b), two recessive genes or_6or_7 (Akhtouch et al. 2002; Fernández-Martínez et al. 2004) and two partially dominant genes Or_6Or_7 (Akhtouch et al. 2016) (Table 3.1). The differences in the background of the genetic material caused different modes of inheritance. Besides dominant resistance genes, previously identified in sunflower breeding programs, for the first time recessive resistance appeared. Recessive genes could influence more, achieving durable resistance to broomrape, but they led to the necessity to incorporate resistant genes into both parental lines in order to develop a resistant hybrid (Akhtouch et al. 2002). While model of dominant resistance is an active process in which the plant synthesizes compounds that interfere with parasites, recessive resistance may result from plant cells that lack certain factors essential to the life cycle of the pathogen (Imerovski et al. 2016). Recently, more virulent populations (G and H) emerged, several new sources of resistance were noted, indicating both dominant and recessive inheritance (Table 3.1).

Recent genetic and molecular studies have revealed a more complex control of broomrape resistance in sunflower. In addition, the race-specific resistance to O. cumana has been reported for quantitative loci (Pérez-Vich et al. 2004a, b; Akhtouch et al. 2016; Louarn et al. 2016; Imerovski et al. 2019). The main advantage of the approach is that, besides major QTL, complementary QTL has minor effects on broomrape resistance, which can be used as donor sources for marker-assisted pyramiding programs. Recent genetic and molecular studies have revealed a more complex control of broomrape resistance in sunflower. In addition, the race-specific resistance to O. cumana has been reported for quantitative loci (Pérez-Vich et al. 2004a, b; Akhtouch et al. 2016; Louarn et al. 2016; Imerovski et al. 2019). The main advantage of the approach is that, besides major QTL, complementary QTL

Table 3.1 Overview available sources of resistance to broomrape races F and G in sunflower (Cvejić et al. 2020)

Genotype name	Source	Resistant to race(s)	Gene(s)	Inheritance	References
P-96	Cultivated sunflower (Yugoslav origin)	F (Spain)	or_6,or_7	Two recessive genes QTL	Fernandez-Martinez et al. (2000), Akhtouch et al. (2002), Perez-Vish et al. (2004), Akhtouch et. al (2016)
R-96	Cultivated sunflower (Yugoslav origin)	F (Spain)	or_6,or_7	Two recessive genes	Fernandez-Martinez et al. (2000); Akhtouch et al. (2002)
L-86	Cultivated sunflower (Russian origin)	F (Spain)	or_6,or_7	Two recessive genes	Fernandez-Martinez et al. (2000); Akhtouch et al. (2002)
K-96	Cultivated sunflower (Russian origin)	F (Spain)	QTL	Recessive	Fernandez-Martinez et al. (2000), Akhtouch et. al. (2016)
KI-534	Unknown	F (Spain)	or_6,or_7	Two recessive genes	Rodriguez-Ojeda et al. (2001)
BR-4 (J1)	Interspecies hybridisation (*H. grosseserratus* and *H. divarticatus*)	F (Spain)	Or_6; Or_6,Or_7	Single dominant gene; Two partially dominant genes	Jan et al. (2002); Rodriguez-Ojeda et al. (2001), Velasco et al. (2007)
LC-1093	Cultivated sunflower	F (Romania)	Or_6	Single dominant gene	Pacureanu-Joita et al. (1998)
AO-548	Inbred line from germplasm collection of Fundulea Institute	G (Romania)	Unknown	Two independent dominant genes	Pacureanu-Joita et al. (2008)
LC-009	Inbred line from germplasm collection of Fundulea Institute	G (maybe new)	Unknown	Unknown	Pacureanu-Joita et al. (2009)
HA267	Selected from the Novi Sad gene-pool	G (Spain, Romania, Turkey)	unknown	Single recessive gene, QTL	Imerovski et al. (2014), Imerovski et al. (2019)

(continued)

Table 3.1 (continued)

Genotype name	Source	Resistant to race(s)	Gene(s)	Inheritance	References
AB-VL-8	Interspecific hybridization with *Helianthus divarticatus*	G (Spain, Romania, Turkey)	$or_{ab\text{-}vl\text{-}8}$	Single recessive gene, QTL	Cvejic et al. (2014), Imerovski et al. (2016, 2019)
LIV-10; LIV-17	Interspecific hybridization with *Helianthus tuberosus*	G (Spain, Turkey)	unknown	Single recessive gene, QTL	Cvejic et al. (2014, 2018), Imerovski et al. (2019)
MS-2161A	Created by AMGAgroselect Company	G (Romania, Moldova)	Unknown	Unknown	Şestacova et al. (2016)
DEB- 2	*H. debilis* subsp. *tardiflorus* (PI468691)	G (Spain)	$Or_{Deb\text{-}2}$	Single dominant gene, QTL	Velasco et al. (2012), Gao et al. (2019)
LR1	INRA	F (Spain) G (Turkey)	QTL	–	Louarn et al. (2016)
H. praecox	Provided from USDA-ARS	G (Posthaustorial resistance)	Or_{pra1}	Single dominant gene	Sayago et al. (2018)
VIR-665 VIR-221 VIR-222 No. 667 No.769 No. 3046	VIR collection	G (Russia)	unknown	Single gene, incomplete dominance	Guchetl et al. (2018)
PHSC1102	Corteva	F_{GV}, G_{TK}	Or_{SII}	Partial dominance	Martin-Sanz et al. (2019)
LSS, S and LSR	Syngenta seeds	F(Spain)	*HaOr7*	Dominance	Duriez et al. (2019)

has minor effects on broomrape resistance, which can be used as donor sources for marker-assisted pyramiding programs. Genotypes with high resistance to most virulent races of broomrape have been identified. However, at the moment, there is no universally accepted set of differential lines for identifying races over F (Martin-Sanz et al. 2016). Each seed companies or research groups use inbred lines from previous studies for racial classification, but broomrape populations in some areas are insufficiently distinguished.

The first broomrape resistance gene that was molecularly analyzed was a major gene Or_5, that confers resistance to race E, mapped on the telomeric region of chromosome *3* (Lu et al. 2000; Tang et al. 2003). A year later, Perez-Vich et al. (2004a; b) reported quantitative resistance to races E and F, and mapped five QTLs for resistance to race E and six QTLs for resistance to race F on 7 different sunflower chromosomes. A major QTL, *or3.1*, was mapped on upper end of the chromosome *3*, associated to the resistance or susceptibility character. On the same chromosome, another *Or* gene was mapped, *orab-vl-8*, which was described as recessive (Imerovski et al. 2016).

This gene was mapped on the lower region of chromosome *3* with the closest SSR, ORS683, being 1.5 cM remote from the gene.

Later on, Imerovski et al. (2019) examined four mapping populations (one was used in the previous study) and mapped two major QTLs on chromosome *3*, *or 3.1* and *or3.2*. *QTLor3.1* was mapped in a similar genomic region as Or_5, while *or3.2* was located in the lower region of the chromosome. Between two and 23 significant QTLs were mapped across sunflower genome, depending on the cross analysed. To facilitate introgression of resistance QTL in the peak region of *or3.2,* CAPS markers were designed. For examination of potential candidate genes for the two major QTLs, Imerovski et al. (2019) identified 123 genes in the region of *or3.1*, between 31.9 to 38.48 Mb, and singled out: *HanXRQChr03g0065841* (TMV resistance protein N-like) and *HanXRQChr03g0065701* (disease resistance protein RPS2-like) (www.heliagene.org) as possible candidate genes. Moreover, in the region of *or3.2*, between 97.13 and 100.85 Mb, 71 genes were identified. A putative defence gene, *HanXRQChr03g0076321*, was singled out as a potential candidate gene.

A TAQMAN® assay (Roche Molecular Systems, Inc.) for detection of the QTL conferring broomrape race H System 2 resistance was patented by Hassan et al. (2011). For, marker design, the authors mapped the locus on chromosome *4*, 3 cM from the SSR HT0664-CA, while SNPs: HT090 and HT0183 were reported as the potential markers of interest.

Louarn et al. (2016) identified QTLs for resistance to race F from Spain and G from Turkey in addition to QTLs for the three stages of broomrape development. The authors reported existence of several mechanisms of quantitative resistance. Seventeen QTLs spread across 9 different chromosomes. On chromosome *13*, a QTL associated to the number of emergences in the field was the only one identified controlling resistance in the field and could be most rapidly used in MAS. A cDNA, *HaT13l034464*, located in the region of this QTL was homologous with gene coding CC- NBS-LRR protein (described by Li and Timko 2009). Recently a major resistance gene, $HaOr_7$, have been mapped on chromosome *7* conferring resistance to race F with a function of coding a complete receptor-like LRR kinase protein (Duriez et al. 2019). Gao et al. (2019) patented marker for detection of a major resistant QTL marker for $Or_{Deb\text{-}2}$ gene conferring resistance to race G on chromosome *4*. This QTL explained 64.4% of the total phenotypic variation.

Broomrape resistant genotypes with incorporated single resistance genes often lose their resistance in a brief period. Therefore, more than one gene and QTL pyramiding into a single genetic background deteriorate the parasite to overcome two or more resistant genes than one controlled by only one single gene. However, gene pyramiding through traditional breeding is difficult to achieve due to linkage drag, which is often challenging to break even after several back crossings (Khan and Khan 2015). Gene pyramiding through MAS is, therefore, a more practical approach for bringing rapid genetic improvement.

3.6.10 Breeding for Pest Resistance

The most widespread and damaging sunflower insect pest in North America is the sunflower head moth, *H. electellum*, while in Europe and Asia it is *H. nebulella.* The most significant achievement in sunflower breeding in Europe concerning insects that cause economic losses to sunflower production is the development of cultivars in which have an armored layer (phytomelanin) induced in the husk from H. tuberosus (Pustovoit et al. 1976). This layer hardens by seed maturity and prevents the seed from being penetrated by larvae of *H. nebulella.* Early studies of inheritance report that phytomelanin layer is controlled by a single dominant gene (Gundaev 1971), while Bochkarev et al. (1991) reported that thickness of the layer varied, indicating control of several modifier genes. Resistance to sunflower moth was incorporated in 1950s and 1960s y through interspecies hybridization and today all sunflower hybrids are resistant to *H. nebulella.*

Besides sunflower head moth, the insects causing most economic damage in North America are sunflower beetle (*Z. exclamationis*), the sunflower stem weevil (*C. adspersus*), the red and grey seed weevils (*S. fulvus* and *S. sordidus*), and banded sunflower moth (*C. hospes*). Over the past decades, many sunflower accessions (cultivated and wild) have been screened for important insect infestations. Observations of these accessions indicate that the resistance to the insects is quantitatively inherited, that is, controlled by several genes. Three resistance mechanisms can mitigate insect damage: antibiosis, antixenosis and tolerance. Therefore, breeding strategies must be directed towards the most effective mechanism in dealing quantitatively controlled traits (Škorić 2012).

Although *Cry1F*-transgenic sunflowers, resistant to *Spilosoma virginicia* and *Rachiplusia nu* were produced (Snow et al. 2003), economic and environmental considerations hampered their introduction into production. Since single genes do not convey resistance equivalent to insecticides, two or more independent types of resistance should be combined whenever possible (Prasifka and Hulke 2012). Furthermore, an effective approach to insect resilience in sunflower includes using existing sunflower defensive traits that is pericarp hardness, terpenoids coumarins, along with the interdisciplinary approaches for efficient insect resistance screening and breeding.

3.7 Future Prospects: Towards Durable Resilience

Sunflower breeding for durable biotic stress resistance is an everlasting challenge, due to the changes in the virulence and aggressiveness of common pests and pathogens, but also the appearance of new biotic stressors due to climate change and spread of sunflower growing area (Miladinović et al. 2019). Since it belongs to a highly diverse genus comprising a number of species that possess different genes for biotic stress resilience, characterization and transfer of valuable genes from wild relatives into the

cultivated sunflower could be a valuable tool for creation of pest and disease resilient genotypes (Warburton et al. 2017). Introduction of molecular tools into the breeding process and the development of the markers for monogenic diseases, such as downy mildew and rust, paved the way for introduction of MAS into sunflower breeding. This enabled the breeders to overcome shortcomings of phenotypic selection, with the targeted introgression of resistance traits and lower linkage drag. MAS also facilitated gene pyramiding and more efficient introduction of recessive resistance (Jocić et al. 2015).

The year that brought a major change in public sunflower molecular research was 2017, when the first assembly of sunflower genome was published (Badouin et al. 2017), followed by publication of sunflower pan-genome sequence (Hübner et al. 2019). This may help speed up the screening of the sunflower and wild relatives' genome to mine for resistance genes, using also information from model species for which considerably more genetic information is available. Combined with the classical genetic studies, newly available genome sequence and sequencing technologies enabled the study of the epigenetic phenomena in sunflower and the application of epigenome profiling and engineering for creation of the genotypes with the durable biotic stress resilience (Varotto et al. 2020). This could be especially the case for the quantitatively controlled resistance, where combination of different -omic tools and approaches could enhance sunflower crop tolerance to a range of economically important diseases, but also different pest insects where little or no progress have been made in elucidation of the resistance mechanisms.

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Chapter 4
Genomic Designing for Biotic Stress Resistant Peanut

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Abstract Peanut is an oilseed crop that is essential for food and nutritional protection around the world. It is a source of livelihoods to smallholder growers of Asia and Sub-Saharan Africa. However, yield losses keep increasing under present climate change accompanied by rising CO_2 levels, erratic rainfall, rising and fluctuating atmospheric temperature, despite a considerable genetic gain in yield since the 1960s. Moreover, climate change and global warming lead to the ocurrence of a number of biotic stresses that severely affect crop yield and productivity. Furthermore, the cultivated peanut's genetic architecture and tetraploid nature have resulted in low genetic diversity for many economically significant traits. Significant achievement in yield and tolerance against biotic stresses has been made by conventional approaches, although time consuming, and laborious. Recent developments in genomics, combined with the use of available genetic resources, have raised the peanut to that of a "genomic

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resource-rich oilseed crop." As a result, a comprehensive approach that includes the application of genomic knowledge and techniques in crop improvement programs is critical for furthering peanut productivity advancement. Molecular markers are the most useful genomic tools for characterizing and harnessing usable genetic variability. Researchers are now moving faster towards traits and their genetic mapping studies. In addition, the existence of a diploid progenitor reference genome, tetraploid genotype, and 58 K SNPs, a high-density genotyping assay have greatly aided high-resolution genetic mapping. There has also been an important progress in developing multiparental genetic mapping populations namely, nested association mapping (NAM) and multi-parents advanced generation intercross (MAGIC) for mapping of quantitative and multiple traits simultaneously with high-resolution. The low cost of sequencing aided the development of mapping techniques based on sequencing especially QTL-sequencing for dissecting complex traits such as resistance to diseases. In peanut, there are a few promising examples of diagnostic markers for biotic stresses being developed and deployed in genetic improvement. In this context, this chapter provides recent information on the various biotic stresses faced by the crop across the globe, progress made through conventional breeding programs, transgenic approaches, and achievements in genomics with a special emphasis on QTL discovery, mapping of desirable traits and molecular assisted breeding approaches. The chapter also offers an overview of the most recent genomic discoveries, methods, and techniques used, as well as their possible applications for peanut improvement.

Keywords Peanut · Biotic stresses · Genomics · Transgenics · Molecular markers · Trait mapping

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4.1 Introduction

Peanut (*Arachis hypogaea L.*), also known as groundnut, is an essential oilseed-food-feed-fodder crop of choice, cultivated in more than 100 countries worldwide. The crop is cultivated as a sole and intercrop on nearly 28.5 million ha area globally, with record production of 45.95 million tonnes and productivity of 1611 kg/ha of pods-in-shell in the year 2018 (http://www.fao.org/faostat/en/#data/QC) (Fig. 4.1). Peanuts are grouped into two sub-species "*hypogaea*" and "*fastigiata*", mainly on the basis of pattern of branching and vegetative and reproductive axes distribution. The subspecies '*hypogaea*' consist of two botanical varieties, '*hypogaea*' (spreading-Virginia runner and semispreading—Virginia bunch types) and '*hirsuta*' (Peruvian runner), whilst the subspecies '*fastigiata*' is grouped into four botanical types ('*fastigiata*'-valencia types; '*vulgaris*'-spanish types; '*peruviana*' and '*aequatoriana*') (Gregory et al. 1973; Krapovickas and Gregory 1994). The cultivated Peanut is an amphidiploid/ disomic tetraploid designated as $2n = 4x = 40$. Peanut is an economically important oilseed crop and its kernels are rich with 45–55% oil, 25–30% protein, and 10–20% carbohydrate (Jambunathan et al. 1985). Peanut haulm contain carbohydrates (38–45%), minerals (9–17%), protein (8–15%) and lipids (1–3%), and has a digestibility of around 53% when fed to cattle. Peanuts are treated as

Fig. 4.1 Healthy peanut crop in the farmers' field

functional food as it is also an important source of minerals such as calcium (Ca), phosphorus (P), iron (Fe), magnesium (Mg), zinc (Zn), potassium (K), vitamins such as vitamin E, thiamine, riboflavin, pantothenic acid, niacin, antioxidants includes primarily *p*-coumaric acid, and bioactive compounds to promote health such as tocopherol, resveratrol, arginine. Over 60% of peanut produced worldwide is crushed for oil extraction while, 40% is used in food purpose and others (Birthal et al. 2010). Several fatty acids are present in peanut oil, of which palmitic, a saturated acid (7–12%), and unsaturated fatty acids viz., linoleic (25–35%) and oleic (40–50%) together make up about 90% of the total fats (Arya et al. 2016; Bera et al. 2018; Kamdar et al. 2020). Also available are high oleic lines with more than 80% oleic acid. There is a growing demand in the international market for peanut and peanut derived products, especially in confectionary use. The most popular peanut commodity in the Australia, Canada and USA, is peanut butter. Peanut kernels can either be eaten raw or roasted or boiled and can also be used to make baked and confectionary products. Peanut, as a legume crop, also helps to improve soil health quality and fertility by leaving organic matter and N_2 back in the soil.

Although the domesticated peanuts originated in region of southern part of Bolivia and north-western Argentina (Simpson et al. 2001), but 95% of peanut area globally is concentrated in Asia and in Africa in the semi-arid tropical regions (SAT) where small and marginal farmers grow the crop under rain-fed conditions (FAO 2017). Moreover, climate change leads to the ocurrence of number of biotic stresses that severely affects crop yield and productivity (Pandey et al. 2015). Nearly 75–80% of the world's peanuts are cultivated in developing countries by smallholder farmers who normally harvestpod yield of 500–800 kg ha^{-1} compared to the ptential yields of more than 2.5 ton per hectare. Low yields are mainly due to various diseases caused by nematodes, bacteria viruses and fungi (Kokalis-Burelle et al. 1997; McDonald et al. 1998). Major fungal diseases that target foliages are rust and leaf spots (early leaf spot and late leaf spot). Major fungal diseases that infect seed and seedlings are crown rot or *Aspergillus* crown rot, dipodia collar rot, yellow mold, damping off by *Rhizoctonia* spp., and smut. The major diseases affecting roots, stems, and pods include *Sclerotinia* root rot, *S.* blight, *Botrytis* blight, pod rot, *Fusarium* wilt, and charcoal rot. The major viral and mycoplasmal diseases are bud necrosis, stem necrosis, peanut mottle, peanut clump, peanut stripe, tomato spotted wilt, peanut rosette and stunt. Two major bacterial diseases are bacterial leaf spot and bacterial wilt. Peanut is also attacked by nematodes and certain insect-pests viz., *Spodoptera*, *Helicoverp*a, leaf miner, white grubs, aphids, thrips and jassids.

Good success has been achieved in peanut by conventional breeding approaches but the process is laborious and time consuming. The improved varieties of peanut with high production potential and resistance against biotic agents were developed and released for cultivation worldwide. A huge repository of variation of the cultivated peanut is present as germplasm accessions in the gene banks. The largest collection of peanut germplasm is being held at ICRISAT, India (15,445 accessions) followed by ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR) with 14,585 accessions; ICAR-Directorate of Groundnut Research (ICAR-DGR) in India with 9024 accessions; 9917 accessions at the U.S. Department of Agriculture

(USDA); Oil Crops Research Institute (OCRI) of the Chinese Academy of Agricultural Sciences (CAAS) with 8083 accessions and 4210 accessions in China at the Crops Research Institute of the Guangdong Academy of Agricultural Sciences. Further, few to medium germplasm collections are held at the North Carolina State University (NCSU) and Texas A & M University (TAMU) in the USA; Brazil, at the Instituto Agronomico de Campinas and EMBRAPA-CENARGEN; and Instituto de Botánica del Nordeste (IBONE) and in Argentina, Instituto Nacional de Technologia Agropecuaria (INTA). Mostly, wide hybridization is being used to tap the usable genes from wild species (Kalyani et al. 2007; Stalker; Malikarjuna; Bera et al. 2010). However, genetic bottleneck in historical origin of the polyploid peanut from natural cross between the diploid ancestors *A. ipaensis* and *A. duranensis* followed by duplication of chromosome limits the available genetic diversity (Kochert et al. 1996). This limits the success of traditional breeding methods. Morever, the unlimited potential of wild species and wild forms, a reservoir of novel and useful alleles, remains under-utilized due to genetic barrier in introgression of genes into elite genotypes, compounded with the transfer of undesirable gene blocks. With the development of genetic linkage maps followed by marker discovery and identification of quantitative trait loci (QTLs) and genetic mapping of the target traits peanut improvement programm has accelerated during the last decade.

However, the impacts of climate change can be seen all over the world, stressing the urgent need for designing climate-smart (CS) crops to be able to cope-up these unfavorable conditions and aid in sustaining agriculture in order to achieve food and nutritional security. For improvement of two or more traits simultaneously, it is important to identify markers for important traits and use them in breeding programme. The cultivated peanut (*A. hypogaea*) is an allotetraploid (AABB) with a total genome size of 2.7 Gb formed from closely related sub genomes (Bertioli et al. 2016). Peanut genomic tools, such as molecular markers (Wang et al. 2012; Bosamia et al. 2015), genetic/linkage maps (Gautami et al. 2012b), and genome sequences of cultivated and progenitors species (Bertioli et al. 2019; Chen et al. 2019; Zhuang et al. 2019), have rapidly developed in the last decade. These advanced genomic tools and resources have facilitated the use of modern genetics and breeding methodologies such as genome-wide association studies (GWAS) for mapping multigenic trait and genomic selection (GS) for improvement of peanut crop. Genomic selection is one approach to broaden the genetic diversity by mining usable alleles from the wild species, landraces or wild relatives. An integrated breeding strategy is needed that will allow multiple desirable alleles to be selected facilitating pyramiding of number of genes as well as the deployment of GS approaches. Moreover, the transgenic approaches are being followed worldwide for the peanut improvement. Several useful genes either from wild species or synthetic genes could be transferred into established cultivars (Tiwari et al. 2008; 2011; Mehta et al. 2013; Sarkar et al. 2014, 2016; Bala et al. 2016; Patil et al. 2017; Bhalani et al. 2019). This chapter describes the major biotic constraints to peanut production (Table 4.1) and reviews the stages and extent of damage, and management options. It also reviews the genetic resources available, and the conventional and molecular breeding approaches to mitigate the

Table 4.1 Major biotic constraints to peanut production

S. No.	Disease	Causal organism	Distribution
1	Early leaf spot	*Cercospora arachidicola*	Worldwide
2	Late leaf spot	*Phaeoisariopsis personata Cercosporidium Personatum*	Worldwide
3	Rust	*Puccinia arachidis*	Worldwide
4	Web blotch	*Phoma arachidicola*, *Didymella arachidicola*	Angola, Argentina, Australia, Brazil, Canada, China, Commonwealth of Independent States, Japan, Lesotho, Malawi, Nigeria, South Africa, Swaziland, USA, Zambia, and Zimbabwe
5	Scab	*Sphaceloma arachidis*	Argentina, Brazil, Japan, and Swaziland
6	*Alternaria* leaf spot and veinal necrosis	*Alternaria alternate*	India, Vietnam, and Thailand
7	*Phyllosticta* leaf sPot	*Phyllosticta arachidis-hypogaea*	Burkina Faso, India, Malawi, Mozambique, Niger, Nigeria, Swaziland, Thailand, and Zimbabwe
8	Powdery mildew	*Oidium arachidis*	India and Israel
9	*Cercospora* leaf blight	*Cercospora canescens*	Thailand
10	*Myrothecium* leaf blight	*Myrothecium roridum*	India and Thailand
11	Zonate leaf spot	*Cristulariella moricola*	India, Thailand, and USA
12	*Sclerotium* leaf spot	*Sclerotium rolfsii*	India, Malawi, and Thailand
13	*Choanephora* wet blight	*Choanephora cucurbitarum*	Thailand and Philippines
14	Pepper spot and leaf scorch	*Leptosphaerulina crassiasca*	Angola, Argentina, Burkina Faso, India, Madagascar, Mauritius, Malawi, Mozambique, Niger, Nigeria, Senegal, Swaziland, Thailand, Taiwan, USA, Vietnam, Zambia, and Zimbabwe
15	Anthracnose	*Colletotrichum arachidis*, *C. dematium*, *C. mangenoti*	India, Niger, Nigeria, Sudan, Senegal, Taiwan, Tanzania, Thailand, Uganda, and USA
16	*Alternaria* leaf blight	*Alternaria alternate*, *A. tenuissima*, *A. arachidis*	India, Nigeria, and Thailand
17	*Pestalotiopsis* leaf blight	*Pestalotiopsis arachidis*	India, Nigeria, and Thailand
18	*Aspergillus* crown rot/collar rot	*Aspergillus niger*	Worldwide

(continued)

Table 4.1 (continued)

S. No.	Disease	Causal organism	Distribution
19	Yellow mold	*Aspergillus flavus*	Worldwide
20	*Diplodia* collar rot	*Lasiodiplodia theobromae*	Australia, India, Israel, South Africa, Thailand, USA, and Venezuela
21	*Rhizoctonia* damping-off	*Rhizoctonia solani*	Worldwide
22	Stem rot	*Sclerotium rolfsii*	Worldwide
23	*Sclerotinia* blight	*Sclerotinia minor*, *S. sclerotiorum*	Argentina, Australia, China, Taiwan, USA, and Zimbabwe
24	*Cylindrocladium* black rot	*Cylindrocladium crotalariae*	Australia, India, Japan, and USA
25	*Botrytis* blight	*Botrytis cinerea*	Australia, Commonwealth of Independent States, Japan, Malawi, Romania, South Africa, Swaziland, Tanzania, USA, Venezuela, Vietnam, and Zimbabwe
26	*Verticillium* wilt	*Verticillium albo-atrum*, *V. dahlia*	Argentina, Australia, Israel, and USA
27	*Fusarium* wilt	*Fusarium oxysporum*	Worldwide
28	Charcoal rot	*Macrophomina phaseolina*	Worldwide
29	Black hull/black pod rot	*Thielaviopsis basicola*, *Chalara elegans*	Israel, Argentina, Italy, South Africa, and USA
30	Pod rot	*Pythium myriotylum*, *Rhizoctonia solani*, *Fusarium solani*, *Fusarium oxysporum*, *Macrophomina phaseolina*	Worldwide
31	Bacterial wilt	*Ralstonia (Pseudomonas) solanacearum*	Angola, China, East Indies, Ethiopia, Australia, Fiji, Indonesia, Sri Lanka, Libya, Madagascar, Malaysia, Mauritius, Nigeria, Papua New Guinea, Philippines, Somalia, South Africa, Swaziland, Taiwan, Thailand, Uganda, USA, Vietnam, Zambia, and Zimbabwe
32	Bacterial leaf spot	*Unidentified bacterium*	India and Vietnam
33	Peanut mottle virus	*Peanut mottle virus*	All peanut-producing countries in Africa, the Americas, Asia, and Oceania

(continued)

Table 4.1 (continued)

S. No.	Disease	Causal organism	Distribution
34	Peanut stripe virus	*Peanut stripe virus*	Most peanut-producing countries in South and Southeast Asia, and USA
35	Peanut clump virus	*Peanut clump virus*	India and West Africa. Probably several other countries in Asia
36	Peanut bud necrosis	*Peanut bud necrosis virus*	South and Southeast Asia
37	Tomato spotted wilt virus	*Tomato spotted wilt virus*	Africa, the Americas, Australia and Europe
38	Stem necrosis	*Tobacco Streak Virus*	India, Australia, Brazil
39	Peanut rosette disease virus	A complex of two viruses (*Peanut rosette assistor virus*, *Peanut rosette virus*) and a satellite RNA	Sub-Saharan Africa, Madagascar
40	Peanut stuntvirus	*Peanut stunt virus*	North America and southern China
41	Peanut streak necrosis virus	*Sunflower yellow blotch virus*	Southern Africa
42	Cowpea mild mottle virus	*Cowpea mild mottle virus*	Asia and Africa
43	Peanut yellow spot virus	*Peanut yellow spot virus*	Thailand and India
44	Witches' broom	*Mycoplasma-like* (*organism MLOs*)	Burkina Faso, China, India, Indonesia, Japan, Niger, Taiwan, Thailand, and USA
45	Root-knot nematode	*M. arenaria*, *M. hapla*, *M. javanica*, *M. incognita*	*M. arenaria*: Egypt, India, Israel, Malawi, Senegal, Taiwan, USA, and Zimbabwe *M. hapla:* Australia, China, India, Israel, Japan, South Africa, South Korea, USA, and Zambia. M. javanica: USA, *M. incognita*: USA
46	Root-lesion nematode	*Pratylenchus brachyurus*	Australia, Benin, Egypt, Gambia, India, Nigeria, Senegal, Thailand, USA, and Zimbabwe
47	Kalahasti malady	*Tylenchorhynchus brevilineatus*	India
48	Peanut smut	*Thecaphora frezii*	Argentina

Source http://oar.icrisat.org/7190/1/IB_PeanutDiseases-2012.pdf

effect of biotic stresses. This chapter provides updates on QTL mapping for economically important traits. In addition, we also discussed identification of SNPs linked to gene/QTLs based on next generation sequencing (NGS) approaches.

4.2 Description of Different Biotic Stresses

4.2.1 Fungal Diseases

4.2.1.1 Foliar Fungal Diseases

Stages and extent of damage

Peanut rust (*Puccinia arachidis* Speg, the causal agent) is a serious foliar disease. The pathogen *P. arachidis* is host-specific and known to produce at both uredial and telial stages. It is, however, almost entirely known for its uredial stage, which is abundant. The pathogen spreads quickly by repeated infection cycles of wind-borne inocula of uredospores (Hennen et al. 1976). It is characterized by orange-red/brown-colored, circular to elliptical pustules (uredinia) ranged in size from 0.3 to 2.0 mm in diameter on the lower surface of the leaves. Though uredia are the main stage of the infection cycle, there are also a few records of the occurrence of the telial stage. Telia chiefly occur on the under surface of peanut leaves (Bromfield 1971). Teliospores are light or golden yellow spores with acute to rounded and thickened apex that are oblong, obovate, ellipsoid, or ovate in shape. They germinate at maturity without a dormancy phase. Rust causes significant yield loss to peanut globally (Subrahmanyam and McDonald 1983). However, disease incidence and severity vary with locations and seasons. The pathogen can cause up to 57% economic damage to the peanut crop when environment is warm and humid (Subrahmanyam and McDonald 1987). Under favorable conditions and the presence of susceptible cultivars, however, rust-related losses can reach to 70% (Subrahmanyam et al. 1985a, b, c; Dwivedi et al. 2002a). Rust losses are compounded if the crop is also affected by leaf spots, such as early leaf spot caused by fungus, *Cercospora arachidicola* and late leaf spot caused by fungus *Phaeoisariopsis personata*, which can result in yield losses of up to 70% (Nutter and Shokes 1995; Shokes and Culbreath 1997). Both pathogens are soil-borne, with conidia produced directly from mycelium in crop debris in the soil, deposited on the first-formed leaves, and then carried to later-formed leaves and other plants by rain splash, wind and insects. Ascospores, chlamydospores, and mycelial fragments, on the other hand, are possible inoculum sources. On volunteer peanut plants and infected crop debris, early and late leaf spot pathogens can survive from season to season. Outside of the *Arachis* genus, no host species has been identified. The early leaf spot pathogen's telemorph and telemorphs of late spot pathogens, *Mycosphaerella arachidis* Deighton and *Mycosphaerella berkeleyi* Jenk, respectively are rarely seen on peanut. Leaf spots damage the plant by causing lesion formation and inducing leaflet abscission, both of which reduce the total photosynthetic area

of the plant (Fig. 4.2). *Cercospora arachidicola* forms subcircular lesions of more than one mm in diameter (Tshilenge 2010). Most sporulation occurs from the lesions on the upper leaf surface where dark brown with always yellow halos, and a lighter shade of brown lesions are formed on the lower leaflet surface. Lesions caused by *Phaeoisariopsis personata* are usually small in size, more nearly circular, and darker (black) and slightly rough than those of *C. arachidicola*, usually do not have yellow halos and most sporulation occurs on the lower surfaces. In addition to leaf spots, these pathogens cause lesions on all above-ground sections of the plant, including stipules, petioles, roots, and pegs (Subrahmanyam et al. 1982a, b).

Fig. 4.2 Wild Arachis sp. infected with *Alternaria* leaf blight

Management

Between successive crops, a fallow period of at least one month should be observed. Crop rotations involving cereals or other non-host crops are successful in preventing disease spread (Mondal et al. 2014a, b). To avoid inoculum buildup and carryover, volunteer peanut plants should be eradicated, sowing times should be planned to avoid contamination from outside, and environmental conditions conducive to the disease should be avoided. Maintaining field sanitation by weeding and proper plant spacing should be added to this (Kokalis-Burelle et al. 1997). Since leaf spot pathogens are primarily soil-borne, crop rotation out of peanuts for 2–3 years and burial of peanut crop residues are used to reduce inoculum load. Leaf rust can be managed with a variety of fungicides and fungicide mixtures. Chlorothalonil, tridemorph, mancozeb-zinc combinations, hexaconazole, strobilurinsterol-inhibitors, and other sulphur-based fungicides are effective in reducing peanut rust incidences (Kokalis-Burelle et al. 1997). Benomyl, chlorothalonil, copper hydroxide, fentin hydroxide, maneb and mancozeb, sulfur, copper/sulpher dusts, propiconazole, and tebuconazole are some chemicals that are being used to reduce the threat due to leaf spot epidemics (Smith and Littrell 1980).

Several biological agents *viz.*, *Acremonium persicinum*, *A. obclavatum*, *Eudarluca caricls*, *Penicillium islandicum*, *Tuberculina costaricana* and *Verticillium lecanii* have been reported significantly inhibiting *invitro* germination of rust spores (Ghewande 1990). Also, pre-treatment with conidia of *T. harzianum* has shown to significantly inhibit germination percentage and germtube growth of *P. arachidis* (Govindasamy and balasubramanian 1989). *Fusarium chlamydosporum*, a mycoparasite that releases chitinase capable of cell wall lysis of fungi can also act as a biocontrol agent (Mathivanan et al. 1998). However, no serious or significant attempts have been made in the field to use any of these species for controlling peanut rust biologically. Mycoparasites, *Dicyma pulvinata* and *Verticillium lecani*, *Acremonium obclavatum*, *Fusarium spp* and *Penicillium spp* are also known to parasitize the leaf spot pathogens. In glasshouse trials, *Pseudomonas* spp., which has broad-spectrum antifungal activity, was also found to significantly reduce late leaf spot (Haas and Keel 2003). Further, foliar spray of chitinolytic bacteria, *B. circulans* and *S. marcescens* for control of LLS of peanut has been documented (Kishore et al. 2005).

4.2.1.2 Fungal Diseases Affecting Stem, Root and Pod

The major fungal diseases attacking root, stems, and pods include *Sclerotium*/Stem rot, *Sclerotinia* blight and *Botrytis* blight, *Fusarium* wilt, pod rot and charcoal rot.

Stages and extent of damage

Stem rot/white mold/southern blight of peanut is caused by a soil dwelling necrotrophic fungal pathogen, *Sclerotium rolfsii*. It is one of the most severe biotic stresses that can affect peanuts, and it is most prevalent in the tropics and subtropics regions and other temperate regions of the world with warm and humid climates

Fig. 4.3 Artificially inoculated peanut field with *Sclerotium rolfsii* for screening resistance to stem rot

(Deepthi and Reddy 2013). *Sclerotium rolfsii* is a deuteromycete fungus belonging to the group "*Mycelia Sterilia*" (Alexopoulos et al. 1962). Although the basidiomycete *Athelia rolfsii* (Cruz) Tu and Kimbrough has been described as the sexual stage of *S. rolfsii*, but it is very rarely seen in the peanut field (Tu and Kimbrough 1978). White mycelia and round, brown sclerotia with diameters ranging from 0.5 to 2 mm distinguish the fungus (Figs. 4.3, 4.4 and 4.5). In the absence of a host, it persists for several years as mycelia in crop debris and as sclerotia in the soil (Punja 1985). The pathogen does not produce any asexual spores. The pathogen primarily infects stems, but it also targets leaves, pods, and other plant parts, resulting in severe damage at all stages of crop growth. Chlorosis and/or wilting of a lateral branch are the first signs of infection; however, if the main stems become infected, the entire plant may appear wilted or chlorotic (Backman and Brenneman 1997). By forming oxalic acid and cell-wall degrading enzymes, stem rot fungus kills plant tissues before colonization (Cilliers et al. 2000; Ganesan et al. 2007). If the fungal pathogen attacks the pods, they develop a brown rot that appears mashed and water-soaked (Punja 1985). Stem rot causes yield losses that typically range from 10 to 40%, but can reach up to 80% in heavily infected fields (Mehan and McDonald 1990; Akgul et al. 2011; Bera et al. 2014a; 2016a).

The soil-borne fungi *Sclerotinia minor* Jagger and *Sclerotinia sclerotiorum* (Lib.) de Bary trigger *Sclerotinia* blight. S*clerotinia* blight is a devastating peanut disease marked by thick tufts of white mycelium and broad, irregularly formed sclerotia. It is a economically significant disease that causes significant yield losses and affects kernels quality. The loss of yield due to disease occurrence is estimated to be 10%,

Fig. 4.4 Sclerotia of *Sclerotium rolfsii* on a heavily infected peanut plant

Fig. 4.5 Peanut plant and pods damaged by *Sclerotium rolfsii*

but in extreme cases, it may be as high as 50% (Porter and Melouk 1997). Sclerotia of *Sclerotinia minor* are often small and abundant, while those of *Sclerotium sclerotiorum* are large and less abundant. Peanut is contaminated by mycelia from germinating sclerotia in majority of the cases. The plant finally dies, and sclerotia proliferate on the dead tissue in large numbers. Some sclerotia are shed from plant tissue into the soil or may be preserved as overwintering inoculum on dead plant tissue. Sclerotia germinate into mycelium or apothecia under ideal conditions. *Sclerotinia minor* and *Sclerotinia sclerotiorum* are ascomycetes. One or more pale orange to white apothecia (sexual stage) may emerge from a single sclerotium. The fruiting body produces ascospores that range in size from 8–17 × 5–7 μm (Porter and Melouk 1997). Watery lesions appear on all infected tissues, including pegs and pods, and the tissues are quickly coated with white fluffy mycelium. On roots, pegs, and pods, yellowish-brown bleached lesions appear after mycelium penetrates the tissues. The stems become girdled and die, and the leaves become chlorotic and necrotic (Backman and Brenneman 1997).

Botrytis blight is also known as gray mold of peanuts and is due to fungus, *Botrytis cinerea* that occurs only sporadically in cold, wet weather. *Botrytis cinerea* Pers.: Fr. (anamorph) belongs to molds/deuteromycete class that rapidly colonizes plants. The fungus can cause plant tissue as well as the entire plant to wilt and die. Blight caused by *B. cinerea* is marked by the abundance of conidia and sclerotia produced on infected plant sections. The fungus overwinters as massive sclerotia, which are irregular structures and colored dark-brown to black (Porter 1997). The ascomycetous stage of *Botrytis* blight, *Botryotinia fuckeliana* (de Bary) Whetzel, is rarely spotted. Mycelium, which comes from germinating sclerotia or conidia, is the primary source of inoculum. *Botrytis* blight is not a serious peanut disease, and the damage it causes is generally minor. Several *Pythium* spp., specifically *P. myriotylum*, *P. irregulare*, and *P. ultimum* (Wheeler et al. 2005), have been found to be associated with diseased peanuts, causing damage to the pod and kernels, as well as substantial yield loss of up to 80% (Beute 1997). Peanut damping-off, root rot and vascular wilt may all be caused by *Pythium* spp. Peanut pod rot is an economically significant disease that affects the quality and yield potentiality of the crop. *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Pythium* spp. are the most common soil-borne mycelial pathogens that cause pod rot (Kokalis-Burelle et al. 1997). *Pythium* spp. caused pod rot is marked by browning and water-soaking of pods in the early stages, accompanied by a brown to black appearance in the later stages (Wells and Phipps 1997). *Pythium* spp. are fungi with white fluffy mycelia that produce sporangia, asexual reproductive structures that germinate by forming motile zoospores. Sexual spores *i.e.*, oospores serve as the primary survival structure of *Pythium* species. Due to the lack of above ground symptoms, it's difficult to estimate yield losses caused by *Pythium* pod rot, but losses of up to 80% have been recorded (Beute 1997). *Rhizoctania solani* Kühn is another soil-borne pathogen capable of causing seed decay, damping off, root rot, limb rot, and pod rot (Garren 1970). The anamorph, *Rhizoctonia solani* Kühn, is a Deuteromycete that does not produce asexual spores and the teleomorph, *Thanatephorus cucumeris*, is a Basidiomycete. Pigmented and septate hyphae, as well as non-differentiated sclerotia, are found on plant debris that germinate to infect host tissues (Brenneman

1997). *Rhizoctonia* pod rot is distinguished by a dry, brown or russet-colored rotted pod, as opposed to *Pythium* spp that form dark greasy-appearing lesions. Pod rot, caused by *R. solani*, can result in yield losses of 22–28% in favorable environmental conditions (Besler et al. 2003). Another soil-borne fungus, *Fusarium solani*, is involved in pod rot, as a predisposing factor as well as one of the saprophytic fungus that aggravates the pod's final breakdown. *Fusarium* spp. reproduces on plant debris and lives saprophytically in soil. Conidia are formed in abundance but are short-lived. Chlamydospores are the long lasting survival structures (Frank 1972; Garcia and Mitchell 1975). *F. solani* makes pods more susceptible to *Pythium myriotylum* infection. Later colonization of pods by *P. myriotylum* is accompanied by rapid increase in pod rot. Finally, pod disintegration is caused by *F. solani* and saprophytic species.

Management

The key technique for controlling stem rot is to prevent inoculum build-up. Disease build-up can be reduced by deep plowing, weed control, and crop rotation with corn or grain sorghum (Backman and Brenneman 1997). Excess canopy growth and irrigation should be avoided because they encourage disease development. Solar heating of moistened soils under a polyethylene tarp, combined with the application of *Trichoderma harzianum*, reduces *S. rolfsii* disease (Grinstein et al. 1979). To reduce *Sclerotinia* disease incidence, it is strongly recommended to minimize damage to peanut plants caused by farm machinery and other mechanical means (Porter et al. 1982). To avoid fungal colonization due to frost damage, *Botrytis blight* should be managed to a large extent by avoiding excessive irrigation, good drainage, mulching, and planting early maturing peanut varieties. Overwatering and flooding should be prevented because *Pythium* spp. forms motile zoospores that travel in water. Peanut rotation with grasses like corn, sorghum, or other pasture grasses may help minimize *Pythium* spp. and *R. solani* (Baird et al. 1995; Brenneman 1997). Rotation of crops has also been shown to minimize *Pythium* spp. inoculum density while having little impact on disease incidence (Beute 1997).

Numerous fungicides are known to inhibit the germination of sclerotia or the mycelia growth of various fungi. To combat stem rot, pentachloronitrobenzene (PCNB) and carboxin have been used. Tebuconazole and other sterol-inhibiting triazole-type fungicides have provided more than 80% control on stem rot (Backman and Brenneman 1997). Propiconazole and flutolanil also offer excellent control of stem rot (Csinos 1987; Grichar 1995). Pruning of peanut vines along with the application of benomyl is reported to control stem rot (Backman 1975). Further, fumigation of soils with methyl bromide, chloropicrin, or metham-sodium is toxic to sclerotia (Elad et al. 1980). Fungicides such as iprodione and fluazinam are known to control *Sclerotinia* blight disease (Bailey and Brune 1997; Butzler et al. 1998). The use of fungicide chlorothalonil against leaf spots should be avoided because it has been shown to trigger *S. minor* to germinate (Beute and Rodriguez-Kabana 1979). However, under conditions conducive to *Sclerotinia blight* chlorothalonil is highly effective and widely used to control the disease. Some protection against *B. cinerea* is

provided by foliar sprays with fungicides including benomyl and chlorothalonil. Iprodione also inhibits the spores germination and inhibit the growth of fungus (Langston et al. 2002). Pesticides such as PCNB and metalaxyl, also have inhibitory activity on *Rhizoctonia* and *Pythium* spp., respectively (Filonow and Jackson 1989). Tebuconazole and Azoxystrobin are systemic fungicides with a wide spectrum of activity that can be used to control R. *solani* (Baird et al. 1991; Brenneman 1997). Metalaxyl and mefenoxam may be effective against oomycetes including *Pythium* spp. (Filonow and Jackson 1989; Lewis and Filonow 1990). High rates of gypsum application at flowering are recommended. In certain areas, the application of high doses of gypsum greatly reduced pod rot caused by *P. myriotylum* (Alva et al. 1989). It is well established that adequate calcium nutrition in the soil is critical for pod rot control (Walker and Csinos 1980; Csinos et al. 1984). Fungicides such as Tebuconazole and flutolanil or fluazinam offer an effective chemical control against *Rhizoctonia* induced pod rot. *Fusarium* populations are selectively suppressed by soil solarization and treatments of soil with biocide metham sodium in sublethal doses. Biological control with antagonistic fungi have also been demonstrated. The fungi *Trichoderma harzianum*, *T. viride*, *T. hamatu*, *T. koningii* and *Pseudomonas fluorescens* have successfully suppressed stem rot severity. They inhibit mycelia growth of the pathogen and suppress sclerotial formation (Karthikeyan et al. 2006; Kwee and Keng 1990). *Talaromyces flavus* parasitized hyphae as well as sclerotia of *S. rolfsii* (Madi et al. 1997). *T. harzianum* proved to be the most efficient biocontrol agent against *S. typhimurium*. When compared to other possible biocontrol agents, *T. harzianum* comes out to be the most effective biocontrol agent to control *S. rolfsii* (Kulkarni and Kulkarni 1994). Further, soil inoculation with *Rhizobium* reduced the population of *S. rolfsii* in the rhizosphere (Bhattacharyya and Mukherjee 1990). *P. fluorescens*, *P. aeruginosa*, *Serratia marcescens* and *B. subtilis* are also antagonistic to stem rot fungus where, *P. aeruginosa* completely inhibited the growth of *S. rolfsii* by producing a siderophore (Podile et al. 1988; Ordentlich et al. 1987). Antagonistic species such as *Gliocladium spp.*, *Penicillium spp., Sporodesmium spp.*,*Talaromyces spp.*, and *Trichoderma spp.*, release compounds such as chitinases, and β-1, 3-glucanases which are enzymes that can pierce the cell walls and cause complete cell death, and also attack on sclerotia of *S. minor* (Sherwood et al. 1995). *Teratosperma oligocladum* and *Sporidesmium sclerotivorum* effectively reduce the survival of sclerotia of *S. minor* in soil (Bullock et al. 1986; Adams 1989; Adams and Wong 1991). *Coniothyrium minitans*, another biocontrol agent, disrupts the life cycle of *Sclerotinia* by targeting the sclerotia and rendering the sclerotia useless as inocula (Jones et al. 1974). *Trichoderma harzianum*, a competitive fungus is also effective against gray mould. A *Gliocladium* species has been known to parasitize conidia, conidiophores and sclerotia of *Botrytis*. The hyperparasites, *Botryotrichum piluliferum*, *Coniothyrium sporulosum*, *Dicyma olivacea*, *Gliocladium catenulatum*, *Stachybotrys chartarum*, *Stachylidium bicolor*, *Stachybotrys elegans*, *Trichothecium roseum*, *Verticillium chlamydosporium*, *V. tenerum*, and *V. bigguttatum* parasitize the hyphae of *Rhioctonia. G. virens*is is known to colonize mycelia as well as sclerotia of *R. solani* (Turhan 1990; Morris et al. 1995; Bertagnolli et al. 1996). In the presence of *T. harzianum*, the growth of *R. solani* was significantly slowed (Tu and Vaartaja

1981). *Pseudomonas fluorescens*, *P. aeruginosa*, *B. subtilis* and *B. megaterium* also inhibit the growth of *R. solani* (Savithiry and Gnamanickam 1987; Podile et al. 1988; Turner and Backman 1991; Badel and Kelemu 1994).

4.2.1.3 Fungal Diseases Affecting Seed and Seedlings

Major fungal diseases that affect seed and peanut seedlings include collar rot or *Aspergillus* crown rot caused by *Aspergillus niger*, yellow mold caused by *Aspergillus flavus*, diplodia collar rot caused by *Lasiodiplodia theobromae* and *Verticillium* wilt.

Stages and extent of damage

Collar rot or seedling blight or crown rot is caused by the fungus *Aspergillus niger* Tiegh., a necrotrophic fungus that exists in an anamorph stage in soil and on crop residues. Soil-borne conidia attack seeds and cause rotting. Infected seeds are covered with masses of conidia and fail to germinate (Subrahmanyam et al. 1992). The pathogen attacks the emerging young seedling and brown discolored spots appear on the collar region. The affected portion becomes soft causing yellowing of lower leaves, blighting of the shoot, finally leading to the death of the crown (Suzui and Makino 1980). While rotting of seeds and preemergence damping-off are general symptoms, infection may also affect mature plants. Large lesions form below the soil line on the stem and spread upwards along the branches, causing leaf drooping and sudden wilting in young plants. The pathogen lives in soil plant litter. The percentage of plants that die as a result of collar rot varies between 28 and 50% (Ghewande et al. 2002).

Yellow mold is a seedling disease caused by the saprotrophic and pathogenic fungus *Aspergillus flavus*. It lives in the soil on organic sources of nutrients in the form of mycelia and resistant structure sclerotia. These structures germinate directly to either produce mycelia or give rise to conidiophores and conidia. Both mycelia and conidia serve as the primary sources of inocula (Scheidegger and Payne 2003). *A. flavus* has an extraordinary ability to colonize seeds. The mold causes pre-emergence rotting of seed, reduce seed viability and germination and causes seedlings to rot (Kumar et al. 2012). After seedlings emerge, infection is mainly confined to the cotyledons. The diseased plants are chlorotic and stunted. Aflatoxin, a form of secondary metabolite produced by the pathogen, is the most toxic carcinogen among known mycotoxins. (Calvo et al. 2002; Klich 2007; Krishnamurthy et al. 2008). As a result, either by killing the plant or by contaminating peanut kernels with aflatoxins, which are then either unmarketable or cause significant health issues to both human and animals that consume contaminated kernels.

Diplodia collar rot of peanut, caused by the soil-borne saprophyte *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl. and by *Diplodia gossypina* are known to cause wilting in immature and mature plants (Porter and Garren 1968). For long periods of time, mycelia and mature conidia of the fungus may be found dormant in soil and plant debris. Heat-stressed peanut plant tissue is more susceptible to *D. gossypina* colonization. Mycelia originating from germinating or mature conidia and

mycelial fragments may cause primary infection. Necrotic areas that are elongated and characterized by light brown centers with dark brown margins are formed on above ground stems. On the surfaces of necrotic tissues, single or compound pycnidia can be seen individually or in groups. Diplodia collar rot occurs infrequently around the world, causing only minor economic losses. Collar rot normally causes yield reductions of less than 1%, but reductions of 25% or more have been recorded (Porter and Phipps 1994).

Verticillium wilt is caused by *Verticillium dahlia* Kleb., which can survive in the soil as microsclerotia for long periods of time. White fluffy mycelia and hyaline single cellular conidia are also produced by the fungus. The fungus infects the host plant systemically by entering the roots and spreading through the xylem, causing vascular discoloration in the crowns, stems, roots, and petioles (Melouk and Damicone 1997). Plant death is preceded by general yellowing, defoliation, leaf necrosis on margins, wilting, general stunting, and dehydration as the disease progresses (Purss 1961; Melouk and Wadsworth 1990).

Management

Irrigation and weed management can be effective in reducing fungal disease. Irrigation alleviating drought stress or early harvest to escape drought are the best control measures for minimizing aflatoxin contamination. Furthermore, planting noninfected, high-quality seeds are the safest way to prevent seed and pre-emergence seedlings rotting caused by A. *flavus.* Diplodia collar rot incidence can be reduced by rotating peanut with crops other than hosts. Furthermore, by manipulating row orientation and maintaining adequate foliage during the growing season, heat induced injury to basal stems of plants can be minimized, and disease severity can be reduced. High temperatures and moisture tension exacerbate the severity of *Verticillium wilt.* As a consequence, infested fields should be irrigated on a daily basis. It's also a good idea to plant V*erticillium*-free seed. Since certain weeds are also susceptible to *V. dahliae*, weed control may help reduce the occurrence of *Verticillium* wilt. Peanuts grown in the presence of nonhost crops like grain sorghum/ Sudan grass produce less wilt than peanuts grown in the presence of susceptible crops like cotton, okra, or peanut. *Verticillium dahliae* has a longer lifetime in the soil than microsclerotia, and short-term crop rotations have no effect on their levels.

Triazole compounds including propiconazole, tebuconazole and difenconazole, carbendazim, carboxin and captan are known to inhibit the mycelial growth and spore production of the collar rot fungus. *Verticillium* wilt cannot be regulated with chemicals. While metham sodium applied via sprinkler irrigation has been effective in controlling the disease in sandy soil (Krikun and Frank 1982).

Biological control has shown to control infection with varying degree of success. *Trichoderma* spp (Harman et al. 1981), *Bacillus* spp. (Capper and Campbell 1986) and *Pseudomonas* spp (Vidyasekharan and Muthamilan 1995) are known to be antagonistic are used to control the crown root fungus with varying degrees of success. In soil treated with *T. harzianum* at both the seedling stage and vegetative growth stage, disease incidence was reduced (Garren et al. 1969; Harder et al. 1979). Further, the treatment of peanut seeds with *Bacillus subtilis* significantly controls crown rot

(Podile and Prakash 1996). *Streptomyces* spp. have a strong antagonistic effect on the growth and development of *Aspergillus* (Zucchi et al. 2008; Zhang et al. 2013). Also, the bio-control agent, *Trichoderma harzianum*, and *T. viride* are known to control *A. flavus* infection as they showed the ability to parasitize *A. flavus* by coiling around its hyphae (Chiuraise et al. 2015). *A. shirousamii* lessen the formation of mycotoxin-aflatoxin by *A. flavus* (Kim and Kim 1986). An atoxigenic strain of *A. parasiticus* is used as a competitive agent to reduce aflatoxin contamination in peanut kernels (Dorner et al. 1992). More recently, pre-harvest aflatoxin contamination of peanut has been effectively be controlled by use of commercial products namely, AflaGuard and Aflasafe derived from atoxigenic strains in the United States (Luis et al. 2017). *A. flavus* produced less aflatoxins in peanut kernels when *Flavobacterium odortum* was present, and *Pseudomonos cepacia* absolutely stopped *A. flavus* from growing (Chourasia 1995; Misaghi et al. 1995). Treatment with a mixture of chitosan or *Bacillus* reduced the growth of *A. flavus* (Cuero and Osuju 1991).

4.2.2 Bacterial Diseases

Two major bacterial diseases are bacterial wilt and bacterial leaf spot.

Stages and extent of damage

Ralstonia solanacearum (Smith) causes bacterial wilt, which is a severe global disease and poses a serious risk to peanut production in many wet and humid regions. *Ralstonia solanacearum* is a aerobic, rod-shaped, and gram-negative bacterium that does not form any spores and accumulate poly-p-hydroxybutyrate as a carbon source (Hayward and Hartman 1994). The phenotypic properties of *R. solanacearum* are heterogeneous, and it has been grouped into five biovars based on its ability to use unique carbon sources. Biovars 1, 3, and 4 have been identified as peanut pathogens. *R. solanacearum* isolates have been tentatively classified into five groups, with race 1 being known in peanut (He et al. 1983). This soil-borne pathogen infects plant roots through lesions/wounds and spreads easily through the conducting system, causing dark xylem and pith discoloration. When the cut ends of stems are immersed in water, milky white ooze with masses of bacteria appears. The roots and pods of infected plants are discolored and rotten. In the advanced stage, drooping and death of branches and the entire plant may occur (Kelman 1953; Mehan et al. 1994; Vasse et al. 1995). In China, Indonesia, and Vietnam, bacterial wilt is a major constraint to peanut production. Yield losses of 10–30% are normal, with losses as high as 60% in heavily infected fields (Mehan et al. 1994).

An unspecified *Pseudomona*s species causes bacterial leaf spot. Small, circular to irregular shaped light-brown water-soaked lesions develop on the leaves in the early stages of infection. Lesions enlarge and grow as chlorotic halos as the disease progresses, resulting in shedding of leaf (Subrahmanyam et al. 1992).

Management

The key sources of bacterial wilt inoculum are susceptible hosts or weed hosts, as well as infected crop residues. Rotation of peanut with non-host crops is effective in reducing losses due to wilt. Seeds infected with fungus are also a possible source of prime inoculum, with seed transmission rates ranging from 4 to 15%. Drying seeds to moisture content below 9% is recommended to control seed borne infection. Flooding fields of peanut for 15–30 days prior to sowing, enhancing soil drainage, preserving sufficient soil moisture, early sowing to avoid high temperatures, burning crop residues, weed reduction, quarantine, and cleaning farm tools after operations in infested fields are all cultural control steps (Mehan et al. 1993).

Some predominant avirulent strains such as *R. solanacearum* and *Pseudomonas* spp., have been found to be antagonistic to the bacterial wilt pathogen, followed by *Acinetobacter* spp., *Bacillus* spp., and *Streptomyces* spp.

4.2.3 Viral Diseases

Viral diseases in peanut caused by cucumber mosaic virus (CMV), peanut bud necrosis virus (GBNV), peanut rosette assistor virus (GRAV), peanut rosette virus (GRV), satellite RNA associated with GRV and/or GRAV, Indian peanut clump virus (IPCV), peanut clump virus (PCV), peanut mottle virus (PeMoV), peanut stripe virus (PStV) and peanut stunt virus (PSV) and tomato spotted wilt virus (TSWV) are the most economically important viral pathogens of peanut and are responsible for serious yield losses globally or regionally.

Stages and extent of damage

Among viruses, peanut rosette disease causes greater yield loss than any other virus disease affecting peanut in the semiarid tropics. Peanut rosette disease has a complex etiology involving three agents: peanut rosette assistor luteovirus (GRAV; Murant 1989), peanut rosette umbravirus (GRV; Murant and Kumar 1990), and a satellite-RNA (sat-RNA; Murant et al. 1988) of GRV. GRV and sat-RNA are packaged within the GRAV coat protein to be transmitted by the aphid, *Aphis craccivora* in a persistent manner. Since none of these agents are carried by seeds, viruliferous aphids are the main vectors of primary infection into the crop. The two predominant symptoms of peanut rosette are “chlorotic” and “green” rosette plants. Due to shortening internodes and decreased leaf size, the virus causes extreme stunting, that cause a bushy appearance. The amount of yield loss due to peanut rosette disease depends on the plant’s growth stage; infection before flowering will result in a 100% loss in pod yield.

Tomato spotted wilt is caused by Tomato spotted wilt virus (TSWV), a species of the genus Tospovirus and family Bunyaviridae. TSWV is transmitted by several species of thrips viz., *Thrips tabaci*, *T. palmi*, *T. setosus*, *Frankliniella spp.*, *Scirtothrips* spp. but the virus is not transmitted through seed or pollen (Mandal et al.

Fig. 4.6 Peanut plants infected with peanut bud necrosis disease

2001; Peters 2003). The most significant species is *F. fusca*, which is the most common vector that reproduces on peanuts. The virus produces a broad range of symptoms from chlorotic and/or necrotic to severe stunting and subsequent death of susceptible peanut plants. It also causes early germination of seeds reducing further crop yield. The disease reduces the number of pods produced, kernel size and yield per plant. Losses up to 100% have been reported due to spotted wilt (Culbreath et al. 2003).

Bud necrosis (Fig. 4.6) is a major problem in dry areas, resulting in yield reductions up to 80% (Chohan 1974; Kamdar et al. 2014). Crop losses worth up to US$89 million from India were reported (Reddy and Devi 2003). The causal virus of this disease was initially identified as tomato spotted wilt virus (TSWV) in India (Ghanekar et al. 1979) but now it is studied to be caused by TSWV or PBNV (Peanut Bud Necrosis Virus) (Reddy et al. 1992; Adam et al. 1993; Satyanarayana et al. 1996). Chlorotic spots on leaves or mottling of immature leaflets or necrotic and chlorotic rings and streaks are formed as a result of viral infection (Bera et al. 2014b). In the later stages of plant growth, petioles bearing infected leaflets become flaccid and droop, finally followed by necrosis of terminal buds (Jasani et al. 2018a). The entire plant shows a highly stunted bushy appearance. Early-infected plants produce thin, shriveled seeds with red, brown, or purple mottling on the testae. Plants that are late infected can produce normal-sized seeds, but the testae are mottled and cracked (Reddy 1991). Both viruses are mechanically transmitted. GBNV is also transmitted by thrips vector, *Thrips palmi* (Reddy and Devi 2003) and TSWV is transmitted probably by vector, *Frankliniella fusca* and *F. occidentalis.*

Peanut clump is caused by two distinct, serologically unrelated viruses viz., peanut clump virus (PCV) mostly confined inwestern Africa, and Indian peanut clump virus (IPCV), virus from India. On newly emerging quadrifoliates of young plants, mottling, chlorotic, and mosaic rings appear. Infected leaves turn dark green, either

with or without faint mottling as a result of the virus infection. Plants that have been infected early are severely stunted, but they may produce flowers. If pods form, they are underdeveloped, and seed weights can be decreased upto 60%. These viruses are transmitted through seed, soil-borne plasmodiophoromycete fungi, *Polymyxa graminis* and mechanically by sap inoculation (Reddy et al. 2005). Since viruses are present on the seed coats of all kernels from infected plants, both viruses are transmitted by seed in peanuts with a frequency of more than 6%. In peanut almost 100% crop loss has been reported if the disease occurs in the early growing season, and up to 60% yield loss in late infected plants (Reddy 1991). The annual loss due to this disease globally is estimated to surpass US$38 million (Reddy and Devi 2003).

Peanut mottle caused by the potyvirus, peanut mottle virus (PeMoV), is another viral disease of economic importance. On young leaflets, the virus produces a faint mottle or a mosaic of irregular size and shapes and islands of dark green colour. The number of pods and root nodules along with size of pods are reduced in plants infected with virus. Also, diseased plants are slightly stunted. Varied symptoms are caused by different strains of the virus as reported by Paguio and Kuhn (1973) and Bijaisoradat et al. (1988). Symptoms caused by chlorosis and necrosis strains of PeMoV are similar to those caused by TSWV (Sreenivasulu et al. 1988). PeMoV is easily transmitted by infected seed and sap at the rates ranging from 0 to 8.5%. PeMoV is spread by *Aphis craccivora*, *A. gossypii*, *Hyperomyzuslactucae*, *Myzus persicae*, *Rhopalosiphum padi*, and *R. maidis*in a non-persistent mode (Paguio and Kuhn 1976; Highland et al. 1981). In Georgia yield losses because of this virus infection were approximated up to 20–70% (Kuhn and Demski 1975), and in India losses may be observed upto 40% in susceptible cultivars.

Peanut yellow mosaic caused by cucumber mosaic virus (CMV) is capable of causing yield losses of upto 40%. CMV, a type species of the genus Cucumovirus and belongs to the family, *Bromoviridae.* Chlorotic spots and rolling of younger leaflets are symptoms of the infection. These spots further coalesce and form large blotches of yellow colour. The leaf lamina of subsequently formed younger leaflets shows yellowing, with green lines running down the lateral veins. The virus is promptly sap transmitted by many aphid species such as *Macrosiphum euphorbiae* in a non-persistent way. Further, it is also observed to be transmitted via the infected seed upto 2–4% (Xu and Barnett 1984). The CMV-CA isolate is peanut seed transmissible and thus the initial spread is probably initiated through the seed-infected with virus. Aphids may play role in secondary spread of virus in peanut fields.

Peanut stripe is caused by PStV, a potyvirus. The characteristic symptoms of a viral disease are intermittent stripes and green bands along lateral veins of peanut leaflets. Striping, mosaic as green islands, and pattern of oak leaf kind can be seen on older leaflets. The plants that have been infected have slightly stunted growth (Demski et al. 1984). Some isolates also result in localized death of tissues on leaves. This leads to stunted growth, severe mosaic patterns and systemic distortion of foliages or stripes symptoms (Chang et al. 1990). The virus is transmitted by sap and is also transmitted through seed up to 37%. Aphids namely, *Myzus persicae*, *Aphis craccivora* and *A. gossypii* transmit the virus in a non-circulative and non-persistent manner.

Shortening of petioles, reduction in the size of leaflets, chlorosis, malformation, and extreme dwarfing of one or more branches or the whole plant are all symptoms caused by the potato stunt virus (PSV). The virus, which belongs to the cucumovirus family, has the potential to cause losses of up to 75%. PSV is spread by three species of aphid namely, *M. persicae*, *A. craccivora* and *A. spiraecola*, by sap inoculation and nature of transmission is non-persistent. It is also transmitted by seeds at the lowest possible frequency of 0.01–0.2% (Xu et al. 1986).

Management

Controlling the virus disease requires cultural practices such as uprooting of all volunteer plants and non-harvested seeds that are infected, sowing of early maturing varieties, manipulating sowing dates, using high-quality pre-treated seed, high seeding rate, and maintaining optimum plant stands. Since, TSWV and PBNV have such wide host ranges, as well as vectors capable of sustaining virus infection and supporting thrips vector multiplication (Reddy et al. 1983), it is not practicable to manage the disease by killing weeds and volunteer peanuts (Reddy et al. 1983). When one row of a fast-growing cereal crop like maize, jowar, or bajra is intercropped with every three rows of peanuts, disease occurrence is reduced (Reddy 1998). Repeated cultivation of dicots and fortuitous hosts like peanut, cowpea, and pigeonpea is likely to reduce the inoculum in the soil (Legreve et al. 1999; Delfosse et al. 2002). Early sowing of the peanut crop prior to monsoon arrival, use of pearlmillet as a bait plants to minimize the inoculum burden in the soil, sowing of peanut during the post-rainy season, avoiding rotation with highly susceptible cereal crops such as maize and wheat, and soil solarization can all help to reduce the incidence of peanut clumps. The initial or early spread of the PeMoV virus is aided by low-level transmission via the infected seed of a few grain legumes (cowpea, mung bean, common bean) as well as peanut (0–8.5%). In nature, substitute crops such as soybean, cowpea, navy bean, clover, peas, French bean, white lupine and weeds (*Desmodium*, *Cassia* spp.) as well as aphids help the virus survive and spread (Demski 1975). The incidence of the virus in young peanut fields appears to be very low (<1%). As the crop reaches maturity, the disease progresses to nearly 80% under congenial conditions that favor vector activity in the fields. So, use of virus-free seed for palnting is important to avoid the disease. Planting should be done with seed lots collected from disease-free areas, as seed is the primary source of PStV virus inoculum. In order to regulate the spread of PStV, the production and subsequent use of virus-free seed should be prioritized. Only certified seeds are permitted to be transported within or outside the countries. The use of plastic film for mulching peanut fields in China is reported to lessen PStV incidence.

Pesticides to reduce vector populations of viruses are available but only little success is achieved. Insecticidal control of thrips vectors is largely ineffective for suppressing spotted wilt in peanut (Culbreath et al. 2003). The use of some insecticides (imidacloprid) was found to increase the disease incidence. Aldicarb, acephate and carbofuran were found to be ineffective. However, chlorophyrifos and phorate (furrow application) reduced spotted wilt in peanut and phorate application is used commercially in the US.

4.2.4 Nematode Diseases

Nematodes are microscopic unsegmented roundworms found in soil. The species of nematodes that cause the most damage to peanuts are peanut root-knot nematodes, root-lesion nematodes, and peanut pod nematodes.

Stages and extent of damage

Among nematodes, the highest loss in peanut is caused by root-knot nematodes *i.e.*, *Meloidogyne arenaria*, *M. javanica*, *M. hapla* and *M. incognita.* Root-knot nematodes result in root galls due to internal swelling of roots and pegs, limit the development of *Rhizobium* nodules, and increase attack by other soil-borne pathogens. Infected pegs and pods may also form galls. Infected plants also exhibit stunting and chlorosis to varying degrees. Root growth is slowed, and vascular elements are disturbed, resulting in poor nutrient and water uptake and transport. Egg masses, infective second-stage juveniles, and adult males of root-knot nematodes can all be found in the soil. Infectious juveniles emerge from the eggs and enter roots, pegs, or pods, moving intercellularly and intracellularly to a location near vascular tissue (McSorley et al. 1992). Under favorable environmental conditions, sedentary juveniles either form males of 1–2 mm length or globose-pyriform shaped mature females that lay large numbers of eggs (about 200–1500 from each female) in a gelatinous matrix. These masses of eggs can either be retained in the roots or squeezed out into the soil. The new second-stage juveniles from hatchecd eggs enter into the soil around the roots. Peanut root nematodes cause yield losses ranging from 20 to 90%. *Pratylenchus coffeae* (Godfrey) Filipjev & Schuurmans-Stekhoven and *P. brachyurus* (Zimmermann) Schuurmanns-Stekhoven (Boswell 1968) are two species of lesion nematodes that target peanut (Chhabra and Mahajan 1976). Lesion nematodes have six life stages, like all nematodes: an embryo, four juvenile stages, and an adult stage and produce. These nematodes are endoparasites that invade the pegs, roots, and pods of peanuts and produce necrotic root lesions and pod lesions followed by discolouration. The infection of pegs also leads to necrotic lesions. The pegs are weakened as a result of these lesions, and pods are shed prematurely. The percentage of sound mature seeds, seed weight, and kernel quality can all be affected by root-lesion nematodes. So, losses results from decreased pod yield and poor yield quality.

Peanut pod nematode (*Ditylenchus africanus* Wendt) is a migratory endoparasite prevalent in limited regions of the world (De Waele et al. 1989). The nematode reaches peanut pegs at the point of pod's attachment and passes through the hull. The nematode reproduces in the hulls and seeds before they are harvested. Approximately 90% of the population of nematode existing within or around a plant is carried inside the pods when they are harvested (De Waele et al. 1989; Basson et al. 1993). A gray, bruise-like soiling of the pod at the point of peg attachment is the first apparent symptom. Premature germination occurs in up to 25% of seeds. The weight of the seeds can also be decreased by 20–50%. The most significant economic effect is the crop's decreased market value as a result of discolored seed (Venter et al. 1991).

Management

Meloidogyne species are holo parasites, and without a host, their populations rapidly decline. Peanut rotation with crops such as maie, cotton, sorghum, and some soybean cultivars will significantly reduce root-knot nematode infestation in soils. Cotton, velvet bean (*Mucuna deeringiana)* and Bahia grass *(Paspalum notatum)* are excellent rotational crops. In addition, since many weeds act as suitable hosts, weed management and volunteer plant eradication are required for a rotating plan to be successful (Taylor and Sasser 1978; Rodríguez-kábana and Canullo 1992; Rodríguez-kábana et al. 1994). However, crop rotation with nonhost crops offers limited success to manage lesion nematode populations, since most *Pratylenchus* species have wide range of hosts that include both dicots and monocots. Nevertheless, crop rotation with the non-host crop *i.e.*, maize reduce the nematode population significantly. The use of nematode- free seed and field-sanitation are important measures. Farmers in *D. africanus*-infested fields are advised to harvest their crops early (Venter et al. 1992).

The fumigant nematicides such as dibromochloropropane (DBCP), ethylene dibromide (EDB), 1,3-dichloropropene (1,3-D) and metham sodium are very effective for the control of root-knot nematodes. Non-fumigants and systemic nematicides that is available for use in peanut are- aldicarb, carbofuran, ethoprop, fensulfothion and phenamiphos (Rodríguez-kábana and King 1985). Phenamiphos at sowing time, aldicarb at sowing or peg formation stage, and oxamyl at peg forming stage are among the registered chemicals for use against the peanut pod nematode (McDonald and Van Den Berg 1991).

Viruses, bacteria, fungi, non-related nematodes, insects, mites, and protozoa, are among the microorganisms and invertebrates that target nematodes. *Pasteuria penetrans*, is one obligate parasite of root-knot nematodes found in many peanut fields. *Arthrobotrys* species and *Monacrosporium* species are the nematophagous fungi that have the potential to control *D. africanus* (Swart and Jones 1994*).*

4.2.5 Insect-Pests

The important insect pests of peanut are aphids (*Aphis craccivora* Koch), many species of thrips (*Frankliniella fusca*, *F. schultzei*, *Thripspalmi*), jassids (*Empoascakerri* and *E. fabae*), leaf miner (*Aproaeremamo dicella*), red hairy caterpillar (*Amsacta albistriga*), and *Spodoptera*. Aphids, thrips and jassids are sap-sucking pests and also carriers of major viral diseases (Fig. 4.7). Termites and white grubs may also cause significant damage to peanuts (Figs. 4.8 and 4.9). Despite the fact that many insect species have been found in the peanut crop, only a few cause major damage and yield losses. Insect pests are responsible for 10–20% of crop losses in general.

Fig. 4.7 Peanut plant infected with sucking pest

4.2.5.1 Sap Sucking Pests

Stages and extent of damage

Peanut aphid, *Aphis craccivora* (Koch), is one of the most serious and injurious pests of peanut of order Hemiptera, with a worldwide distribution. The aphid is ovoviviparous; females retain eggs inside their bodies and give birth to small larvae. Males are alate and sexual form. Crop losses are caused by *A. craccivora* either directly or indirectly, mainly through the transmission of plant viruses. *A. craccivora* attacks plants at their seedling stage, vegetative stage, and reproductive stage. Aphids tend to feed on immature pods, shoots, young and tender leaves, and fruits. The highest losses in yield due to direct damage are incurred when aphid colonies target developing tips of plants in the spring. Large numbers of aphids feeding directly on peanuts can cause partial sterility of the plants (Mayeux 1984). Peanut yield losses

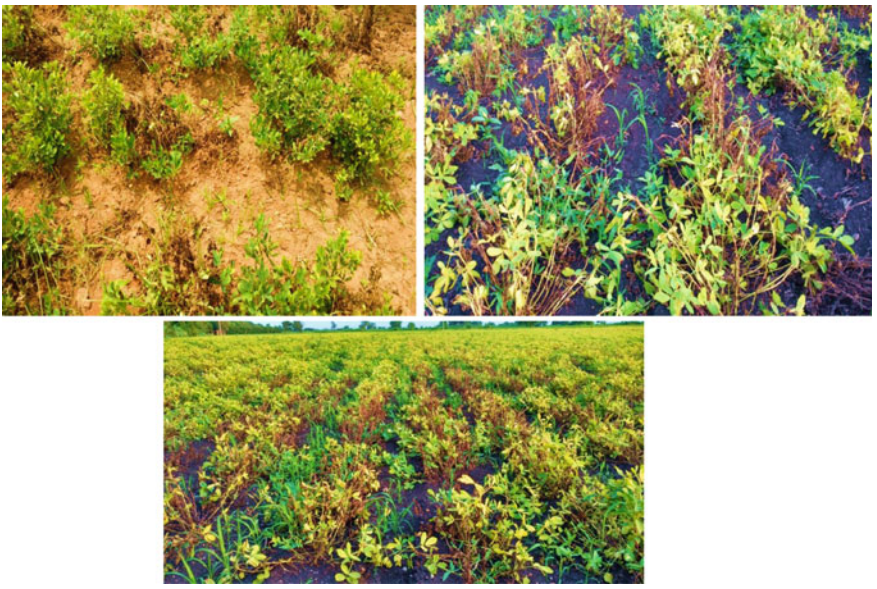

Fig. 4.8 Peanut crop damaged by termite

Fig. 4.9 Peanut pods damaged by termite

of 16% have been reported in India due to insect pests, the most common of which is *A. craccivora* (Jagtap et al. 1984). The development of honeydew, which serves as a substrate for growth of fungus, and the spread of plant viruses such as peanut rosette, peanut (peanut) mottle, and peanut stunt viruses cause indirect damage from *A. craccivora.* Thrips, from order Thysanoptera are small in size (less than 2 mm long) and slim insects having fringed wings that live in the flowers and folded leaflets of peanut plants. The most important thrips on peanut are *Scirtothrips dorsalis*, *Thrips palmi* and *Frankliniella schultzei* (Amin 1985; Ekvised et al. 2006). They are hemimetabolous insects that go through four stages: embryo, larvae, nymphs (two nymphal, and the 'prepupal' and 'pupal' instars), and adult. Adults and larvae are mobile, and adults have wings of their own (Lewis 1997). The sap is sucked from the surface of the leaflets by nymphs and adults. This causes white patches on the upper surface of the leaves, known as silvering, and necrotic patches on the lower surface, known as necrotic patches. As the leaflets expand they split as newly developing leaflets are distorted due to formation of patchy necrotic areas that puncture eventually. Seedlings are often injured. Thrips are vectors for many viruses like PBNV, TSWV, and stem necrosis virus, all of which can lead to widespread yield loss. Jassids (leafhoppers) are another important foliage-sucking pest of peanut and act as limiting factors in the successful cultivation of the peanut crop. *E. kerri* Bachlucha is the most common jassid that attacks peanuts in Asia, and it can be found in abundance in western India, mainly Gujarat. In Africa, *E. facialis* and *E. dolichi* are common jassid species on peanut, and *E. fabae* is widely distributed in the Americas. Both the nymphs as well as adults suck the sap from the tender leaf and mostly from the lower surface of the leaflet causing whitening of the veins, yellowing in the form of patches of the leaflets, leaf curling and necrosis (necrosis of leaf tips in V shape known as hopper burn), stunted growth and eventually death of plants. Jassids also act as a vector of leaf curled, tomato spotted and other viruses (Amin and Palmer 1985; Singh et al. 1990).

Management

Early and dense sowings are highly recommended to control aphids. Early sowings enable plants to initiate flowering before aphids' arrival, while dense sowings provide a barrier to aphids entry into the field (Mayeux 1984). Sanitary measures are important within crops and between seasons to prevent the transmission of viruses by *A. craccivora.* Virus-infected plant materials should be eliminated after harvest and any volunteer plants or weeds that harbour viruses should be destroyed. Thrip populations in peanuts can be substantially reduced by cultural practices. Lower thrip densities are achieved by manipulating sowing dates to avoid peak thrips dispersal and during the susceptible seedling period (McKeown et al. 2001; Culbreath et al. 2010). Likewise, heavy plant residue from conservation tillage systems, increased plant density and twin-row planting reduces thrips infestation on peanut (Brown et al. 1996; Culbreath et al. 2008; Tubbs et al. 2011).

The insecticides such as chlorinated hydrocarbons, organophosphates, carbamates, and pyrethroids have all been used against *A. craccivora.* Systemics that have a high level of persistence during the plant's growth stage are favored. Furthermore,

neem formulations have been shown to be effective against *A. craccivora*, making them a viable alternative to use of insecticides (Egho et al. 2009; Baidoo et al. 2012; Chaudhari et al. 2015). The most regulary used category of insecticides against thrips are carbamates, neonicotinoids, organophosphates, phenylpyrazole and pyrethroids (Todd et al. 1996; Mandal et al. 2012; Marasigan et al. 2016; Srinivasan et al. 2017). Insecticides from newer groups, such as diamides and spinosyns have also been discovered to be effective against thrips (Marasigan et al. 2016, 2018). Seed treatment with Imidacloprid protects for almost a month against sucking pests. If more than 10% of leaves have the typical 'hopper burn' symptoms of thrips, dimethoate can be sprayed during the initial crop development, which is up to 30 days after emergence. However, chemicals should not be used indiscriminately and should be used depending on the economic threshold level of insect population. In India and Africa, coccinellids, *Cheilomenes sexmaculata*, is recommended as a significant natural agent in peanuts (Agarwala and Bardhanroy 1999). Release of the reduviid predators namely, *Rhynocoris marginatus* (Sahayaraj and Martin 2003), *R. kumraii* (Sahayaraj and Ravi 2007), and *Chrysoperla zastrowi sillemi*, a chrysopid predator (Baskaran and Rajavel 2013) and spraying fungus *Verticillium lecanii* reduced populations of *A. craccivora* in Indian fields of peanuts (Sahayaraj and Namachivayam 2011).

4.2.5.2 Foliage Feeders or Defoliators

Many leaf eating insects species are found in peanut crop, of which *Spodoptera*, hairy caterpillar and leaf miner are of economic importance.

Stages and extent of damage

Spodoptera litura (Fab.), tobacco caterpillar/tobacco armyworm and *Spodoptera littoralis*, cotton leaf worm are the two dominant leaf worm species. The adults are light brown moths and lay eggs in group of hundreds, primarily on the upper leaf surfaces. There are six larval instars, which disperse from egg batches. Larvae are regarious feeder and eat leaves, bulbs, and fruits, and are considered a significant defoliator. As a result, *S. litura* is one of a number of pests that can be problematic during the peg initiation stage, pod development stage, and maturation stages of crop growth (Singh and Sachan 1992). The red hairy caterpillar, *Amsacta albistriga* Walk. *and Amsacta moori* Butler, are the most common hairy caterpillars that target peanuts. At the start of the southwest monsoon, the brownish white adults emerge from the soil. They eat all plant bits, including buds, flowers, and leaves and are voracious feeders. They often move from one field to another for food after destroying the vegetation and hatching in one field, resulting in a significant reduction in yield. Peanut leaf miner, *Aproaerema modicella* (Deventer) is a usual pest of peanuts in South and South-East Asian contries and a major pest of India. Young larvae dig into the leaves of hatcheries, depositing single gleaming white eggs on the underside of the leaflets. There are five larval instars stages and pupation takes place inside webbed leaves. For peanut, yield losses of >50% have been reported due to feeding

on the leaves (Islam et al. 1983). From a point, a heavily attacked field appears to be 'burned,' and epidemics can result in complete crop loss.

Management

To expose pupae of *Spodoptera* to natural enemies and adverse weather-related factors, clean cultivation and deep plowing are recommended. Sunflower, taro and castor plants allure *Spodoptera* and thus, may be sown to collect egg masses and larval instars both around and within fields, as trap crops (Zhou 2009). Light traps or pheromone traps can be used to collect moths of defoliators. Crop rotation with sorghum, pearlmillet or maize should be followed. The migration of larvae of red hairy caterpillar can be avoided by digging deep trenches. To reduce the larval densities of leaf miner intercropping of peanut with sorghum, millet or cowpea is preferred. Also, cotton-sorghum-peanut is the best crop rotation combination to give better yields and reduce the incidence of leaf miner. Removing the alternative hosts and weeds viz., lucerne, amaranthus, berseem and *Indigofera hirsuta* can be effective to control the growth of the leaf miner population.

S. litura and other defoliators have gained resistance to most of the available pesticides used commercially (Ramakrishnan et al. 1984; Naeem Abbas et al. 2014), so control is becoming increasingly difficult, although, spraying of dimethoate, fenthion, phosphomidon, Imidacloprid, carbaryl, dichlorovos, and Quinalphos, is practiced. Chlorantraniliprole, spinosad, and emamectin benzoate, are among other new chemicals that have shown optimistic results against *S. litura* (Gadhiya et al. 2014). When adult stage of leaf miners is discovered in the attacked area, fruit powder extract of neem can be used to effectively reduce oviposition. Insecticides, ideally dimethoate or imidacloprid can be used.

Telenomus remus, egg-larval parasitoid and larval parasitoid species namely, *Apanteleruficrus*, *A. kazak*, *Cotesia marginiventris*, *Campoletes chloridae*, and *Hyposoterdidymator* are some biological controls reported but have varying efficiency (Braune 1982; Michael et al. 1984). *Trichogramma* parasitize on eggs and young larvae of red hairy caterpillar. Spraying of bioinsecticides based on Nuclear Polyhedrosis virus (NPV) or *Bacillus thuringiensis* can manage spodoptera effectively.

4.2.5.3 Root and Pod Feeders

Stages and extent of damage

White grub species, *Lachnosterna* (=*Holotrichia) consanguinea* (Blanch.) and *L. serrata* are the two most important soil inhabiting polyphagous pests of peanut. Adults are dark brown and emerge out of the soil within 3–4 days after the onset of rain. The eggs are white and round in shape, while larvae are whitish yellow in colour, fleshy and C-shaped. The young grubs in their second, third and fourth instar larval stages feed on organic matter and fine rootlets while mature grubs feed on both roots and pods. Wide patches of dead plants can be found in heavily infested

fields, and the remaining plants are often stunted and wilting. The damage to peanut crops in endemic areas varies from 20 to 80%. Peanut plants are harmed by termites, mostly *Microtermes* spp. and *Odontotermes* spp. They burrow within the root and stem, killing the plant; they make holes in the pods, damaging the kernels; and they cause scarification (stripping of the soft corky tissue between the pods veins). As a result, pods are more vulnerable to *Aspergillus* species infection.

Management

Summer ploughing exposes the pupae to scorching solar radiation and predation by birds. Crop rotation with sorghum and pearl millet, early sowing, and use of light traps and pheromone traps should be practiced. Clearing mounds of termites around peanut fields and injecting chlorpyriphos into the termite mounds are two cultural operations that can effectively reduce termite populations in cropping areas. Termite control was also found to be successful when peanuts were harvested at the optimum maturity stage and debris was removed from the field. Although, soil insects are expensive and difficult to manage insecticides namely, carbofuran, chlorpyrifos and phorate can be incorporated in soil prior to sowing and seed treatment with chlopyriphos and imidacloprid can be practiced.

4.3 Genetic Resources and Trait Discovery

Genetic resources are important sources of variability and serve as repository of many desirable alleles for current and future programmes for peanut improvement. Genetic variability preserved in gene banks are important sources of variability and harbor many useful genes for utilization in breeding programs. Thousands of peanut accessions are conserved in national and global gene banks around the world, including ICRISAT, the United States, Brazil, India, and China, where biotic stress variations can be seen (Ntare et al. 2006; Pandey et al. 2012a, b). Furthermore, cultivated peanut accessions, gene banks have a large number of wild peanut accessions. Since cultivated peanuts are the result of a single hybridization among diploid ancestors, they have a narrow genetic base and genetic variability in response to biotic stresses. Wild *Arachis* species, on the other hand, have been reported to have higher tolerance/resistance to a variety of stresses (Figs. 4.10 and 4.11). In addition, several interspecific hybridization lines have been established to create new variability (Fig. 4.12), and some improved varieties have also been released. The genus *Arachis* has 80 species (Valls and Simpson 2005). Initially, Krapovickas and Gregory in the year 1994 grouped the genus *Arachis* into nine sections based on cross compatibilities, morphology, phylogeny and geographic distribution namely, *Arachis* with 31 species., *Erectoides* 14, *Extranervosae* 10, *Procumbentes* 10, *Rhizomatosae* 4, *Heteranthae* 6, *Caulorhizae* 2, and *Trierectoides* 2 and *Triseminatae* with single species. The *A. hypogaea*, a cultivated and tetraploid peanut, *A. monticola*, another non-cultivated tetraploid species, and 29 diploid species make up the *Arachis* section.

Fig. 4.10 Wild *Arachis* sp. maintained in field conditions

Fig. 4.11 Wild Arachis sp. resistant to foliar fungal diseases

Fig. 4.12 Synthetic amphidiploid maintained under field conditions

Genetic diversity in the peanut is grouped into different gene pools as suggected by Singh and Simpson (1994). Breeders benefit from the idea of gene pools because it helps them choose germplasm to use in hybridizations to widen the genetic base of crop and enhance the crop's genetics. Landraces and typical cultivars of peanut from 1° as well as 2° centres of genetic diversity, along with wild *A. monticola*, make up the primary gene pool (GP1). Hybridization within the GP1 results in routine chromosome pairing and thus, fertile progeny, so gene transfer from GP1 to *A. hyogaea* is easy. The secondary gene pool (GP2) consists of diploid species of the *Arachis* segment that are congenial in cross with *A. hypogaea* but contain sterile to partly fertile hybrids because of ploidy variations. The tertiary gene pool (GP3) consists of species from section *Procumbentes*, which are compatible in cross with diploid species of *Arachis* section (Mallikarjuna 2005; Mallikarjuna and Hoisington 2009), section *Erectoides*, whose species have low cross-compatibility with and *A. hypogea* (Singh 1998); and *Rhizomatosae*, whose tetraploid species can be crossed both with diploid species of section *Arachis* and *A. hypogea* (Gregory and Gregory 1979; Mallikarjuna and Sastri 2002). The remaining *Arachis* species that are incompatible or weakly compatible with *A. hypogaea* and other *Arachis* species are included in the Quaternary Gene Pool (GP4). The most open sources GP1 and GP2, which have been successfully used in peanut improvement, and their probable benefit

is now much more efficient and predictable. However, the use of biotechnological techniques is needed to exploit tertiary and quaternary gene pools. The use of GP1 for many traits has been restricted, and wild *Arachis* species have frequently shown desired variability and a higher degree of resistance than GP1. For example, in the case of PStV, despite screening 9000 accessions, no resistant source was established in cultivated peanuts, but a negative reaction was observed in many wild *Arachis* accessions (Culver et al. 1987; Prasada Rao et al. 1991). Wild *Arachis* spp., such as *A. batizocoi*, *A. correntina*, *A. cardenasii*, *A. duranensis*, *A. diogoi*, *A. pusilla* and *A. villosa*, have higher resistance and tolerance to peanut-rust (Abdou et al. 1974; Subrahmanyam et al. 1982a, b; 1985a, b, c), but their pods are catenate and small. Many wild species from the *Arachis* section that are cross-compatible with the cultivated species displayed either an immune response or highly resistant response to the late leaf spot pathogen, including *A. diogoi*, *A. cardenasii*, *A. glabrata*, *A. stenosperma*, *A. repens*, *A. appressipila*, *A. paraguariensis*, *A. villosulicarpa* and *A. hagenbeckii*, were among the highly resistant species found in other sections (Subrahmanyam et al. 1985a, b, c). Further, several resistance sources to ELS were identified in *A. hypogaea* and two diploid wild species, *A. stenosperma* and *A. diogoi* were also scored as highly resistant (Foster et al. 1981). Also, considerable genetic variation for virus resistant was found in wild species. *A. cardenasii A. diogoi*, *A. correntina*, and *A. pusilla* showed no infection to TSWV under field conditions. Two species namely, *A. diogoi* and *A. pusilla* also exhibited no infection from Peanut mottle virus (Subrahmanyam et al. 1985a, b, c; Demski and Sowell 1981). Both reproductive resistance and hypersensitive necrosis to *Meloidogyne* spp. have been reported recently in tetraploids derived from complex crosses of *A. hypogaea* (Nelson et al. 1989; Holbrook and Noe 1990) comprising of three species viz., *A. batizocoi*, *A. cardenasii*, and *A. diogoi* Hoehne that are resistant to nematode. There was considerable variation for resistance in different accessions of wild species (Sharma et al. 2003). *A. batizocoi*, *A. diogoi*, *A. correntina*, *A. villosa*, *A. spegazzini*, *A. cardenasii*, *A. stenosperma*, *A. duranensis*, *A. rigonii*, *A. paraguariensis*, *A. pusilla*, *A. glandulifera*, *A. ipaensis* and *A. repens* are species that possess resistance to thrips (Yang et al. 1993; Michelotto et al. 2017; Srinivasan et al. 2017). *A. cardenasii*, *A. duranensis*, *A. kempff-mercadoi*, *A. monticola*, *A. stenosperma*, *A. paraguariensis*, *A. pusilla*, and *A. triseminata* showed multiple resistances to the leaf miner and thrips. *A. cardenasii*, *A. appressipila A. ipaensis* and *A. paraguariensis* showed antibiosis to *Spodoptera* and also resistance to leaf feeding (Sharma et al. 2003).

Fertility obstacles triggered by species incompatibilities and ploidy level differences; association of desirable traits with traits that are agronomically unadapted and undesirable; and monitoring introgressed segments have all hampered the transfer of genes from wild species. Many methods are being used for the introgression of wild genes in cultivated peanut with varied success of which the hexaploid and tetraploid routes are most successful. In the hexaploid route, a triploid hybrid derived from a cross between the cultivated allotetraploid species and the diploid wild species is colchicine treated to produce a hexaploid plant, followed by generations of selfing to select tetraploid plants with resistance to multiple disease resistances (Stalker et al. 1979; Stalker and Beute 1993; Reddy et al. 1996). In tetraploid route as suggested

by Simpson et al. (2001) firstly, an A genome hybrid was made by crossing *A. cardenasii* with *A. diogoi*. Then, the B genome species *A. batizocoi* was crossed with the A genome hybrid to create a sterile AB hybrid. This sterile hybrid was treated with colchicine to double the chromosome number and restore fertility. This tetraploid, also known as amphidploid [*A. batizocoi* × (*A. cardenasii* × *A. diogoi)*], was registered as TxAG-6, that has a strong resistance to nematodes and later used as a source in breeding two cultivars, COAN and NemaTAM. More recently, amphidiploids were developed using *A. duranensis* and *A. ipaensis* (Fávero et al. 2006) and *A. gregoryi* and *A. linearifolium* (Simpson and Starr 2001; GCP 2005; Simpson et al. 2003). Further, considering the potential use of amphidiploids ICRISAT has developed many tetraploids and amphidiploids peanuts using wild species. Synthetic amphidiploids, such as ISATGR 278–18 (*A. duranesis* × *A. batizocoi)* and ISATGR 5B *(A. magna* × *A. batizocoi*), were developed by ICRISAT and have been used in backcross breeding program to transfer useful genes into elite cultivars/genotypes that possess many traits of interest, including resistance to foliar diseases (Kumari et al.2014). The sterile diploid hybrids from *A. magna* V 13,751 and *A. kempff-mercadoi* V 13,250 were treated with colchicine for polyploidization, and the amphidiploids were crossed with *A. hypogaea* cv. IAC OL 4 to initiate the introgression of the wild genes for pest resistance into the cultivated peanut (de Paula et al. 2017). Furthermore, the release of an Indian variety (GPBD 4) with foliar disease resistance due to chromosome segments from *A. cardenasii* is an example of achievement from wide hybridization. Further, with the advent of marker technologies and biotechnological tools, prebreeding activities have been accelerated. Molecular markers are being used to test hybridity, to characterize the introgression lines for wild genes and molecular diversity analysis. To overcome the problems of barriers between the cultivated species and the wild species and to get rid of undesirable gene blocks genetic engineering techniques would be an ideal option in peanut improvement.

4.4 Conventional Breeding Methods for Biotic Stresses Resistance

Many of the biotic stresses can be controlled to a lesser degree by adopting appropriate cultural practices and chemical control measures. However, farmers can afford to use very little pesticides in general and still less for controlling biotic stresses. So, using disease-resistant cultivars is one of the most effective and cost-effective ways to reduce disease-related crop losses. Peanut breeding for biotic stresses involves the identification of sources of resistance either from existing variability in cultivated germplasm accessions, from wild *Arachis* species or creating new variability by mutation breeding and their introgression into elite genotypes. This approach has resulted in the development of many disease resistant cultivars coupled with higher yield. Availability of potential donors, understanding of genetic control of resistance and proper screening methods are prerequisite to begin any disease resistance breeding

program. The general approach includes the screening of germplasm, crossing and development of hybrids, and effecting selections in segregating generations advanced through pedigree, bulk method, single seed descent, backcross or their modifications. The pedigree method enables breeders to concentrate on high-heritability traits, while the bulk-pedigree methodology, a simplified variant of the bulk method aimed at enhancing traits with low heritability (Wynne and Gregory 1981). The single seed descent method is gaining popularity because it saves both space and money (Isleib et al. 1994). In 1927, a Dutch scientist from East Java (Indonesia), made the first effort to use genetic resources to order to develop a disease resistant peanut and as a result, Schwarz 21, a variety resistant to bacterial wilt was developed (Buddenhagen and Kelman 1964). Despite these early achievements in leveraging host-plant heterogeneity, biotic stresses resistance breeding was not given much attention until the late of 1970s. Most of the resistant germplasm lines against foliar fungal diseases are primeval and land races that have unwanted pod and kernel characteristics. Rust resistance sources presently used by peanut breeders have factors for "slow rusting" and reported to have either recessive inheritance or dominant with duplicate recessive or partial dominant, or polygenic inheritance. Some sources of rust resistance governed by a few major genes are relatively easy to transfer into agronomically adaptable and desirable types. GPBD 4 is a most popular rust-resistant variety produced at UAS, Dharwad, from the parental genotype ICGV 86855, which is an interspecific derivative derived from cross, *A. hypogaea* × *A. cardenasii* (Stalker 1997). Some tetraploid lines or nearly-tetraploid lines originated from crosses of cultivated allotetraploid peanuts with wild *Arachis* species have shown a high level of resistance to ELS and LLS (Subrahmanyam et al. 1985a, b, c). Genetic resistance shows complex inheritance and factors including initial infection, sporulation, size of lesions, and defoliation, all play a role (Green and Wynne 1986; Chiteka et al. 1988a, b; Anderson et al. 1993; Waliyar et al. 1993, 1995). Rate-reducing resistance to leaf spots is quantitative and governed by both additive and non-additive gene effects along with maternal effects (Anderson et al. 1986a; Dwivedi et al. 1993). Some of the released cultivars that are tolerant to early leaf spot (ELS) in India and USA are BG 3, Bailey, C-99R, CSMG 84-1, DP 1, GG 7, Florida 07, Georganic, ICGS 44, M 335, ICGS 76, M 522, Prutha, Somnath, Sugg and VA 81B. LLS tolerant cultivars released from India are ICGV 86590 and ICGV 86325, ICG (FDRS) 10, Girnar 1, K 134, GBPD 4, ALR #s 1, 2, and 3, BSR 1, R 8808, VRI (Gn) 5, CSMG 84-1 and RG 141. In the USA, C-99R, Florida 07, Florida MDR 98, Southern Runner, TUF Runner, TM '727', and others were released (Gorbet et al. 1987).

In order to integrate resistance to both leaf spots in a single line, two strategies are being used. Selecting for LLS resistance among germplasm lines that has already been screened for ELS resistance is one approach. A strategy is to combine individual sources for resistance to LLS and ELS in a single cultivar. Genes for resistance to LLS and ELS are inherited singly and can be consolidated into a single genotype (Kornegay et al. 1980; Anderson et al. 1986b). Multiple foliar fungal disease resistant cultivars namely, ALR 1, ALR 2, DOR 8-10, Girnar-l, GPBD4, ICGS (FDRS) 10, and ICGV 86590 were developed in India but are not popular because of poor kernel and pod characteristics. Partially resistant cultivars can also be cultivated to decrease

the inocula build up and rate of spread of leaf spot epidemics, but this resistance is not complete and stable (Subrahmanyam et al. 1982a, b).

Resistance to soil-enduring fungi is difficult to breed for, and progress has been slow. Until recently, low to average levels of resistance to stem rot is reported in peanut germplasm. To date, resistance to soil-borne fungus is attributed to polygenic with minor but additive effects (Fry 1982), and is thought to be similar to horizontal or field resistance. However, integrating this form of field resistance into germplasm with desirable agronomic traits has proven difficult. If soil-inhabiting fungus of peanut is to be controlled using the available sources that is incomplete, extensive cooperative breeding and pathology research is needed. Peanut cultivars viz., Virginia 81B, Virginia 93B, Southwest Runner, Tamspan-90, and Tamrum OL07 possess considerable resistance to pathogen, *S. minor* (Akem et al. 1992; Baring et al. 2006). Some cultivars in USA are known to show partial resistant to *S. rolfsii* namely, Southern runner, Toalson, Pronto, Tamrun 96 and Georgia Browne (Simpson et al. 1979; Banks and Kirby 1983; Gorbet et al. 1987; Branch 1994; Smith et al. 1998; Backman and Brenneman 1997). Moderately resistant cultivars such as VA-98R, VA 93B, and Perry are being utilized commercially (Chappell et al. 1995). Certain peanut lines have been confirmed to have high production potential along with average resistance to *Pythium* spp. Georgia Browne, a runner peanut, has been found to have partial resistance to *R. solani.* Resistance to both *Pythium* spp. and *R. solani* may be found in Spanish cultivars, mainly Toalson (Beaute 1997; Brenneman 1997).

Preharvest resistance, resistance by seed coat against *invitro* seed colonization (IVSC), and cotyledons aversion to aflatoxin formation are all independently inherited resistance mechanisms against *Aspergillus flavus*, provide future achievement from gene pyramiding (Upadhyaya et al. 2002). But to date, no effective efforts have been made because the genetics and mechanisms of resistance are complex and not fully understood. One released variety, J 11 is reported to have resistance to initial infection and subsequent colonization by the fungus *A. flavus*, and this resistance is associated with the hardening of its hypocotyl tissues (Hadwan and Bhowmik 1991; Nayak et al. 1992). Yueyou 9 and Yueyou 20 are *A. flavus* resistant cultivars released from China (Liang et al. 2009). ICRISAT has identified some germplasm with limited resistance in their Minicore collection (Waliyar et al. 2016). The Senegal variety 55-437 is reported to have some resistance (Clavel 2004). More recently, two accessions, Zh.h0551 and Zh.h2150 resistant to aflatoxin production were identified from China's minicore collections (Yu et al. 2020).

Southern Runner' was the first released cultivar of peanut with average resistance to TSWV (Culbreath et al. 1992a, b, 1994, 1996). Further, additional cultivars having TSWV resistance similar to Southern Runner including 'Georgia Browne', and 'Georgia Green' 'C 99R', 'Florida MDR 98' and 'Tamrun 96', were released (Branch 1996; Culbreath et al. 1994, 1996). All currently grown cultivars in the southeastern region of the U.S. have higher resistance to TSWV.

Excellent resistance sources to rosette disease are available in several genotypes from different maturity groups (Bock et al. 1990; Subrahmanyam et al. 1998; Naidu et al. 1999). Subramanyam et al. (2001) have identified several wild *Arachis* species resistant to all the three causative agents of peanut rosette. Resistance to

rosette virus is controlled by a monogenic dominant or two independent recessive genes, so these resistances are relatively easy to transfer into agronomically desirable types (Nigam and Bock 1990; Olorunju et al. 1992). GRD resistance sources were first discovered in Senegal in the year 1952, and subsequently they were used as parents in developing high-yielding, rosette-resistant peanut varieties, RMP 91, RG1, RMP12. In Nigeria, UGA2 (Samnut21), M572.80I (Samnut22), and ICGV-IS96894 (Samnut23), medium duration and resistance to GRD were released in 2001, and following three early maturing varieties with GRD resistant Samnut24, Samnut25, and Samnut26, were released more recently (Ajeigbe et al. 2015). Rosette resistance is successfully introgressed by backcrossing with a commercial cultivar, 28–206(R) (Mauboussin et al. 1970). Also, GBNV resistant peanut cultivars viz., ICGS 11 and ICGS 44 were released in India.

The higher resistance in the cultivar Schwarz 21 to bacterial wilt was first identified in Indonesia. A series of resistant cultivars have been released commercially in China since 1980s (Mehan et al. 1994). Bacterial wilt resistant sources from wild *Arachis* species (Tang and Zhou 2000) and cultivated species (Liao et al. 2005) were used as sources to develop and release resistant peanut cultivars viz., Zhonghua 4, Tianfu 11, Zhonghua 6, and Zhonghua 21 in China (Yu et al. 2011) and in other countries.

Garcia et al. (1996) reported that resistance to nematode in *A. cardenasii* was governed by two genes, dominant in nature, where one gene designated as *Mag*, is responsible for inhibiting root galling and another gene named as *Mae*, is responsible for hindering egg production by nematode, *M. arenaria.* In complex hybrids (tetraploid) of *A. hypogaea* (Nelson et al. 1989; Holbrook and Noe 1990) derived from three species, *A. batizocoi*, *A. cardenasii*, and *A. diogoi* Hoehne, resistant to nematode, both hypersensitive and necrotic cell death and reproductive resistance to *Meloidogyne* sp. have been identified. As a result, the first breeding line (TxAG-7), resistant to *Meloidogyne* was commercially released for cultivation (Simpson et al. 1993). TxAG-7 was originated from a backcross of *A. hypogaea* cv. 'Florunner' with TxAG-6 (Simpson et al. 1993). A backcross program was also used to introduce root-knot nematode resistance from TxAG-7 into Florunner, resulting in the release of 'COAN,' the first peanut cultivar with *M. arenaria* resistance (Simpson and Starr 2001). The resistance in this cultivar was governed by a single gene of dominant nature. Subsequently, introgressing genes from TxAg-6 to *A. hypogaea*, resulted in release of two cultivars, NemaTAM (Simpson et al. 2003) and Webb (Simpson et al. 2013).

When resistance to multiple biotic stresses is needed, it is hard to accumulate enough polygenes, inherited independently with conventional breeding approaches to provide good resistance levels to all diseases. Exceptions to this will happen if the same genes/or set of genes confer resistance to more than one diseases, for example several genotypes resistant to *Pythium* pod rot also shows resistant to *S. rolfsii* (Smith et al. 1989). One successful example is Tifguard, a peanut variety bred with resistance to nematode, root-knot nematode and virus, TSWV released from USA (Holbrook et al. 2008). However, the lack of major or complete resistance sources for biotic stresses may partly be the reason for the slow gain in breeding for disease-resistant cultivars in peanut (Allen 1983).

Due to the difficulty in screening a huge number of germplasm accessions and segregating populations under erratic and variable insect strains, insect resistance breeding has received little attention. Repellent, antibiosis, immunity, physical structures, and avoidance are some of the resistance mechanisms that can be used alone or in combination. Many genotypes with insect pest resistance have also been reported (Nigam et al. 1991). Resistance to thrips and jassids is related to high trichome density, distribution, and length, as well as thick leaf cuticles. Antibiosis works by reducing growth and fecundity in aphid resistant genotypes (Padgham et al. 1990). Resistance against *A. craccivora* was reported in the breeding line, ICG 12991, governed by a single recessive gene (Minja et al. 1999). ICGV 87160 (ICG (FDRS), Serenut 10R, SGV0023, SGV 002, SGV 0053, SGV 0084, Samnut 22 and 23 are released cultivars reported to a have higher yield in leaf miner infested fields. A higher tolerance to leaf miner and *Spodoptera* in a breeding line ICGV 86031 is seen as an enhanced ability of the vegetative tissue to regrow after defoliation (Wightman and Rao 1994).

Traditional breeding programs has been successful in some areas but has failed in others due to a lack of improved and more efficient screening methods and techniques, as well as a lack of knowledge about the underlying mechanisms of resistance. Before starting any breeding program, we need to know about the inheritance/genetics of certain traits. Furthermore, in breeding programs, greater diversification of parental resources is needed to expand the genetic base and produce new cultivars that will perform better under adverse conditions. To access genes from GP3 and GP4 pools, recombinant DNA technology with a cis-transgenic approach must be used. Emerging molecular tools offer a way to improve the efficiency, effectiveness and gain from traditional breeding programs, especially for complex polygenic traits. A comprehensive approach incorporating traditionaland molecular breeding, with transgenics techniques would offer solutions to the complex problems presently confronting the peanut improvement.

4.5 Molecular Breeding in Peanut

Marker-assisted breeding implies the application of molecular markers in combination with genomics tools and techniques to improve traits in the desired direction using modern breeding strategies such as marker-assisted selection (MAS), marker-assisted recurrent selection (MARS), marker-assisted backcrossing (MAB), and genomic selection (GS). For the application of markers in breeding program availability of markers/marker techniques along with dense genetic linkage maps are necessary.

Progress in marker work has been heavily dependent on advances in marker technology. Initially, molecular marker discovery in peanut was focused on proteins and isozymes, followed by rapid progress on discovery of DNA-based markers such as RFLP, RAPD, AFLP, SSR and SNPs. The earlier genomics studies were focused on the use of polymorphic RFLP and RAPD markers for screening interspecific

breeding lines and cultivated peanuts genotypes (Burow et al. 1996, 2001; Subramanian et al. 2000; Dwivedi et al. 2001, 2002b; Garcia et al. 1995). *Eco*RI/*Mse*I and *MIu*1/*Mse*I primer pairs initially observed polymorphisms within cultivated peanut accessions and interspecific tetraploid derivatives in AFLP assays (He and Prakash 1997; Herselman 2003). However, use of these markers is not suitable for the application in MAS. Although RFLP is co-dominating and highly reproducible marker, method is more time consuming, laborious and based on radioactive-based probes. Further, dominant marker RAPD is distributed in whole genome but have less reproducibility. Whereas, assays of STS (PCR-based sequence tagged site markers derived from closely linked RFLP markers) and SCAR (sequence characterized amplified region originated from polymorphic RAPD bands) are more accurate, co-dominant in nature and can be used for high-throughput genotyping (Olson et al. 1989; Paran and Michelmore 1993). Similarly, dominating nature of AFLP can be more suitable for diversity analysis compared to MAP. This marker can be converted into co-dominating markers namely, STS and SCAR (Konieczny and Ausubel, 1993; Negi et al. 2000; Huaracha et al. 2004). Due to multitude characteristics of SSRs (simple sequence repeats) such as reproducibility, polymorphism, multiallelic, genome distribution, co-dominance inheritance, simple assay and transferability across species, SSRs are markers of choice for the molecular breeding (Weber 1990). As a result, several novel SSRs have been found in peanut and utilized in breeding program. In recent years, more than 2500 SSR markers have been produced in peanut using methods such as the construction and subsequent sequencing of SSR-densed genomic DNA libraries, the sequencing and mining of Bacterial Artificial Chromosome (BAC)-end sequences (BES) for repeats motifs, and the mining of transcript sequences developed either by Sanger method of sequencing or more advanced developed next-generation sequencing (NGS) approaches (Mace et al. 2007; Cuc et al. 2008; Gautami et al. 2009; Pandey et al. 2012a, b). Efforts by several researchers to develop SSRs markers for peanut have resulted in more than 9000 repeats (Guo et al. 2016). The degree of polymorphisms in cultivated peanuts, however, remains low. The use of more robust techniques such as SNPs, kompetitive allele-specific PCR or KASPar and genotyping by sequencing (GBS) approaches are required due to the lower genetic variation at molecular level. There have been major developments over the last decade, with the discovery of massively parallel technology, next generation sequencing technology (NGS). Several multiple approaches to bioinformatics, whole genome study using de novo assembly, resequencing have enabled the development of large numbers of SNPs and SSRs (Bertioli et al. 2016). In addition, NGS and data mining have made it easier to discover cost-effective, large-scale generation of EST-SSRs and SNPs (expressed sequence tags) (Pandey et al. 2012a; b; Zhao et al. 2012; Guimaraes et al. 2012; Nagy et al. 2012; Zhang et al. 2012; Bosamia et al. 2015). With the advantages of most abundance and widely distribution of SNP throughout genome, cost efficient SNP genotyping platform are not freely available for the tetraploid peanut and microsatellites are still considered as best choice as markers for tetraploid peanuts because it is co-dominant and easy to score (Pandey et al. 2012a; b). Miniature Inverted-Repeat Transposable Elements

(MITEs) based markers have also been developed in peanut (Bhat et al. 2008; Shirasawa et al. 2012) and a large number of polymorphic *AhMITE*1 markers have recently been identified from the peanut genome re-sequencing data (Gayathri et al. 2018).

4.5.1 Genetic Linkage Maps

The development of genetic mapping populations by crossing genetically divergent parents is the first step in developing linkage maps and the identifying QTLs/genes linked to the trait of interest. Several genetic populations for mapping traits have been developed including F_2 population, $F_{2:3}$ populations, recombinant inbred lines (RILs), backcross introgression lines (BILs), near isogenic lines (NILs), and association mapping populations based on natural populations, nested association mapping (NAM), and multi-parent advanced generation inter-cross (MAGIC) populations (Pandey et al. 2012a, b; Varshney et al. 2013; Janila et al. 2013). Higher levels of polymorphism greatly encourage the development of more saturated genetic linkage maps that form the basis for identifying markers of economically significant characteristics closely linked to governing QTLs. Based on F_2 mapping population derived from *A. stenosperma* (AA) × *A. cardenasii* (AA), the first linkage map of 11 LGs consisting 117 RFLP markers loci was constructed (Halward et al. 1993). Later, population derived from cross between synthetic amphidiploids [*A. batizocoi; BB* × *(A. cardenasii; AA* × *A. digoi; AA*] and cv. Florunner were used to construct linakge map that comprised of 370 RFLP loci on 23 LG (Burow et al. 2001). The first incomplete/partial linkage map based on population derived from cultivated peanutwas made, which had 12 AFLP markers distributed on five linkage groups (Herselman et al. 2004). Further, agenetic 88 BC_1F_1 individuals from cross of synthetic amphidiploids (*A. ipaënsis* × *A. duranensis*) with *A. hypogaea*cultivar Fleur11 was constructed using 298 SSRs loci that distributed on 21 LGs. (Fávero et al. 2006). Thes elow-density maps have minimal use in QTL mapping. Later, several SSR based genetic maps have been constructed by various research groups including 131 SSR loci map distributed on 20 LGs from the population of cross between Yueyou 13 and Zhenzhuhei (Hong et al. 2008), 135 loci on 22 LGs, from a RILs population derived from crossing parents, ICGV 86031 and TAG 24 (Varshney et al. 2009), composite map of 175 SSR in 22 LGs (Hong et al. 2010), 101 SSRs in 17 LGs (Zhang 2011) and integrated composite map of 897 SSRs distributed on 20 LGs was constructed by Gautami et al. (2012b). In a similar vein, two other genetic maps based on RIL derived from TAG24 × GPBD4 (188 SSR loci) and TG26 × GPBD 4 (181 SSR loci) were created and used to generate a 225 SSR loci consensus map (Sarvamangala et al. 2011; Sujay et al. 2012). In addition to these maps, two linkage maps are generated one with 119 SSR loci from the RILs of ICGS 76 3 × CSMG 84-1 and another with 82 SSR loci from RILs derived from cross, ICGS 44 × ICGS 76 (Gautami et al. 2012a) along with consensus linkage map population derived from TAG 24 × ICGV 86031. More recently, Qin et al. (2012) built individual genetic maps consisting of 236 and 172 EST-SSR marker loci, respectively,

from the two RILs populations, one from cross, Tifrunner × GT-C20 and other from cross, SunOleic 97R X NC94022. A consensus map consisting 324 marker loci spanning 1352 cM of genetic distance was then constructed (Qin et al. 2012). Wang et al. (2012) constructed linkage map based on single mapping population with a total of 318 SSRs mined from BAC-end sequences (BES) covering 1674.4 cM map distance. Shirasawa et al. (2012a) used sequence data from the parental lines to mine marker in silico and mapped 1114 loci in 21 LGs. Later, 897 marker loci (895 SSRs and 2 CAPS) were mapped on 20 LGs spanning a total genetic distance of 3607.97 cM, followed by 3693 marker loci mapped on 20 LG with total map distance spanning 2651 cM (Gautami et al. 2012b; Shirasawa et al. 2013).

Nearly all maps, however, constructed using low-throughput markers, including RFLPs, SSRs have produced comparative low density map and are unable to provide reliable information of complex trait. In contrast, the most abundant marker, SNPs was used to construct genetic map for the "A" genome for the first time in 2012. With advent of high-throughput sequencing technologies, different methods have been established to genotype the mapping population of peanut such as restriction site-associated sequencing (RAD-seq) double digest RAD-seq, genotyping by sequencing (GBS) and high density SNPs or insertion/deletions (InDel) (Miller et al. 2007; Peterson et al. 2012; Poland et al. 2012; Zhou et al. 2014; Han et al. 2018). The first genetic map based on SNPs for cultivated peanuts was constructed using ddRAD seq with 1621 SNPs (Zhou et al. 2014). Recently, SLAF-seq technology (specific length amplified fragment sequencing (SLAF-seq) was used to construct high density linkage map in peanut (Wang et al. 2018a, b; Hu et al. 2018). These dense genetic maps would have a greater effect on genetic studies in peanuts and marker-assisted selection programs to improve traits. Table 4.2 provides a list of genetic maps constructed using various molecular markers for the *Arachis* species.

4.5.2 Marker Trait Associations and QTLs Discovery

4.5.2.1 Mapping Populations and Approaches

The two prerequisites for molecular breeding are the discovery of linked markers associated significantly with traits to be improved and the identification of QTLs by genetic mapping. Trait mapping can be done by various approaches including linkage mapping, linkage disequilibrium (LD) based association mapping and joint use of linkage and LD based, linkage-cum- association mapping (JLAM). In linkage mapping, bi-parental populations (RILs, NILs, BILs and $F_{2:3}$) are commonly used however, recent advances in the area of marker trait association, linkage disequilibrium based association mapping like candidate gene-based association (CGAS) and GWAS were also used in natural populations (Zhu et al. 2008). Bi-parental populations have high trait mapping ability, but have disadvantages in being able to have few traits and low resolution with allelic variation. In contrast, association mapping has advantages of use of large number of germplasm to cover huge amount

Table 4.2 Comprehensive list of genetic maps developed in peanut

Populations used	Markers used	No of loci mapped	Coverage (cM)	References
Genome AA				
A. stenosperma × *A. cardenasii*	RFLP	132	1063.00	Halward et al. (1993)
[*A. stenosperma* × (*A. stenosperma* × *A. cardenasii*)]	RAPD, RFLP	206	800	Garcia et al. (2005)
A. duranensis (K7988) × *A. stenosperma* (V10309)	SSR	204	1230.89	Moretzsohn et al. (2005)
A. duranensis (K7988) × *A. stenosperma* (V10309)	SSR, anchor, AFLP, NBS profiling, SNP	369	–	Leal-Bertioli et al. (2009)
A. duranensis (PI 475,887) × *A. duranensis* (Grif 15,036)	SNP, SSR, SSCP, RGC	1724	1081.30	Nagy et al. (2012)
A. duranensis (K7988) × *A. stenosperma* (V10309)	SSR, TE	597	544.00	Shirasawa et al. (2013)
A. duranensis (K7988) × *A. stenosperma* (V10309)	SNP, SSR	384	705.10	Bertioli et al. (2014)
A. duranensis (K7988) × *A. stenosperma* (V10309)	SNP, SSR, RGA	502	1004.10	Leal-Bertioli et al. (2016)
Genome BB				
A. ipaensis (K30076) × *A. magna* (K30097)	SSR	149	1294.00	Moretzsohn et al. 92009)
A. ipaensis (K30076) × *A. magna* (K30097)	SSR, TE	798	461.00	Shirasawa et al. (2013)
A. ipaensis (K30076) × *A. magna* (K30097)	SSR, TE	399	678.00	Leal-Bertioli et al. (2015)
K 9484 (PI 298,639) × GKBSPSc 30,081 (PI 468,327) of *A. batizocoi*	SSR	449	1278.60	Guo et al. (2012)
Genome AABB				
Florunner × TxAG-6 {[*A. batizocoi* K9484 × (*A. cardenasii* GKP10017 × *A. diogoi* GKP10602)]4 × }	RFLP	370	2210.00	Burow et al. (2001)
ICG 12991 × ICGVSM 93541	AFLP	12	139.4	Herselman et al. (2004)
[Fleur 11 × (*A. ipaensis* × *A. duranensis*)4 ×]	SSR	298	1843.70	Foncéka et al. (2009)
Yueyou 13 × Zhenzhuhei	SSR	131	679.00	Hong et al. (2008)

(continued)

Table 4.2 (continued)

Populations used	Markers used	No of loci mapped	Coverage (cM)	References
TAG 24 × ICGV 86031	SSR	135	1270.50	Varshney et al. (2009)
TAG 24 × ICGV 86031	SSR	191	1785.40	Ravi et al. (2011)
Yueyou 13 × Zhenzhuhei	SSR	132	684.90	Hong et al. (2010)
Yueyou 13 × Fu 95-5	SSR	109	540.69	Hong et al. (2010)
Yueyou 13 × J11	SSR	46	401.70	Hong et al. (2010)
TAG 24 × GPBD 4	SSR	56	462.24	Khedikar et al. (2010)
TAG 24 × GPBD 4	SSR	188	1922.40	Sujay et al. (2012)
TG 26 × GPBD 4	SSR	45	657.90	Sarvamangala et al. (2011)
TAG 24 × GPBD 4	SSR	181	1963.00	Sujay et al. (2012)
ICGS 44 × ICGS 76	SSR	82	831.40	Gautami et al. (2012b)
ICGS 76 × CSMG84-1	SSR	119	2208.20	Gautami et al. (2012b)
SunOleic 97R × NC94022	SSR, CAPs	172	920.70	Qin et al. (2012)
SunOleic 97R × NC94022	SSR, CAPs	206	1780.60	Pandey et al. (2014)
Tifrunner × GT-C20	SSR	318	1674.40	Wang et al. (2012)
Tifrunner × GT-C20	SSR, CAPs	239	1213.40	Qin et al. (2012)
YI-0311 × Nakateyutaka	SSR, TE	326	1332.90	Shirasawa et al. (2012a)
Satonoka × Kintoki	SSR, TE	1114	2166.40	Shirasawa et al. (2012b)
VG 9514 × TAG 24	SSR	95	882.90	Mondal et al. (2012)
A. hypogaea "Runner IAC 886" × (*A. ipaensis* × *A. duranensis*)4×	SSR, TE	1469	1442.00	Shirasawa et al. (2013)
Tifrunner × GT-C20	SSR, CAPs	378	2487.40	Pandey et al. (2014)
Tifrunner × GT-C20	SSR	418	1935.40	Pandey et al. (2014)
A. hypogaea "Runner IAC 886" × (*A. ipaensis* × *A. duranensis*)4×	SNP, SSR	772	1487.30	Bertioli et al. (2014)
Zhonghua 5 × ICGV 86699	SNP, SSR	1685	1446.70	Zhou et al. (2014)
VG 9514 × TAG 24	SSR, ISSR, TE, RGC	190	1796.70	Mondal et al. (2014a; b)

(continued)

Table 4.2 (continued)

Populations used	Markers used	No of loci mapped	Coverage (cM)	References
Zhonghua 10 × ICG12625	SSR	470	1877.30	Huang et al. (2015)
Zhonghua 10 × ICG12625	SSR, TE	1219	2038.75	Huang et al. (2016)
TAG 24 × GPBD 4	SSR, TE	289	1730.80	Kolekar et al. (2016)
SunOleic 97R × NC94022	SSR	248	1425.90	Khera et al. (2016)
Fuchuan Dahuasheng × ICG 6375	SSR	347	1675.60	Chen et al. (2016)
Xuhua 13 × Zhonghua 6	SSR	228	1337.70	Chen et al. (2016)
Florida-EP™ "113" × Georgia Valencia	SSR, SNP	30	157.80	Tseng et al. (2016)
ICGV 00350 × ICGV 97045	DArT, DArTseq	1152	2423.12	Vishwakarma et al. (2016)
79266 × D893	SSR	231	905.18	Li et al. (2017)
Florunner × TxAG-6 {[*A. batizocoi* K9484 × (*A. cardenasii* GKP10017 × *A. diogoi* GKP10602)]4 × }	SSR	91	1321.90	Wilson et al. (2017)
Yuanza 9102 × Xuzhou 68-4	SSR	743	1232.57	Luo et al. (2017)
Yuanza 9102 × Xuzhou 68-4	SSR	830	1386.19	Luo et al. (2017)
ICGV 07368 × ICGV 06420	DArT, SSR	854	3526.00	Shasidhar et al. (2017)
ICGV 06420 × SunOleic 95R	DArT, DArTseq	1435	1869.00	Shasidhar et al. (2017)
ICGV 06420 × SunOleic 95R	SNP	1211	–	Liang et al. (2017)
TMV 2 × TMV 2-NLM	TE	91	1205.66	Hake et al. (2017)
GG20 × CS19	SSR	12	558.74	Bera et al. (2016b)
ZH16 × sd-H1	SNP	3630	2098.14	Wang et al. (2018a; b)
Xuhua 13 × Zhonghua 6	SNP	2595	2465.62	Liu et al. (2020)
TG37A × NRCG CS85	SNP	266	1092	Dodia et al. (2019)
Tifrunner × NC 3033	SNP, SSR	1524	3382	Chavarro et al. (2020)
NC 3033 × Tifrunner	SNP, SSR	1524	3381.96	Luo et al. (2020a, b)
Consensus				

(continued)

Table 4.2 (continued)

Populations used	Markers used	No of loci mapped	Coverage (cM)	References
3 populations	SSR	175	885.40	Hong et al. (2010)
3 populations	SSR	293	2840.80	Gautami et al. (2012b)
2 populations	SSR	225	1152.90	Sujay et al. (2012)
13 maps	SSR, TE	3693	2651	Shirasawa et al. (2013)
8 populations	SSR, TE	5874	2918.62	Lu et al. (2018)

of allelic variation in nature which can provide high resolution mapping, however, QTL detection power is very low. Further, multiparent populations namely, MAGIC population, training population and recombinant inbred advanced intercross line (RIAIL) populations (Morrell et al. 2012) are being exploited. MAGIC populations involve recombination of alleles from multiple parents and provide a high mapping resolution and high power of detecting QTL (Cavanagh et al. 2008). By choosing different founder parents and creating a wide collection of interrelated RILs populations, NAM population captures genetic diversity, which allows achieving high resolution mapping by using power of ancestral meiotic recombination. In addition to that, whole-genome average interval mapping (WGAIM) along with the joint association mapping approaches have been developed to analyses QTL accurately (Verbyla et al. 2014). Further, WGAIM method concurrently integrates all probabilities at each marker for all individuates. Two NAM populations have been developed for peanut, *i.e.*, one each in Spanish type (cross of ICGV 91114 with 22 testers) and other in Virginia type (cross of ICGS 76 with 21 testers) and could be used for higher resolution of mapping (Varshney 2016; Pandey et al. 2016). Sixteen populations have been developed in a community wide project in the US and numerous QTLs have been identified for biotic stresses in a limited subset of these populations (Chu et al. 2018).

4.5.2.2 Trait Mapping and QTLs Discovery for Biotic Stresses

For most biotic stresses, various types of markers have been identified. Stalker and Mozingo (2001) established an association between ELS sporulation and RAPD marker AM 1102 in a peanut population derived from a cross between an *A. hypogaea* and *A. cardenasii* introgression line with 'NC 7'. Mondal et al. (2008) identified RAPD marker J 7 (1300) as a suitable genetic marker associated with rust. Genetic linkage maps with 188 and 181 loci respectively, were constructed from population derived from TAG 24 × GPBD 4 and TG 26 × GPBD 4. Morever, RILs mapping populations were used to associate SSR markers (IPAHM103, GM2009, GM1536,

GM2301 and GM2079 with major QTLs for rust. Using genotyping and phenotyping data, 13 QTLs for rust and 13 QTLs for late leaf spots were discovered from these RILs populations, explaining 2.54 to 82.96% and 10.07 to 67.8% phenotypic variance, respectively (Sujay et al. 2012). In $F_{2:3}$ progenies of cross between two contrasting parents, TMV 2 (susceptible) × COG 0437 (resistant), Shoba et al. (2012) identified SSR marker, PM384 associated with LLS and rust. Shoba et al. (2013) also reported a QTL for LLS in the same mapping population with 37.9% phenotypic variation. However, large QTLs that contribute > 20% phenotypic variation and must be confirmed should be targeted for active QTL introgression in elite breeding lines (Varshney et al. 2013). Mondal et al. in the year 2012 reported two EST derived SSR markers named as SSR HO115759 and SSR GO340445 and these were appropriate candidates for use in marker-assisted selection as they are closely linked to rust resistance. Two transposable element (TE) based markers, TE 498 and TE 360, were reported to be in association with the rust resistance in a RIL population of VG 9514 × TAG 24. But, these linked markers need further validation to speed up the process of introgressing resistance into megavarieties (Sujay et al. 2012; Gajjar et al. 2014).

Lei et al. (2006) detected an AFLP named as, E45/M53-440 originated SCAR primer, AFs-412 to be closely associated with resistance to infection by *A. flavus.* For protection against *A. flavus* invasion, Liang et al. (2009) idebtified six QTLs, each of which is located on a separate linkage group and can explain phenotypic variance of 6.2 to 22.7%. Two large QTLs for TSWV resistance were discovered by Qin et al. (2012). The AFLP marker was used by Herselman et al. in 2004 to map aphid resistance in ICG12991. A number of DNA markers linked to root-knot nematode resistance were also discovered. For the root-knot nematode, *Meloidogyne arenaria*, RAPD markers (Z3/265, RKN410, KKN229 and RKN440), RFLP loci (*R2430E* and *R2545E)* and SSR markers were found to be linked tightly to dominant resistance genes, *Mae* (for restricting egg number) and *Mag* (for restricting gall formation) (Burow et al. 1996; Garcia et al. 1996; Church et al. 2000; Wang et al. 2008; Carpentieri-Pípolo et al. 2014). This marker was cloned and SCAR (197/909) and RFLP (R2430E, R2545E and S1137E) probes obtained from cDNA libraries further confirmed linkages with nematode resistance (Burow et al. 1996; Chu et al. 2007). Nagy et al. (2010) used high-resolution mapping for nematode resistance to establish another SSR marker, GM565. Later, another tool, single base pair extension (SBE) was discovered to be efficient for high-efficient SNP mapping in peanut, and the genetic map revealed five candidate genes conditioning resistance to biotic stresses (Alves et al. 2008). Later, Khera et al. in the year (2013) used a collection of 96 explanatory SNPs to establish KASPar assays, named as GKAMs (Groundnut KASPar Assay Markers), and validated 90 GKAMs against different biotic stresses. Clevenger et al. (2017) used QTL-seq approach to identify KASP markers from an RIL population segregating for quantitative field resistance to LLS. QTL analysis from cross,'Tifrunner × GT-C20' derived F_2 genetic population detected two QTLs for thrips, 15 for TSWV, and 37 QTLs for LS. However, in the advanced F_5 population, one for thrips, nine for TSWV, and 13 for leaf spots have been identified. This is the first research to report new QTLs for thrips, TSWV, and leaf spots, and it will

need to be improved and validated in the future (Wang et al. 2013, 2014). Using a common RILs population derived from cross, VG 9514 X TAG 24, two main QTLs, qTDP-b08 for total development period and qAE2010/11-a02 for adult emergence with 57–82% and 13–21% PVE respectively, were detected for bruchid resistance (Mondal et al. 2014a; b). A mapping population derived from the SunOleic 97R x NC94022 cross yielded 155 QTLs, including one and three significant QTLs for TSWV and LLS resistance, respectively (Guo et al. 2013). Further, many marker-trait associations (MTAs) for *Aspergillus flavus* (01, 24.69% PV), ELS (06, 9.18–10.99% PV), LLS (01, 18.10% PV) and GRD (31, 10.25–39.29% PV) were discovered using GWAS approach (Pandey et al. 2014). Recently, Jasani et al. (2021) reported one major QTL from cross JL-24 x NRCGCS-85 for PBND resistance. Details of some main QTLs that have been reported in peanut to be associated with disease stresses are given in Table 4.3.

Table 4.3 QTLs reported for biotic stresses in peanut

S. No.	Traits/biotic stress	Marker system	Source/population	QTLs identified	PVE (%)	References
1	Rust and LLS	SSRs	GJG17 × GPBD4	Two	29.06–70.52	Ahmad et al. (2020)
2	*Sclerotinia blight*	SNPs	Tamrun OL07 × T × 964117	Seven	6.6–25.6	Liang et al. (2020)
3	*Aspergillus flavus*	SNPs	Yueyou 92 × Xinhuixiaoli	Two	5.15–19.04	Khan et al. (2020)
4	Bacterial wilt	SSRs and SNPs	Xuhua 13 × Zhonghua 6	One	37.79 -78.86	Luo et al. (2020a, b)
5	Stem rot	SSRs and SNPs	Tifrunner × NC 3033	33	4.76–20.01	Luo et al. (2020a, b)
6	Stem rot	SNPs	Tifrunner × NC 3033	Two	9–13	Cui et al. (2020)
7	PBND	SSRs	TAG 24 × ICGV 86031	5	3.92–12.57	Jadhav et al. (2019)
8	ELS and LLS	SNPs	Florida-07 × GP-NC WS16	6	5–41	Chu et al. (2019)
9	Tomato Spotted wilt virus	SNPs	SunOleic 97R × NC94022,	One	36.51	Agarwal et al. (2019)

(continued)

Table 4.3 (continued)

S. No.	Traits/biotic stress	Marker system	Source/population	QTLs identified	PVE (%)	References
10	Aflatoxin	SSRs	Zhonghua 10 × ICG 12625	12	9.32–21.02	Yu et al. (2019)
11	Bacterial wilt	SNPs	Xuzhou 68–4 × Yuanza 9102	4	7.72–23.33	Wang et al. (2018a; b)
12	ELS, LLS and TSWV	SNPs	Tifrunner × GT-C20	35	6.32–47.63	Agarwal et al. (2018)
13	PBND	SSRs	JL-24 × NRCGCS-85	2	12.38–16.88	Jasani et al. (2018b)
14	Stem rot	SSRs	GG-20 × NRCGCS-319	1	25.36	Kamdar et al. (2018)
15	ELS and LLS	SNPs	Florida-07 × GP-NC WS 16	15	4.93–16.60	Han et al. (2018)
16	ELS, LLS and TSWV	SSRs	Tifrunner × GT-C20	42	6.36–15.6	Pandey et al. (2017a)
17	Leaf spot	SNPs	Tamrun OL07 × Tx964117	Six	11- 24	Liang et al. (2017)
18	Bacterial wilt	SSRs and SNPs	Xinhuixiao × Yueyou 92	Two	12–21	Zhao et al. (2016)
19	LLS	SNPs	Zhonghua 5 × ICGV 86699	20	3.41–19.12	Zhou et al. (2016)
20	Rust and LLS	SSRs and TE	TAG24 × GPBD4	Five	10.2–53.7	Kolekar et al. (2016)
21	Root-knot nematode	SNPs	*A. duranensis* × *A. stenosperma*	Eight	5.70–43.70	Leal-Bertioli et al. (2016)
22	ELS, LLS and TSWV	SSRs and ESTs	SunOleic 97R × NC94022	48	3.88–29.14	Khera et al. (2016)
23	TSWV	SSRs	Florida EPTM "113" × GeorgiaValencia	2	10.02–22.70	Tseng et al. (2016)
24	Stem rot	SSRs	GG-20 × CS-19	1	17.15	Bera et al. (2016b)
25	Rust	SSRs and TE	*A. ipaënsis* (accession K 30076) × *A. magna* (accession K 30097)	13	5.8–59.3	Leal-Bertioli et al. (2015)

(continued)

Table 4.3 (continued)

S. No.	Traits/biotic stress	Marker system	Source/population	QTLs identified	PVE (%)	References
26	Bruchid	SSRs	VG 9514 × TAG 24	44	11.00–82.00	Mondal et al. (2014a; b)
27	Root-knot nematode	RFLP	Florunner × TxAG-6	10	-	Burow et al. (2014)
28	LLS	SSR	TMV 2 × COG 0437	1	20.2–24.1	Shoba et al. (2013)
29	TSWV, LS, Thrips	SSRs	Tifrunner × GT-C20	77	5.20–34.92	Wang et al. (2013)
30	TSWV	SSRs	Tifrunner × GT-C20 and SunOleic 97R × NC94022	2	12.90–35.80	Qin et al. (2012)
31	Rust and LLS	SSRs	TAG 24 × GPBD 4 and TG 26 × GPBD 4	43	2.54–82.96	Sujay et al. (2012)
32	LLS and Rust	SSRs	TAG 24 × GPBD 4	23	1.70–55.20	Khedikar et al. (2010)

4.5.2.3 Advanced Trait Mapping Approaches

In addition, advanced-backcross QTL (AB-QTL) is proposed by Tanksley et al. (1996) to save the time and increase the precision of identifying associated markers and simultaneous ingression of desirable traits from wild species and wild forms to cultivated genotypes. Some QTLs for root-knot nematode resistance (Fonceka et al. 2012; Burow et al. 2014), LLS and rust resistance (Varshney et al. 2013) was identified using the same approach. Further higher resolution towards mapping efforts can be gained with NGS methods and mapping by sequencing approaches (Huang et al. 2009; Schneeberger and Weigel 2011). Furthermore, QTL-seq, MutMap, and BSR-seq are three new trait mapping methods that have demonstrated for rapid recognition of candidate genomic regions and diagnostic markers for the targeted traits. The DNA samples pooled from F_2 segregating progeny derived from a cross between a mutant type and corresponding wild type are used in the MutMap method to conduct whole-genome re-sequencing (WGRS) (WT). The SNP index is used to identify new SNPs, and then the sequence of bulk DNA is compared to the reference sequence. The SNPs that have sequence reads containing only the mutant sequences (SNP index = 1) are assumed to be related to the causal SNP responsible for the mutant phenotype. MutMap strategy was conceptually integrated to the standard F_2 and RIL populations in the QTL-seq technique (Takagi et al. 2013). For accelerated detection of agronomically significant QTLs, a combination of BSA and whole genome re-sequencing is used. BSR-Seq uses RNA-Seq reads for mapping traits effectively, even in populations in which no molecular polymorphic survey have previously been

conducted (Liu et al. 2012). Allele-specific functional markers and SNPs markers for rust resistance and LLS resistance were identified in peanut using the QTL-seq method (Pandey et al. 2016, 2017b). ICRISAT recently released a 10-SNP panel with related SNPs for two foliage fungal diseases (rust and LLS) mapped on chromosomes A02 (LLS) and A03 (rust).

4.5.3 Molecular Breeding for Disease Resistance

Some of the diagnostic markers reported to be linked with QTLs of significant effect have been validated and established for use in marker-assisted selection (MAS) and marker-assisted backcross (MABC) breeding programme. MABC is most commonly employed to introgress transgene or loci with major effect into a commercial cultivar. (Figs. 4.13 and 4.14). Further, to improve the genotype MARS and genomic selection (GS approaches are now days are being used to accumulate desirable alleles with small effects). Using MABC approach first variety with resistance to root-knot nematode,-NemaTAM was released in the USA (Simpson et al. 2003). Since then, several other cultivars with the use of *A. cardenasii*, as a source of resistance have been released in the USA named as, Tifguard (Holbrook et al. 2008), Webb (Simpson et al. 2013), Georgia-14 N (Branch and Brenneman 2015) and TifNV-High O/L (Holbrook et al. 2017). Major QTLs governing rust and LLS explaining up to 82.62% and 67.98% phenotypic variation respectively, was transferred from

Fig. 4.13 Peanut plants tagged for genotyping in early generation in the field

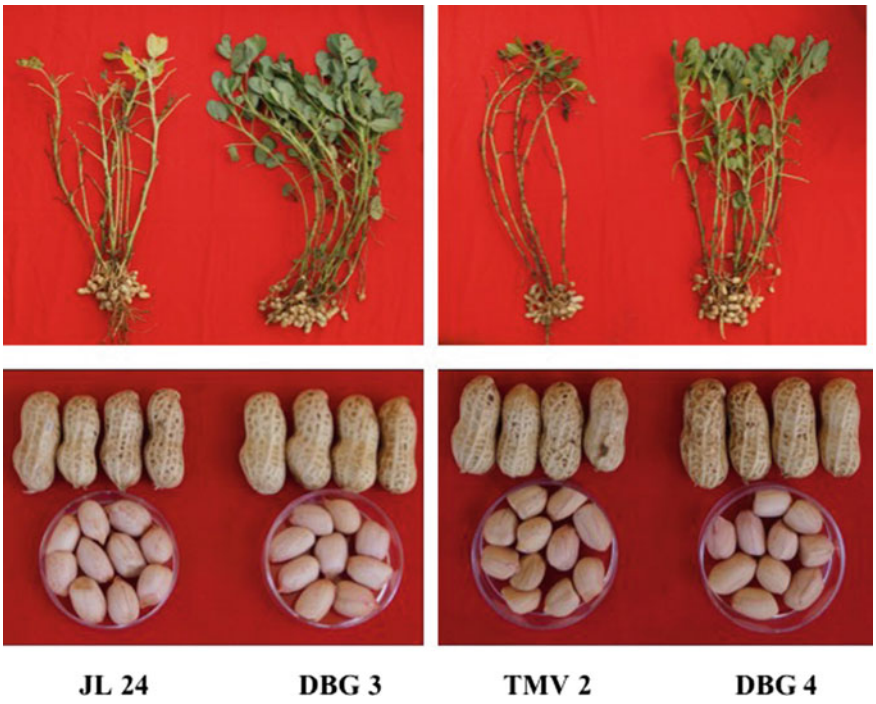

Fig. 4.14 Late leaf spot resistant marker-assisted backcross breeding lines DBG 3 and DBG 4 developed from JL 24 and TMV 2, respectively (Yeri and Bhat 2016; Kolekar et al. 2017)

'GPBD 4' into three rust susceptible varieties viz., ICGV 91114, TAG 24 and JL 24 by using four linked markers namely, IPAHM103, GM2301, GM2079 and GM1536 in MABC program (Varshney et al. 2014). Two developed amphidiploids synthetics from ICRISAT, one is ISATGR 278-18 derived from cross, *A. duranensis* × A. *batizocoi* and other is, ISATGR 5B derived from cross, *A. magna* × *A. batizocoi* were utilied to introgress resistance to foliar diseases in five mega-varieties namely, ICGV 91278, ICGV 91114, ICGS 76, JL 24 and Dh86 using backcrosses (Kumari et al. 2014). Furtherefforts to use the linked markers for resistance to foliar diseases for pyramiding desirable QTLs in the three popular peanut cultivars viz., GJG 9, GG 20 and GJGHPS 1 are underway (Fig. 4.15).

Marker-assisted selection (MAS) aims to improve tolerance against biotic stresses by targeting major QTLs and eventually omits the possibility of stacking minor effect and epistatic QTLs. Thus, combining the desirable genes or pyramiding of minor and epistatic QTLs through the MABC is a big challenging task (Peleman and Voort 2003). To accumulate beneficial alleles with small phenotypic effects in a single genotype, the MARS and GS approaches can be used. GS is a kind of MAS that at a time predicts all loci, haplotype, or marker effects across the genome to calculate Genomic Estimated Breeding Values (GEBVs). It is a tool in plant breeding to predict the genetic value of untested lines based on genome-wide marker

Fig. 4.15 High yielding peanut breeding lines with huge pod bearing

data. Estimated GEBVs are then used for selecting desirable types for advancing the breeding cycle without need of phenotyping. Unlike MABC and MARS, GS or genome wide selection (GWS) aims to sort out superior lines with higher breeding value in a breeding program using marker profile data of whole genome and high throughput genotyping. As a result, GS appears to be a possible strategy for breeding complex traits in the near future. But these approaches in peanut have not been widely explored. However, more recently initial GS usage attempts have identified four GS-models and suggested the use of the best models to achieve higher accuracy in predicting characters with large G × E effects in peanut (Pandey et al. 2020).

4.6 Transcriptomics and Proteomics

Transcriptomic analysis has been employed to identify the differentially expressed genes for resistance to ELS (Gong et al. 2020), LLS (Han et al. 2017) and leaf rust (Rathod et al. 2020a, b). The results suggest that a few major genes and several factors

mediate the resistance to ELS disease, showing the characteristics of quantitative trait in defense responses. Most of these studies identified the defense-related genes. Molecular responses of the wild peanut challenged with the LLS pathogen were studied using cDNA-AFLP and 2D proteomic study. A total of 233 differentially expressed genes, involved in cell wall strengthening, hypersensitive reaction and resistance related proteins were identified in wild peanut, *A. diogoi* (Kumar and Kirti 2015). Transcriptomic analysis in the *A. flavus* resistant peanut cultivar J11 led to the detection of 663 differentially expressed genes. Further functional analysis revealed that these genes encoded a wide range of defense or PR- proteins (pathogenesis related proteins). Changes in the expression patterns of these genes might contribute to peanut resistance to *A. flavus* (Zhao et al. 2019). Bosamia et al. (2020) used RNA-Seq to unravel the mechanisms of resistance to stem rot caused by *Sclerotium rolfsii* using a resistant (NRCG-CS85) and susceptible (TG37A) genotype. Differentially expressed genes and translated proteins in wild peanut indicate its defense mechanism upon interaction with pathogen and provide initial breakthrough of genes possibly involved in sensing or recognizing and early signalling responses to fight the infection through subsequent development of resistance.

4.7 Transgenic Approaches for Genetic Improvement of Peanut Against Biotic Stresses

As a consequence of ploidy barrier between the cultivated species and the wild species, introgression of stress-related genes from the diploid progenitors by conventional breeding becomes complex. Further, introgression lines developed by crossing wild species with cultivated peanuts carried undesirable gene blocks. To overcome the problem of lack of beneficial genes within crossable germplasms, genetic engineering/recombinant DNA techniques such as *Agrobacterium tumefaciens* mediated or direct transfer of desired genes from wild species would be an ideal option to impart resistance against diseases (Vasavirama and Kirti 2012).

Resistance to several fungal and virus diseases has been achieved through the use of transgenes coding for cell wall components such as chitinase, glucanase etc., PR proteins, coat proteins, bacterial chloroperoxidase, oxalate oxidase, RNA interference (RNAi), and crystal proteins. Sunkara et al. (2013) reviewed the use of chitinase, glucanase, Rs-AFP2 (*Raphanus sativus* antifungal protein-2) and SniOLP (*Solanum nigrum* osmotin like protein) for LLS and ELS, oxalate, chitinase and glucanase for *S.* blight, chitinase for rust, and anionic peroxidase, glucanase, stilbene synthasesynthetic peptide D4E1, chitinase, mod1, nonheme chloroperoxidase (cpo), LOX 1, and Pn LOX 3 against *A. flavus* infection and aflatoxin production. When compared to the parent variety, transgenic lines of the Okrun cultivar harboring chitinase gene from rice and glucanase genes from alfalfa showed a 43–100% reduction in *S. blight* incidence (Chenault et al. 2005). Two genes viz., Rchit and CHI coding for chitinase

enymes against *Fusarium* wilt and leaf spots fungi have been evaluated for inheritance in peanut transgenic events (Rohini and Sankara 2001; Iqbal et al. 2011, 2012). Late leaf spots incidence was decreased in transgenic lines of peanut expressing a defensin gene, BjD from mustard (Anuradha et al. 2008). Transgenics with cDNA sequence of barley oxalate oxidase conferred enhanced resistance to blight by *Sclerotinia* (Livingstone et al. 2005). Transgenics developed using bacterial non-heme chloroperoxidase gene from *Pseudomonas pyrrocinia (cpo-p)* and rice chitinase gene (*Rchit*) showed hyphal growth inhibition of *A. flavus* (Niu et al. 2009; Prasad et al. 2013).

The complete nucleotide sequence (4019 nts long) and genome organization (4 ORFs) of GRV are known (Taliansky et al. 1996). Because the coat protein gene of virus, GRAV has been sequenced and transformation constructs is created, the chances of producing rosette-resistant cultivars by inserting the coat protein genes into peanut have improved significantly (Taliansky et al. 1998). Peanut cultivar JL 24 was transformed with the GBNV nucleoprotein gene at ICRISAT, and T2 transgenic events were tested for virus resistance. If these events are successful, they will provide reliable GBNV resistance that can be bred into other peanut cultivars through back-cross breeding programs. Also, the genomes of viruses namely, PCV and IPCV is sequenced, so there are excellent chances of using viral coat protein genes to cuase resistance in peanut using unorthodox methods (Sharma and Anjaiah 2000). At ICRISAT, peanut cultivar JL24 was transformed with IPCV-H coat protein and replicase genes to induce pathogen-derived resistance. Genetically modified peanut cultivars that carry viral coat protein gene exhibited high levels of resistance to PStV (Franklin et al. 1993). Further, transgenic peanut plants of Gajah and NC 7 that contained untranslatable full length sequence (*CP2*) and translatable *CP* gene with an N-terminal truncation (*CP4*) of PStV, offered resistance to virus (Higgins et al. 2004). Insertion of viral nucleocapsid protein-coding gene (*tswvnp)* in peanut genome has resulted in resistance to TSWV (Brar et al. 1994). Furthermore, by activating RNA silencing, a natural virus defense mechanism, high-level resistance or immunity can be induced in plants (Waterhouse et al. 2001). RNAi technology such as,RNA silencing or homologous gene cosuppression are powerful methods for developing resistance to viruses in peanut genotypes (Wang et al. 2000). At ICRISAT, an RNAi-mediated approach is being used to counteract the effect of the PBNV genome's nonstructural silencing suppressor gene (NSs gene). Transformed plants with specific small RNAs, the products of RNA silencing were highly resistant to PStV infection and the resistance was stably inherited over atleast five generations (Dietzgen et al. 2004). Resistance derived from pathogens by introducing GRAV or GRV genes/ genome sequences, or SatRNA-derived sequences that inhibit/slow down GRV replication is a possible strategy against GRD via transgenic plant generation (Taliansky et al. 1996). Cry1 EC gene against *S. litura* (Tiwari et al. 2008) and cry1 X gene against *H. armigera* and *S. litura* (Entoori et al. 2008) are two synthetic genes that have shown promise against their respective insect pests. When the trypsin inhibitor gene from cowpea was introduced into peanuts, it increased tolerance to insects (Xu et al. 2003). The success and achievement of transformation techniques is still poor due to its allopolyploidy, genotype specificity, low transformation and

regeneration efficiency and low level of transgene expression. Although many transgenic lines have been developed against biotic stresses, to date no transgenic cultivars of peanut is released commercially. Targeted genome editing technology for functional genes is an exciting new advancement. It has the potential to be an effective tool in driving disease-fighting varietal development. Plant targeted genome editing has proven to be effective using zinc finger nucleases (ZFNs) and transcriptional activator-like effector nucleases (TALENs), which involve two DNA binding proteins flanking a sequence of interest (Lloyd et al. 2005; Wright et al. 2005; Cermak et al. 2011; Li et al. 2012; Mahfouz et al. 2011). Furthermore, CRISPRs (clustered regularly interspaced short palindromic repeats), a high-throughput genome editing technology focused on the prokaryotic immune system, offer a promising hope for further peanut improvement. Recently, CRISPR/ Cas9 technology has become very popular for genome editing, trait discovery and manipulating genome in desired direction. However, utilization of CRISPR based genome modification in peanut is challenging, because of complexity of genome. Also, CRISPR/Cas9 technology does not transfer DNA sequences from one species to another. However; CRISPR/Cas9 technology has the ample scope for enhancing the limited resistance available against biotic stresses.

4.8 Future Prospects

Peanut is a high nutritional value, multipurpose food-feed-fodder crop that has gained global significance. The key to maintain competition and meet the potential future demand is the genetic enhancement of peanuts for increased yield and enhanced tolerance to biotic and abiotic stresses. Knowing the presence of higher diversity, allelic variations and presence of novel alleles in wild *Arachis* species, more conserted multiinstitutional and multidisciplinary efforts with greater investment are required to intensively evaluate and properly characterize the desirable quest in wild *Arachis* and their use in breeding program supported with modern genomic technologies. New genetic and genomic innovations have given tremendous optimism to achieve higher genetic gains with high precision and accuracy in less time and resources. Peanuts now have enough genomic and genetic resources required to speed up the process of peanut improvement. There are presently few but successful examples of molecular breeding products available in peanut; however in the coming years there will be more of such successful tales. In genomics research, still, more efforts are required to saturate the peanut linkage map so that MAS can be deployed for peanut improvement. At the same time, new breeding technologies such as genomic selection and genome editing are also being implemented to develop next-generation model peanut varieties that can give better performance under changing climatic conditions. Moreover, to combine conventional breeding and molecular breeding approaches, a comprehensive approach is needed to improve complex traits governed by multigenes and other problems that peanut is currently facing.

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Chapter 5
Genomic Designing for Biotic Stress Resistance in Rape and Mustard

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Abstract Oilseed *Brassica* species (mainly *Brassica napus* and *Brassica juncea*) are economically important crops, cultivated worldwide for their edible oil and protein-rich livestock feed. Major diseases of Brassica crops such as Alternaria leaf spot, white rust, blackleg, Sclerotinia stem rot, clubroot, downy mildew, and powdery mildew results in significant yield and economic losses in Brassica growing countries the world over. The frequency and severity of biotic stresses have intensified due to global climate change which requires urgent research efforts to tackle them effectively. For providing resistance against diseases, genetic resources are being exploited using conventional and tissue culture techniques (embryo rescue and somatic hybridization). Advanced sequencing technologies enabled the sequencing of all the six species of the U's triangle i.e. *B. rapa, B. nigra, B. oleracea, B. napus, B. juncea,* and *B. carinata.* The emerging omics technologies such as genomics, proteomics, transcriptomics, and metabolomics have elucidated the function of genes and molecular intricacies between the host and pathogen at a deeper level. In this chapter, we discuss the genetic resources, mapping, and cloning of R-genes, omics approaches, transgenics, gene editing, and bioinformatics tools in context to biotic stresses in rapeseed-mustard. A holistic approach involving genetics, genomics resources, genetic engineering, gene editing, and bioinformatics tools will facilitate the development of Brassica crops which can combat various biotic stresses.

Keywords Brassicas · Gene pool · Molecular markers · Genetic mapping · Quantitative trait locus · Cloning · Resistance gene analogues

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5.1 Introduction

5.1.1 The Brassica Crops

The Brassicaceae (*Cruciferae*) is one of the ten most economically important plant families encompassing 3709 species and 338 genera (Warwick et al. 2006). Brassicas exhibit enormous diversity, as they are being cultivated as oilseed, vegetables, mustard condiments, and fodder crops (Dixon 2007). Mustard (*B. juncea*), rape (*B. rapa*), rapeseed (*B. napus*), and Ethiopian mustard (*B. carinata*) are mainly categorized as oilseed crops (Raymer 2002; Rakow 2004), while *B. oleracea* is raised as cole-crop, the genera *Raphanus* for edible roots and *Sinapis* along with *B. nigra* for condiments.

Among the several species, six cultivated species of *Brassica* are considered agriculturally important. Three of these are diploids, i.e. *B. rapa* (AA genome, $n = 10$), *B. nigra* (BB, $n = 8$) and *B. oleracea* (CC, $n = 9$) and three are allopolyploids namely, *B. juncea* (AABB, $n = 18$), *B. napus* (AACC, $n = 19$), and *B. carinata* (BBCC, $n = 17$). These allopolyploid species are ancestrally derived from the natural hybridization between the diploid species. The genomic relationship within these species has been described by U in the form of the U's triangle model (U 1935).

B. napus and *B. juncea* are among the important crops grown across the world for edible oil and protein-rich livestock feed. Historically, *B. juncea* has been grown in India and China and more recently in Australia (Rakow 2004). *B. juncea* has been derived from multiple independent hybridization events between wild forms of *B. rapa* and *B. nigra* (Vaughanet al. 1963; Axelsson et al. 2000; Prakash et al. 2009; Kaur et al. 2014). According to the molecular marker-based phylogenetic analysis, the *B. juncea* has been divided into the exotic (East European/Chinese) gene pool and the Indian gene pool (Pradhan et al. 1993; Burton et al. 2004). *B. napus* is derived from the hybridization of *B. rapa* and *B. oleracea* (Allender and King 2010) but since its true wild relative has not been identified so far, it is difficult to comment on its origin (Gomez Campco and Prakesh 1999).

5.1.2 Production Status

Globally, oilseed Brassicas ranks second after soybean (USDA 2018). During 2018–19, the area and production of rape and mustard in the world were estimated to be 36.59 million hectares (mha), 72.37 million tonnes (mt) with a productivity of 1980 kg/ha. India ranks fourth in the edible oil sector after the USA, China, and Brazil. Globally, India accounts for 19.8% and 9.8% of the total acreage and production, respectively (USDA 2018). A substantial increase in production from 61.64 mt to 72.42 mt and productivity from 1840 kg/ha to 1980 kg/ha has been observed during the last 8 years (2010–11 to 2018–19; DRMR-www.drmr.res.in). In India, rape and

mustard crops are grown under varied agro-climatic conditions ranging from north-eastern/north-western hills to down south under timely/late sown, irrigated/rain-fed, mixed cropping, and saline soils conditions. Indian mustard accounted for about 75–80% of the 6.23 mha under these crops in the country during the 2018–19 crop seasons (DRMR-www.drmr.res.in). Despite, having a substantial area under oilseeds cultivation, India is one of the major importers of edible oil worth >Rs. 0.73 trillion annually (Ministry of Agriculture and Farmers Welfare, Government of India, 2017). Declining oilseed production and shifting of acreage to other crops are some of the reasons for widening this demand-supply gap, leading to enforced huge import of edible oils in India resulting in remarkable foreign exchange drain (USDA 2018).

The main factors restricting the productivity of oilseed Brassica are mainly the non-availability of high-yielding varieties appropriate for high input conditions, weather fluctuations, and many abiotic and biotic stresses. Abiotic stresses include salt, drought, heat, frost, and heavy metal stress. At the seedling stage, plants are more prone to salt and high (>30 °C) temperature stress while frost and drought pose a major challenge during the flowering to grain filling stage. The presence of heavy metals in the soil induces several physiological and biochemical responses, leading to a reduction in the growth and development of the plant. Besides abiotic stresses, the Brassica crop is afflicted by several biotic stresses, which include fungal diseases, bacterial, and viral diseases, nematodes, and aphid pests (Table 5.1).

5.2 Major Pathogens and Pests Causing Yield Losses in Mustard

Fungal diseases play an important role in causing significant yield loss and affect the crop at different crop growth stages from seedling up to maturity. The major diseases covered in this chapter are Alternaria leaf spot (ALS), white rust (WR), Sclerotinia stem rot (SSR), Blackleg (BL), powdery mildew (PM), along with aphid attack (Fig. 5.1)

5.2.1 Alternaria Leaf Spot and Blight (ALB) in *Brassica* plants are mostly caused by *Alternaria* species (*Alternaria brassicae*, *Alternaria brassicicola*, *Alternaria raphani*, and *Alternaria alternata*). The global quantitative and qualitative yield losses of oilseeds and vegetable *Brassicas* due to *Alternaria* infections range from 11 to 100% depending upon environmental conditions, the stage of crop and management strategies (Kolte 1985; Tewari and Conn 1993; Verma and Saharan 1994; Seidle et al. 1995; Meah et al. 2002; Prasad et al. 2003; Mondal et al. 2007). Premature ripening, shrivelling of seeds, shattering, reduction in siliqua length, seeds per siliquae, 1000-seed weight, oil content, germination, and changes in the chemical composition of seeds are some of the major manifestations of severe *Alternaria* infections (Nijhawan and Hussain 1964; Bandyopadhya et al. 1974; Degenhardt et al. 1974; Chahal and Kang 1979; Kaushik et al. 1984; Kolte 1987; Tripathi et al.

Table 5.1 Major diseases and insect-pests of oilseed Brassicas

Major diseases	Causal agent	Distribution and yield losses
Fungal		
Alternaria leaf blight	*Alternaria brassicae* and *A. brassicicola*	Global; up to 47% depending on the region and season
Sclerotinia stem rot	*Sclerotinia sclerotiorum*	Global presence; up to 100% depending on the stage of infection, recently emerged as a major threat in India
Blackleg	*Leptosphaeria maculans*	Prevalent in Canada, Europe, and Australia
Powdery mildew	*Erisiphe cruciferarum*	*Global presence*
Oomycete		
White rust	*Albugo candida*	Spread Worldwide; yield losses vary from 28–90%
Downy mildew	*Hyaloperonospora parisitica*	Spread worldwide
Bacterial disease		
Black rot	*Xanthomonas campestris*	The incidence reported worldwide mostly on vegetable *brassicas* More likely in warmer climates
Clubroot	*Plasmodiophora brassica*	Reported globally mainly on vegetable brassicas
Viral diseases		
	Cauliflower mosaic virus	Globally on vegetable *brassicas*
	Turnip mosaic virus (TuMV)	Globally on vegetable *brassicas*
	Turnip yellow virus (TuYV)	Globally on vegetable *brassicas*
Parasitic plant		
	Orobanche aegyptiaca	Broad host range, emerging as a major problem in India
Aphid Pests	*Brevicoryne brassicae*; *Lipaphis erysimi* and *Myzus persicae*	Worldwide, losses due to *L. erysimi* vary from 10 to 90%

1987). Losses due to silquae infection are comparatively more in *B. campestris* (*B. rapa*) than other *Brassica* spp.

5.2.2 White Rust (WR) is another major limiting factor in achieving the yield potential of oilseed Brassica worldwide and especially in the Indian subcontinent. The disease is caused by an obligate oomycete *Albugo candida*. The fungus can affect both the vegetative and the reproductive phases of the plant. The symptoms appear as white pustules on the abaxial surface of leaves/cotyledons while infection of the flowering stem and pods results in staghead formation leading to major economic losses (Fig. 5.1a, b). Yield losses due to white rust range from 17 to 34% in Indian mustard (Bains and Jhooty 1979; Bisht et al. 1994) and 34% in *Toria* (Kolte et al. 1981). The extent of yield losses reported worldwide go as high as 60% depending

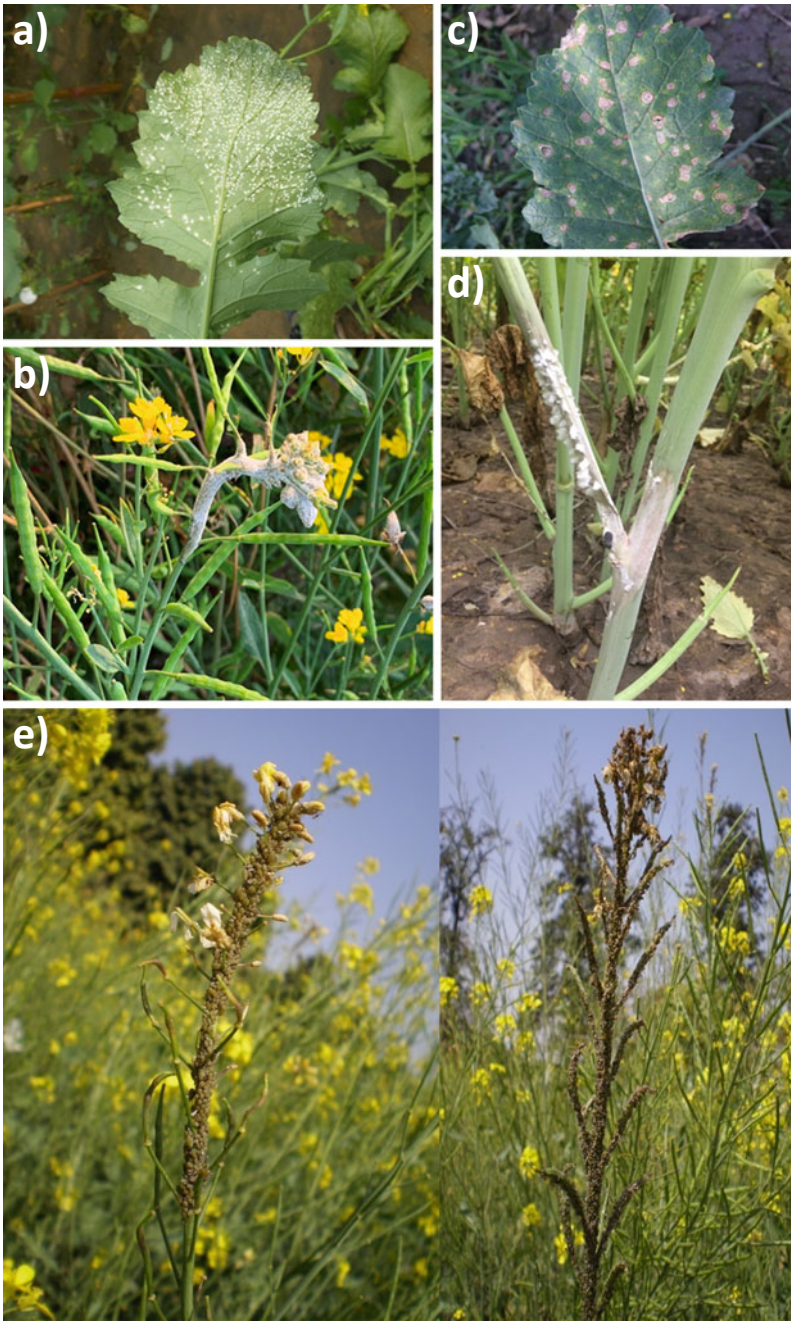

Fig. 5.1 Major biotic stresses in Brassica production **a**, **b** white rust, staghead (*Albugo candida*) **c** Alternaria leaf blight (*Alternaria brassicae*); **d** Stem Rot (*Sclerotinia sclerotiorum*) and **e** Aphid infestation

upon the severity of the infection and environmental conditions (Harper and Pittman 1974; Petrie and Vanterpool 1974; Kolte 2002; Khunti et al. 2003; Sachan et al. 2004; Kumar and Kalha 2005).

5.2.3 Blackleg (BL), yet another serious disease affecting Brassica production worldwide is caused by a hemi-biotrophic fungus *Leptosphaeria maculans,* belonging to the class Ascomycetes. The disease is prevalent in Australia, Canada, and Europe where *B. napus* (Canola) is extensively cultivated (McGee and Emmett 1977; Gugel and Petrie 1992). On the other hand, the incidence of blackleg in India and China is very low presumably due to the widespread cultivation of *B. juncea* which is inherently resistant to blackleg. Evans et al. (2008) predicted a possible increase in the range and severity of blackleg disease with the projected global climate change. The disease appears as white/buff-colored lesions on the leaves and white or grey lesions with a dark border on stems. Under severe infection conditions, the fungus girdles the stem at the crown thereby leading to the lodging of the plant. Blackleg infection on pods results in premature pod shattering and seed infection (Raman et al. 2013).

5.2.4 Sclerotinia Stem Rot (SSR) a disease of minor importance earlier, has emerged as a new threat to Brassica cultivation worldwide. The cosmopolitan appearance of the pathogen, broad host range due to adoption of multiple pathogenicity strategies, makes it truly a devastating, and difficult to manage pathogen (Bolton et al. 2006). The Sclerotinia infection initially appears as water-soaked patches on leaves and/or stems especially during the flowering stage. These spots gradually turn into whitish brown enlarged patches of cottony growth followed by girdling of the stem, drooping leaves, and premature drying of plants. The sclerotial development is commonly observed inside and outside the dried lesion (Saharan and Mehta 2008). The severity of SSR in Brassica crops is highly influenced by the time of infection, climatic conditions, and microenvironment in the plant canopy. It is a menace for the oilseed crop right from the seedling stage to the end of the flowering stage accounting for 10–100% economic damage based on the environmental conditions and plant growth stage at infection. There are no known varieties with complete resistance to Sclerotinia.

5.2.5 Powdery Mildew (PM): PM is caused by *Erysiphe curciferarum* once considered a minor disease is emerging as a major devastating disease worldwide mainly due to the rising temperatures as a result of global warming. Drifting climatic conditions, pathogen variation, and high-intensity cropping practices have led to considerable disease outbreaks posing threats to Brassica production (Honghao et al. 2020). The disease appears as powdery growth on the leaves, stems, and pods and spreads rampantly under favorable environmental conditions (Meena et al. 2014). In the Indian sub-continent powdery mildew of Brassicas results in sporadic yield losses ranging from 10 to 43%, whereas in rapeseed-mustard the disease accounts for 10–90% yield losses besides reduction in oil quality and quantity (Meena et al. 2018).

5.2.6 Aphid: Besides fungal pathogens, the Brassica crops are hosts for several aphid pests that cause significant damage. The damage is caused by both nymphs and adults of aphid. Three species viz. *Brevicoryne brassicae*; *Lipaphis erysimi* and *Myzus*

persicae cause serious damage to Brassica crops in one or other parts of the world. *L. erysimi,* the most important pest of oilseed Brassica in the Indian subcontinent, is native to eastern Asia (Blackman and Eastop 2000). The damage caused by *L. erysimi* has been reported to vary from as low as 10 to as high as 90% which is influenced by varying population levels, prevailing agro-climatic conditions and phenological stage of the crop (Rohilla et al. 2004; Rana 2005; Ahuja et al. 2009; Kular and Kumar 2011; Deka et al. 2017; Kumar 2017). In addition to direct feeding damage, *L. erysimi* is also a vector of 10 non-persistent viruses including turnip mosaic virus, cauliflower mosaic virus, and cabbage black ringspot virus (Rana 2005), though no virus problem is reported in oilseed Brassica in India.

5.3 The Pathogens and Pests: Knowing Thy Enemy

ALB is caused by the necrotrophic fungus belonging to the genus *Alternaria*. The large, multicellular, melanized conidia with longitudinal as well as transverse septa constituted the major taxonomic features of *Alternaria.* Strains of *A. alternata*, *A. brassicae,* and *A. brassicicola*, have been defined as formae speciales or 'pathotypes' as they have pathological differences inspite of sharing similarity in morphological features (Nishimura and Kohmoto 1983; Verma and Saharan 1994; Saharan et al. 2003; Thomma 2003). Woudenberg et al. (2013) subclassified *Alternaria* clade into 121 strains representing the Alternaria complex based on DNA sequence in combination with morphological features. Distribution of *A. alternata* and *A. raphani* is widespread in the Northern hemisphere while *A. brassicae* and *A. brassicicola* are cosmopolitan. The pathogens in this genus have a wide spectrum of hosts including almost all crucifers. Host specificity varies in different species of *Alternaria* on *Brassica* species (Dixit et al. 2020).

WR and staghead are caused by the fungal pathogen *Albugo candida* an obligate oomycete that can attack Brassicaceae, Convolvulaceae, and other plant genera (Agrios 1988). Being a member of class Oomycete, it is characterized by affecting only or primarily the above-ground tissues of its hosts, in particular the leaves, young stems, and fruits (Streets 1982). Morphological variation within *A. candida* for the first time was demonstrated by Savulescu and Rayss 1930. Based on host specialization and morphology, 10 varieties of *A. candida* were established. Three different biological forms of *A. candida* were confirmed on *Raphanus sativus, B. juncea,* and *B. rapa* sp. *chinensis* (Hiura 1930). Pound and Williams (1963) proposed the concept of races in *A. candida* based on species relationships. Six races of *A. candida* specific to the species they infect were classified. The race I, 2, 3 4, 5, and 6 were identified based on their specific reaction to *Raphanus sativus* var. Early Scarlet Globe, *B. juncea* var Southern Giant Curled, *Armoracia rusticana* var Common, *Capsella bursa-pastoris, Sisymbrium officinale,* and *Rorippa islandica* respectively (Pound and Williams 1963). Later, two new races—race 7 and 8 from *B. rapa* Turnip or Polish rapeseed and *B. nigra* were added (Walker et al. 1975; Delwiche and Williams 1977). Williams (1985) reported three new races, races 9, 10, and 11 from

B. oleracea, Sinapis alba, and *B. carinata* respectively. Bhardwaj and Sud (1988) identified nine new biological races from nine hosts comprising 26 cultivated and wild cruciferous hosts, viz, *B. rapa* var. Brown Sarson cv. BSH 1, *B. rapa* var. Toria cv. OK-I, *B. juncea* cv. Varuna, *B. chinensis. B. rapa* var. Pekinensis cv. Local, *B. rapa* cv. PTWG, *Raphanus sativus* cv. Chineses Pink, *Raphanus raphanistrum,* and *Lepidium virginicum.* Based on the specificity of a race to different crucifers at least 13 races of *A. candida* have been identified globally, of which race 2 predominantly infects *B. juncea* (Verma et al. 1999).

BL of *Brassicas* is caused by a species complex comprising of *Leptosphaeria maculans–L. biglobosa* of which *L. maculans*is associated with the severe cankers formed at the base of the stem, causing early senescence, lodging, and severe yield losses (Fittet al. 2006). *L. maculans*is a polycyclic fungus that undergoes several reproductive cycles on the host plant species in one growing season with production both asexual and sexual spores. The sexually produced ascospores are the primary inoculum and the source of variability. Various classifications have been made to catalog the existing variability in *L. maculans.* Initially, McGee and Petrie (1978) divided the *L. maculans* isolates as virulent and avirulent based on the contrasting pathogenicity on a differential set comprising of Westar, Quinta, and GlacierIt. Koch et al. (1989) categorized the isolates as aggressive and non-aggressive while Johnson and Lewis (1994) categorized them as A group (Aggressive) and the B group (non-aggressive) based on the reaction on a couple of different Brassica hosts (*B. napus, B. rapa, B. oleracea, B. juncea, B. carinata, B. nigra, Thlaspi arvense* and *Raphanus sativus).* It was Balesdent et al. (2005) who systematically characterized a worldwide collection of *L. maculan* isolates into several defined races based on the composition of the *Avr* genes within the isolates. Balesdent et al. (2006) analyzed 1797 field isolates from France and could identify only 11 races. Most of the European isolates shared *ArvLm6* and *ArvLm7*, whereas *ArvLm3* and *ArvLm9* were absent. On the other hand, Australian isolates were highly diverse in terms of *Avr* allele combinations and were therefore highly virulent, capable of overcoming the resistance in *B. juncea and B. carinata.* Liban et al. (2016) analyzed the diversity of *Avr* genes in *L. maculans* isolates collected from Canadian commercial fields to conclude that *AvrLm*6 and *AvrLm*7 were predominantly present in >90% of the isolates whereas *AvrLm-3*, *9*, and *LepR2* were detected in less than 10% of isolates screened. Thus far, at least 14 *Avr* genes of *L. maculans* have been identified and atleast seven i.e *AvrLm1* (Gout et al. 2006), *AvrLm2* (Ghanbarnia et al. 2015), *AvrLm3* (Plissonneau et al. 2016), *AvrLm4-7* (Parlange et al. 2009), *AvrLm5* (Van de Wouw et al. 2014), *AvrLm6* (Fudal et al. 2007), and *AvrLm11* (Balesdent et al. 2013) have been cloned.

SSR*: Sclerotinia sclerotiorum* is the most notorious pathogen associated with stem rot of Brassicas. It is non-specific, cosmopolitan, omnivorous and a highly successful pathogen that can infect nearly 400 other dicot species besides *Brassica.* The fungus acts both, as a soilborne and airborne pathogen capable of infecting both above and below-ground plant parts (Abawi and Grogan 1979). Several attempts have been made to record the genetic diversity/variability within the Sclerotinia isolates collected from a crop plant within a geographical region (Mert-Turk et al. 2007), isolates collected from different geographical locations (Sun et al. 2005;

Aldrich-Wolfe et al. 2015; Lehner and Mizubuti 2017; Lehner et al. 2015), and also in isolates collected from different crop species (Attanayake et al. 2019). Several reports on epidemiology and population biology of *S. sclerotiorum* infecting canola are available (Atallah et al. 2004; Sun et al. 2005; Sexton et al. 2006; Sharma et al. 2018). Predominantly clonal mode of reproduction is revealed by several studies with very limited outcrossing (Kohli and Kohn 1998). Njambere et al. (2008) observed very little variability in the ITS sequences from *Sclerotinia* isolates collected from multiple hosts. Most of the diversity studies are based on morpho-physiological parameters (Sharma et al. 2013), and molecular markers like microsatellite markers (Sirjusingh and Kohn 2001), AFLP (Cubet et al. 1997) also reveal clonal reproduction most of the time. Further, mycelial compatibility groups (MCG), another method for analyzing the diversity has also been used to establish the kinship within *S. sclerotiorum* isolates collected from multiple geographical locations, and different crops (Li et al. 2008). However, recently the reliability of MCG in studying genetic diversity has been questioned (Kamvar et al. 2017). Although genetic diversity has been observed in the isolates of *S. sclerotiorum* from different hosts yet no clear correlation could be drawn between genetic diversity and virulence.

PM*: Erysiphe cruciferarum* causing the PM of crucifers is an obligate biotrophic fungus belonging to the family *Erysiphaceae.* It has a polycyclic life cycle with very short incubation and latent periods. The *Erysiphe* taxonomic studies have been elaborated by Braun (1981, 1987, 2012). Conidial morphology and germination have been utilized for constructing the identification keys for different powdery mildew genera and species. Globally, more than 873 species of PM affecting as many as 10,000 plant species have been documented (Braun 1981, 1987, 2012; Kuhn et al. 2016). Despite being the presence of both asexual and sexual modes of reproduction, the pathogen variability has not been extensively evaluated for powdery mildew.

Aphids: Several aphid species have been reported to cause a variable level of damage to the Brassica crops. Among these, three species viz. *B. brassicae, L. erysimi,* and *M. persicae* cause serious damage in one or other parts of the world. Of these, *L. erysimi* is the most important in the Indian subcontinent (Blackman and Eastop 2000). *B. rapa* and *B. juncea* are generally preferable hosts as compared to other *Brassica* species (Rana 2005). It is cosmopolitan in distribution and is found wherever Brassica plants are grown. Host range may include many species and genera of Brassicaceae, including *Brassica, Barbarea, Capsella, Erysimum, Iberis, Lepidium, Matthiola, Nasturtium, Raphanus, Rorippa, Sinapis, Sisymbrium,* and *Thlaspi* (Capinera 2001; Kumar 2015).

5.4 Methods of Control

Changing climate and land use patterns have increased the risk of epidemics and underline the importance of better and robust integrated disease management measures. With the growing consciousness about the harmful effects of pesticides,

the integrated use of various agricultural practices including optimum spacing, sanitation, and integrated nutrient management with less and timely fungicidal sprays are gaining importance in recent years. Apart from fungicides and pesticides, many cultural operations have been utilized extensively for the management of diseases and pests. In this section, we have elaborated on the control methods adopted for each of the biotic stresses.

ALB: The integrated management strategy of ALB includes tolerant varieties, timely sowing, seed treatment, fungicides, biocontrol agents, and recommended doses of fertilizers. Since *Alternaria* leaf spot is also soil and seed-borne disease, chemical treatment of seeds with Bavistin, Difolatan, and Dithane for atleast 24 h was found effective for control of pathogen in seeds (Kolte and Tewari 1978; Kumar and Singh 1986). Seed-borne inoculums can be minimized by the aging of Brassica seeds at temperatures more than 35°C for about 5–6 months (Chahal 1981; Kolte 1985). The use of biofertilizers such as Azatobacter, PSB along with recommended doses of fertilizers helps in the establishment of vigorous plants and thereby minimizing the incidence of *Alternaria* blight and white rust. Biocontrol agents including *Chaetomium globosum, Fusarium* spp., *Streptomyces griseoviridis, S. rochei, S. hygroscopicus*, *Trichoderma harzianum,* and *T. koningii*were found effective for ALB (Vannacci and Harman 1987). Essential oils and various phytochemical extracts have also been shown to inhibit spore germination and the growth of *Alternaria* pathogens. Various plant extracts including *Azadirachta indica*, *Allium sativum,* and *Zingiber officinale* have been used for the management of ALB (Mahapatra and Das 2013). Latif et al. 2006 used garlic extract for the management of seed-borne pathogens in mustard.

WR: *Albugo candida* can infect plants at various stages of the life cycle starting as early as the seedling stage. Seed treatment with Metalaxyl followed by foliar spray of Ridomil MZ is effective in managing WR up to 65% at leaf stage and 40% at stag-head stage respectively. The stage of the crop, the number of sprays, and the time interval between foliar sprays are crucial for the effective control of the WR (Saharan et al. 2014). Foliar sprays with chemical fungicides in combination with biological agents like *T. harzianum* and *P. fluorescence* can reduce the white rust disease severity. Seed treatment with *T. harzianum* and *P. fluorescence* followed by foliar spray of Ridoml-MZ, Captan, and Mancozeb effectively reduced pressure of *Albugo candida* (Rohilla et al. 2001; Rathi and Singh 2009).

BL: Effective management of blackleg mainly depends on the use of resistant cultivars along with cultural practices that contribute to the reduction in the inoculum load thus minimizing the disease risk. As *L. maculans* survive as a saprophyte on the stubbles present in the field, therefore, many cultural practices like burning the stubbles and flooding the field have been adopted in the past to reduce the inoculum load (Petrie 1995). This also helps in keeping the highly virulent isolates in check and prolongs the effectiveness of the genetic resistance (Kutcher et al. 2010). According to Aubertot et al. (2004), blackleg can be reduced by early sowing and avoiding high nitrogen availability in the early stages of plant development. Since this is a seed-borne fungus, using clean seeds treated with fungicides like benzimidazole, dicarboximide, and morpholineare more economical and effective compared to foliar

sprays (West et al. 2001). Furthermore, low-density planting prevents the build-up of high humidity conditions from building up under the crop canopy thus stemming the disease spread.

SSR: In the absence of any resistant variety available, the farmers largely depend on cultural practices and strategic application of chemical fungicides to control the damage caused by *S. sclerotiorum*. Although none of the fungicides offers complete control yet some commonly used foliar fungicides from the group methyl benzimidazole carbamate (Attanayake et al. 2011), anilinopyrimidines (Benigni and Bompeix 2010), succinate dehydrogenase inhibitor (Stammler et al. 2007), and demethylation inhibitor (Li et al. 2015a) have been deployed to impede the establishment and reduce the damage. The time of fungicide application is very critical for its effectiveness and maximum success is recorded when the fungicide application is made close to or during flowering as maximum multiplication of inoculum (ascospores) is witnessed during this period. Crop rotation with non-host monocots coupled with an efficient weed control program is highly recommended to keep the inoculum build-up under control (Kharbanda and Tewari 1996). Tillage of farmland has a direct impact on SSR incidence. Deep tillage tends to reduce the disease incidence by accelerating the degradation of sclerotia from the upper layers (Gracia–Garza et al. 2002; Wu and Subbarao 2008). Similarly, maintaining low moisture conditions under the crop canopy especially during the critical period of infection (flower initiation) helps to reduce infection. Biocontrol agents like mycoparasitic fungus *Coniothyrium minitans* capable of parasitizing the sclerotia are efficient in controlling the initial inoculum in the field. It is commercially available under the trade name of **Contans**. Additionally, oxalate degrading bacteria—*Bacillus cereus* and *B. subtillis* (Kamal et al. 2015), *Trichoderma* (Geraldine et al. 2013) have also been demonstrated to be effective in limited field trials.

PM: *Erisiphe cruciferarum,* an obligate biotrophic fungus belonging to the family—*Erysiphaceae* causes powdery mildew. The profound effect of sowing date, intercropping and plant density affects the severity of PM (Devi and Chhetry 2017). Delayed sowing of oilseed Brassicas increases the incidence of powdery mildew up to 71.5% as compared to 20.5% in timely sown conditions. Thus, a timely sown crop (during October) escapes infection by early maturity. However, in a non-traditional area of India (Jharkhand and Bihar), the disease appears early as the temperature remains above 20°C in the mustard growing season (Mukerji et al. 1999). Fungicides such as Sulfex, Karathane, and Calixin were found effective to reduce the disease intensity (Singh and Solanki 1974; Saharan and Sheoran 1985; Sharma et al. 1990). Additionally, biotic agents like *Trichoderma harzianum*, *Pseudomonas fluorescens* alone or in combinations with garlic extractcan effectively control PM (Meena et al. 2013).

Aphids: The two major strategies adopted and practiced widely to control the aphid infestation are timely sowing of the crop and optimal nutrient application. Early sown crops (mid-October) lead to phenological asynchrony between the most susceptible crop stage and peak aphid activity (Ali and Rizvi 2011; Kular et al. 2012; Saeed and Razaq 2014). Additionally, the optimum nutrient application is an essential component for the management of *L. erysimi.* Excessive use of nitrogenous

fertilizers can make plants more succulent (Khattak et al. 1996) and susceptible to insect attacks (Yadu and Dubey 1999). On the other hand, an increase in K application adversely affects the reproduction and honeydew excretion by *L. erysimi* (Bhat and Sidhu 1983). Increased application of P and K reduced aphid incidence on mustard plants (Ram and Gupta 1992).

Biological agents like fungus *Verticillium lecanii,* neem seed kernel extract, extracts from *Lantana camara, Melia azedarach, Solanum xanthocarpum* exhibited variable toxicity against *L. erysimi* (Pandey et al. 1977). Hawkins et al. (1999) proposed the deployment of natural enemies for effective pest control. Many coccinellids, Syrphids chamaemyiid, and hemerobiid have been reported as natural enemies of the pest. Despite their abundance, these natural enemies fail to provide satisfactory control of mustard aphid due to phenological asynchrony between the peak activity period of *L. erysimi* and its natural enemies (Sarwar 2009; Kumar 2015). In addition to predators, small aphid parasitoids, *Diaeretiella rapae,* and *Encyrtu* ssp. are also associated with mustard aphid. Just like predators, the parasitoids also appear late in the season (around mid-February).

Due to the lack of effective and promising pest management programs against aphids, insecticides, particularly neonicotinoids, have been used extensively as the principal method of aphid management (Dewar 2007). Although a very high level of aphid control is obtained by the use of synthetic insecticides, high fecundity and short generation time of aphids lead to rapid population growth. Thus, the aphid population in the treated fields attains a level similar to that in the untreated fields in just 2–3 weeks (Singh et al. 1984). Therefore, a stable resistance source is the need of the hour to provide an effective, economical, and environmentally friendly option for aphid management.

5.5 Sources of Disease Resistance in the Primary, Secondary, and Tertiary Gene Pools

Management of biotic stresses using agronomic and chemical methods has been far from satisfactory/Thus, looking for a stable resistance gene within or across the genepool appears to be the long-term and sustainable way for effective biotic stress management. In this section, we have summarised the established resistance sources identified and illustrated so far.

ALB: Inter—specific variation exists for reaction to ALB**.** Digenomic *Brassicas*, *B. napus,* and *B. carinata* show a better response to *Alternaria* than their common monogenomic parent *B. rapa.* Among cultivated oilseeds Brassicas, *B. carinata* shows the highest level of resistance to *Alternaria.* Several genotypes of oilseed Brassicas were characterized for their response to *Alternaria* under uniform disease trials. Some of the Brassica genotypes, viz. CSR 43, CSR I42, CSR- I42-2, CSR 343, CSR 448, CSR 622, CSR 741, Gulivar, KRV-Tall, Midas, PHR 1, RC-781, TMV2, Tower, and YRT3 have shown field resistance under uniform disease nursery trials

(reviewed in Saharan et al. 2016). Pathotype-specific resistance to *A. brassicae* has been recorded in the genotype GS-05-1 of *B. napus* and RH-8544, Pusa Swarnim, and HC-9605 of *B. juncea* also showed a moderately resistant reaction (Kumar et al. 2014).

A high level of resistance against *A. brassicicola* and *A. brassicae* has not been identified among the cultivated *Brassica* species. However, related species such as *B. alba*, *B. carinata*, *B. desnottessi*, *B. elongata, B. fruticulosa*, *B. maurorum,* and *B. spinescenes* have shown resistance to *A. brassicae* (Brun et al. 1987a, b; Tewari and Conn 1993; Hansen and Earle 1997; Chrungu et al. 1999; Sharma et al. 2002). Resistance against *A. brassicae* and *A. brassicicola* has been reported among other wild members of the Brassicaceae family viz. *Alliaria petiolata*, *Barbarea vulgaris*, *Capsella bursa-pastoris, Coincya* spp., *Diplotaxis catholica*, *D. berthautii*, *D. creacea*, *D. erucoides, D. tenuifolia*, *Erucastrum gallicum*, *E. vesicaria* subsp. *sativa*, *Hemicrambe fruticulosa*, *H. matronalis*, *N. paniculata*, *R. sativus*, and *S. arvensis* (Conn and Tewari 1986; Conn et al. 1988; Sharma et al. 2002; Tewari 1991; Tewari and Conn 1993; Warwick 2011). Rajarammohan et al. (2017) reported a huge variation in the response of a collection of *Arabidopsis* accessions to *A. brassicae* ranging from highly resistant to highly susceptible.

WR: As mentioned above *B. juncea* is highly susceptible to white rust race 2, and its 2 variants, 2A (Gurung et al. 2007) and 2V (Kaur et al. 2011a), the main pathotypes of *A. candida* affecting *B. juncea.* The other species of oilseed *Brassica* viz., *B. nigra*, *B. napus, B. carinata,* and *B. rapa* have been reported to be comparatively tolerant to this *Ac* race 2 (Gulati et al. 1991; Liu and Rimmer 1991). The great variability of reactions has been recorded within the primary gene pool. The germplasm of the Indian gene pool of *B. juncea* is highly susceptible whereas resistant reaction has been observed in east European germplasm (Panjabi-Massand et al. 2010). *B. juncea* var. Cutlass showed a resistant response to the mixture of *A. candida* isolates derived from *B. juncea* and *B. rapa* except for 2V (Canadian isolate). Resistance response to all the Indian isolates and 2V has been found in the progenies of genotypes, RESJ -1052, RESJ-1004, RESJ-1005, RESJ-1033, and RESJ-1051 earlier selected for Indian isolates (Awasthi et al. 2012). More recently, Donskaja IV was also reported to be resistant to several Ac race 2 isolates collected from India (Arora et al. 2019). Further, varying levels of leaf and/or inflorescence ('staghead') resistance have been reported across genotypes from Australia, China, and India. Chinese and Australian genotypes generally gave better resistance than those from India (Li et al. 2007; Li et al. 2008b). Resistance to white rust has also been observed in wilds. *B. fruticulosa, C. abyssinica,* and *T. arvense* are generally free from white rust. *B. tournefortii* and some species of genus *Diplotaxis* and *Sinapis* gave moderately tolerant reaction (Saharan et al. 1988; Dang et al. 2000). Bansal et al. (1997) identified *Eruca sativa* as a potential source of white rust resistance and all the tested accessions of this genus were reported to be resistant to race 2. These species can be exploited to diversify the resistant source.

BL: Resistance for blackleg has been generally evaluated based on disease reaction recorded either under controlled greenhouse conditions or field conditions. Since the disease causes losses at both the seedling stage as well as the adult stage screening

has been carried out to identify resistant sources for different stages. It is observed that the resistance to Blackleg is both qualitative involving the canonical R- Avr interactions and quantitative which is race non-specific and governed by a complex interaction of multiple genes (Delourme et al. 2006). Genetic variation for resistance to blackleg has been reported in *B. napus* (Rimmer and Berg 1992), *B. rapa* ssp. *sylvestris* (Crouch et al. 1994). Significant deployable resistance has been reported in the *Brassicas* having the B genome—*B. nigra*, *B. carinata,* and *B. juncea* (Rimmer and Berg 1992). The B genome resistance, thus, has been used to improve the existing *B. napus* varieties (Chevre et al.1996, 1997; Gaebeleinet al. 2019). Resistance has also been reported in *B. oleracea* (Mithen et al. 1987) although in general, the C genome species of the *Brassicas* are susceptible to blackleg (Sjödin and Glimelius 1988). Besides the primary and secondary gene pool resistance to *L. maculans* has also been observed in *Arabidopsis*, *Diplotaxismuralis*, *D. tenuifolia, Eruca vesicaria,* and *Sinapis arvensis* (Siemens 2002; Delourme et al. 2006). Although, these sources of resistance can be used to further expand the genetic diversity of the elite cultivars, yet very limited success has been achieved in this direction.

SSR: Various approaches have been used to screen the germplasm for resistance against *S. sclerotiorum.* Disease resistance has been assessed under controlled conditions on young plants, detached adult leaf, detached stem, and petiole, or under the field conditions using steminoculations with or without injury. Despite rigorous screening, the identification of sources of complete/strong resistance against *Sclerotinia* has been a major challenge. All the cultivated rapeseed-mustard crops are susceptible to this pathogen. The maximum level of field tolerance in rapeseed reported from China was observed in Chinese *B. napus* cultivar Zhongyou 821 and Zhongshuang No. 9 (Li et al. 1999; Wang et al. 2004). Additionally, partial resistance has been identified in some of the *B. napus*—ZY004 and 06-6-3792 from China (Zhao et al. 2006; Gyawali et al. 2016; Wu et al. 2016a) and in some genotypes of *B. juncea* from China, Australia (Li et al. 2009b) and India (Singh et al. 2008). Navabi et al. (2010a, b) reported a usable level of resistance in *B. nigra* and *B. carinata* lines. *B. oleracea* germplasm has been screened for leaf and stem resistance and moderate resistance was identified in *B. rupestris*, *B. incana*, *B. insularis*, and *B. villosa* (Mei et al. 2011, 2013). Additionally, a promising high level of resistance to stem rot has been reported in the related wild crucifer species *B. fruticulosa*, *B. oxyrrhina*, *B. parachinensis*, *B. tournefortii* (Uloth et al. 2013), *E. cardaminoides*, *E. abyssinicum, D. tenuisiliqua* (Garg et al. 2010), *Capsella bursa-pastoris* (Chen et al. 2007).

PM: Resistance against powdery mildew has been identified in cultivated species. *B. napus, B. juncea,* and *B. carinata* showed resistance response while *B. rapa* is comparatively more susceptible (Bradshaw et al. 1989; Singh et al. 1997). Nanjundan et al. (2020), screened 1020 accessions of *B. juncea* for resistance to powdery mildew under natural epiphytotic conditions. RDV 29, an Indian mustard variety was identified to be highly resistant to powdery mildew infection. Resistance to *E. curciferarum* has also been reported in *B. carinata* (Dang et al. 2000; Mehta et al. 2008), *B. napus,* and *B. rapa* (Dang et al. 2000; Mehta et al. 2008) and all the tested genotypes of *Sinapis alba* (Mehta et al. 2008). Additionally, Australian canola and mustard (Uloth et al. 2016), Swede rape (Bradshaw et al. 1989) have also been characterized for their

reaction to powdery mildew infection. X-ray mutant resistant to powdery mildew has been found in a variety of *Abacus* and the resistance is attributed to enhanced levels of unsaturated fatty acids (Petkova et al. 2014). Various natural accessions of *Arabidopsis* (Kas-1, Ms-0, Su-0, SI-0, Stw-0, Te-0,Wa-1) also showed a resistance response to powdery mildew infection (Adam and Somerville 1996). *Arabidopsis*-powdery mildew pathosystem has been extensively used to understand the molecular genetics mechanism underlying host-pathogen interactions. Host resistance is operated both at the pre- and post-penetration stages of pathogen infection. The plant defense system is activated through the deposition of callose, pectin, cellulose, waxes, silicon, ion fluxes, formation of the papilla, phenolic compounds, overexpression of R, and activation of defense-related genes (Reviewed in Saharan et al. 2019). The triple mutants of *Arabidopsis*, AtMLO2, AtMLO6, and AtMLO12 induce programmed cell death and provide resistance against powdery mildews (Acevedo-Gracia et al. 2014). Enhance disease resistance mutants (EDR) of *Arabidopsis* also showed a link between mitochondrial function, SA-mediated resistance, and programmed cell death (Ausubal 2005). Higher levels of camalexin contribute to the enhanced resistance to powdery mildew in Cyp83 a1-3 mutants of *Arabidopsis* (Liu et al. 2016). Resistance genes, pathogen-related protein 1 (PR1), Beta 1,3glucanase (PR2), plant defensin 1.2 (PDF1.2), basic chitinase (PR3), pathogenesis-related 4 (PR4), and pathogenesis-related 5 (PR5) are involved in defense mechanism against powdery mildew infection in *Arabidopsis* (Gu et al. 2002; Zhu et al. 2013).

Aphid: Inter-varietal hybridization, induced mutagenesis, or autotetraploidy failed to provide significant resistance against aphid. Kumar et al. (2011) identified *B. fruticulosa as resistant* to *L. erysimi* after screening of a diverse array of wild crucifers. *B. juncea—B. fruticulosa* introgression Lines (IL) carrying genes for aphid resistance have been developed. Further, monitoring of feeding behavior of *B. brassicae* by electrical penetration graph (EPG) revealed a large reduction in duration of passive phloem uptake in *B. fruticulosa* compared to *B. oleracea* var. capitata cv. 'Offenham Compacta'. Aphids either showed a quick withdrawal of stylets from sieve tubes or there was disrupted phloem uptake (Cole 1994). The mechanism of resistance was a combination of both antixenosis and antibiosis (Ellis and Farrel 1995). In addition to resistance against aphid pests, *B. fruticulose* has also been reported to possess resistance (antibiosis) against *Delia radicum* (Jenson et al. 2002).

5.6 Mapping and Cloning the Resistance Genes and QTLs

With diverse sources of resistance against the major pathogens available in the primary, secondary, or tertiary gene pool several studies have been carried out to not only understand the pattern of genetic inheritance and but also dissect the genetic architecture of disease resistance. These studies have primarily used approaches like forward genetics, Quantitative trait locus (QTL) analysis more recently genome-wide association studies (GWAS). Molecular mapping relies on the traditional molecular

markers random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), cleaved amplified polymorphic sequences (CAPS), amplified fragment length polymorphism (AFLP), sequence characterized amplified region (SCAR), inter-simple sequence repeat (ISSR), and simple sequence repeat (SSR) to name a few and several different kinds of segregating mapping populations- F_2 population, doubled haploid (DH), backcross (BC) population, recombinant inbred lines (RILs), and near-isogenic lines (NILs) to map the chromosomal location of resistance loci. The generation of high-density linkage maps has improved the speed of identifying genomic regions contributing to disease resistance. The mapping of resistance genes has been more straight forward where the gene-for-gene resistance interaction are in play such as the white rust, powdery and downy mildew, and blackleg resistance. On the other hand, the reproducibility of identifying the quantitative trait loci (QTLs) imparting quantitative resistance against *Sclerotinia* stem rot and blackleg has been more challenging. Table 5.2 summarizes the QTLs associated with resistance against these diseases in *B. napus* and *B. juncea.*

WR: *A. candida* race 2 is primarily associated with the white rust disease of *B. juncea/B. napus.* The white rust resistance has been reported to be governed mostly by a single dominant gene (Delwiche and Williams 1974; Ebrahimi et al. 1976; Tiwari et al. 1988; Kole et al. 1996), two dominant genes (Verma and Bhowmik 1989; Santos et al. 2006) or minor genes (Edwards and Williams 1987; Kole et al. 1996, 2002b). Although isolation and cloning of resistance genes have been challenging but tightly linked molecular markers have been widely reported and adopted in marker-assisted selection (MAS). Prabhu et al. (1998) identified the *Ac2*$_1$ locus imparting resistance against *A. candida* associated with the two RAPD markers, WR2 and WR3, in mustard. Mukherjee et al. (2001) mapped *Ac2(t)* locus for white rust resistance with two RAPD markers, OPN011000 and OPB061000 in the RIL population in Indian mustard. Varshney et al. (2004) mapped *Ac2(t)* locus with CAPS and AFLP markers at recombination distance of 3.8 cM and 6.7 cM, respectively in *B. juncea. Acr*, another resistance locus identified in *B. juncea* co-segregates with the flanking RFLP marker (X140a) and (X42 and X83) (Cheung et al. 1998). In *Brassica rapa* ssp. *Oleifera,* white rust locus has been mapped on linkage group (LG) 2 near RAPD marker Z19 in an F_2 population (Tanhuanpaa 2004), whereas, resistance locus (*ACA1*) mapped on LG 4 in *B. rapa* (Kole et al. 1996). Ferreiraet al. (1995) found a linkage between the ACA1 locus and nine RFLP loci on linkage group 9 of *B. napus.* Panjabi-Massand et al. (2010) identified two independent qualitative resistance loci, AcB1-A4.1, AcB1-A5.1 conferring partial and complete resistance in two east European *B. juncea* lines Heera and Donskaja-IV respectively. Tightly-linked intron polymorphic (IP) markers, At2g36360, At5g41560 have also been validated in different populations of Indian mustard (Singh et al. 2020). These markers are being deployed in MAS for introgressing the resistance into elite Indian *B. juncea* varieties. Recently, Arora et al. (2019) fine mapped AcB1-A5.1 locus to successfully cloned and functionally validated *BjuWRR1* the first white rust resistance gene to be cloned thus far. The gene encodes a canonical R gene belonging to the CC-NB-LRR class of receptors. Importantly, *BjuWRR1* confers complete resistance to a range of Indian isolates of *A. candida.* Additionally, a new resistance-conferring

Table 5.2 Summary of disease resistance QTLs identified in Brassicas

Species	Population used	Markers used	Mapped gene/QTLs	References
White rust				
B. napus	Major X Stellar F1 derived DH	RFLP	*ACA*1	Ferreria et al. (1995)
B. rapa	Cultivar Per X R500 F2 and F3 populations	RFLP	*ACA*1	Kole et al. (1996)
B. juncea	F_1-derived doubled halpoid (DH)	RAPD	*Ac2a1*	Prabhu et al. (1998)
	Donskaja × Jubilejnaja F1 derived DH	RFLP	*Acr* = *Ac2a1*, monogenic dominant gene (race 2A)	Cheung et al. (1998)
	BEC144 X Varuna (F_7; F_2) RIL populations	RAPD, CAPS, AFLP	AC2 (t)	Mukherjee et al. (2001), Varshney et al. (2004)
	Heera X Varuna **(F1 Derived DH)**	IP marker AFLPs, SSRs	AcB1-A4.1	Panjabi-Massand et al. (2010)
	Donskaja IV X TM-4	IP marker, AFLPs, SSRs	AcB1-A5.1	Panjabi-Massand et al. (2010)
	Tumida X Varuna F1 derived DH	IP markers, SSR, SNP	*BjuWRR2*	Bhayana et al. (2020)
Sclerotinia stem rot				
B. napus	F2 population (128 lines)	SSR, RFLP, AFLP, RAPD	LR- A3, C2, C7 SR-A10, C5, C7	Zhao et al. (2003)
	DH population	RFLP	A2, A3, A5, C2, C4, C6, C9	Zhao et al. (2006)
	DH population (72 lines)	SSR, RAPD, SRAP, RFLP, EST	MTI-A4, A7, A10, C1, C2 IPI-C2 MPI-A1, A3, A4, A6, C7, LG11	Yin et al. (2010)
	DH population (190 Lines)	SSR MARKERS	LR-A3, A9, C5 SR-A1, A2, A3, A6, A8, A9, C6, C7, C8	Wu et al. (2013)

(continued)

Table 5.2 (continued)

Species	Population used	Markers used	Mapped gene/QTLs	References
	Used earlier developed and published populations (F2, DH lines)	SSR MARKERS	LR-A9, C1, C9 SR-A1, A2, A6, A8, A9, C2, C4, C5, C6, C7, C8, C9	Li et al. (2015)
	DH population (H1, H2, H3-106, 84, 58 lines)	SRAP, Microsatellite markers	H1-A7, C6, C7 H2-A3, A9 H3-A2, C6	Behla et al. (2017)
	F10 RILs (244 lines)	SNPs	Single environment-A1, A2, A7, A8, A9, A10, C2, C3, C6, C8 Multi environment A2, A4, A9, C2, C3	Zhang et al. (2018)
	DH population (181 lines)	SNP Array Genotyping	A2, A9, C2, C3, C4, C6	Qasim et al. (2020)
B. juncea	*B. juncea—B. fruticulosa* Introgression lines	SNP and SSR	A01, A03, A04, A05, A08, A09, and B05	Rana et al. (2017)
	B. juncea—B. fruticulosa Introgression lines	GBS and GWAS	A01, A03, A04, A05, A08, A09 and B05 ad 20 candidate genes	Atri et al. (2019)
	B. juncea—E. cardamonides Introgression lines	GBS and GWAS	A03 and B03	Rana et al. (2019)
B. oleracea	F2 population (149 lines)	SSR, SRAP, AFLP	C09	Mei et al. (2013)
Blackleg				
B. napus	Maxol/S006 (140 DH)	RAPD	A07—(*Rlm 1*)	Delourme et al. (2004)
	Maxol/Westar-10 (96 DH)	SSR, DArT	A07b—(*Rlm1*)	Raman et al. (2012)
	Glacier/Score (110 F_2)	RAPD	A7—(*Rlm2*)	Delourme et al. (2004)
	Darmor/Samourai (133 DH)	RAPD	A7—(*Rlm2*)	Delourme et al. (2004)
	F2 population (221) 2311.1/Darmor	RAPD	A7 (*Rlm 7*)	Parlange et al. (2009)

(continued)

Table 5.2 (continued)

Species	Population used	Markers used	Mapped gene/QTLs	References
	DH Population Damor-Yudal	RAPD	A7 (*Rlm9*)	Delourme et al. (2004)
	Surpass400/Westar	SSR	A10 (*LepR3*)	Yu et al. (2008)
	Surpass400/Westar	SRAP, SNP	A10 (*BLMR2*)	Long et al. (2011)
	Seven *B. napus* seven donor parents for introgression lines	SNP	Genomic background of individual varieties and multiple defense-related gene interactions influence the resistance levels	Larkan et al. (2015, 2016)
	243 *B. napus* accessions from Canada and China	GBS, GWAS	A08 with 25 RGAs identified consisting of NBS, RLK, RLP, and TM-CC type R genes	Fu et al. (2020)
B. juncea	Recombinant Lines (*B. napus* and *B. juncea*	RAPD, RFLP	B8 (*Rlm6*)	Chevre et al. (1997)
	AC Vulcan/UM3132 (F_2)	RFLP, SSR	*LMJR1*	Christianson et al. (2006)
	AC Vulcan/UM3132 (F_2)	RFLP, SSR	*LMJR2*	Christianson et al. (2006)
B. nigra	NILs Damor X Junius	Isozyme RAPD	B4 (*Rlm 10*)	Delourme et al. (2008)
B. napus	Springfield X 6279) DH population	RFLP	A2 (*LepR1*)	Yu et al. (2005)
	6279 X 3027	RFLP	A10 (*LepR2*)	Yu et al. (2005)
Powdery mildew				
B. napus	F10 RILs (244 lines)	SNPs	Single environment-A1, A2, A7, A8, A9, A10, C2, C3, C6, C8 Multi environment A2, A4, A9, C2, C3	Zhang et al. (2018)

locus *BjuWRR2* from *B. Juncea* var. Tumida was mapped on LG A6. Similar to the previously identified *BjuWRR1*gene, *BjuWRR2* also encodes a CC-NBS-LRR (CNL) type protein (Bhayana et al. 2020).

Three loci (*RAC1*, *RAC2*, and *RAC3*) conferring resistance to *A. candida* have been identified in two accessions Ksk-1, Ksk-2 of *A. thaliana* (Borhan et al. 2001). Only two R genes, *RAC1* and *WRR4* conferring resistance to *A. candida* race *Acem* have been identified and cloned from the model crucifer species *Arabidopsis thaliana* (Borhan et al. 2004, 2008). Both these genes encode for receptor proteins belonging to the TIR-NB-LRR subfamily.

BL: *Rlm1*, was the first R gene identified to be involved in the gene for gene type of resistance interaction against the blackleg pathogen in *B. napus* (Ansan-Melayah et al. 1998). It provides resistance against isolates carrying the corresponding avirulence factor *AvrLm1*. Since then directed efforts from several labs have led to the identification of 18 different qualitative race-specific R genes from different *Brassica* sources (Reviewed in Hayward et al. 2012, and references therein). Although, it remains unclear if some of the genes are the same with different nomenclature used by researchers using different -crosses, -isolates, and -marker systems. In *B. napus* the R gene Rlm 10 has been mapped to chromosome A10, while *Rlm1*, *3*, *4*, *7*, and *9* have been mapped to chromosome A07. Recently, it was observed that *AvrLm4* and *AvrLm 7* recognized by *Rlm4* and *7* respectively represent the allelic variation of the gene *Avr4-7* and can trigger a hypersensitive response in *B. napus* lines carrying *Rlm 4* or *Rlm 7, Rlm 3*, *Rlm 4*, and *Rlm 7* all clustering on LG A07 may be allelic variations of the same gene (Parlange 2009; Larkan et al. 2016). *Rlm5* and *Rlm6* have been identified in *B. juncea* (Balesdent et al. 2002; Fudal et al. 2007) *Rlm8* and *11* have been identified from *B. rapa* (Balesdent et al. 2002) and *Rlm10* in *B. nigra* (Chevre et al. 1996). Additionally, *LepR1*, *LepR2*, *LepR3*, and recently *LepR4* have been identified in *B. rapa* ssp *sylvestris* (Yu et al. 2005, 2008).

Besides, the R (resistance) genes governing qualitative resistance against *L. maculans*, several QTLs for quantitative resistance have also been identified in different *Brassicas* using the traditional bi-parental mapping populations. As the B genome *Brassicas* show significant resistance to *L. maculans*, they have been used to transfer resistance into elite *B. napus* cultivars (Plieske et al. 1998). Roy (1984) and Chevre et al. (1996) have introgressed blackleg resistance present on the B08 chromosome of *B. juncea* to *B. napus* through interspecific crosses. The R genes from *B. rapa*—*LepR1*, and *LepR2* have also been introgressed into *B. napus* varieties carrying *LepR3* locus through interspecific crosses (Crouch et al. 1994). The blackleg resistance in *B. nigra* is localized to the LG B04; this is different from the B genome resistance present in *B. juncea.* Chevre et al. (1996) have introgressed the B04 chromosome from *B. nigra* into *B. napus* variety 'Damor' a highly susceptible genetic background. Introgression lines carrying the B04 chromosome from *B. nigra* perform significantly better upon blackleg infection and the resistance holds against multiple races of *L. maculans.*

SSR: Resistance to Sclerotinia in oilseed Brassicas has been reported to be governed by a complex interplay of multiple minor genes. Several QTLs have been identified, however, the phenotypic variation explained by these QTLs is generally

very small thereby making it difficult to utilize them effectively in breeding programs (Disi et al. 2014). Zhao and Meng (2003) identified distinct QTLs associated with leaf and stem resistance suggesting that the mechanism regulating resistance is different in the two tissues. Using the traditional biparental DH/RIL/F_2 segregating populations derived from different cultivars have led to the identification of a considerable number of the resistance-related QTLs that have been found associated with the A genome (A02, A03, A09) or C genome (C02, C04, C06, C07, C09) of *B. napus* (Yin et al. 2010a, b; Wu et al. 2013; Wei et al. 2014). Behla et al. (2017) used three different DH mapping populations derived by crossing the semi-winter partially tolerant cultivar Zhongyou 821 with three different susceptible lines. They were able to identify for the first-time common QTLs across three populations, localized on A07, C06, and A09. Analyzing two segregating populations Zhao et al. (2006) identified 10 QTLs while Qasim et al. (2020) identified 17 QTLs.

Additionally, various agronomic crop traits including canopy architecture, flowering time (FT), maturity date, and stem thickness (Kim and Diers 2000; Miklas et al. 2001) have been shown to affect *S. sclerotiorum* incidence. FT is an important development stage that may play role in plant-pathogen interaction (Kazan and Lyons 2016). A negative correlation between SSR resistance and flowering time has been reported and co-localization of QTLs of SSR resistance and flowering times further strengthens the observation (Wei et al. 2014; Wu et al. 2019). In addition to flowering time, stem width also may affect disease incidents, especially when artificially induced. In recent decades, progress has been made in mapping of QTLs for SSR and FT in several crops, including *Glycine max* (Kim and Diers 2000), *Helianthus annuus* (Bert et al. 2002), *B. napus* (Zhao et al. 2006), and *B. oleracea* (Mei et al. 2013). However, the co-localization of QTLs of SSR resistance and FT is still not clear.

Although, several QTLs associated with SSR resistance have been identified yet surprisingly very few common QTLs have been detected repeatedly. Several factors account for this lack of reproducibility (a) small, additive contribution of individual QTL to resistance, (b) low heritability of the trait, (c) variable disease assays employed for QTL analysis and poor correspondence between these assays due to environmental influence, (d) unaccounted pathogen variability as different isolates are used in different studies.

PM: Resistance to powdery mildew in the Brassica crops is mostly governed by a single dominant gene with modifiers. Arabidopsis- powdery mildew pathosystem has been extensively worked on to understand the mechanisms of resistance imparted by the atypical resistance gene, *RPW8*.

Xiao et al. (2004) identified two distinct loci for homologous resistance (HR) genes in *Brassica* species. Both loci are represented by tandemly arrayed HR regions in both *B. napus* and *B. oleracea.* The first locus consists of three, whereas the second locus consists of one tandemly arrayed HR region. All these HR genes in Brassica shared a high level of similarity to the Arabidopsis *HR3* gene (Xiao et al. 2004). Multiple evolutionary events including insertion, deletion, point mutation gene loss, and intragenic recombination were involved in *B. napus HR* genes. The involvement

of *HR* genes in cell death provides resistance to powdery mildew in *B. napus* (Li et al. 2016b).

5.7 Application of the Omics Technologies in Brassica-Pathogen Interactions

QTL mapping based identification of disease resistance genes has been extensively used but the limited inferences can be drawn due to (a) a limited number of alleles studied at a time from two parents, (b) a small number of recombinant events analyzed, (c) a limited number of markers are used to develop the linkage map. The sequencing of crop genomes has yielded unprecedented insights into the genomic footprints of its evolution, gene content, and has facilitated the development of molecular markers for accelerating breeding efforts. The long-read sequencing technologies (Pacific Bio and Oxford Nanopore) in conjugation with Nanobio optical mapping have tremendously advanced the field of Brassica genomics. All the six Brassica genomes in the "triangle of U" model have been assembled to date. *B. rapa* (A genome) was the first *Brassica* species to be sequenced. A Chinese cabbage morphotype of *B. rapa* (Chiifu- 401-42), was used to generate the reference genome (Wang et al. 2011). Later on, the genome assembly of *B. rapa* was updated using long-read sequencing data from PacBio sequencing and chromosome conformation capture technology to yield much higher contiguity and completeness of the genome (Zhang et al. 2018b). Additionally, an oilseed type *B. rapa* (*B. rapa* ssp. *trilocularis* Z1) has also been sequenced using Nanopore sequencing technologies and scaffolded using optical mapping to yield a highly contiguous genome with N50 > 5 Mb (Belser et al. 2018). The genomes of two *B. oleracea* (C genome) varieties have been assembled similarly using both short read and long-read sequencing technologies (Parkin et al. 2014; Belser et al. 2018). Two genome assemblies of *B. nigra* (B genome) are currently available in the public domain (Perumal et al. 2020; Paritosh et al. 2020b). For the allopolyploid *B. napus* (AC genome), four reference-quality genomes are available, three of which were developed using short-read technologies and one using long-read technology (Chalhoub et al. 2014; Bayer et al. 2017; Sun et al. 2017; Lee et al. 2020). For Indian mustard, *B. juncea*, two genome assemblies are currently available. The first genome assembly is of leafy type of *B. juncea* (var. Tumida) (Yang et al. 2016) and the second genome assembly, which is more contiguous is of the oilseed type (*B. juncea* var. Varuna) (Paritosh et al. 2020a). Most recently, Song et al. (2021) have reported the sequencing of *B. carinata* the Ethiopian mustard. Apart from these reference-quality genome assemblies for the six species, various accessions of these species have been re-sequenced to obtain diversity information and to generate markers for GWAS (Cheng et al. 2016; Wu et al. 2019). These genomic resources have played a pivotal role not only in delineating the evolutionary trajectories within the Brassicaceae but have also enabled gene discovery for various important agronomical traits.

In addition to the available sequences, the advances in bioinformatics tools have allowed high throughput genome-wide discovery of SNPs in various polyploid species including the *Brassicas* (Delourme et al. 2013). Whole-genome resequencing, genotyping by sequencing (GBS), and *Brassica* 90K Illumina Infinium SNP array provide diverse platforms for high throughput genotyping of large sets of germplasm/diversity panels. These NGS technologies have been used for the mapping of QTLs/genes in Brassicas.

5.7.1 Association Mapping

Genome-wide association studies (GWAS) have become a widely used technique for dissecting the genetics of complex traits in the post-genomics era. Genome-Wide Association (GWA) mapping relies primarily on naturally occurring genetic variation arising due to historical recombination events that prevail in the diverse germplasm (Atwell et al. 2010). GWA study aims to identify polymorphisms that are associated with the phenotypic variation observed for a trait, here we focus on examples of GWAS for disease resistance. The success of GWAS depends on (a) the number of genetic markers, (b) the size of the population used for phenotyping (c) accuracy of scoring the disease reaction (d) the extent of genetic variation in the population/population structure. The presence of population structure can confound the associations determined in GWAS and therefore several statistical methods are employed for considering the population structure and kinship (Zhang et al. 2010). The robustness of scoring the disease can be achieved by using automated platforms with image-based quantification of disease symptoms (Laflamme et al. 2016; Barbacci et al. 2020). The advantage of the GWAS approach is that one can identify either the causative gene or tightly linked markers that can be used in MAB to transfer the promising loci into the elite varieties. Nevertheless, very limited GWAS studies have been carried out in Brassica- disease resistance field.

Broad sense heritability of blackleg resistance is reported to be high suggesting that a large percentage of phenotypic variation is due to the genetic component. Besides the traditional bi-parental mapping studies, GWAS have been attempted to identify the resistance genes and also to study genetic architecture for quantitative resistance against *L. maculans*. Raman et al. (2016) mapped *Rlm12* on ChrA01 using a *B. napus* diversity panel comprising of 179 lines. Additionally, they identified the previously mapped *Rlm4* on A07, and several new SNPs showing a strong association with the blackleg resistance trait (Raman et al. 2020). In a similar study, Fikere et al. (2020) used larger diversity sets of *B. napus* for identifying robust novel QTL/genes for blackleg resistance. Fu et al. (2020) carried GWAS studies using a set of Canadian and Chinese *B. napus* germplasm to identify novel genomic regions on A08, A09, and A03 associated with blackleg resistance. Kumar et al. (2018) integrated the multi-year data available for three bi-parental populations and the GWAS association panel to identify complementary genomic regions across the two approaches. Identification

of causal genes within these stably associated regions in the future would help in improving durable resistance against blackleg (Raman et al. 2018).

A similar approach has also been used to identify allelic variation, present within the *Brassica* natural germplasm, that contributes to *Sclerotinia* stem rot resistance. A GWA study using a diversity panel comprising of 337 *B. napus* accessions identified 17 genomic regions showing significant associations with SSR resistance (Wei et al. 2016). Following year, Wu et al. (2016a) used an association panel of 448 *B. napus* lines to identify 26 SNPs associated with 3 genomic loci on C04 C06 and C08 contributing to SSR resistance.

Introgressive breeding, an important technique, to incorporate useful genes into cultivated popular varieties from wild species, has been exploited for transferring disease resistance loci from exotic and wild relatives. For *Sclerotinia*, two sets of introgression lines, *B. juncea—B. fruticulosa and B. juncea-E. cardamonides* have been developed, which revealed significant variation for stem rot. GWAS of *B. juncea–E. cardamonides* introgression lines allowed the detection of 10 significant marker-trait associations on LG A03, A06, and B03. The maximum no. of SNPs for resistance were present on LG A03 and A06 (Rana et al. 2019). In the *B. juncea—B. fruticulosa* introgression lines, Rana et al. (2017) mapped resistance genes against *Sclerotinia* on LG A01, A03, A04, A05, A08, A09, and B05. Annotations of the linked region revealed the possible role of anti-fungal proteins, metabolites, hypersensitive reaction, and signal transduction pathways in defense against SSR. Atri et al. (2019) mapped resistance responses against stem rot on seven *B. juncea* LGs: A01, A03, A04, A05, A08, A09, and B05 through GWAS in the set of introgression lines of *B. juncea—B. fruticulosa* (Atri et al. 2019). Annotations of genomic regions revealed the role of TIR-NBS-LRR class, Chitinase, Malectin/receptor-like protein kinase, defensin-like (DEFL), desulfoglucosinolate sulfotransferase protein, and lipoxygenase. Similar attempts have been made to introgress the robust resistance against blackleg available in the B-genome species (*B. carinata*) into *B. napus* (Fredua-Agyeman et al. 2014). Tonguc and Griffiths (2004) reported similar efforts of transferring resistance against powdery mildew available in *B. carinata* into *B. olereacea.*

Rajarammohan et al. (2018) reported extensive variation for resistance to *A. brassicae* in a panel of 123 *A. thaliana* natural accessions. GWA mapping revealed multiple genomic regions to be associated with Alternaria blight resistance in *Arabidopsis.* Many of the associated candidate genes identified in this study were validated using the T-DNA knockout mutant resource available in *Arabidopsis.*

5.7.2 NGS-Based Bulked Segregant Analysis

NGS-based BSA is another recent application of omics for fine mapping and cloning of resistance genes. In this technique, DNA or RNA from contrasting segregated phenotypes are bulked to form pools. These pools were genotyped followed by

detection of QTLs through SNPs calling (Liu et al. 2012; Takagi et al. 2013). NGS–BSA has been used for fine mapping and cloning of the blackleg resistance gene, *Rlm1*, in *B. napus*. *Rlm 1* shares homology with *STN7* (*B. rapa*, *B. oleracea,* and *Arabidopsis*) encoding a serine/threonine-protein kinase which is involved in triggering the systemic immune responsevia the production of reactive oxygen species. It encodes a serine/threonine kinase protein (Fu et al. 2019). Similarly, BSR-Seq has also been used for cloning TIR-NBS-LRR encoding genes, Rcr7 (*B. oleracea* cultivar "Tekila") and Rcr2 (in Chinese cabbage) involved in resistance against clubroot. Additionally, QTL-Seq has been instrumental in detecting two QTL regions on A07 and A08 in *B. chinensis* (Zhu et al. 2019), and another region on A03 in *B. rapa* also involved in clubroot disease resistance (Pang et al. 2018).

5.7.3 Transcriptomics and Proteomics

Several biological and physiological processes are associated in the host with the biotic stress responses and a better understanding of these at the molecular level can contribute immensely to designing novel and efficient disease management strategies and approaches. In recent years, many studies have used the omics approaches to unravel the molecular intricacies underlying host responses to pathogen attack. RNAseq-based comparative transcriptome analysis has emerged as an efficient way to assess the variation in global gene expression in response to pathogen infection. Additionally, it provides scope to discover host signaling pathways involved during pathogen challenge.

Qasim et al. (2020) reported the involvement of genes with diverse functions including TIR-NBS-LRR, genes involved in the synthesis of hormone and secondary metabolites, transcription factors, in *Sclerotinia* resistance in *B. napus.* The role of JA and ET signaling and cellular redox signaling was also described in response to resistance against *Sclerotinia* and *P. brassicae* in *B. napus* (Wu et al. 2016b; Girard et al. 2017; Galindo-González et al. 2020). Transcriptomic studies on transgenic lines expressing NPR-1 like genes in *B. napus* further strengthened the contribution of SA and JA signaling. Downregulation of the NPR-1 like gene facilitated the invasion of the fungus into the host cell (Wang et al. 2020a). Additionally, the role of secondary metabolite, glucosinolates, and its degradation products, was also indicated during transcriptome analysis of *B. napus* for the reaction against *S. sclerotiorum* (Zhang et al. 2015; Wu et al. 2016b).

Comparative RNA sequencing analysis of *B. napus*-pyramided line with two genes (*PbBa8.1* and *CRb*, for clubroot resistance) displayed a multi-gene resistance mechanism. The salicylic acid and reactive oxygen species were involved in the defense mechanism (Shah et al. 2020). The transcriptome analysis of *B. rapa* with *CRb* gene confirmed salicylic acid-mediated pathway along with upregulation of transcription factors (MAPK, WRKY) for resistance against *P. brassicae* (Chen et al. 2016). Involvement of NLR and PR genes, SA signalling, chitinases, and calcium-binding proteins were observed (Chhikara et al. 2012; Ji et al. 2018, 2020).

Transcriptome analysis also confirmed the role of signaling and metabolism pathways of plant defence hormones of JA and ET, biosynthesis of indole-containing compounds, and callose deposition in *B. rapa*- clubroot pathosystem (Chu et al. 2014). Similarly, genome-wide expression profiling in cabbage discovered the pathogen-induced down-regulation of genes involved in photosynthesis (Zheng et al. 2020), metabolism, photosynthetic carbon cycle (Xiao et al. 2016) during defense response against *H. peronospora.*

Besides screening for differential expression, RNA seq data is a valuable asset for identifying exonic SNPs that can be converted to functional markers. **Associative Transcriptomic**, another RNA-based approach simplifies the integration of the transcriptome data (levels of gene expression) with GWAS (allelic variation at the associated loci) thus increasing the power to identify loci associated with the traits (Harper et al. 2012; Havlickova et al. 2018). A very limited application of this has been reported in the *Brassica*—pathogen field. Hejna et al. (2019) used expression QTLs (eQTLs) to interpret the SNP associations from GWAS with the gene expression changes to identify candidate genes involved in Clubroot resistance.

Proteomics has also facilitated the identification of proteins expressed during the host-pathogen interaction. For example, enzymes involved in H_2O_2 scavenging, RuBisCO for CO_2 fixation, and redox metabolism were upregulated and formed the basis for resistance during *B. napus—L. maculans* interaction (Sharma et al. 2008). Similarly, the involvement of ubiquitin-related proteasome system, lignin biosynthesis along with activation of ROS, MAPK signaling pathway proteins expressed in *B. rapa–P. Brassicae* patho system has been unraveled using comparative proteomics (Song et al. 2016). In another study of the same pathosystem, proteins involved in the biosynthesis of tryptophan and glutathione and cytokinin signalling were observed (Lan et al. 2019). Moon et al. (2020) used a proteomic approach to identify the thioredoxin enzyme to be responsible for the defense response in *B. oleracea–P. brassicae* pathosystem. Sun et al. (2014) depicted the role of ROS—mediated defense through Ca2+ signaling in Chinese cabbage against *H. parasitica.* Kaur et al. (2011b) reported the role of superoxide dismutase, glutathione, plant-thaumatin-like protein, S-transferase, cysteine synthase, and red chlorophyll catabolite reductase during *B. juncea—A. candida* interaction.

5.7.4 Genomics Aided Identification of Candidate R Genes

Disease-resistance (R) genes impart resistance towards various pathogens including bacteria, viruses, fungi, oomycetes, and nematodes (Wan et al. 2012b; Dangl et al. 2013). Based on structural similarities and predicted domains, R genes could be classified into five different classes (Staskawicz et al. 1995; Liu et al. 2007a). The largest class encodes proteins with a putative nucleotide-binding site (NBS) and leucine-rich repeats (LRRs). The NBS-LRR consists of a variable N-terminal domain followed by the central NBS domain and C-terminal LRR domains. NBS-LRR genes could be divided into TIR-NBS-LRR (TNL) and CC-NBS-LRR (CNL)

based ontoll/interleukin-1 receptor and coiled-coil domain at the N-terminus (Cannon et al. 2002; Meyers et al. 2003). These subfamilies are involved in defense against numerous pathogens. The presence of several conserved motifs with a high degree of sequence similarity could be used to identify NBS-LRR genes (Meyers et al. 1999; Wan et al. 2012a). R-genes or NBS-LRR genes are further grouped into resistance gene analogs (RGAs) along with pattern-recognition receptors (PRRs; Sekhwalet al. 2015). PRRs consist of two classes of proteins viz. surface-localized receptor-like protein kinases (RLKs) (Walker 1994) and membrane-associated receptor-like proteins (RLPs).

Using conserved motifs and several structural features of the canonical R genes, genome-wide analysis of NBS-LRRs has been performed in numerous plant species (Zhou et al. 2004; Bayer et al. 2019). Besides, several bioinformatics pipelines to identify RGAs on a genome-wide scale for example NLR-PARSER, DRAGO, RGAugury, NLR-Annotator, NLGenome sweeper have been developed and used for multiple crop species (Steuernagel et al. 2015, Li et al. 2016a; Osuna-Cruz et al. 2018; Steuernagel et al. 2020; Tirnaz et al. 2020; Toda et al. 2020; Zhang 2020). Despite numerous studies that have identified RGAs across different plant species, only ~140 R genes have been cloned due to the complexity of fine mapping of R genes, which is partially due to the lack of information about their genomic structure and distribution (Tirnaz et al. 2020).

Recently, there has been analysis of NBS-LRRs in several *Brassica* species, both diploid and amphidiploids, including *B. rapa*, *B. napus*, *B. oleracea*, and *B. juncea* (Alameryet al. 2018; Bayer et al. 2019; Inturrisi et al. 2020a, b; Mun et al. 2009; Wu et al. 2014; Yu et al. 2014). In general, a greater number of R genes were identified in the diploid progenitors than in the individual sub-genomes of the amphidiploids (Inturrisi et al. 2020a). Varying numbers of NBS-LRR genes have been predicted in *B. napus (*641), *B. oleracea* (443), and *B. rapa* (249) (Alamery et al. 2018). Similarly, a total of 289 NLR genes were identified in *B. juncea*, 202 in *B. rapa,* and 282 in *B. nigra* (Inturrisi et al. 2020a). Recently, Bayer et al. (2020) identified a total of 1989 RGA candidates in the *B. oleracea* pangenome assembly. In another study, 34,065 RGAs were predicted in the Brassicaceae family of which, a majority (21691) was represented by RLK, followed by 8588 NLRs and 3786 RLPs (Tirnazet al. 2020).

Linking the predicted RGA with the already identified QTLs can help in identifying potential candidate resistance genes. Bayer et al. (2019) identified 37 RGAs in the already defined QTLs for *Sclerotinia*, black rot, and Fusarium wilt resistance. These comparative analyses provide a better understanding of their function, structure, and distribution, which can be used to aid the identification and cloning of RGAs from previously untapped sources and can subsequently be used in resistance breeding.

The availability of several *Brassica* reference genomes and pangenomes assemblies has helped immensely in predicting RGAs in Brassicas. Since multiple reference genomes are not available, resistance gene enrichment and sequencing (Renseq) approach has also been used for targeted resequencing of leucine-rich repeats (NLRs) genes from the Brassica diversity panel (Jupe et al. 2013). Van de Weyeret al. (2019) used RenSeq in combination with Pac Bio to study the variability of NLR genes in

64 accessions of Arabidopsis which helped in constructing a species-wide pan-NLR-ome. This pan-NLR-ome represents variability among all the NLR genes. A similar type of approach can be used for developing NLR- pangenome for Brassicaceae. RenSeq in combination with PacBio sequencing was applied to study the R gene sequence variants of the white rust resistance (WRR) gene against *A. candida* (Jupe et al. 2013).

5.8 Transgenic Approaches to Improve Brassica Biotic Resistance

In the past few decades, disease resistance has been an attractive target trait for genetically modifying Brassicas especially in cases where the natural genetic resistance is not available, e.g. Alternaria leaf blight, Sclerotinia Stem Rot, etc. Mostly, researchers have relied on insights from the transcriptome and proteome data to select the important candidate genes which play an important role in defense responses either in the Brassica crop or in the wild relative Arabidopsis, Sinapis, or in other crop plants. Functional validation of these candidate genes is done either by overexpression, knockdown by RNAi, and more recently targeted knockout by CRISPR-Cas9 (genome editing). Additionally, candidate genes showing promising results in improving disease resistance in other crop systems have also been tested in the Brassicas. Table 5.3 summarizes a few examples of transgenic plants developed in Brassicas for enhanced disease and insect-pest resistance.

5.9 Gene Editing Technologies

The new gene-editing tool CRISPR/Cas9 has been explored for functional analysis as well as introducing precise genome modification for crop improvement (Ma et al. 2016). A few reports of success at gene editing using CRISPR/Cas 9 in the *Brassicas*, *B. napus*, *B. campestris,* and *B. oleracea* has been published recently although genome and gene redundancy is a limiting factor (Lawrenson et al. 2015; Braatzet al. 2017; Yang et al. 2017; Maet al. 2019; Xionget al. 2019; Zhaiet al. 2019). Recently, Murovec et al. (2018) reported genome editing in *B. oleracea* and *B. rapa* using the DNA-free CRISPR/CAS approach with success. The utilization of the CRISPR/Cas9 system for the development of biotic stress-tolerant rapeseed is still in infancy. For example, roles of WRKY transcription factors *BnWRKY11*, and *BnWRKY70* in disease resistance/susceptibility to SSR were elucidated using the CRISPR- Cas9 approach to induce nucleotide specific mutations in these genes. Mutant lines of *BnWRKY70* showed increased resistance to *S. sclerotiorum* while lines overexpressing *BnWRKY70* were susceptible (Sun et al. 2018).

Table.5.3 Summary of transgenic brassicas developed for improved biotic stress tolerance

Category	Genes used	Source	Transgenic plants	Resistance against phytopathogens/pests	References
Antimicrobial peptides	*Pm*AMP1	*Pinus monticola*	*B. napus*	*A. brassicae, L. maculans,* and *S. sclerotiorum*	Verma et al. (2012)
	Msra1	*Bacteria/synthetic peptide*		Resistance against Alternaria blight and stem rot fungi	Rustagi et al. (2014)
Signaling pathways	*Bj*NPR1	*B. juncea*	*B. juncea*	*A. brassicae, E. cruciferarum,* and	Ali et al. (2017)
	*Os*PGIP2	*Oryza sativa*	*B. napus*	*S. sclerotiorum*	Wang et al. (2018)
	MPK4, MPK3, MPK6	*B. napus*	*B. napus*	*S. sclerotiorum*	Wang et al. (2009, 2019, 2020b)
	RLP48	*B. rapa*	*B. rapa*	*Hyaloperonospora parasitica*	Zhang et al. (2018a)
	GDSL1	*A. thaliana*	*B. napus*	*S. sclerotiorum*	Ding et al. (2020)
Transcription factors	*WRKY6*	*B. oleracea*	*B. oleracea*	*H. parasitica*	Jiang et al. (2016)
Germin and germin like proteins	*gf*-2.8	*Triticum aestivum*	*B. napus*	*S. sclerotiorum*	Thompson et al. (1995), Zou et al. (2007), Dong et al. (2008)
	HvOxO	*H. vulgare*	*B. napus* *B. juncea*	*S. sclerotiorum*	Liu et al. (2015), Verma and Kaur (2021)
PR proteins	Chitinase, glucanases	*Solanum lycopersicum, Barley*	*B. juncea*	*A. brassicae*	Mondal et al. (2007), Chhikara et al. (2012)
	β-1,3-glucanase (*Bgn13.1*)	*Trichoderma virens-10*	*B. napus*	*S. sclerotiorum*	Kheiri et al. (2014)
	BoDFN Defensin gene	*B. oleracea*	*B. oleracea*	*Peronospora sparsa*	Jiang et al. (2012)

(continued)

Table.5.3 (continued)

Category	Genes used	Source	Transgenic plants	Resistance against phytopathogens/pests	References
Thaumatin or Osmotin like protein	Thaumatin like tlp gene	*Secale cereal L.*	*B. napus*	*S. sclerotiorum*	Zamani et al. (2012)
	DRR206	*Pisum sativum*	*B. napus*	*Leptosphaeria maculans*	Wang et al. (2001)
R genes	WRR4	*Arabidopsis thaliana*	*B. juncea* and *B. napus*	*Albugo candida*	Borhan et al. (2010)
	WRR1	*Brassica juncea*	*B. juncea*	*A. candida*	Arora et al. (2019)
	Lm1	*B. nigra*	*B. napus*	*L. maculans*	Wretblad et al. (2003)
Lectins	Hevein	*Rubber tree*	*B. juncea*	*A. brassicae*	Kanrar et al. (2002a)
	Agglutinin	*Triticum*	*B. juncea*	*L. erysimi*	Kanrar et al. (2002b)
	Agglutinin ACA	*Allium cepa*	*B. juncea*	*L. erysimi*	Hossain et al. (2006)
	Agglutinin	*Colocasia esculenta*	*B. juncea*	*L. erysimi*	Das et al. (2018)
	Fusion lectin + protease inhibitor	*A. sativum*	*B. juncea*	*L. erysimi*	Rani et al. (2017)
Protease inhibitors	HvCPI-6	Barley	*Arabidopsis*	*A. pisum*	Carrillo et al. (2011)
	oryzacystatin I (OC I)	Oryza	*B. napus*	*M. persicae*, *A. gossypii* and *A. pisum*	Rahbe et al. (2003)
Metabolic engineering of raffinose-family oligosaccharides	*Galactinol Synthase, Raffinose Synthase and Stachyose Synthase*	Wild-type Arabidopsis	*Arabidopsis, Tobacco*	*M. persicae*	Cao et al. (2013)
RNAi against *Rack1*	Rack1 protein located in gut and C002 protein located in the salivary glands of *Myzuspersicae*	*M. persicae*	Tobacco and *Arabidopsis thaliana*	*M. persicae*	Pitino et al. (2011)

5.10 Conclusions

The global climate change, resulting in the rapid evolution of pathogens and pests, and the ever-growing population presents a great challenge for Brassica breeders aiming for improved crop yields. Since major yield losses occur due to biotic stresses imposed on the crop, therefore, disease management and improving resistance are the top priorities in the breeding programs. Although the conventional breeding methods form an integral part of the crop improvement program, the advances in genetic and genomics technology have accelerated the progress in developing varieties with improved resistance. *Brassica* species with improved resistance for major fungal phytopathogens have been discussed in this chapter. The omics tools along with bioinformatics pipelines have facilitated to comprehension of Brassica–pathogen interactions and in silico-identification for candidate genes. Additionally, the availability of genomes and pangenome assemblies of several *Brassica* species has facilitated the genetic study of disease resistance mechanisms and allowed cloning of R genes. It has also led to the identification/ prediction of new RGAs thereby expanding the repertoire of R genes that can be evaluated for stable and robust field resistance and utilized in disease resistance breeding programs. The establishment of databases for resistance genes with their function will facilitate the deployment of gene-editing technologies for improving Brassica varieties for biotic stress tolerance. Also, knowledge gained from model species -*Arabidopsis*, could be translated into Brassicas for designing innovative strategies for resistance breeding.

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Chapter 6
Genomic Designing for Resistance to Biotic Stresses in Sesame

Hongmei Miao, Hongyan Liu, Yinghui Duan, and Haiyang Zhang

Abstract Sesame (*Sesamum indicum* L.) is an important oilseed crop unique with high contents of unsaturated fatty acids and antioxidants. Sesame originated from tropical regions and possesses high tolerance to drought and infertility conditions, while the resistance level to biotic stresses (especially fungal diseases) is relatively low. We describe here the major diseases and pests causing significant damage to sesame production and the progresses made in the genetics and breeding research for disease resistance in sesame. The application of genomics-assisted breeding in sesame is also deliberated.

Keywords Sesame · *Sesamum* · Biotic stress · Disease resistance · Breeding · MAS · Genomics-assisted selection

6.1 Description on Different Biotic Stresses in Sesame Production

6.1.1 Economic Important of Sesame

Sesame (*Sesamum indicum* L., $2n = 26$) is an annual oilseed crop (Fig. 6.1) and is widely cultivated in tropical and subtropical regions in the world. Its cultivation history could be traced back to the Early Bronze Age (3,000 BC) (Bedigian 2004). Sesame belongs to the *Sesasum* genus, Pedaliaceae family, and is the sole cultivated

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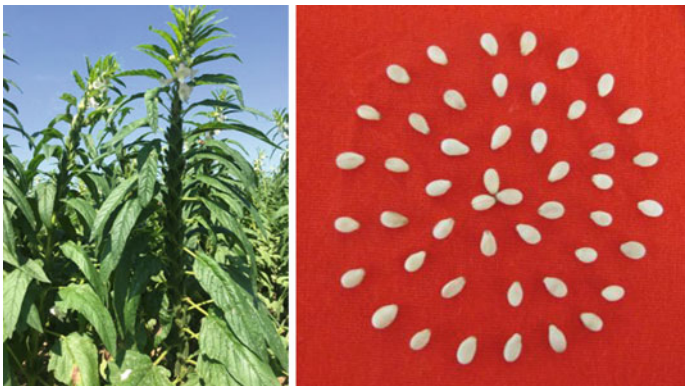

Fig. 6.1 Sesame plant and mature seeds. Left image is a Chinese sesame variety Yuzhi Dw609 cultivated in field. Right side image is sesame seeds with white seed coat color (Provided by H. Zhang)

species of the *Sesamum* genus (Zhang et al. 2013a; b). Sesame seed contains abundant oil (50–55%), proteins (18–20%), carbohydrate (13–25%), and antioxidants (0.3–1.5%) (Zhang et al. 2019), and is named as the queen of the oilseed crops for the specific and high seed quality. Especially, in China, sesame is also used as a medicinal ingredient for health care.

At present, sesame is widely cultivated in about 75 countries. The total harvested area of the world reaches to 10.5 million hectare. The total annual production is about 6 million tons. Sudan, India, Myanmar, Tanzania, and China are the leading countries for sesame production in the world. However, the average yield of the world sesame keeps low to 577.9 kg per hectare according to FAO statistics data. Besides the traditional and extensive cultivation styles, biotic stresses specially diseases are the main factors affecting the yield and seed quality of sesame (Zhang et al. 2019).

6.1.2 Reduction in Yield and Quality Due to Biotic Stresses

There are tens of pests and diseases which make huge damages to sesame growth and development and the final seed yield. At least eight fungal diseases cause economic losses to sesame in the world production areas (Kolte 1996; Khalifa 2003; Didwania 2019). Especially, the top three fungi diseases including Fusarium wilt, charcoal rot, and Alternaria leaf spot can cause a loss of about 25–40% of seed harvest (El-Bramawy and Wahid 2006; El-Bramawy et al. 2008). Prathuangwong and Yowabutra (1997) explored the relationship between the severity of bacterial leaf spot disease caused by *Pseudomonas syringae* pv. *sesami* and the yield loss under both greenhouse and field conditions. As the severity of disease increased by 1% for all sesame seeds, the average yield of the 14 sesame varieties reduced by 1.30% and 0.92%, respectively.

To reveal the damage of pathogens on sesame yield and quality, Li et al. (2017) systematically investigated on the yield related traits including plant height, capsule zone length, capsule node number, the capsule number per plant, seed yield, and seed quality traits of the three sesame varieties TP4816-0, JDP12-0, and JDP21 cultivated in the artificial Fusarium wilt disease nursery. Five *Fusarium* strains with high pathogenicity were cultured and evenly applied into nursery soil. For the samples with disease grade 3 syndrome, the value of seed yield and seed quality traits significantly changed ($p < 0.05$). The seed quality related traits including seed appearance traits, seed nutrition components, and oil quality were affected accordingly. For grade 4 plants, no mature seeds could be harvested (Li 2017; Yuan et al. 2018). Especially, three indicators (L, a*, and b*) of the seed coat color traits varied with the change of grade level from 0 to 3, and could be used to indicate the occurrence of the Fusarium wilt disease. Meanwhile, the results showed that the polysaccharide content is significantly affected by pathogen invasion and disease occurrence. For grade 3 samples of the tested three varieties, the acid values increased by 36.48–68.27% and the peroxide value decreased by 15.04–51.92%, which finally reduced the oil quality and taste.

6.1.3 Taxonomy of Causal Agents of the Diseases

6.1.3.1 Fusarium Wilt Disease and Host Resistance Evaluation

Sesame Fusarium wilt (SFW) disease is one of the most important sesame diseases in the world and has been detected and determined since 1920s (Armstrong and Armstrong 1950; El-Shakhess et al. 2007). SFW is caused by *Fusarium oxysporum* f. sp. *sesami* (FOS) (Fig. 6.2) (El-Shazly et al. 1999; Li et al. 2012; Miao et al. 2019;

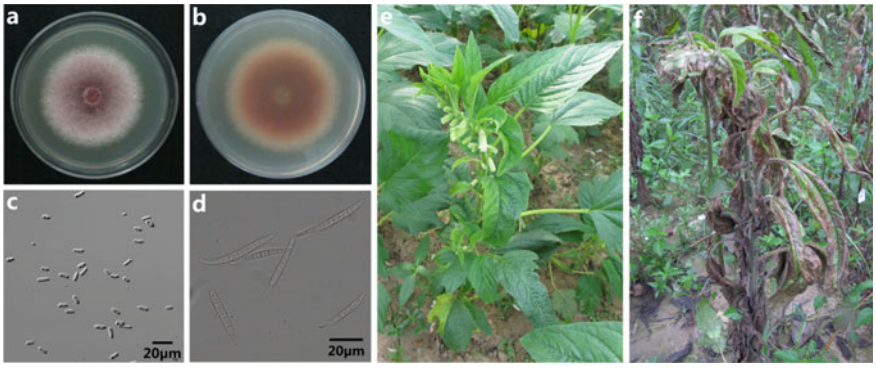

Fig. 6.2 FOS pathogen and sesame plant inoculated by Fusarium wilt disease. **a** FOS colony front side; **b** FOS colony back side; **c** FOS conidium. Bar = 20 μm; **d** FOS chlamydospore. Bar = 20 μm; **e** FW seedling with low disease grade; **f** FW sesame plant with high disease grade (Provided by Hongmei Miao)

Duan et al. 2020). SFW is a worldwide disease and usually occurs on 15% plantlets in the ordinary sesame field and causes considerable loss of seed yield (Wang et al. 1993; Elewa et al. 2011; Li et al. 2017; Wei et al. 2018). Inoculation experiment indicated that the sesame plantlets presented the Fusarium wilt symptom in 1–2 weeks later after inoculated with 1×10^6 microconidia/mL FOS suspension in greenhouse (Miao et al. 2019). However, field investigation indicates that the FOS pathogen commonly infects the root tissue, causes damping-off (such as leaf chlorosis, abscission, stem necrosis, and internal vascular browning), and results in whole plant wilting and death at seedling and adult stages in sesame (Li et al. 2012; Miao et al. 2019).

In the past a few years, hundreds of *Fos* isolates have been identified from wilted sesame plants in China. The morphological and pathogenic characteristics and the genetic diversity of the Fos isolates have been further described (Li et al. 2012; Qiu et al. 2014; Duan et al. 2020). Duan et al. (2020) compared the 69 *Fos* isolates collected from major sesame production regions in China. Based on the inoculation evaluation on the three differential sesame hosts, the 69 isolates were grouped into the three pathogenic groups. Correspondingly, 10 *SIX* (secreted in xylem) genes that translate one family of effectors of FOS were detected and found to reflect the difference of the three pathogenic groups for the first time. Further transcriptome analysis showed that most homologues of *Fos SIX* genes presented the specific expression profiles in sesame during the *Fos* infection. The results supported that most of the *Fos SIX* genes play important roles in the virulent genotypes.

To evaluate the resistance level of sesame accessions to FOS disease, the resistance evaluation indicator with the five ratings under natural infection conditions were used in sesame (El-Bramawy and Wahid 2006; Qiu et al. 2014; Miao et al. 2019). The disease index (DI) was calculated and the five DI value scale was determined for resistance evaluation (Qiu et al. 2014).

Based on the above standards, Miao et al. (2019) established the evaluation method of sesame resistance to Fusarium wilt disease at vegetative stage and evaluated 40 cultivated accessions. The results showed that 57.5% samples were highly susceptible (HS, DI > 70), while 27.5% were showing high- or medium-resistance ($15 < \text{DI} \leq 55$) and proved presence of low percentage of elite germplasm accessions with high resistance to FOS strains.

To reflect the genetic basis of the resistance to FOS in sesame, some researchers evaluated the Fusarium wilt resistance of sesame populations under the natural or artificial field condition (Wang et al. 1993; El-Bramawy and Wahid 2006; Sìlme and Çarğirgan 2010). Only a few dominant genes should determine the resistance level of sesame to FOS (Wang et al. 1993; Bakheit et al. 2000; El-Bramawy and Shaban 2007). Recently, with the aid of genome data and the above concise evaluation method, Miao et al. (2017) evaluated disease resistance in an F_2 population derived from the parent DS899 (DI 6.1%) and JS012 (DI 100%) to FOS pathogen no. HSFO09095 and located one major quantitative trait locus (QTL), which indicated that the resistance of the population to FOS is controlled by single gene pair.

6.1.3.2 Charcoal Stem Rot Disease and Host Resistance Evaluation

Charcoal stem rot disease is the most important and soil-borne disease for sesame because of the huge loss of the seed yield. Charcoal stem rot disease in sesame is caused by the fungus *Macrophomina phaseolina* (Tassi.) Goid. (MP) and also widely occurs in the world (Mihail and Taylor 1995; Rajput et al. 1998; El-Bramawy and Wahid 2006). Charcoal rot disease can occur in all stages of life cycle of a sesame plant and the incidence rate reaches to 10–25% with high yield loss (Vyas 1981; Wang et al. 2017). The common symptoms of the charcoal stem rot disease include the sudden wilting and spot blight on plant stem (Fig. 6.3). The stems get brown and black and the syndrome gradually extends upward. Subsequent extension of the black and infected stem results in the death of the plant. The peak stage of the disease occurs at the final flowering stage. After infected by MP pathogen, the root becomes brittle and black. On the infected plants, the capsules change to black and crack prematurely, and the seeds shrivel with low yield and quality.

For charcoal rot disease, the diversity of host species and the geographic range are wide (Jana et al. 2005; Babu et al. 2010; Saleh et al. 2010). Zhang and Feng (2006) made the charcoal rot resistance standard with five grades (Zhang and Feng

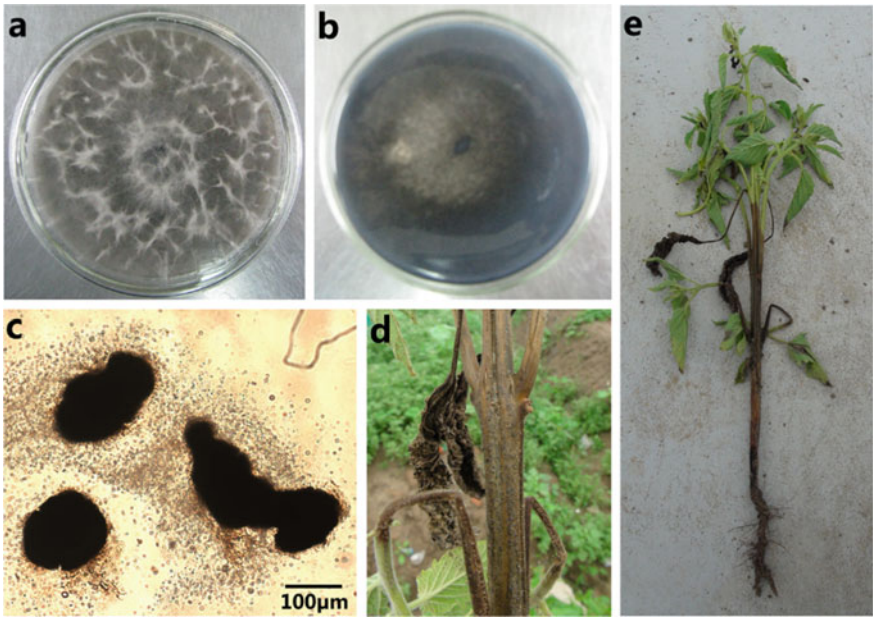

Fig. 6.3 Symptoms of sesame charcoal rot disease in sesame. **a** *Macrophomina phaseolina* strain colony front side; **b** *Macrophomina phaseolina* strain colony back side; **c** Sclerotia of *Macrophomina phaseolina* strain. Bar = 100 μm; **d** dark-colored sclerotia mass of *Macrophomina phaseolina* strain on the stem of diseased plant; **f** Symptom of charcoal rot disease in sesame (Provided by Hongyan Liu)

2006; Wang et al. 2017). The resistance level to charcoal rot disease is calculated based on the relative resistance indicator. Meanwhile, Thiyagu et al. (2007) used the five grades with different infection percentage (Dinakaran and Mohammed 2001) and established the artificial screening and sick plot methods to assay the resistance to the charcoal rot disease. Similar to the resistance to FOS, the charcoal rot disease resistance in sesame was controlled by a few dominant genes (El-Bramawy and Shaban 2007).

6.1.3.3 Leaf Diseases and Host Resistance Evaluation

For sesame, leaf diseases are also common diseases (Fig. 6.4), as the main leaf diseases such as leaf blight can occur on 30–40% of the plants and cause the loss of above 30% seed yield (Zhao et al. 2014). There are many types of leaf diseases in sesame caused by different pathogens. For instance, leaf blight disease is caused by Fungus *Helminthosporium sesami* Miyake (Poole 1956; El-Fawy et al. 2018); Alternaria leaf spot (or black spot) disease is caused by fungus *Alternaria sesami* (Kawamura) Mohanty et Behera (Dolle and Hegde 1984b); Nigrospora leaf blight disease is caused by fungus *Nigrospora sphaerica* (Dutta et al. 2015); brown spot

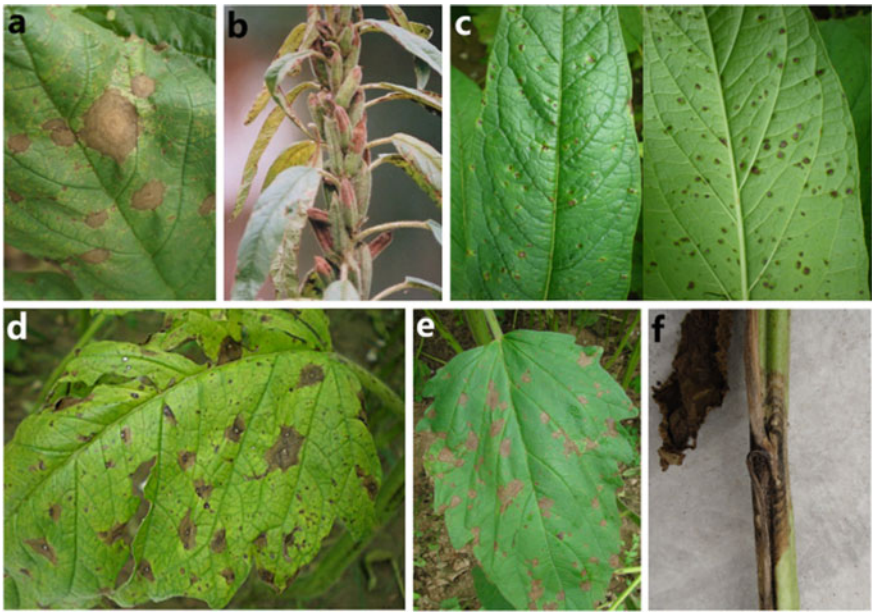

Fig. 6.4 Symptoms of the main leaf diseases in sesame. **a** Sesame leaf blight in leaf; **b** Sesame leaf blight in capsule; **c** Sesame Alternaria leaf spot in leaf; **d** Sesame brown spot in leaf; **e** Sesame Corynespora leaf spot in leaf; **f** Sesame *Corynespora* leaf spot in stem; **g** Nigrospora leaf blight in leaf (Provided by Hongyan Liu)

disease is caused by fungus *Ascochyta sesami* Miura; and Corynespora blight (or Corynespora blight spot) disease is caused by fungus *Corynespora cassiicola* (Berk and Curt) (Shivas et al. 1996). All the above five diseases are the most common leaf diseases in sesame (Zhao et al. 2014; Zhang et al. 2019).

Alternaria leaf spot disease is very common and could infect some wild species (Mehta and Prasad 1976; Dolle and Hegde 1984a). As to Corynespora blight, the pathogens can infect between plants under suitable conditions, and spread to stem, leaf petiole, and capsule, and cause disease aggravation. At early stage, the disease spots are angular brown with circular speckle lesions (5–15 m in diameter) and scatter on both surfaces of the infected leaf. Subsequently, the diameter of the lesions would expand. Previous report indicated that *Corynespora cassiicola* pathogen can exist in soil for more than two years (Qi et al. 2011). The fungus can be spread through seeds both internally and externally, and can survive in the plant debris. Therefore, the primary spread medium of the pathogens in the field should be seeds and plant residues. The secondary medium may be wind borne conidia.

In sesame field, some of the leaf diseases often occur simultaneously in population and are affected by both sesame genotype and environments. The complication of the disease occurrence limits the genetic inheritance analysis of the resistance to leaf diseases in sesame. Some studies reported that the seed color trait was related with the disease resistance (El-Bramawy et al. 2008; Li et al. 2014). As to the morphological characters of the sesame resistance to leaf disease, El-Bramawy and Shaban (2007) investigated the disease syndrome of 45d old sesame plants infected by *Alternaria* leaf spore suspension (2×10^3 spore per mL) under greenhouse conditions according to Karunanithi (1996). After inoculated by *Alternaria* leaf spore suspension for 1 week, the leaf spot percent of the samples ranged from 1.32 to 18.54% and presented the mostly additive variance. Inheritance analysis results of the crosses of RT-273 (resistant) and Gulbarga Local Black (susceptible) showed that the resistance to Alternaria blight in sesame was controlled by single dominant gene pair (Eshwarappa 2010). For the advanced generations F_3 and F_4 under field condition, the resistance to Alternaria blight disease was controlled by the single dominance of gene action (Goudappagoudar et al. 2014).

6.1.3.4 Other Diseases in Sesame

Besides the above diseases, powdery mildew (*Erysiphe cichoracearum*) and phyllody are also common and worldwide diseases in sesame (Venkata et al. 2013). Powdery mildew occurs in the season with low temperature. In addition, viral diseases are caused by virus ions and are transmitted by insects (mainly aphids). Viral diseases mostly occur in the seedling stage, with symptoms such as plant dwarfing, mosaic leaves, narrowing leaves, yellow-green spots, and deformed stems and leaves which finally result in fewer capsules and lower yield.

6.1.3.5 Disease Control

Plant diseases are the interaction results between susceptible host plants and virulent pathogens under different environments. To control disease occurrence, some methods including agronomic management, pharmaceutical treatment, and cultivation management have been widely applied (Elad and Pertot 2014). Agronomic management is a traditional management method which could be traced back to the ancient times. The main management solutions include seed disinfection, reasonable close planting, crop rotation, water and fertilizer management, and soil preparation. Of which seed treatment such as seed priming can reduce the early infection of pathogens and is regarded as an effective and key method to control the disease spread. Moreover, harvesting crop on time is also an effective solution to reduce seed loss and control the fungal diseases.

As to the soil-borne pathogens such as *F. oxysporum* isolates, to realize the disease prevention prior to infection is the best choice (Lievens et al. 2008; Ghini et al. 2011). Cleaning up the infected left over debris and soil in time is necessary. Especially for susceptible crops, planting in the fields infected by pathogens should be avoided. In addition, giving the balanced nutrition with abundant potassium, nitrogen, and other vital nutrients is effective.

At present, spraying chemical fungicides is still the first choice for farmers to prevent and control crop diseases for the easy adaptability, convenient use, and immediate therapy. Powdery mildew occurs frequently in sesame disease, Kabiet al. (2019) reported that powdery mildew disease could be prevented by dusting sulfur and other chemicals. Moreover, biological control is another safe and effective tool for sesame disease control (Sunita et al. 2019). Till now, several strains with high biological control function to FOS and MP pathogens have been found and are being tested on sesame (Unpublished data, Haiyang Zhang). Therefore, studies on the effective control of fungal infection provide the promising methods for biological control in sesame. As far as the use of pesticides is concerned, in the case of spraying pesticides on a single leaf surface, 50 EC 0.1% profenofos with foliar spraying method has the better effects than 5% NSKE plant-derived insecticides on capsule stem borer, leafhoppers, and larvae control (Nayak et al. 2019).

Biocontrol techniques with the natural products and biological agents are ecofriendly and important for improving the seed yield and quality of sesame (Didwania 2019). Especially for the soil-borne pathogens, the use of biological agents is a promising method to realize the disease control (Deacon and Berry 2010). In the past few decades, some potential biological organisms have been isolated, identified, and gradually commercialized. Lubaina and Murugan (2015) evaluated the efficiency of *Trichoderma* species as biocontrol against *Alternaria sesami* in sesame. The results showed that *T. harzianum* can colonize and inhibit the growth of *Alternaria* pathogen which exerted an obvious biological control effect. Moreover, applying microorganisms is also a biological control method to inhibit the phytopathogens (Baker and Paulitz 1996). *VA mycorrhizal* fungi can protect plants from damage via increasing the activity of the antifungal chitinase enzymes in roots (Zeng 2006). Ziedan et al. (2011) developed biotic agents such as *Trichoderma* and *VA mycorrhiza* to control

sesame wilt and root rot diseases. The results showed that the protection effects were great and the seed yield significantly increased. The mixed inoculation using *mycorrhizal* symbiotic bacteria and biological control agent was more effective than applying single strain (Ziedan et al. 2011).In order to understand the role of silicon in the prevention and treatment of sesame charcoal rot, the effects of different silicon sources on the mycelial growth of *M. phaseolina* were performed in vitro (Siddiqet al. 2019).

At present, biological control is attracting more and more attention for agricultural practices. Integration of biological control agents, plant botanicals, and organic amendments can reduce the use of farmland fungicides and ensure the safety of agricultural ecology (Nayan et al. 2019). Considering the demand for green and healthy seed products, safe alternatives to chemical fungicides should be emphasized and applied in the future.

6.1.4 Insect-Pests and Their Management

Sesame yield is easily affected by insect-pests. Insect-pests are one of the main factors restricting sesame production. Twenty nine pests attacking sesame in India (Delhi) have been reported (Baskaran et al. 1997). Especially, leaf webber and capsule borer (*Antigastra catalaunalis*), gall midge (*Asphondylia sesami*), pod sucking bug (*Elasmolomus sordidus*), sphingid moth (*Acherontia styx*), leaf hopper (*Orosius albicinctus*), and white fly (*Bemisia tabaci*) are the main pests for sesame (Baskaran et al. 1997). Of which sesame leaf webber and capsule borer (Lepidoptera: Pyralidae) are the most harmful pest for sesame and can cause the loss up to 90% and 80.42%, respectively (Gnanasekaran et al. 2010; Wazire and Patel 2016).

Many insect-pests have a negative impact on crop yield and quality. Insect-pests in the field are difficult to control. In agricultural production, pesticides are often used to control insect-pests. However, the hidden dangers of drug residues often exist. Ecofriendly pest control methods have been widely used and present the privileges in protecting the agricultural product quality and the surrounding ecosystem. For sesame, the diseases and insect-pests control solutions are being changed from the traditional chemical method to the biological control method now. Some research results have confirmed that salicylic acid can promote plant growth and inhibits the infestation of *M. persicae*, *B. tabaci*, *E. lybica*, *Creontiades* sp., *N. viridula*, and phyllody pathogens (Mahmoud 2013). Liu et al. (2017) integrated a set of comprehensive prevention and control technology system for sesame diseases and insect-pests, based on the individual prevention and control techniques in Northwest China. The control system presented the high effects on insect-pests, diseases, and weeds in sesame field (Liu et al. 2017).

6.2 Disease Resistance Breeding in Sesame

6.2.1 Traditional Breeding for Disease Resistance

Crop varieties with high disease resistance inherit the resistance to diseases and enhance the protection from pathogen infection. In the past 50 years, sesame scientists in the world used the simple hybridization, physical and chemical mutagenesis, and heterosis breeding methods in sesame breeding and bred hundreds of new sesame varieties. The resistance of the varieties to pathogens is improved accordingly. With the development of agriculture and crop production, aggregating more elite genes with high resistance to pathogens using new modern breeding techniques seems more necessary.

In the previous studies, some researchers proposed to create male sterile lines and to breed hybrid varieties using the heterosis character of sesame (Pal 1945; Tu et al. 1995; Zhao and Liu 2008). The first sesame male sterile line was found by an Indian scientist and was introduced to the world (Tu et al. 1995). In 2003, the first sesame hybrid 'Yuzhi 9' was bred by Chinese scientists and the two-type sterile lines hybrid technique was formed accordingly (Zheng et al. 2003). Meanwhile, mutagenesis technique was also applied to obtain a wide range of male sterile mutants (Li and Chen 1998; Zhao and Liu 2008). However, the male sterile lines currently used in China still have some disadvantages including environmental sensitivity, incomplete sterility, and the high manual cost for 50% of male fertile seedlings removal during producing hybrid seeds (Zheng et al. 2003).

6.2.2 Rationale for Molecular Breeding

Traditional breeding methods are relatively time-consuming and labor-intensive, and require high personal work experience for breeders. In recent years, several modern biological techniques such as genome sequencing and genetic modification rapidly developed. The application of the modern biological techniques, especially the next generation sequencing technique reinforces the modern plant breeding techniques. Modern molecular breeding technology represented by molecular marker breeding, genetically engineering, and molecular design breeding is gradually becoming the mainstream of crop breeding methods around the world. Compared with the traditional breeding techniques, molecular breeding has the advantage of increasing yield and saving labor. With the support of big data, molecular breeding techniques can significantly improve breeding efficiency. The breeding cycle is significantly shortened accordingly.

To further increase the yield level and the total sesame production, and to narrow the gap between demand and supply, new elite varieties with high and stable yield level are more requisite. In a recent few years, the Chinese scientists bred a series of sesame varieties such as Wanzhi, Yuzhi, and Zhongzhi. For example, Wanzhi

No. 10 is a new hybrid bred by the Crop Research Institute, Anhui Academy of Agricultural Sciences, China. The disease index of the variety to stem spot blight and Fusarium wilt diseases was 5.07 and 0.85, respectively. The average yield reached to 1261.65 kg/hm^2. The oil and protein content of the seeds were 58.18 and 20.97% (Li et al. 2018). Gnanasekaran et al. (2010) comprehensively considered the genetic effects of the disease resistance, combining ability, and the yield contributing traits and selected more excellent lines with high yield and resistance potential. In 1984, a new variety 'Ahnsankkae' was bred via 20 krad X-ray irradiation, and has become the main sesame variety in Korea for the high yield and strong resistance. Meanwhile, 'Sunwonkkae' is a hybrid derived from an X-ray mutants and Korean local varieties in 1991 (Kang et al. 1994). The goal of traditional crop breeding is clear and mainly focuses on the yield, seed quality or disease resistance of the varieties through various breeding techniques to maximize the advantages of breeding improvement.

Modern biological technology improves the development of molecular breeding techniques including screening disease resistance related genes and molecular markers and genetic transformation or gene editing, and provides an effective method to realize the high breeding efficiency with more new varieties with high disease resistance (Zhang et al. 2012). Marker assisted selection is being applied in breeding projects and for aggregating more disease resistance genes. For example, a new dwarf sesame variety 'Yuzhi Dw609' (Fig. 6.1) was bred from the first dwarf variety Yuhzi Dw607 with short internode length trait by the Henan Sesame Research Center, Henan Academy of Agricultural Sciences (HAAS) in the past a few years. With the aid of hybridization technique and molecular marker assisted selection, Yuzhi Dw609 inherits both the short internode length trait from the parent Yuzhi Dw607 and the high resistance character from the other parent Ganzhi 9 (Unpublished data, Haiyang Zhang).

6.2.3 Breeding Objectives

Breeding disease-resistant varieties requires the knowledge of genetics and inheritance of the disease resistance. The success of any plant breeding program largely depends on the selection of appropriate parents. A few wild *Sesamum* species such as *S. malabaricum* and *S. mulayanum* seem to possess high tolerance to powdery mildew (Venkata et al. 2013). However, elite genes introgressing into the cultivars from wild relatives is still difficult for sesame because of lacking the effective interspecific hybridization techniques. Developing innovative breeding techniques needs long-term work in the future.

6.3 Genetic Diversity Related to Disease Resistance in Sesame

6.3.1 Phenotype-Based Diversity Analysis

Sesame has a long cultivation history in China, India, Near East, and Central Asia. These regions are diversity centers for sesame germplasm. Germplasm exchange and selection provides a huge breeding space for sesame scientists (Laurentin and Petr 2006; Mahajan et al. 2007). Thus, assessing the morphological and the genetic diversity of the cultivated accessions is meaningful for genetic base expansion and genetic resources protection during breeding new varieties (Amini et al. 2007). For sesame, the morphological and agronomic traits of a great deal of germplasm accessions have been investigated and the genetic diversity is evaluated based on various morphological and agronomic characteristics, isozyme analysis, and molecular marker polymorphism (Liu et al. 1997). In early stage, the expressed sequence tag-simple sequence repeat (EST-SSR) markers were used to evaluate the level of genetic variation among different sesame genotypes. However, the number of SSR markers used for research on diversity analysis and sesame linkage map construction was very limited before the initiation of the sesame genome project (Zhang et al. 2019).

6.3.2 Genetic Background of the Resistance to Diseases

The primary aim of breeding for sesame scientists is to improve the seed yield. In the breeding genotypes, the resistance to sesame blight, charcoal rot and black spot differs greatly (Kavak and Boydak 2006; El-Shakhess and Sammar 1998). Breeding methods depend to a large extent on the nature and number of the genes that control the genetic behavior of most research traits. In addition, understanding the nature and extent of the gene effects on yield and yield components, as well as the resistance to major diseases (such as Fusarium wilt, charcoal rot, and black spot), is useful for formulating effective breeding strategies for genetic improvement in sesame (Zhang et al. 2019).

6.3.3 Relationship with Geographical Distribution

In the worldwide sesame production regions, at least eight economically important fungal diseases commonly occur (Kolte 1996). In Iran, *Macrophomina phaseolina* is an important soil pathogen, causing charcoal rot in many important crops including sesame (Salahlou et al. 2016). In the history of sesame cultivation, some genotypes from different geographical regions were clustered in the same group. Thus, Pissard et al. (2008) proposed that geographic distribution is an important parameter for

germplasm collection, even though the geographic origin character is not always able to predict the genetic difference. Selecting genotypes with suitable genetic distance and excellent agronomic traits in crosses can help breed excellent varieties (Parsaeian et al. 2011). Therefore, in order to improve the disease resistance of sesame varieties, both plant materials exchange and selective crossing between the parents with different geographic distributions and disease resistance levels should be encouraged.

6.3.4 Relationship with Other Cultivated Species and Wild Relatives

As we all know, the use of the host plants with high resistance to biotic stresses is a more practical strategy to realize the high yield and seed quality. During breeding new varieties with high disease resistance, understanding the genetic patterns of the disease resistance or drug resistance in hosts is necessary. Even though the wild sources such as *S. malabaricum*, *S. mulayanum*, and *S. prostratum* always have high resistance to pests and diseases, no significant research progress in interspecific hybridization breeding has been achieved so far. The application of the advanced technologies such as molecular markers assisted breeding with resistance genes is a prerequisite for the success of breeding programs (Venkata et al. 2013). Mehetre et al. (1994) reported that *S. mulayanum*, a wild relative of sesame has the high resistance to powdery mildew as well as phyllody (Mehetre et al. 1994). Krishnaswami et al. (1983) studied the heredity of powdery mildew resistance in F_2 offspring derived from the susceptible and the resistant parents. The results showed that the resistance to powdery mildew is controlled by the action of two major genes and complementary genes, which provide the foundation for the improvement of powdery mildew-resistant varieties (Krishnaswami et al. 1983).

6.3.5 Artificial Induction and Incorporation of Resistance Traits

The morphological and agronomic traits are greatly influenced by environmental factors. However, to change the genetic character and collect the elite traits is the basic solution to improve the agronomic traits in crops. For sesame, breeding is done by using mutagenesis by physical and chemical mutation, genetic transformation, and interspecific hybridization (Zhang et al. 2019). For example, South Korean scientists have bred 14 sesame varieties since 1955. Of these two varieties were bred through mutation breeding. From 1989 to 1992, 2625 mutant pedigree lines and 89,200 plants were planted and investigated (Kang et al. 1994). In addition, sodium azide (NaN)-treatment was also used to create new lines. A dwarf mutant line, ‘Suwon 128’, which

was unique in the dwarf shape and strong lodging resistance, was obtained and used for variety breeding (Kang et al. 1994).

In addition, the development of high-throughput sequencing technology greatly promotes the detection of a great amount of sequence-based molecular markers and the application in genetic variation research in sesame.

Root rot is the most harmful disease for the sesame industry. At present, there is no effective prevention and management approach available for root rot resistance. Study of host resistance is the key to solving this disease. In addition, due to the lack of understanding of the molecular mechanisms of *M. phaseolina* interacting with the host, it is not feasible to develop resistant genotypes through genetic engineering. On the basis of genome-wide research, cultivating varieties with inherent resistance is one of the most effective and economical means to control sesame root rot. However, there are currently little data on the genetic analysis of sesame root rot resistance (El-Bramawy and Shaban 2007). Recently, Yu et al. (2013) compared the NBS (nucleotide binding site) type resistance gene analogs (RGAs) and EST sequences and cloned 16 full-length RGAs from 10 different resistant cultivars against *Macrophomina phaseolina* (GenBank accession number: KC477692-KC477707). All these RGA gene sequences belong to the non-TIR-NBS type R genes and contain the specific NBS domains. This result laid the foundation for further screening of resistance genes for *Macrophomina phaseolina* in sesame (Yu et al. 2013).Wang et al. (2017) developed new molecular markers with the aid of genome data and constructed molecular genetic maps. As a result, the genetic maps were applied to determine the QTLs related to the resistance to charcoal rot disease.

6.4 Genetic Analysis and Association Mapping Studies

6.4.1 Molecular Marker Development in Sesame

Compared with the phenotypes and isoform proteins, molecular markers have more advantages for germplasm assessment (Pissard et al. 2008). In the early stage, many studies have been performed to develop the universal molecular markers such as random amplified length polymorphism (RAPD), amplified fragment length polymorphism (AFLP), and inter-simple sequence repeat (ISSR) and revealed the application of the molecular markers in the genetic diversity analysis and association mapping of agronomic traits in sesame (Isshiki and Umezake 1997; Bhat et al. 1999; Ercan et al. 2002, 2004; Kim et al. 2002; Hernan and Petr 2006; Ali et al. 2007; Zhang et al. 2010; Shinwari 2011; Li et al. 2014; Daret al. 2017). Parsaeian et al. (2011) studied the genetic variation in 18 sesame genotypes from Iran and the six foreign genotypes from the Asian countries. The results showed that the genotypes of the various agricultural morphological traits were significantly different, and the genetic variation coefficients of the number of capsule branches per plant, capsule number, plant height, and seed yield per plant were relatively high. According to

the polymorphism of RAPD markers, large genetic differences existed between the foreign and Chinese domestic germplasm accessions (Zhang et al. 2004).

Subsequently, the specific markers, such as the SSRs and EST-SSRs were developed and applied for genetic map construction, and genetic diversity and trait association analysis in sesame (Powell et al. 1996; Dixit et al. 2005; Wei et al. 2009). Wang et al. (2017) detected a total of 110,495 genomic SSRs from the sesame genome (var. Zhongzhi No. 13) and provided a plenty of polymorphic SSR markers. In the recent years, construction of the fine genome map for sesame and the genomic analysis has significantly improved the molecular breeding techniques in sesame (Laurentin et al. 2008; Zhang et al. 2019). Wei et al. (2014) combined the three RNA-Seq datasets and compared the single nucleotide polymorphism (SNP) and insertion/deletion (InDel) variants in the three transcriptome sets for the first time. With the abundant genome and transcriptome information, Zhang et al. (2016) constructed the first high-solution SNP genetic map with millions of SNPs in the cultivated sesame varieties and located a gene controlling the inflorescence determinacy. Till now, so many SNP and InDel markers have been detected and applied in the genetics research in sesame (Wei et al. 2014; Wu et al. 2014b; Zhang et al. 2016; Zhang et al. 2019).

6.4.2 Molecular Genetic Linkage Maps and QTLs Related to Disease Resistance in Sesame

For sesame, the first molecular genetic linkage map was constructed by HAAS using an F_2 population and eight EST-SSRs markers, 25 AFLP markers, and 187 RSAMPLs (random selective amplification of microsatellite polymorphic loci) markers in 2009 (Wei et al. 2009). Of which the 30 linkage groups carrying the 220 markers were formed. Subsequently, the map was saturated with more SSR markers and using larger F_2 population with 260 individuals (Zhang et al. 2013b). Four QTLs linked to the seed coat color trait were detected. Till now, to our knowledge, at least eight molecular genetic linkage maps have been constructed for sesame (Zhang et al. 2013c; Wu et al. 2014a; Wang et al. 2017; Uncu et al. 2016; Zhang et al. 2016; Mei et al. 2017; Du et al. 2019; Liang et al. 2021). Of these two SSR genetic maps constructed using a recombinant inbred lines (RIL) population were used to locate the QTLs for the waterlogging tolerance and charcoal rot disease resistance traits (Zhang et al. 2013b; Wang et al. 2017).

In order to improve the genome map construction, the first ultra-dense SNP genetic map was developed using the Illumina genome re-sequencing data of an F_2 population (Zhang et al. 2016). A total of 3,041 bins representing 30,193 SNPs distributed on the 13 linkage groups (LGs) were developed and the even correspondence of LG and chromosome (Zhang et al. 2016) was realized. The ultra-dense SNP genetic linkage map has been utilized for genome assembly and gene location because of the saturated SNP density.

Meanwhile, to explore the genetic inheritance of the key agronomic traits, the above molecular genetic maps have been used to locate the QTLs related to yield, seed quality, and disease resistance traits (Miao et al. 2017; Zhang et al. 2019; Liang et al. 2021). For example, Wang et al. (2017) detected 10 QTLs significantly associated with the resistance to charcoal rot disease on five LGs using an RIL population. The phenotypic variance explained of the 10 QTLs varied from 5 to 14%. With the aid of the first ultra-dense SNP genetic map and the resistance variation of the $F_{2\text{-}3}$ families to Fusarium wilt disease, Miao et al. (2017) determined a QTL location on LG8 (111.5–112.7 cM). The explanation ratio reached to 51.66% ($P < 0.001$). As a result, the first marker *SiFWR2145* associated with the resistance to Fusarium wilt disease was developed and applied for elite SNP selection during disease resistance breeding.

6.5 Genomics-Aided Breeding for Disease Resistance

6.5.1 Sesame Genome and Resistance Genes

In order to improve the sesame breeding techniques, the Sesame Genome Working Group (SGWG) initiated the Sesame Genome Project in 2010 (Zhang et al. 2013a). Based on the complicated sequencing and assembly platform, the chromosome scaled genome map was constructed for sesame (Zhang et al. 2016, 2019). The fine genome is 335.19 Mb in size and contains 31,462 genes (Zhang et al. 2013a; Zhang et al. 2019). At present, the fine genome version 3 for *S. indicum* (var. Yuzhi 11) has been uploaded to NCBI public database. The sesame genome information and the genomics results will be published soon.

In the fine genome version 3, a total of 31,462 genes were annotated (Unpublished data, Haiyang Zhang). Based on the GO database (Gene Ontology Consortium 2004) (http://www.geneontology.org/), all the 31,462 genes were classified into three main categories (i.e., 'Molecular function', 'Biological process', and 'Cellular component' categories) (Fig. 6.5). About 41.53% genes were classified into the category 'Biological process', of which 21.86% genes belonged to the 'metabolic process' group, followed was the 'cellular process' (21.50%). In the second category of 'Molecular function', 31.81% genes were further classified into the 15 groups. The top group was 'binding' (15.32%). Meanwhile, the third category of 'Biological process' contained 26.66% genes.

Besides the Illumina sequencing platform, Wang et al. (2014) assembled and published a sesame genome draft (var. Zhongzhi No. 13). Uncu et al. (2015) performed the genome sequencing of var. MMuganli 57 using the Roche 454 GS-FLX technique and obtained 65 Mb sequences with the genome coverage of 19.3%. All the sesame genome data supplies precious information for genomics, genetics, and breeding research in sesame and other crops.

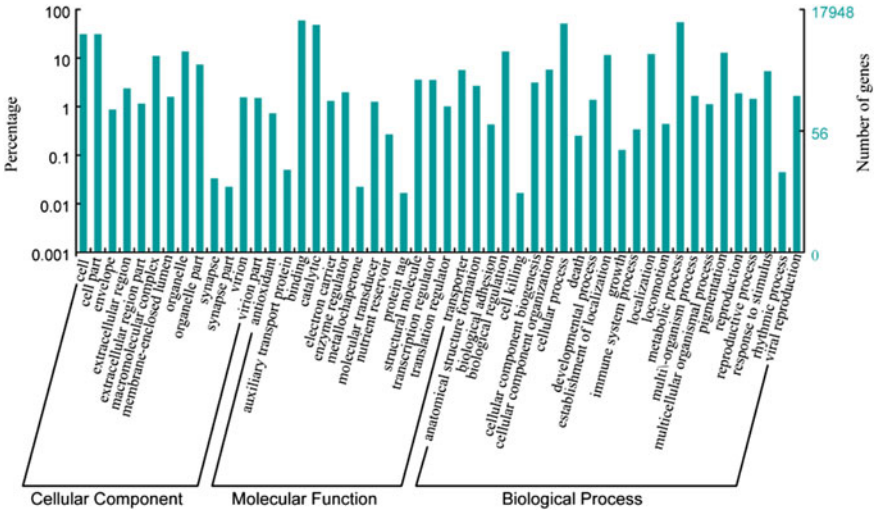

Fig. 6.5 GO classification of the sesame genes. The left vertical axis indicates the gene percentage. The right vertical axis indicates gene number. The horizontal axis indicates gene group type and category (Provided by Haiyang Zhang)

6.5.2 Disease Resistance Gene Digging

Genome data and the genome re-sequencing techniques stimulated the genome wide association studies (GWAS) of the key agronomic traits in sesame (Wei et al. 2015; Li et al. 2018; Zhang et al. 2019). As to identify molecular markers associated with the resistance to stresses, Wei et al. (2015) performed the GWAS using 705 sesame accessions (Wang et al. 2014). A total of 549 loci associated with the 56 agronomic traits were detected in the population under four environments. Of these 13 loci were related to the susceptibility of phyllody disease. With the GWAS analysis method, one SNP associated with Fusarium wilt resistance using 560 germplasm accessions was determined recently and confirmed the resistance character of dominant gene control in sesame Fusarium disease (Unpublished data, Haiyang Zhang). We believe the genomics research should significantly contribute to the marker assisted breeding in sesame in near future.

In sesame, there are some key orthologous gene families of the AP2/ERF transcription factors and Hsfs (heat shock transcription factors) genes which are probably related with the resistance and tolerance to the biotic and abiotic stresses (Wei et al. 2015; Dossa et al. 2016a, b). Orthologous gene analysis indicated that all these genes probably play the role in regulating drought tolerance and disease resistance in sesame. In sesame, there are about 132 AP2/ERF orthologous genes (Dossa et al. 2016b). The transcription indicated that AP2/ERF gene family was regulated under the drought stress in sesame. In addition, comparative genomics analysis between *S. indicum* and wild *Sesamum* species and other oilseed crops was performed using the

fine genome maps. The results indicated that some key disease resistance gene families presented the expansion or constriction characters. The variation of the several R resistance genes or families might be related with the relative low resistance to diseases in the cultivated sesame (data now shown, Haiyang Zhang). Thus, introducing new gene resources from the wild species using modern molecular breeding methods is a prosperous direction for future sesame breeding.

6.5.3 Genome Assisted Breeding in Sesame

In the last two decades, the key objective of sesame breeding was to breed new lines with high and stable yield potential. A few genes involved in regulating the synthesis of fatty acids, seed storage proteins, and secondary metabolites and the tolerance to salt stress have been determined using the gene mapping and homolog analysis (Yukawa et al. 1996; Jin et al. 2001; Lee et al. 2005; Chyan et al. 2005; Hsiao et al. 2006; Kim et al. 2007, 2010; Hata et al. 2010; Zhang et al. 2019). The Sesame Genome Project provides the necessary information for identification of genes and genomics assisted breeding in sesame. With the application of the sequencing technologies and based on the genetics and genomics research in sesame, more than one hundred QTLs, candidate genes, and associated molecular markers were detected (Zhang et al. 2013a, b; Miao and Zhang 2014; Wang et al. 2014; Wei et al. 2015; Dossa et al. 2016a; Miao 2016). We thus believe that the resistance genes to the main diseases, such as Fusarium wilt and charcoal rot should be identified in near future (Zhang et al. 2019).

6.6 Future Perspectives and Potential for Boosting Up of Sesame Productivity

In the past two decades, the modern biological technologies and high-throughput sequencing platform accelerated the molecular genetics and breeding research in sesame. However, we have to know that the efficiency of the interspecific hybridization and transgene techniques in sesame is still low and limits the innovation of the breeding techniques. Gene editing technique is immature in sesame. To realize the molecular design with the new varieties with high resistance to environmental conditions is also a huge task for sesame breeders. Before performing the concise molecular breeding technique, more new breeding materials with multiple elite traits should be found or created; more precise molecular markers and genes should be determined and utilized for target screening. Meanwhile, the regulation network of the multiple marker loci and the interaction in sesame genome should be clarified. Therefore, large-scale gene cloning and gene function research are still the main tasks (Zhang H. personal communication). With advance of the sesame genomics

research, we believe that the genome designing for sesame resistance breeding and new elite line creation should be carried out in near future.

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Chapter 7
Biotic Stresses in Castor Plant

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Abstract *Ricinus communis* is a kind of crop with high economic value in the world. It has been paid more and more attention because of its high economic value. In the process of growth and development, it is subjected to a variety of biotic stresses, especially from diseases and pests. In this paper, the stress on castor was discussed in consideration of diseases including bacterial, fungal and viral diseases, and a variety of pests. It is suggested that more attention should be paid to the identification and utilization of resistant resources, resistance genetic mechanism research and breeding for resistant varieties.

Keywords *Ricinus communis* · Biotic stress · Disease · Insect · Resistant germplasm resources · Disease resistance breeding

7.1 Introduction

Castor bean cultivation has a long history, however, compared with the main crops, its genetic research lags behind, the improvement degree is low, coupled with its own genetic basis is narrow, the breeding level is not high. One of the most serious problems is that the existing varieties are almost all susceptible to disease and insect pests, resulting in a production loss of 20–30% generally, in severe cases, more than 50% even the whole field died. Whether the research of genetic mechanism of resistance or the resistant germplasm resources selection or the resistance variety breeding has a long way to go.

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7.2 Diseases of Castor

The decline in longevity and reduction in productivity of *Ricinus communis* are caused by many factors, especially fungal pathogens, bacteria, or viral disease, among which Fusarium root rot, Fusarium wilt and leaf spot are the most serious. The persistence of disease in some areas may render crops unprofitable and lead to periodic migration of crops to new areas. This review will cover the major diseases of castor beans, their symptoms, etiology, and management.

7.2.1 Bacterial Diseases

7.2.1.1 Bacterial Leaf Spot

Xanthomonas ricinus caused castor leaf spot disease, which is an occasional seed-borne disease. The typical symptoms of the disease are brown or black, round, or angular leaf lesions. Occasionally, the fleshy twigs are attacked, but the vascular tissue is not. In the case of serious disease, spraying of streptocycline at (1 g/10 L water) could be adopted to control it (Basappa 2003).

7.2.1.2 Bacterial Wilt

The early symptoms are that the leaves on one side of the plant show similar symptoms of water loss and wilting, which recover sooner or later, and wilt during the day; after repeated several times, the withered leaves began to turn yellow and fall off; at the later stage, the whole plant wither and die; After cutting the stem, the vascular bundle appear dark brown necrotic spots, which is preliminarily diagnosed as *Ralstonia solanacearum* (Yuelian Liu Unpublished).

7.2.2 Fungal Diseases

7.2.2.1 Charcoal Rot of Castor

Charcoal rot of castor, also known as Macrophomina root rot, is caused by *Macrophomina phaseolina*n, and occurred in most castor-growing countries (Araújo et al. 2007; Rajani and Parakhia 2009). One criteria for the development of resistance to charcoal rot has been established (Grezes-Besset et al. 1996), and some tolerant genotypes have been developed (Anjani et al. 2004; Anjani 2005b). Control of the disease is primarily based on cultivar resistance, but crop rotation and organic matter amendments can also reduce the severity of the disease (Rajani and Parakhia 2009).

7.2.2.2 Fusarium Wilt

Castor Fusarium wilt is a serious vascular disease, is caused by the soil borne fungus, *Fusarium oxysporum* f. sp. *Ricini*. The disease was spreading in India (Desai and Dange 2003) and in China. Vascular wilt generally appears in patches and at all growth stages of the crop. The blight of castor can occur at any growth stage of castor bean, including root, stem, leaf, and ear. It can cause root necrosis, stem and leaf wilt, plant dwarfism, ear thinness, reduced yield and even no harvest. The typical symptoms of castor wilt are that light pink or powdery white mildew layer can be seen on the back of leaves or the surface of the diseased side of stalks under suitable conditions. Corresponding prevention and treatment measures can be taken according to whether the symptoms of castor wilt appear in the field (Anjani 2006, 2010).

7.2.2.3 Fusarium Root Rot of Castor

This disease was study by the authors. Fusarium root rot of castor, which was caused by *Fusarium solani* species complex, has been observed in Zhanjiang (21.17 N, 110.18 E), Guangdong Province, China since 2016. The results of the investigation showed that about 1800 plants in field (0.66 ha) were affected, and the disease incidence reached 26.2%. Early symptoms comprised the wilt of lower leaves and the darkening of vascular tissue of roots, which turned brown. Progressively, whole plant wilted, the roots rotted and the plant ultimately died (Zhou et al. 2019).

7.2.2.4 Gray Mold of Castor

Castor gray mold is one of the most severe disease, was caused by *Botryotinia ricini*. The disease was first epidemic outbreak in the Florida Experiment Station, Gainesville, Florida (Godfrey 1919, 1923). Then, in October 2000, the disease occurred in Woniu and Okcheon of Korea (Hong et al. 2001). Gray mold mainly damages young flowers, young fruits, tender stems, and leaves. Infected leaves, water-stained disease spots spread along the veins of the leaves, causing early leaf fall. Infected stems, the initial disease spots on the leaf scars gradually expand, the diseased parts lose gloss, dry up and appear black sclerotia, often cause the upper tissues wither. Flower and fruit susceptibility are often affected by diseased stems and vines, causing the flowers, buds, and capsules to brown and fall off, and seeds immature so that the seeds mildew. In the tropics, the primary source of inoculation would be the spores produced by wild castor. The first flower infected by the fungus could produce a large number of spores. Re-infections could occur since the spores is spread by wind, or by rain and possible by insects (Soares 2012).

7.2.2.5 Leaf Rust of Castor

Melampsora ricini, has been documented on castor plants as a rust disease. The disease spread in Asia, Africa, and southern Europe (Punithalingam 1968). Severe leaf rust have been investigated in India and Italy. Uredinia and telia could be observed in the infected leaves. The dense coverage of *Uredinia vulgaris* on the back of the leaves resulted in the appearance of spots on the surface of the leaves, which led to premature wilting, premature senescence, and the decline of host vigor. Some Uredinia occurs on the front and on the cotyledons (Shivas et al. 2000).

7.2.2.6 Leaf Spot of Castor

Alternaria leaf spot The disease caused by *Alternaria ricini* is, is one of leaf spot diseases in India (Hiremani et al. 2012). The symptoms are irregular spots on the leaves covered with a layer of mold that appears to be concentric rings. In the case of the disease spot expands and fusions, it can cause the leaf to shed prematurely. In severe cases, seeds can also be infected (Gahukar 2018).

Cercospora leaf spot A Cercospora leaf spot of castor, which is caused by *Cercospora coffeicola*, has been observed in Brazil. Symptoms of the disease are black and purple necrotic lesions with pale yellow halos and pale white centers. Strong sporulation occurs in the center of the lesion (Souza and Maffia 2011).

Cladosporium leaf spot This disease was study by the authors. Since 2016, leaf spot of castor, which is caused by *Cladosporium tenuissimum*, has been observed in Zhanjiang (21.17 N, 110.18 E), Guangdong Province, China, with disease incidence of about 20.5%. Early symptoms appeared on affected leaves as light brown or gray necrotic spots. Successively, a mold appeared on both sides of the spots. The spots were irregular in shape and increased in size and coalesced (Liu et al. 2019).

Phyllosticta leaf spot This disease was study by the authors. Since 2016, leaf spot of castor, which is caused by *Phyllosticta capitalensis*, has been observed in Zhanjiang (21.17 N, 110.18 E), Guangdong Province, China, with disease incidence of about 22.5%. Initial leaf symptoms were round spots with gray centers, surrounded by yellow halos. Ultimately, the spots then gradually spread and merged (Tang et al. 2020a).

Corynespora leaf vein spot This disease was study by the authors. In July 2019, a new leaf vein spot disease, which is caused by *Corynespora cassiicola*, was observed on castor in a field in Zhanjiang (21.17 N, 110.18 E), China, with an incidence of 30%. Initial leaf symptoms consisted of small dark brown spots along leaf veins or leaf midribs surrounded by yellow halos. The lesions eventually became necrotic and spread into triangle or irregular shapes with grayish-white centers. The spots were emerged in mesophylls also. Either in veins or mesophylls, the grayish-white centers were typical of the spots (Tang et al. 2020b).

7.2.2.7 Powdery Mildew of Castor

Leveillula powdery mildew In August 2010, leaves of *Ricinus communis* with typical symptoms of powdery mildew were collected in south Khorasan province, eastern Iran. The pathogen was identified as the anamorphs of *Leveillula taurica.* Symptoms show white patches in large areas on the underside of older leaves. The white patches was covered with mycelium and conidiophores. And the upper surface of leaves is chlorosis and necrosis (Mirzaee et al. 2011).

Podosphaera powdery mildew In September 2016, symptoms of powdery mildew caused by *Podosphaera xanthii* were observed in a private garden in Gimcheon, South Korea (36° 07′ 09′ N, 128° 07′ 46′ E). White Patches of mycelium, conidiophores, and conidia were distributed on both sides of the leaves. The infection is weak and does not cause early defoliation or leaf deformation (Zhao et al. 2018).

7.2.2.8 Seedling Blight

The disease caused by *Phytophthora parasitica* have caused considerable economic losses in the past decade (Basappa 2003). The disease often occurs during the rainy season and is particularly prevalent in low-lying areas and poorly drained fields. The infected leaves and stems appeared dark green patches on the surface, the disease became worse, the stems withered and the seedlings died.

7.2.2.9 Seed Bud Rot of Castor

This disease was study by the authors. Since 2016, a new seed bud rot disease which is caused by *Fusarium solani* species complex, occurred on castor seed bud in a field in Zhanjiang (21.17 N, 110.18 E), China, with an incidence of 10–40%. Disease symptoms are mainly manifested as the browning and rotting of hypocotyls and cotyledons, and the trend of development is aggravated. First, water-stained lesions appeared on the hypocotyls of the seed buds. Then, the lesions gradually expanded and turned dark brown. Finally, the cotyledons turned yellow and the seed buds rotted badly (Tang et al. 2020c).

7.2.2.10 Stem Rot of Castor

***Rhizoctonia* stem rot of castor** In March 2006, symptoms of the disease include stem rot and root rot, which eventually leads to plant death. The disease was first found in the castor bean plant in São Paulo, Brazil. White mycelium was observed in dead plants and necrotic tissue. The pathogen was identified as *Rhizoctonia* spp. (Sneh et al. 1991; Basseto et al. 2008).

***Lasiodiplodia* stem rot of castor** Stem Rot in the Brazilian castor plant was reported to have been caused by *Lasiodiplodia theobromae* (Lima et al. 1997) and *L. hormozganensis* (Fábio et al. 2018). Investigations into castor-oil plant diseases in the states of Bahia and Paraiba, revealed symptoms of stem, neck and root rot that eventually led to plant death. Regardless of soil type, adult plants usually exhibit symptoms at capsule maturity, but more frequently under water stress.

7.2.3 Cucumber Mosaic Virus

Castor bean in four commercial glasshouses around Yazd, Iran, was caused by CMV. Symptoms show leaf deformation and distortion, blisters, and severe mosaic (Raj et al. 2010). The incidence of symptomatic leaves is between 5 and 10% (Mirhosseini et al. 2017).

7.3 Pests of Castor

In India, the pests that caused higher economic losses are castor semilooper (*Achaea janata*), castor shoot borer (*Conogethes punctiferalis*), capsule borer (*Dichocrosis punctiferalis*), tobacco caterpillar (*Spodoptera litura*), red hairy caterpillar (*Amsacta* spp.), and leafminer (*Liriomyza trifolii*) (Basappa 2007; Anjani et al. 2010). In Brazil, the principal pests include stink bug (*Nezara viridula*); leafhopper (*Empoasca* spp.); defoliators including armyworm (*Spodoptera frugiperda*), *A. janata*, and black cutworm (*Agrotis ipsilon*); and the mites *Tetranychus urticate* and *Tetranychu sludeni* (Soares et al. 2001; Ribeiro and Costa 2008). In Colombia, cotton lace bug (*Corythucha gossypii*) was reported as a pest of castor plants (Varón et al. 2010). In China, the major pests of castor are *Achaea janata* (Linnaeus), *Agriotessubrittatus* (Motschulsky), *Belippa horrida* (Walker), *Clania variegata* (Snellen), *Ergolis ariadne* (Pendlebury), *Geometridae*, *Gibbium psylloides* (Czemp), *Monolepta hieroglyphica* (Motschulsky), *Philosamia cynthia* (Walker), *Spodoptera litura* (Fabricius), *Serica orientalis* (Molsech), *Tetranychus cinnabarinus* (Boisduval), and *Xylinophorus mongolicus* (Faust) (Liu and Liu 2002).

7.3.1 Leafhopper, Empoasca flavescens

Green leafhopper, *Empoasca flavescens*, which was observed as light green or greenish yellow nymphs and adults, is one of serious sap-sucking pest. This pest has been reported to be much injurious to young plants, sucking the juices to such an extent that the plants fade, curl and eventually die. Nymphs and adults of *E. flavescens* damage the plant by sucking the sap of young leaves and tender shoots. Attacked

leaves become dry, uneven, curls downward in the shape of an inverted boat and their margins turn brown. This characteristic symptom is known as "Rim Blight" or "Hopperburn" (Agyenim-Boateng et al. 2018). Damage to castor is another species, *E. notata,* in China.

7.3.2 Scirtothrips dorsalis

Including six species of thrips attack on castor beans (Basappa 2003). The studies showed that only cotton thrips and *Scirtothrips dorsalis* have reached the status of major pests. A severe pest on castor, *S. dorsalis* attacks shoots, leaves, flowers and young fruits. Parts of infected plant become brown to black. Plant show deformation and defoliation under severe damage. Although pests occur throughout the year, they peak in dry months. Nymphs and adults scrape off leaf surface and suck sap from leaves that curl into wrinkles (Gahukar 2018).

7.3.3 Castor Whitefly, Trialeurodus ricini

Castor whitefly, *Trialeurodus ricini* (Misra) (Hemiptera: Aleyrodidae) is a severe pest of castor in Africa and Asia (Mound and Halsey1978; Bink-Moenen 1983; Vora et al. 1984; Abd-Rabou et al. 2000). It has been recorded as a minor pest in Iran (Farahbakhsh 1961). Larvae and adults suck sap from lower leaves, resulting in honeydew deposits that lead to the development of sooty mildew. Heavy infestations can produce a large amount of honeydew and sooty moulds can cause a significant reduction in photosynthesis, which reduces plant growth. Castor seed yield and oil yield may be reduced by sooty mold (Patel et al. 1986). The excessive sap loss caused by a large number of insect pests reduces the vitality of the host.

7.3.4 Castor Semilooper, Achaea janata

Castor semilooper, *Achaea janata* (Lepidoptera: Noctuidae) is an important pest in all regions where castor is the main upland crop (Basappa and Lingappa 2001). Larvae are voracious leaf-eaters, causing the larvae to shed their leaves completely, although the larvae eat only the lower leaves that leave the venation (Gahukar 2018).

7.3.5 *The Lace Bug,* Corythucha gossypii

The lace bug, also called the "cotton or bean lace bug", *Corythucha gossypii* (Fabricius) (Hemiptera: Tingidae) is common pests on cotton. However, it has been reported that the pest has another 24 plant hosts, including castor beans. However, this plant as well as other hosts of the lace bug *C. gossypii*, when suffering its attack, show a delay in their growth, especially during dry conditions, due to the loss of sap caused by the suction on the leaves that they carry adults and nymphs of this insect, showing first a creamy white dotting, followed by the appearance of yellow or bright brown areas on the leaves (Varón et al. 2010).

7.3.6 *Sagotylus confluens*

R. communis was severely affected by *Sagotylus confluens* (Say) (Hemiptera: Coreidae), present during both seedling and adult stages. This insect mainly harms castor leaves (terminal shoots and recent leaves). Because of their large size and the high loss of latex, their excrement helps build up the fungus on the terminal bud, drying it out (Valdés-Rodríguez et al. 2015).

7.3.7 *Tobacco Caterpillar,* Spodoptera litura

Tobacco caterpillar, *Spodoptera litura* Fb. (Lepidoptera: Noctuidae) is a polyphagous insect that occasionally attacks leaves and inflorescences, causing serious damage (Tnau 2015). The larvae remain below the leaves but disperse in later stages. The larvae feed on leaves at night, destroy plants and hide in the soil during the day.

7.3.8 *Red Hairy Caterpillar,* Amsacta albistriga

Red Hairy caterpillar, *Amsacta albistriga* The red hairy caterpillar (RHC) is an important pest infesting castor crop in the Telengana region of Andhra Pradesh. Any stage of plant growth can be attacked and result in severe defoliation. Larvae of all ages eat plants. Leave veins on the adult plant and the seedlings will be destroyed (Prabhakar et al. 2010).

7.3.9 *Leafminer,* Liriomyzatrifolii

Leafminer (*Liriomyza trifolii* Burgess), Diptera, Agromyzidae, is one of the pests that cause serious damage to the leaves of castor, from cotyledon stage to crop maturity. The female leaf miner stabs into the upper surface of the leaf to feed and lay eggs. The larvae feed on the mesophyll tissue and form snake-like veins on the surface of the leaves, which reduce the photosynthetic area and cause the premature shedding of the leaves (Anjani et al. 2007).

7.3.10 *Shoot and Capsule Borer,* Conogethes punctiferalis

Shoot and capsule borer, *Conogethes punctiferalis* is the main pest in the development of castor plant. The pests begin with flowering and continue until the crops are mature. There are few vermin in the pollution-free convenience store. There are purple stems, or very loose spikes, and small and non-prickly capsules (Basappa 2003; Lakshminarayana 2005). The survival rate of larvae was decreased by regularly collecting and destroying damaged shoots and pods (Basappa 2003).

7.3.11 *The Green Stink Bug,* Nezara viridula

Nezara viridula is a pest of castor in the tropics. In the infected fields, the plants were severely damaged, the pods shed more, the pods and seeds decreased, and the seed yield decreased (Conti et al. 1997).

7.3.12 *Gram Caterpillar,* Helicoverpa armigera

The polyphagous pest *Helicoverpa armigera*, infested castor as defoliator, also caused considerable damage to castor crops by boring castor capsules in addition to simply feeding on foliage (Basappa 2003).

7.3.13 *Bihar Hairy Caterpillar,* Spilosoma obliqua

Bihar Caterpillar, the obliquely spotted Caterpillar, appears between October and December, and most recently in July. In recent years, it has also become an important pest of peanuts. The adults are reddish brown with black spots and wings pink with black spots. They are polyphagous, feed on leaves and cause loss by way of

defoliation. In severe cases only stems are left behind. In defoliated crops it also feeds on capsules (Basappa 2003).

7.3.14 Slug Caterpillar, Parasalepida

Slug caterpillar, *Parasalepida*, damage the castor plant sporadically. It is most common in the southern regions of India especially Madras and has been recorded from Ceylon also. Larva feeds on leaves voraciously leaving only the midrib and veins resulting in severe defoliation. To begin with, they feed gregariously on the leaves of castor and later spread over to the entire plant (Basappa 2003).

7.3.15 Hairy Caterpillar, Euproctis fraterna

Hairy caterpillar, *Euproctis fraterna*, is active throughout the year but its activity is reduced in winter. Defoliation is the main symptom (Basappa 2003).

7.3.16 Spiny Caterpillar, Ergolis merione

Spiny caterpillar, *Ergolis merione*, is a serious though sporadic pest. Insect attacks the crop at an early stage. Insects feed on the leaf tissue and cause defoliation (Basappa 2003).

7.3.17 Wooly Bear, Pericallia ricini

Wooly bear, *Pericallia ricini*, feeds on leaves resulting in defoliation. The damage is caused by caterpillar (Basappa 2003).

7.3.18 Castor Gallfly, Asphondylia ricini

Castor gallfly, *Asphondylia ricini*. The damage is caused by maggots. As a result of feeding by them, the buds develop into galls and produce no fruits and seeds. This pest is active from September to March (Basappa 2003).

7.3.19 Agriotessubrittatus *(Motschulsky)*

The larva can bite the seed and make it unable to germinate. If the seedling has already emerged, it will damage the fibrous root, the main root, and the underground part of the stem, causing the seedling to wither gradually.

7.3.20 Belippa horrida *(Walker)*

The larvae mainly bite the leaves of caster and eat up all the leaves. The damage was obvious.

7.3.21 Clania variegata *(Snellen)*

The larvae bite the leaves of castor to form a lot of holes and gaps in leaves. In serious cases, the larvae can eat up all the leaves of the host plants and cause the plants to die.

7.3.22 Ergolis ariadne *(Pendlebury)*

The larvae damage the leaves and young fruits of castor. In severe cases, it can eat up all the castor leaves and fruitlets of the whole field (Liangzhen Guo et al. Unpublished).

7.3.23 Geometridae

The larvae of geometrid damage the leaves and cause baldness in severe cases. When it is still, it is often used to grasp the branches with gastropods and tail feet to make the insect body stretch forward obliquely, rather like a dead branch. When frightened, it spins silk and droops (Liangzhen Guo et al. Unpublished).

7.3.24 Gibbium psylloides *(Czemp)*

When injuring castor seeds, both adults and larvae like to nibble on the parts around the seed coat, which makes the seeds decrease or lose their germination ability.

7.3.25 Monolepta hieroglyphica *(Motschulsky)*

The adults chew on the leaves and flowers of castor bean, and the larvae eat the root of the seedlings, and even cause the death of the whole plant.

7.3.26 Philosamia cynthia *(Walker)*

The larvae feed on leaves and shoots. When the damage is light, the leaves will be gnawed into holes or to be incised, and when the damage is serious, the leaves will be eaten up.

7.3.27 Serica orientalis *(Molsech)*

The adults are greedy for the leaves of castor bean seedlings, resulting in many defects and holes in the leaves, resulting in the destruction of the leaves, thus affecting the growth and development of castor. Castor leaf has peculiar smell and toxin, often send cash, tortoise shell has dead phenomenon. The larvae of the beetle also damage the root of castor.

7.3.28 Spodoptera litura *(Fabricius)*

It is mainly caused by larvae. The larvae eat a large amount of food. The newly hatched larvae damage the back of the leaves, feeding on the mesophyll, leaving only the epidermis. After the 3rd instars, the leaves completely damaged, and even all of them are eaten up. The larvae eat the flower buds and cause defects, which is easy to cause disaster (Liangzhen Guo et al. Unpublished).

7.3.29 Tetranychus cinnabarinus *(Boisduval)*

Tetranychus cinnabarinus gathered on the back of the lower leaves of castor, concentrated near the veins of the leaves, spun silk and formed a net. The net was covered with dust. It fed under the net and sucked the juice from the leaves with a piercing mouthpiece. At first, yellow and white spots appear on the leaves, and then the leaves turn red. In serious cases, the leaves droop, wither and fall off, and the plants become bare stalks, which seriously affect the growth and development of castor.

7.3.30 Xylinophorus mongolicus *(Faust)*

The adults gnaw on the tender buds and cotyledons of castor bean seedlings, preferring to eat the seeds and seedlings just sprouting, and the larvae damage the roots. When the damage is serious, the leaves can be eaten up and the cultivation will be destroyed.

7.4 Cultural Methods of Control

The prevention and control of castor diseases and insect pests should be carried out from different angles of agricultural development. It not only fundamentally eliminates the impact of diseases and insect pests on castor, at the same time, it also provides sufficient time for soil restoration to ensure soil nutrition. The balance of agricultural control is the main feature of agricultural control (Rajput and Kasana 2020). The main agricultural control methods are as follows: before sowing should be as far as possible. The results showed that the varieties with strong resistance to disease and insect should be selected. In order to ensure that the soil is nutritious enough, it is necessary to adopt the method of rotation, sowing time adjustment upon the climate conditions for castor to effectively avoid the peak of pest occurrence (Marcos and Leon 2019; Mandeep et al. 2019).

7.5 Chemical Methods of Control

This method is a relatively obvious method for the control of diseases and pests. But it also has certain shortcomings in the process of using. If chemical agents are used for disease control for a long time, it would not only lead to the enhancement of resistance to diseases and insect pests, but also the chemical spraying attached to the surface of castors, and will cause serious harm to human body (Sivarajah 2019). Therefore, in the process of using chemical control method to control diseases and pests, we should pay attention to the following aspects: the search for pesticides must be scientific and reasonable. The selection of pesticides is very important in the control of castor diseases and insect pests. If a pesticide is used for a long time, it will lead to the increase of resistance to diseases and insect pests. Therefore, it is not possible to use a pesticide repeatedly for a long time to prevent and control field diseases and insect pests. Meanwhile, in the process of using pesticide, it is necessary to use the pesticide based on the actual temperature and weather conditions, so that the effect of pesticide on diseases and pests could be fully exerted; The pesticide spraying must be appropriate. In the process of castor growth, it could not be appropriate that as many pesticides as possible must be sprayed. The concentration and number of spraying pesticides must be strictly controlled according to the requirements to ensure the

normal growth of castors and avoid the adverse effects of pesticide use on the growth of castors (Yusuf 2016; Varfolomeev et al. 2019).

7.6 Biocontrol Methods with Natural Products and Biotic Agents

The biological control of diseases and insect pests is the use of biological inhibitors produced by the natural enemies of diseases and insect pests in nature. The biggest feature of this control method is that it not only ensures the normal growth of castors, but also protects the ecological environment to the greatest extent, and fundamentally reduces the cost of agricultural production (Mohamed et al. 2020). But in the process of practical application, there are also some defects, such as the number of natural enemies of diseases and insect pests is relatively small, if imported from abroad, it is likely to lead to the phenomenon of wild growth of animals and plants, so the use of this method must be strictly controlled. However, pest control mainly refers to the use of natural enemies of diseases and insect pests for placement (Murray et al. 2019). Generally, there are two kinds of natural enemies of pests: predatory and parasitic. The predatory natural enemies are ladybugs, which prey on mites. Parasitic natural enemies mainly rely on the body fluids or organs of pests to control diseases and insect pests. The biological inhibitors are the use of plant components and insect hormones to control the development of diseases and insect pests. For example, pepper water can be sprayed on castors, which can effectively reduce the damage caused by pests to castors. The biggest characteristic of biological control method is that its duration is relatively long, and it will not affect human health. Although the operation method of chemical control method is relatively simple, its impact on the ecological environment is relatively large. Therefore, the combination of biological control and chemical control not only solves the problem of single control method of castor diseases and insect pests, but also ensures the normal growth of castors and protects the ecological environment (Gray et al. 2018; Bamisope et al. 2018; González-García et al. 2019).

7.7 Integrated Pest Management

Scientific and reasonable use of a variety of methods for diseases and insect pests' control on castor, not only can effectively protect the natural ecological environment, improve the efficiency of agricultural production, but also avoid the harm of castor pest control on human body. Therefore, it is necessary to further strengthen the research on the integrated pest management (IPM) of castor diseases and insect pests, and improve the existing methods in depth, to fundamentally reduce the impact of

diseases and insect pests on the growth of castors (Leeland et al. 2019; Imane et al. 2020; Rakes et al. 2021).

7.8 Breeding for Biotic Stress Resistance in Castor

7.8.1 Traditional Breeding for Biotic Stress Resistance

7.8.1.1 Resistance Breeding for Antifungal Disease

There are few reports on the genetic research of resistance to castor antifungal disease, and the results are far from meeting the requirements of breeding, mainly focusing on the selection of resistance resources and the genetic analysis of resistance, QTL mapping is even less reported.

Immune materials against major fungal diseases have not been found, and only some germplasm resources with moderate tolerance have been found (Araujo et al. 2007; Anjani 2012; Yin et al. 2019a). India has developed a number of hybrids and inbred lines resistant to blight and carbon rot (Anjani et al. 2004; Anjani 2005a, b, c, 2012; Patel and Pathak 2011).

Genetic studies on resistance to castor wilt have not been concluded, with multiple and single gene controls reported (Desai et al. 2001; Lavanya et al. 2011; Patel and Pathak 2011).Anjani and Mohammada (2014) reported the interaction of two pairs of genes, including dominant gene overlap (15 resistance: 1 susceptible), recessive gene overlap (9 resistance: 7 susceptible) and a interaction between 1 pair of dominant genes and 1 pair recessive genes (13 resistance: 3 susceptible).

Mutation breeding has achieved great success in some traits such as dwarfness and pistillate character. However, it failed to breed genotypes with high resistance to biotic stresses (Rojas-Barros et al. 2005).

7.8.1.2 Insect Resistance Breeding

Castor bean is affected by more than 100 insects. It is often used as an insect trap plant in some crops. Available resistance sources to some insect pests have been identified. For example, Jayaraj (1966, 1967) reported that the accessions, RC1098 Baker, RC1094, RC1092 Italy and RC1096 Cimmerron Coonoor, were available resistant sources and C3 Pakistan was a tolerant source against leafhopper (*Empoasca flavescens*). It is reported that double and triple-bloom types are more resistant to leafhopper than no-bloom and single-bloom types in castor (Jayaraj 1968; Srinivas Rao et al. 2000; VijayaLakshmi et al. 2005). Lakshminarayana and Anjani (2009) identified dozens of stable resistant sources against leafhopper from Indian collections (Lakshminarayana 2003; Lakshminarayana and Anjani 2009). Some resistant sources has resistance to multiple insect pests, for example, the Indian accession RG

43 is resistant to leafhopper, wilt and nematode. Anjani et al. (2018) reported that five accessions, viz., RG-43, RG-631, RG-1621, RG-3037, and RG-3067 showed resistance to leafhopper. The accessions HY1 and HY2, wild genotypes collected from sourthen China, kept resistance to leafhopper, without any hopper burn, when all leaves of other materials died, so did some purple types (Xuegui Yin unpublished).

Ramanathan (2004) reported that an exotic accession EC 103745 was whitefly-resistant material. In addition, forty-three Indian accessions were reported to be possible sources of resistance to whitefly (Lakshminarayana 2003; Anjani and Jain 2004). Among the defoliators in castor, tobacco caterpillar and semilooper are the most destructive. Thanki et al. (2001) observed the moderate resistance of cultivar CO-1 to tobacco caterpillar and tolerance of five accessions (RG 5, 33, 221, 224 and RG 449) to semilooper. The resistance against capsule borer of five Indian accessions (RG 1934, 2546, 2770, 2543 and 2786) were confirmed under caged conditions (Lakshminarayana 2003; Lakshminarayana and Anjani 2010).

Two morphological types of castor were reported to be resistance to leaf miner, one is purple color morphotype and the other is papaya leaf type morphotype. For example, Indian resistant accessions RG 1930 and RG 2008 behaved with dark purple color morphotypes and the Indian resistant accessions RG 1766 and RG 1771 showed papaya leaf type morphotypes. Anjani et al. (2007) reported that the leafminer resistant purple color accession RG 1930 belonged to maternal inheritance, only when it was used as a female parent did it show resistance. Other study revealed the relationship between the resistance to leafminer and the concentration of total phenols. Resistant genotypes were observed to have high concentration of total phenols (Prasad and Anjani 2000; Anjani et al. 2010).

7.8.2 Molecular Breeding for Antibiotic Stress

7.8.2.1 SSR Markers and Genetic Maps in Castor

Molecular markers and genetic maps are the basic tools for molecular breeding.

The development of sufficient genome-specific simple sequence repeat (SSR) markers in castor beans was very late. Bajay et al (2009, 2011) exploited 23 SSR markers and used them to identify castor accessions. Qiu et al (2010) developed 118 polymorphic expressed sequence tag (EST)-SSRs from EST library. Pranavi et al. (2011) developed 92 polymorphic EST-SSRs and applied them to identify the purity of hybrid verities.

The publication of the castor draft genome sequence (Chan et al. 2010) laid a foundation for the mining of SSR markers based on genomic information. Tan et al (2014) reported 1435 SSR primer pairs with genome sequence information, among which 670 (46.7%) were polymorphic between six accessions. Liu et al (2016) developed 3000 SSR primer pairs using the castor genome sequence information, with a polymorphic rate of 27.38% and 59.6% in biparent populations and a population composed of 10 accessions randomly selected respectively.

The first molecular genetic map of castor was constructed in 2016 (Liu et al. 2016). It contained 10 linkage groups (LGs) and covered 1164.73 cM, composed of 331 markers, with an average interval between markers of 3.63 cM. Tomar et al. (2017) constructed another genetic map composed of 261 markers of different types assigned to 10 LGs, with a total length of 1833.4 cM and an average interval between markers of 6.93 cM. The first high-density genetic map in castor bean was constructed using specific length amplified fragment (SLAF) sequencing in 2017, containing 10 LGs, composed of 4300 SLAF markers and 120, covered 1547.41 cM of genome, with an average marker interval of 0.35 cM (Yin et al. 2019b). The above genetic maps laid the foundation for the mapping of disease resistance and insect resistance genes in castor.

7.8.2.2 QTL Mapping for Resistance to Biostress Character

Anjani (2005a) confirmed the resistance of purple castor bean RG 2008 and RG 1930 to Fusarium wilt, put forward that the purple could be used as a morphological marker for selection of Fusarium wilt. Singh et al (2011) established three random amplified polymorphic DNA (RAPD) molecular markers linked to Fusaria wilt resistance, RKC231375, RKC211080 and OPBE18900, the genetic distances from the quantitative trait loci (QTLs) were 5, 10.7 and 7.6 cM respectively. Reddy et al. (2011) established OPH-124973 and OPJ-154268, two RAPD molecular markers linked to QTLs underlying the resistance to Fuslight wilt, with a genetic distance from QTL of 5 cM and 7 cM respectively. Tomar et al. (2016) established two molecular markers linked to he resistance to Fusarium wilt, CST73 (SSR marker) and R83 (RAPD marker). Mir (2014) found a marker RCM9109 linked to Fusarium wilt resistance by association mapping. Tomar et al. (2017) found three pairs of molecular markers associated with three QTLs for resistance to carbon rot located on LG 2, 6, and 9 respectively. Mhaske et al (2013) identified 12 LOX (lipoxygenase) candidate genes by qRT-PCR, and believed that *Rc-LOX5* might play an important role in wilt disease resistance.

7.8.3 Genomic Selection and Future Prospects

Though few genes controlling the resistance to disease and insect pest were cloned and identified functionally in castor bean, some gratifying achievements have been made. Some useful resistance sources have been identified and can be used in breeding. There are already some inbred lines and hybrids that have some resistance to disease and insect pest. Nearly hundreds of disease resistance genes have been identified or prospected by genome and transcriptome analyses, which contained resistance gene analogue (RGA) familys, defense-response related transcription factors. In addition, many genetic markers linked to disease resistance genes have also established which can be used to marker-assisted selection and pyramiding breeding. It

is prospected that morden biotechnological approaches such as genomics, transcriptomics, proteomics, and genome editing will be able to enhance the disease resistance in castor bean. Recently, Xuegui Yin et al. assembled the high-quality genomes of two accessions after genomic sequencing of the second and third generations (unpublished). The size of the genomes are 332.4 Mb and 305.5 Mb respectively, each genome has only 21 contigs in total, compared to more than 26,000 contigs in previously published genome, which solved the worries of castor gene annotation.

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Chapter 8
Genomic Designing for Genetic Improvement of Biotic Stress Resistance in Flax

Frank M. You, Khalid Y. Rashid, and Sylvie Cloutier

Abstract Biotic stresses attributable to various pathogens such as fungi, bacteria and viruses are external threats to plant growth, development and ultimately productivity. To date, genetic improvement of varieties continues to be the most powerful, sustainable and eco-friendly way to overcome these continuously evolving biotic-based threats in plans. The development of genomewide molecular markers and the identification of quantitative trait loci and genes which are linked to biotic stress resistance have the potential to efficiently and genetically enhance the biotic stress resistance of varieties by marker-assisted selection, genomic selection and precision breeding via genome editing. Powdery mildew, Fusarium wilt, pasmo, and rust are major fungal diseases threatening flax production. This chapter briefly reviews the genomic designing for genetic improvement of biotic stress related traits in flax, with a particular emphasis on genomic studies of pasmo resistance, including methodology, outcomes and potential application in breeding.

Keywords Flax · Biotic stress · Genomewide association study (GWAS) · Genomic selection (GS) · Pasmo resistance · Precision breeding · Quantitative trait loci (QTLs)

8.1 Introduction

Flax (*Linum usitatissimum* L.) is a valuable source of linseed and stem fiber. Linseed, also known as flaxseed is rich in omega(ω)-3 essential fatty acids (α-linolenic acid

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or ALA), lignans, and soluble and insoluble fibers, making it one of the most healthy plant foods (Fofana et al. 2010; Touré and Xueming 2010; Kim and Ilich 2011; Leyva et al. 2011). Linseed oil also has various industrial uses such as soap, vehicle paints, linoleum, printing inks, oil clothing, textiles, patent leather, shoe polish and others (Juita et al. 2012). Flax fiber extracted from the skin of the flax stem is mainly used for linen, the manufacture of twine and rope and as raw materials for some high quality paper products (Deyholos 2006).

Flax has been grown worldwide, but primarily in temperate and subtropical regions, such as Canada (linseed), China (fiber and linseed), USA (linseed), India (linseed), Russia and Europe (fiber and linseed) and Kazakhstan (linseed) (Foulk et al. 2004; Liu et al. 2011; Worku et al. 2015; You et al. 2016b). In these growing regions, the biotic stresses primarily involve various diseases produced by fungi, viruses and mycoplasma like organisms, with fungal diseases including rust (*Melampsora lini*), anthracnose (*Colletotrichum lini*), pasmoor spharella linorium (*Septorialinicola, Mycosphaerella linicola*), wilt (soil-borne fungus *Fusarium oxysporum* f.sp. lini), seedling blight and root rot, and stem break and browning (*Aureobasidium pullulan* var. lini or *Polyspora lini*) (seedborne and soil-borne fungi *Rhizoctonia solani*, *Fusarium* spp., or *Pythium* spp. etc.) being predominant (https://flaxcouncil.ca/growing-flax/chapters/diseases/). These diseases damage flax plants, affect plant growth and development, and ultimately reduce seed and fiber yield and quality. To control these biotic stresses, rotations with other crops such as cereals (spring and winter wheats, barley and oat), oilseed (canola and mustard) and pulse (peas, lentils and soybean) crops are an effective agronomic practice in Canada. Seed treatment with suitable fungicides is another useful practice to kill seed borne pathogens (Bradley et al. 2007).

Incorporating genetic differences to develop agronomic characteristics and add long-term disease tolerance in flax has traditionally been done by conventional breeding methods (You et al. 2016b). A successful example is the genetic improvement against flax rust which has the potential to be the most destructive disease affecting flax. The rapidity with which rust races can evolve represents a challenge in breeding new resistant varieties. Over the last 70 years, more than 500 flax rust races have been recorded. Fortunately, flax rust resistance to different races is controlled by several major genes (Lawrence et al. 1995; Anderson et al. 1997; Ellis et al. 1999; Dodds et al. 2001a, b; Lawrence et al. 2010) that have been successfully pyramided in elite varieties by conventional breeding in Canada. Currently, all Canadian modern cultivars are immune to the locally existing rust races.

However, resistance to other major diseases such as wilt, pasmo, and powdery mildew is quantitative and controlled mostly by minor-effect polygenes (You et al. 2017a; He et al. 2019b), which poses a challenge to the widely used conventional breeding methods. To date, all flax cultivars registered in Canada are moderately resistant to powdery mildew, wilt, and pasmo (You et al. 2016b). The development of advanced genomic tools, such as quantitative trait locus (QTL) mapping, genomewide association study (GWAS) and genomic selection (GS) allows the rapid identification of QTLs that control complex quantitative traits, contributing to more efficient offspring selection and assisting candidate gene isolation whose validation

can now be accurately performed via gene editing (GE), all of which contribute to accelerating crop genetic improvement.

This chapter briefly introduces genomic design strategies for genetic improvement of biotic stresses with special emphasis on pasmo as an example to describe methodology, outcomes and potential applications in breeding.

8.2 Genomic Design for Genetic Improvement of Biotic Stress Traits

With the development of QTL markers associated with biotic stress resistance, including functional markers, conventional breeding techniques are being revolutionized. Marker-assisted selection (MAS) has been used for traits controlled by major genes such as rust (Kumar et al. 2011; Miedaner and Korzun 2012). GS has been used for complex quantitative traits controlled by numerous polygenes such as resistance to Fusarium wilt, powdery mildew and pasmo (He et al. 2019a), and precision breeding using GE has been used for improving traits controlled by known genes (Nekrasov et al. 2017). Therefore, the identification and characterization of QTLs and causal genes are now an integral part of modern flax breeding programs.

8.2.1 Identification of QTLs

While classical quantitative or statistical genetics is capable of estimate genetic variances of polygenes for quantitative traits at the phenotypic level (Falconer and Mackay 1996), combining suitable genomic design with molecular markers provide a precise way to identify individual polygenic loci or QTLs on chromosomes, estimate their effects and predict co-located candidate genes related to the traits.

Two types of the QTL mapping strategies have been developed and successfully used for QTL identification: linkage mapping (LM) and GWAS (Sehgal et al. 2016). LM use segregating biparental populations, such as F_2, backcross (BC), recombinant inbred line (RIL), and doubled haploid (DH) populations, to create a recombination-based genetic map using molecular makers that is suitable to find QTLs responsible for the characteristics that segregate in the population (Price 2006). The statistical methods and software tools for QTL mapping in biparental populations have been well developed (Kulwal 2018). The major statistical methods to detect additive, dominant and epistatic QTLs include simple interval mapping (SIM), composite or inclusive composite interval mapping (CIM/ICIM), multiple interval mapping (MIM), Bayesian interval mapping (BIM), and multiple trait mapping (MTM) (Kulwal 2018). These methods are implemented in many software tools, such as R/qtl (Arends et al. 2010), MAPMAKER/QTL (Lander et al. 1987), and QGene (Joehanes and Nelson 2008). QTLIciMapping may be mostly recommended because it provides

functions of both construction of genetic maps and QTL mapping for additive, dominant, and digenic epistasis as well as interaction of QTLs with environments for various biparental and nested association mapping (NAM) populations (Meng et al. 2015). Traditional statistical methods primarily detect large-effect QTLs and have limited power to identify small-effect and linked QTLs. Recently, Zhang et al. (2020c) proposed a genomewide composite interval mapping (GCIM) for segregating biparental populations and developed a corresponding R package with a command line version called QTL.gCIMapping (v3.2) and a graphical user interface version named QTL.gCIMapping.GUI (v2.0). This method has been effective in identifying small-effect and associated QTLs in biparental populations (Wang et al. 2016b; Wen et al. 2019, 2020).

GWAS is based on linkage disequilibrium (LD) between molecular markers and QTLs in a diverse genetical panel, as opposed to biparental populations, in order to overcome the limitations of the latter. Many population types can be used for GWAS, including natural germplasm collections, diversity panels of both genetic germplasm and breeding lines, and multi-parent breeding populations such as nested association mapping (NAM) (Yu et al. 2008; Monir and Zhu 2018; Ren et al. 2018) and multi-parent advanced generation intercross (MAGIC) populations (Mackay and Powell 2007; Cavanagh et al. 2008; Camargo et al. 2018; Ongom and Ejeta 2018).

GWAS advantages over linkage-based QTL mapping include high genetic variation among individuals, high density molecular markers, and high resolution of QTLs and causal genes on chromosomes (Goutam et al. 2015; Ogura and Busch 2015). Many statistical models have been developed to identify large- and small-effect QTLs that can simply be grouped into two categories: single- and multi-locus models. General Linear Model (GLM) (Price et al. 2006) and Mixed Linear Model (MLM) (Yu et al. 2006) are two traditional single-locus statistical models implemented in many software tools such as TASSEL (Bradbury et al. 2007) for example. Single-locus approaches search the genome in one dimension and measure the significant marker-trait associations one by one. To control for false positives, the stringent Bonferroni correction for multiple tests (*P* value divided by the number of markers in the model) is frequently used, usually resulting in the exclusion of many false negative loci. This drawback can be particularly acute in crop genetics for traits measured from field experiments that are often plagued by large inherent experimental errors (Zhang et al. 2019). Thus, these types of methods have a restricted capability to detect polygenes with small effects that control the bulk of quantitative traits.

Multi-locus statistical methods that simultaneously test multiple markers include early proposed models such as Multi-Locus Mixed-Model (MLMM) (Segura et al. 2012), and more recent powerful methods to identify quantitative trait nucleotides (QTNs) with small effects. The latter include mrMLM (Wang et al. 2016a; Li et al. 2017), FASTmrMLM (Zhang and Tamba 2018), FASTmrEMMA (Wen et al. 2018), pLARmEB (Zhang et al. 2017a), ISIS EM-BLASSO (Tamba et al. 2017), and pKWmEB, which have been implemented in the R package "mrMLM", thus called "mrMLM models" (Table 8.1). These multi-locus models use LOD score ($\geq$3), rather than the stringent Bonferroni correction to identify significant QTNs, which substantially increases the statistical power to detect small effect QTNs and reduces Type 1

Table 8.1 Some single- and multi-locus statistical methods for genomewide association study (GWAS)

Statistical model	Threshold for significant QTNs	R package	Reference
Single-locus models			
GLM	Bonferroni	MVP v1.0.1	Price (2006)
MLM	Bonferroni	MVP v1.0.1	Yu and Buckler (2006)
GEMMA	Bonferroni	GEMMA v0.96	Zhou and Stephens (2012)
Multi-locus models			
mrMLM	LOD ≥ 3	mrMLM v3.0	Wang et al. (2016a)
FASTmrEMMA	LOD ≥ 3	mrMLM v3.0	Wen et al. (2018)
ISIS EM-BLASSO	LOD ≥ 3	mrMLM v3.0	Tamba et al. (2017)
pLARmEB	LOD ≥ 3	mrMLM v3.0	Zhang et al. (2017a)
pKWmEB	LOD ≥ 3	mrMLM v3.0	Ren et al. (2017)
FASTmrMLM	LOD ≥ 3	mrMLM v3.0	https://cran.r-project.org/web/packages/mrMLM/index.html
RTM-GWAS	Default[b] or Bonferroni	RTM-GWAS[a]	He et al. (2017)
FarmCPU	Bonferroni	MVP v1.0.1	Liu et al. (2016)

Bonferroni: $P = 0.05$ followed by Bonferroni correction for multiple tests. [a] A standalone software tool. [b] Default threshold for significant QTNs is $P = 0.05$ for preselection of markers using single-locus model and $P = 0.01$ for multi-locus and multi-allele models

errors and running time (Wang et al. 2016a; Li et al. 2017; Ren et al. 2017; Tamba et al. 2017; Zhang et al. 2017a; Wen et al. 2018). FarmCPU, a multi-locus model implemented in the MVP R package, is an exception because it still relies on the Bonferroni correction to declare significance of association (Liu et al. 2016).

The haplotype block based multi-locus GWAS method RTM-GWAS (He et al. 2017) is implemented in a standalone software (https://github.com/njau-sri/rtm-gwas). This two-step method first groups highly correlated SNPs into LD blocks (called SNPLDBs) to define bi- or multi-allelic haplotypes. This is then followed by a two-stage association analysis to identify QTNs: (1) pre-screening haplotype markers using a single-locus model, and (2) identifying significant QTNs using a multi-locus and multi-allele model with stepwise regression (He et al. 2017).

We have evaluated these single and multi-locus models in several studies for agronomic traits, abiotic and biotic traits in flax and wheat (He et al. 2019b; Fatima et al. 2020; Lan et al. 2020; Sertse et al. 2020). Our results demonstrate that the single-locus models detected mostly large-effect QTNs, while the multi-locus models were capable of identifying QTNs with smaller effects. Some QTNs were identified by multiple models, but, generally speaking, the models identified different subsets of QTNs, indicative of the uniqueness and complementarity of these algorithms (He et al. 2019b). Therefore, both single and multi-locus models resulted in the

identification of a more comprehensive set of QTNs that has been shown to increase prediction ability of GS, and hence is recommended (Lan et al. 2020).

In flax, several biparental populations have been developed to identify QTLs for biotic stress resistance. For Fusarium wilt resistance, a DH population of 143 lines was developed from a cross between the resistant variety Linola and the susceptible Australian flax variety Glenelg, from which two independent and additive genes were identified under greenhouse and field conditions (Spielmeyer et al. 1998). Using a RIL population of 160 lines derived from the resistant cultivar Aurore and the susceptible cultivar Oliver, two independent and recessive genes were also identified for wilt resistance (Edirisinghe 2016). For powdery mildew resistance, three QTLs were detected from F_3 and F_4 families derived from an F_2 population of a cross between the susceptible cultivar NorMan and the resistant cultivar Linda (Asgarinia et al. 2013). Additional biparental populations have also been developed for QTL mapping of flax biotic stress resistance, for example, a Bison/Novelty population of 704 RILs segregating for Fusarium wilt and a Linda/Norman (LNm) population of 160 RILs segregating for powdery mildew (unpublished). These populations have been evaluated for field resistance in multiple years and locations and also re-sequenced using a genotyping-by-sequencing method.

GWAS have been successful in identifying QTLs for agronomic and seed quality traits in flax (Soto-Cerda et al. 2014a, 2014b; Xie et al. 2017; You et al. 2018b). The strength and effectiveness of GWAS using the flax core collection (You et al. 2017a) to detect QTNs for biotic stress traits have been shown for pasmo (He et al. 2019b), powdery mildew (unpublished) and Fusarium wilt (You et al. 2017b).

8.2.2 Candidate Gene Prediction

QTL mapping and GWAS are used to find causal genes underlying traits of interest. Prediction of candidate genes linked to QTNs first requires genomewide gene scans along chromosomes to pinpoint the co-located genes. Although QTNs can be located within coding regions, QTL mapping and GWAS do not provide sufficient resolution to pin the QTLs to accurate intragenic locations or genetic features responsible for controlling the traits. Most QTNs are located in intergenic regions. To infer causal genes linked to a QTN, a logically reasonable method is to check whether the LD correlation (r^2 or D') between the QTN and the markers on neighboring genes is sufficiently high (e.g., >0.8) or, alternatively, to partition the whole genome into haplotype/LD blocks based on the genomewide markers of the diversity panel (Purcell et al. 2007; He et al. 2017; Kim et al. 2019) and then perform candidate gene searches within haplotype blocks harboring significant QTNs. An obvious limitation of this method is that LD blocks or correlations depend on the genetic diversity and the structure of a population. For example, the size of LD blocks in the diversity panel for GWAS are much smaller than that of a biparental population because the former represents a greater number of historical recombination events of the GWAS panel. Thus, GWAS may find a candidate gene of a higher resolution.

A straight forward approach for prediction of candidate genes is to find related genes on the fixed-size flanking regions within a QTL, such as a window of 100–200 kb downstream and upstream of a QTL (Kumar et al. 2015; He et al. 2019b; Sertse et al. 2019; You and Cloutier 2019). The fixed window size may be estimated through analysis of LD decay curve (You et al. 2018b). However, this method has a disadvantage in that the fixed block size does not reflect the differential recombination rates across the genome. Therefore, no matter the methods used to identify candidate genes, all must be validated through functional genomics.

Resistance gene analogs (RGAs) are candidates of resistance genes in plants. They can be identified based on known structural features. RGAs can be clustered as either nucleotide-binding site leucine-rich repeat (NBS-LRR) or transmembrane leucine-rich repeat (TM-LRR) (Hammond-Kosack and Jones 1997). NBS-LRR can be further divided into toll/interleukin receptor (TIR)-NBS-LRR (TNL) or non-TNL/coiled coil-NBS-LRR (CNL) (Hammond-Kosack and Jones 1997). Similarly, TM-LRRs could be classified into two classes: receptor-like kinases (RLKs) and receptor-like proteins (RLPs) (Hammond-Kosack and Jones 1997). Genome-wide RGAs can be identified through software tools (Li et al. 2016) or manually using basic local alignment search tool (BLAST) against annotated gene sequences (You et al. 2018a). Using these approaches, we identified 1327 RGAs in the flax genome which constitute a useful subset to investigate co-localized QTLs associated with biotic stresses (You et al. 2018a).

8.2.3 Genomic Selection

Genomic selection (GS) is a promising breeding selection method that employs prediction models constructed using a training population that is both genotyped with genomewide markers and phenotyped, to predict genomic estimated breeding values (GEBVs) of genotyped but unphenotyped breeding lines. GS promises to increase selection accuracy, shorten breeding cycles, and reduce breeding cost. To date, GS has been implemented in many breeding programs to improve yield, quality, abiotic and biotic stresses, in a wide-range of crop plants such as wheat (Rutkoski et al. 2012, 2014, 2015; Daetwyler et al. 2014), rice (Spindel et al. 2015), flax (You et al. 2016a; He et al. 2019a; Lan et al. 2020), and others. GS is most often used for progeny selection in a breeding program but it can also be applied to evaluation of germplasm and parents, and to predict general combining ability (GCA) and specific combining ability (SCA) of crosses (Bernardo 2015; Lado et al. 2017; Yao et al. 2018). However, the performance of GS depends on (1) choosing a proper statistical model to construct a prediction model; (2) choosing a proper marker set to construct the prediction model; and (3) choosing a proper training population closely related to the test populations.

To evaluate the prediction accuracy or ability of GS models, cross-validation schemes which randomly split the whole population into several subsets (or folds) are frequently used, e.g., five subsets would be called five-fold cross-validation scheme.

For a given random sample, each subset or fold is in turn used for a test data set, and the remaining four subsets are merged to be a training data set. This process is iterated multiple times, e.g. 100 to generate a set of random samples. In this case, a total of 500 permutations of training data sets are generated to construct GS models, which are then used to predict GEBVs. Each of these random sample data sets is used for GS modeling and GEBV prediction. The prediction accuracy or ability is defined using a Pearson's correlation between the GEBVs and the observed phenotypes (You et al. 2016a).

Various genomic models have been developed to optimize prediction models for numerous complex traits. These models include classical parametric statistics based models such as best linear unbiased prediction (RR-BLUP) (Henderson 1975), and genomic BLUP (GBLUP) (Daetwyler et al. 2014); Bayesian statistics based parametric methods such as Bayesian LASSO (BL) (Park and Casella 2008), Bayesian ridge regression (BRR) (Campos et al. 2009), BayesA, BayesB and BayesC; and machine learning based non-parametric methods such as support vector machine (SVM), random forest (RF), radial basis function neural network (RBFNN) and some deep learning methods (Gonzalez-Camacho et al. 2018; Montesinos-Lopez et al. 2018; Fukuoka 2019; Lo-Ciganic et al. 2019; Grinberg et al. 2020; Gupta et al. 2020). These models have been implemented in some popular software tools, especially in some useful R packages (Table 8.2).

GS parametric statistical models are usually built on additive genetic models and their prediction abilities differ depending on genetic architecture of the traits that are examined. However, because some non-additive effects such as dominance and epistasis interactions are common in quantitative traits, these effects are also considered in some GS models (Varona et al. 2018). Besides genomic prediction for individual traits, multi-trait models in GS have been evaluated (Covarrubias-Pazaran et al. 2018; Fernandes et al. 2018; Montesinos-Lopez et al. 2019b). Providing significant genetic correlation between the target traits, the multi-trait GS models outperform those for individual traits. Nevertheless, construction of multi-trait models is computation-intensive, especially for a large molecular marker and phenotypic data set. Recently, some computation-efficient GS models and R packages have been developed for modeling of multiple traits (Montesinos-Lopez et al. 2019a).

Although many GS models have been implemented and evaluated in a variety of crops and traits, RR-BLUP is the most widely used because of its high-caliber capability (Arruda et al. 2015; Rutkoski et al. 2015; Poland and Rutkoski 2016; Dong et al. 2018; Liabeuf et al. 2018). For example, RR-BLUP effectively identified complicated patterns with additive effects and conveyed effective genomic prediction in wheat disease resistance (Ornella et al. 2012). RR-BLUP also has a distinct benefit as well in the performance of computing compared with most of the alternative statistical models (Piepho 2009; Endelman 2011; Arruda et al. 2015; Liabeuf et al. 2018).

GS was initially suggested by Meuwissen et al. (2001). The main idea behind GS is the use of genomewide markers to train statistical models without prior knowledge of genes or QTLs associated with the traits. With the development of high-throughput genotyping technology, high-density genomewide molecular markers can be readily

Table 8.2 Some popular R packages for modeling of genomic selection

R package	Features	Reference
rrBLUP	A classical and efficient maximum—likelihood algorithm based model	Endelman (2011)
BGLR	Bayesian regression models; GBLUP; continuous and categorical traits	Perez and Campos (2014)
BWGS	A pipeline wrapper package integrating random cross-validation, imputation and 15 statically models	Charmet et al. (2020)
BMTME	Bayesian models for multi-trait and multi-environment	Montesinos-Lopez et al. (2019a)
sommer	GBLUP, rrBLUP, faster algorithms	Covarrubias-Pazaran (2016)
G2P	A wrapper package integrating 16 statistical models (BayesA, BayesB, BayesC, BRR, BL, RKHS, RR, rrBLUP, SPLS, LASSO, BRNN, AI, NR, EM, EMM and bigRR) and four machine learning models (RFC, RFR, SVC and SVR) which are provided by other R genomic selection packages; cross-validation	https://github.com/cma2015/G2P
BGGE	Genomic selection for genotype by environment	Granato et al. (2018)
DeepGP	A deep learning pipeline implementing deep learning models of multilayer perceptron networks (MLP) and Convolutional neural network (CNN)	https://github.com/lauzingaretti/DeepGP
DeepGS	A deep learning model	https://github.com/cma2015/DeepGS

obtained and breeding populations can be genotyped at low costs. Several popular genotyping methods are available, such as genotyping by sequencing (GBS), array-based genotyping (e.g., iSelect 90 K array for wheat), and target sequence based genotyping (Bekele et al. 2020; Zhang et al. 2020a). To date, most GS models are constructed based on genomewide random markers. Though some studies have discussed the use of QTLs as markers, only major QTLs were used and the outcome was only a minor improvement in prediction accuracy. Our recent studies revealed that combining single and multi-locus GWAS methods can effectively detect both large and minor effect QTLs that can be used to build GS models, thereby significantly improving genomic prediction accuracy (He et al. 2019a, b; Lan et al. 2020).

8.2.4 Genome Editing (GE) and Precision Breeding

GE is a genome-engineering technology that facilitates precise and efficient targeted modification of genomes to characterize the functions of genes and create novel genetic resources for the genetic improvement of plants (Langner et al. 2018; Chen et al. 2019). GE starts with the creation of site-specific double-strand breaks (DSBs) at the target loci by sequence-specific nucleases. Then the DSBs are repaired by the plants endogenous DNA repair mechanisms, either error-prone non-homologous end joining (NHEJ) or homology-dependent recombination (HDR). NHEJ generates small random insertions, deletions and substitutions, preferably causing a gene knockout, whereas HDR is able to generate accurate point mutations, deletions, or gene knock-in especially useful for plant precision breeding but with low editing frequencies (Langner et al. 2018). Broad-sense genome editing techniques include reverse genetic tools such as induced mutagenesis (Rowland 1991; Chantreau et al. 2013; Fofana et al. 2017), oligonucleotide directed mutagenesis (Sauer et al. 2016), epigenome editing (Miglani et al. 2020), transposons, RNA interference (RNAi), and typical genome editing tools such as zinc-finger nucleases (Bibikova et al. 2002; Shukla et al. 2009; Osakabe et al. 2010), Transcriptional Activator Like Effector Nucleases (TALENs) (Malzahn et al. 2017), and Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR/Cas9) systems (Langner et al. 2018; Chen et al. 2019). In particular, the CRISPR/Cas9 system with CRISPR-associated protein 9 (Cas9) is presently the most commonly used approach for plant genome editing due to its ease and robustness.

GE has been successfully applied to improve disease resistance against various plant pathogens, such as in rice (Li et al. 2012), wheat (Wang et al. 2014; Zhang et al. 2017b), tomato (Nekrasov et al. 2017), citrus (Peng et al. 2017), watermelon (Zhang et al. 2020b) and virus (Chandrasekaran et al. 2016).

Fusarium wilt (*Fusarium oxysporum*) and powdery mildew are destructive diseases in many crops, including flax. Examples of GE applications for these two diseases are listed (Table 8.3). *F. oxysporum* is a soil-borne fungus that exists as pathogenic and non-pathogenic strains (Leslie and Summerell 2006). Three Fusarium mitogen-activated protein kinase (MAPK) signaling genes (*FMK1*, *HOG1* and *PBS2*) are associated with plant surface hydrophobicity (sensing) and pathogenesis (Di Pietro et al. 2001). The RNAi-mediated silencing of these three genes in *F. oxysporum* resulted in decreased mycelial growth on tomato fruits, leading to reduced pathogenicity compared to the unsilenced fungus (Pareek and Rajam 2017). The *F. oxysporum* species complex (FOSC) is an economically important group of pathogenic filamentous fungi that are able to infect both animals and plants. Wang et al. (2018) developed an *F. oxysporum*-optimized Cas9 ribonucleoprotein (RNP) and a protoplast transformation method to generate a mutant *bik1* of *BIK1*, a gene in a secondary metabolite biosynthetic cluster, confirming that this polyketide synthase was involved in the synthesis of the red pigment bikaverin.

Mildew resistance locus O (*Mlo*) harbors a gene associated with powdery mildew resistance. Its wild-type alleles confer susceptibility to fungi resulting in the powdery

Table 8.3 Some applications of genome editing in improving biotic stress resistance

Crop	Enhanced disease resistance	GE method	Target genes	Type of modification	Reference
Tomato	Fusarium wilt against *F. oxysporum*	RNAi-mediated silencing	*FMK1*, *HOG1*, *PBS2*	Silencing	Pareek and Rajam (2017)
Cotton	Fusarium wilt against *F. oxysporum* f. sp. *vasinfectum*	CRISPR-Cas9	*URA5*, *URA3*, *BIK1*	Insertion	Wang et al. (2018)
Wheat	Powdery mildew against *Blumeria graminis* f. sp. *tritici*	TALEN, CRISPR-Cas9	*TaMLO-A1, TaMLO-B1 TaMLO-D1*	Knockout	Wang et al. (2014)
Tomato	Powdery mildew against *O. neolycopersici*	CRISPR-Cas9	*Mlo*	Knockout, deletion	Nekrasov et al. (2017)
Tomato	Powdery mildew against *O. neolycopersici*	CRISPR-Cas9	*SlPMR4*	Knockout	Koseoglou (2017)
Tomato	Powdery mildew against *O. neolycopersici*	RNAi-mediated silencing	*SlPMR4*	Silencing	Huibers et al. (2013)

mildew disease (Acevedo-Garcia et al. 2014), while its homozygous knockout mutations (*mlo*) lead to resistance to powdery mildew. Nekrasov et al. (2017) reported a non-transgenic tomato variety resistant to powdery mildew (*Oidium neolycopersici*) using the CRISPR/Cas9 technologyto edit the *Mlo* gene (*SlMlo1*) which is based on the Cas9 DNA nuclease guided to a specific DNA target by a single guide-RNA (sgRNA). PMR4 encodes a callose synthase and its loss-of-function mutants are resistant to powdery mildew in *Arabidopsis* and tomato. The CRISPR/Cas9-mediated knockout mutants of the *PMR4* ortholog (*SlPMR4*) in tomato showed partial resistance against the powdery mildew pathogen *O. neolycopersici* (Koseoglou 2017). RNA silencing of *SlPMR4* also enhanced the resistance to powdery mildew in tomato (Huibers et al. 2013).

The new technology represented by the CRISPR/Cas-based GE opens a new era in plant precision breeding and is expected to drive the second green evolution (Chen et al. 2019). This technology is considered a novel plant breeding technique that could provide an alternative to the strict regulations applied to 'genetically modified organisms' (GMOs). Technically, GE can be employed in precision breeding in many ways (Chen et al. 2019): (1) knocking out genes that confer undesirable traits; (2)

knock-in and replacement to introduce new favorable alleles without linkage drag or generating allelic variants that do not exist naturally; (3) nucleotide editing to alter SNPs in either coding or noncoding regions; (4) fine-tuning gene regulation by altering gene expression, mRNA processing, and mRNA translation; and (5) development of high-throughput mutant libraries for functional genomics and genetic improvement.

In flax, the first application of GE aimed to develop an herbicide tolerant version of CDC Bethune, the most popular flax variety in Western Canada, by precisely editing the *ENOLPYRUVYLSHIKIMATE-3-PHOSPHATE SYNTHASE* (*EPSPS*) genes using single-stranded oligonucleotides (ssODNs) and CRISPR/Cas9 (Sauer et al. 2016). Attempts to create a new flax variety tolerant to the herbicide glyphosate are being made by CIBUS (https://www.cibus.com/), a precision gene-editing company located in San Diego, using their proprietary GE method.

8.3 QTL Identification and Genetic Improvement for Pasmo Resistance in Flax

Pasmo disease affects flax production worldwide. This fungal disease caused by *Septoria linicola* (Speg.) Garassini is widespread through all flax growing regions and infects flax plants during the entire growth season (Halley et al. 2004). Rainfall accumulation from June to August increases the incidence and severity of the disease (Halley et al. 2004). High humidity and high temperature conditions during ripening mostly promote disease incidence. The major symptoms are brown circular lesions on leaves and brown or black banding patterns interspersed with green healthy tissues on stems. Pasmo negatively impacts both seed yield and fiber quality (Hall et al. 2016).

Pasmo resistance is a quantitatively heritable trait. The genetic improvement of pasmo resistance is hindered by the scarcity of highly resistant germplasm and a poor understanding of its complex genetic architecture. To date, no flax cultivars are truly highly resistant to pasmo (Diederichsen et al. 2008). Current flax cultivars developed in Canada are only moderately resistant and show a narrow genetic base (You et al. 2016b). To broaden the genetic base of flax cultivars, a core collection of 407 flax accessions has been assembled from a world collection of approximately 3,500 accessions of cultivated flax maintained by Plant Gene Resources of Canada (PGRC) (Diederichsen et al. 2012; Soto-Cerda et al. 2013). We previously evaluated pasmo resistance of the flax core collection and found significant variation associated with the geographical origin (You et al. 2017a). The most pasmo-susceptible accessions originate from India and Pakistan, whereas the accessions from Europe possessed the highest levels of resistance. Of the accessions from North America, most were moderately susceptible and susceptible. Even though CN101536 was evaluated as the most resistant Canadian linseed breeding line in the flax core collection, it was

just moderately resistant to pasmo with a rating of 4.4 (You et al. 2017a). Therefore, pyramiding additional favorable alleles into current elite varieties is considered an efficient first step to develop highly resistant varieties. The *a priori* identification of QTLs associated with pasmo resistance is not only a prerequisite to perform such marker-assisted backcrossing but could also be applied to screen advanced flax breeding germplasm.

8.3.1 Genetic Panel and SNP Set for GWAS

The flax core collection of 407 accessions is a diverse genetic panel. The entire collection was re-sequenced using GBS methodology and generated 100-bp Illumina paired-end reads to an average of 17 × genome coverage using the Illumina HiSeq 2000 platform (Illumina Inc., San Diego, USA). The reads were mapped to the CDC Bethune reference (Wang et al. 2012) using BWA v0.6.1. The mapped reads were analyzed as described (He et al. 2019b) and 1.7 M SNPs were obtained. These SNPs were remapped to the chromosome-scale reference (You et al. 2018a; You and Cloutier 2019). From this unfiltered SNP data set, 258,873 SNPs were extracted using the following filtering criteria: minor allele frequency (MAF) $\geq$ 0.05, genotyping rate $\geq$ 60% and pairwise correlation coefficients (r^2) among neighboring SNPs > 0.8 (International HapMap Consortium et al. 2005; Huang et al. 2010). Imputation was performed utilizing Beagle v.4.2 with default parameters (Browning and Browning 2007) to predict some of the 14.13% missing SNPs.

8.3.2 Pasmo Field Resistance of the Core Collection

Evaluation of flax accessions to pasmo resistance was carried out in a pasmo nursery that was established in the 1960s. To assure sufficient pasmo infection in the nursery, additional pasmo-infested chopped straw was spread from past growth periods as inoculum between rows when plants were roughly 30-cm tall. In addition, a misting system was applied to spread water for five minutes every half hour for four weeks, except on rainy days, to ensure conidia dispersal and disease infection and development. The 391 accessions were rated for pasmo resistance in the same nursery for five consecutive years from 2012 to 2016 at the farm of Agriculture and Agri-Food Canada, Morden Research and Development Centre, Morden, Manitoba, Canada. The field trial data was adjusted using a type-2 modified augmented design (MAD2) (Lin and Poushinsky 1985).

Pasmo severity, rated on a 0–9 scale, was evaluated based on symptoms on leaves and stems of all plants in a single row plot. Evaluation was conducted at four growth stages, i.e., the early (P1) and late flowering stages (P2), the green boll stage (P3), and the early brown boll stage (P4). To group the resistance of accessions, a rating of 0–2 is categorized as resistant (R), 3–4 as moderately resistant (MR), 5–6 as moderately

Table 8.4 Field evaluation criteria for pasmo severity on a scale of 0–9

Severity score	Criteria	Resistance class
0	No symptom of pasmo	R
1	<10% leaf or/and stem area affected	R
2	10–20% leaf or/and stem area affected	R
3	21–30%leaf or/and stem area affected	MR
4	31–40% leaf or/and stem area affected	MR
5	41–50% leaf or/and stem area affected	MS
6	51–60% leaf or/and stem area affected	MS
7	61–70% leaf or/and stem area affected	S
8	71–80% leaf or/and stem area affected	S
9	>80% leaf or/and stem area affected	S

susceptible (MS), and 7–9 as susceptible (S) (Table 8.4). Statistical analyses for pasmo ratings were previously described in You et al. (2013).

We observed that pasmo infection increased with growth stages and peaked at the final evaluation stage every year, which followed a nearly normal distribution (Fig. 8.1) (You et al. 2017a); thus, only the data observed at the final growth stages (P3 or P4) was used for GWAS. Although significant correlation existed among years, significant differences between years and significant genotype × year interactions were also observed, indicating that the individual year data sets could be used for GWAS to identify environment-specific QTLs.

8.3.3 QTL Identification

A total of 370 accessions from the 391 pasmo evaluated accessions, which have both quality SNP and phenotype data, were used for GWAS. We employed three single-locus models (GLM, MLM and GEMMA) and seven multi-locus models (six implemented in mrMLM and one in FarmCPU) (Table 8.1) to identify QTNs from the 370 accessions with 258,873 SNPs. Six pasmo rating data sets were independently analyzed for GWAS: five individual year data set and the 5-year average dataset. Significant QTNs associated with the traits were detected at $\alpha = 0.05$ followed by a Bonferroni correction ($1.93 \times 10^{-7} = 0.05/258{,}873$ SNPs) for GLM, MLM and

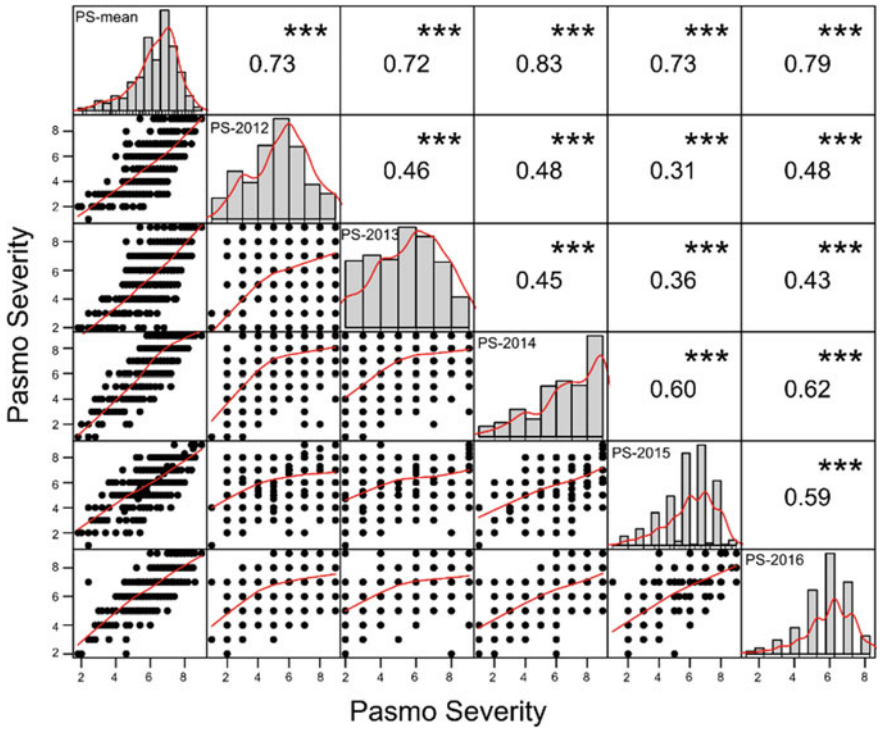

Fig. 8.1 Pearson correlations (upper triangle), scatter plots (lower triangle), and histograms (diagonal) between six pasmo severity datasets. Fitted curves are displayed in scatter plots and histograms. *** represents significance at the <0.001 probability level. *Source* He et al. (2019a)

FarmCPU, and a log of odds (LOD) score threshold of 3.0 for the remaining models. The pipeline for QTL identification and annotation is described in Fig. 8.2.

There were a total of 719 QTNs detected using the ten statistical models for the six pasmo rating datasets. These QTNs were further filtered by removing the QTNs for which the allele effect was not significant, and then grouped into 500 QTN clusters or QTLs based on LDs of contiguous markers as shown in Fig. 8.3. When there was more than one QTN in a cluster, the tag QTN with the largest QTL effect among all QTNs in the cluster was chosen to represent the QTLs. Hereafter QTN and QTL are interchangeably used.

Of these 500 QTNs, 14.4% (72) had large QTN effects ($R^2 > 10\%$), i.e., QTNs explaining a major portion of the phenotypic variance, while 24% (120) had minor effect ($R^2 < 1\%$). Several notably large-effect QTNs were identified, including Lu1-9232234 ($R^2 = 16.17\%$), Lu8-23104696 ($R^2 = 16.53\%$), Lu9-1896658 ($R^2 = 17.12\%$), andLu9-4333365 ($R^2 = 23.39\%$).

QTN detection power varies depending on statistical models used. Single-locus models mostly identified large-effect QTLs. Of the three single-locus models, MLM identified only one large-effect QTN with $R^2 = 15.02\%$, GEMMA identified six with

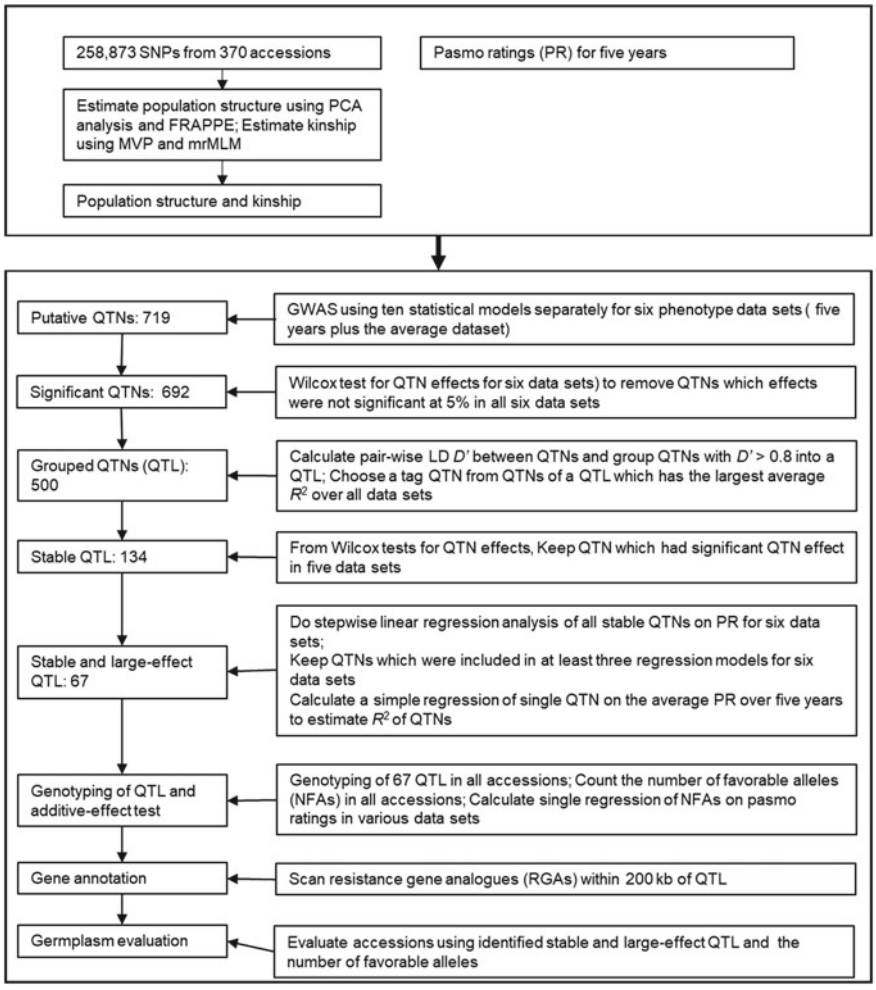

Fig. 8.2 Pipeline of quantitative trait loci (QTLs) identification using genomewide association study (GWAS) and annotation for flax pasmo resistance. *Source* Modified from He et al. (2019b)

an average R^2 of 11.13%, whereas GLM detected 209 QTNs that had an average R^2 of 5.57% and a range from 0.48 to 15.02%. Multi-locus models identified more small-effect QTNs than single-locus models. In addition, the six mrMLM models detected more QTNs with smaller effects (average R^2 of 2.80%) than FarmCPU (average R^2 of 5.09%), because the high stringency of the Bonferroni correction was applied to FarmCPU.

The stability and reliability of the QTNs identified correlated with the number of statistical models (NSMs) and the number of pasmo phenotype datasets (NPDs) to display significant allele effects for the QTNs (Fig. 8.4). A total of 127 QTNs were identified by two or more statistical models, but most of them (373) were detected by

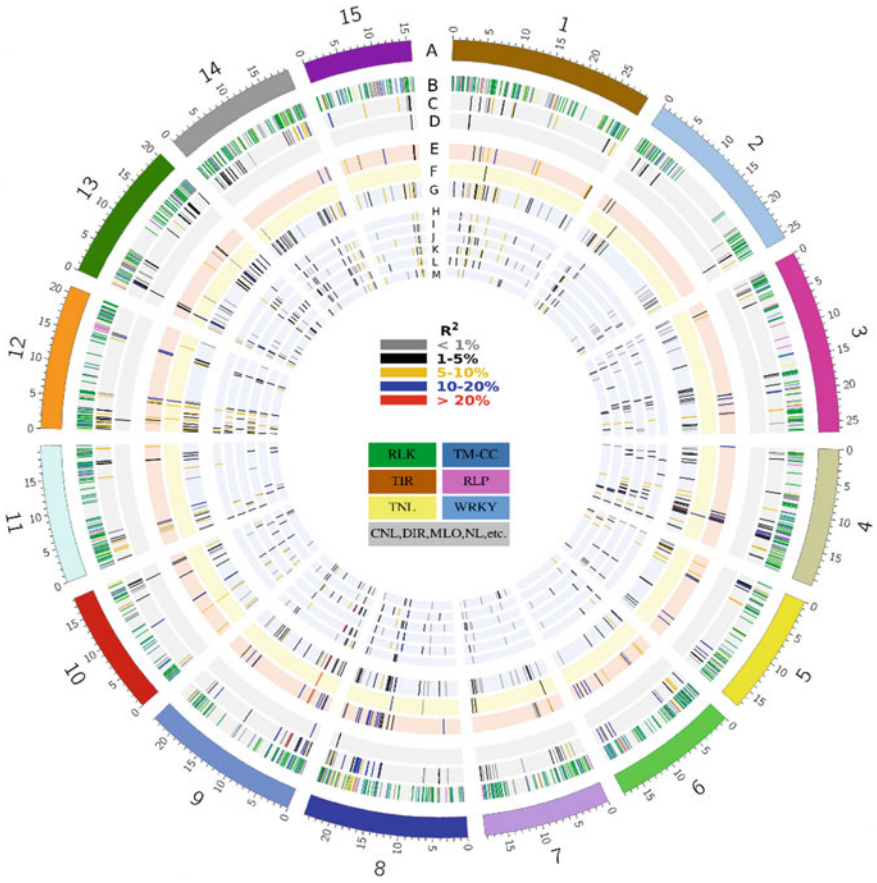

Fig. 8.3 Circos map of 500 QTNs associated with pasmo resistance measured in the field for five consecutive years and identified using ten single- and multi-locus models. Track **A**: flax genome chromosomes; **B**: 1599 resistance gene analogs (RGAs); **C**: 372 putative candidate RGAs for pasmo resistance; **D**: 8 RGAs co-located with identified QTNs; **E**: 209 QTNs identified by GLM; **F**: 22 QTNs identified by FarmCPU; **G**: 281 QTNs identified by all six "mrMLM models" (from **H** to **M**); **H**: 60 QTNs identified by FASTmrEMMA; **I**: 125 QTNs identified by FASTmrMLM; **J**: 97 QTNs identified by ISIS-EM-BLASSO; **K**: 97 QTNs identified by mrMLM; **L**: 95 QTNs identified by pKWmEB; **M**: 118 QTNs identified by pLARmEB

a single model. However, the effect size of QTNs was not necessarily associated with the NSMs (Fig. 8.4a), though the large-effect QTNs Lu4-14738243, Lu9-4333365 and Lu8-14317356 were all detected by more than five or all six models (Fig. 8.4a).

Nevertheless, the effect size of QTNs significantly correlated with NPDs (Fig. 8.4b), indicating that QTNs detected by a greater number of data sets were more reliable and associated with larger effect than QTNs identified in fewer data sets. Inversely, small-effect QTNs were usually identified in only one or two phenotypic datasets (or environments), indicative of their environment-specific associations.

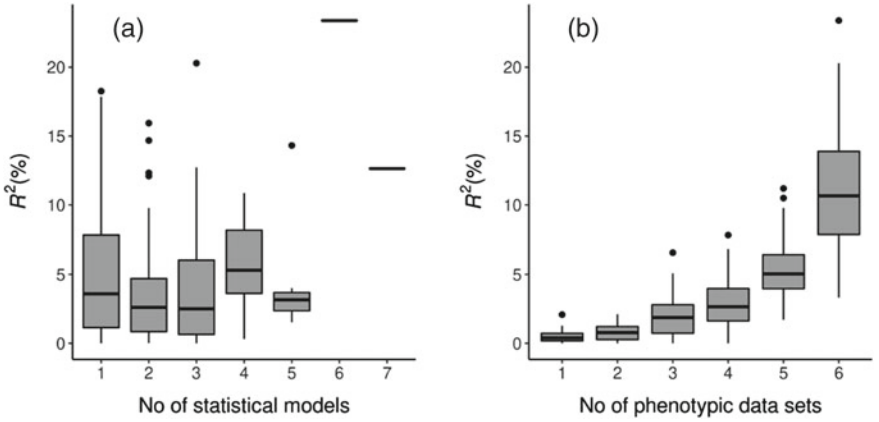

Fig. 8.4 Relationship between R^2 (phenotypic variance explained by a QTL, %) with the number of statistical models that detected the QTLs (**a**) and the number of pasmo phenotypic datasets that showed significant allele effects for the QTLs (**b**)

Based on the QTN effect size and the number of pasmo phenotypic datasets that showed significant QTN effect, two QTN subsets were generated from the 500 QTN set associated with pasmo resistance in flax. The first subset was defined based on 134 stable QTNs that have significant QTN effects in all six phenotypic datasets and explained 27.4–60.9% of the total variation. The second subset of 67 QTNs represented the non-redundant and stable QTN subset, which were identified by the construction of forward stepwise multiple regression models and retained in at least three models. This subset contributed 31.5–64.2% of the total variation in the six phenotypic datasets, a range comparable or moderately larger than that of the 134 QTL subset, indicating that the latter retained redundant markers.

The 500 QTN set appeared to be primarily additive for pasmo resistance. Significant negative correlation between the number of favorable alleles (NFAs) and pasmo ratings were observed ($R^2 = 0.73$) (Fig. 8.5), signifying that NFA is a good indicator or criterion to evaluate pasmo resistance of accessions.

8.3.4 Candidate Genes

To find candidate resistance genes that are co-localized with the detected QTNs, we first identified 1599 RGAs on the 15 chromosomes (Fig. 8.3, Track B), including the 1327 initially detected in the flax pseudomolecule (You et al. 2018a). We then performed genomewide scans along chromosomes to locate all the RGAs within a 200-kb window of the QTN's flanking regions. A total of 372 RGAs co-locating with 314 QTNs were thus detected. Among them, Lu1-3420323, Lu2-23730537, Lu8-22525597, Lu9-1067536, Lu10-16054459, Lu12-1874446, Lu13-2227366 and Lu15-14719354 were located in the following RGAs per se: *Lus10042324* (RLK), *Lus10030634* (RLK), *Lus10015350* (TNL), *Lus10028975* (TM-CC), *Lus10022900*

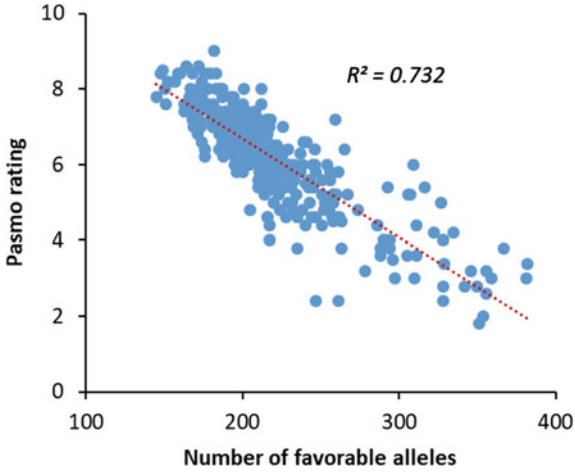

Fig. 8.5 Relationship between the number of favorable alleles and the average pasmo ratings of 370 flax accessions evaluated in the field for five consecutive years

(CNL), *Lus10023329* (TN), *Lus10026988* (RLK), and *Lus10014810* (RLK), respectively (Table 8.5, Fig. 8.3).

We further analyzed the 67 stable and large-effect QTN subset and found that 45 QTNs co-localized with 85 RGAs (Table 8.5), representing all four types, i.e., RLP, RLK, NBS coding genes, and those encoding transmembrane coiled-coil proteins (TM-CC) (Sekhwal et al. 2015). RLKs accounted for 36.47% of RGAs, while TNLs for 22.35% (He et al. 2019b).

Table 8.5 Quantitative trait nucleotides (QTNs) and putative candidate genes associated with pasmo resistance

Tag QTN	Chr	Position	SNP	Favorable allele	Effect	R^2	Gene/annotation
Lu1-3420323	1	3420323	G/A	A	0.28	2.89	*Lus10042324*/RLK[a]
Lu1-28707496	1	28707496	G/A	G	−0.54	5.7	*Lus10006052*/RLK, *Lus10006056*/RLK, *Lus10006057*/RLK, *Lus10006067*/RLK
Lu2-23730537	2	23730537	A/T	T	0.56	1.24	*Lus10030634*/RLK[a]
Lu3-19643168	3	19643168	G/A	G	−1.97	12.82	*Lus10008221*/TNL, *Lus10008222*/TNL, *Lus10008230*/RLP
Lu3-22688547	3	22688547	C/G	C	−0.89	8.98	*Lus10033291*/RLK
Lu4-14576826	4	14576826	A/G	G	0.42	7.99	*Lus10041509*/RLK, *Lus10041512*/TM-CC
Lu4-14615685	4	14615685	A/T	A	−0.65	10.85	*Lus10041509*/RLK, *Lus10041512*/TM-CC

(continued)

Table 8.5 (continued)

Tag QTN	Chr	Position	SNP	Favorable allele	Effect	R^2	Gene/annotation
Lu4-17204590	4	17204590	C/A	A	0.64	5.17	*Lus10004040*/RLK, *Lus10009107*/TNL, *Lus10009108*/TX, *Lus10009109*/NBS, *Lus10020794*/TM-CC
Lu4-17214936	4	17214936	G/T	T	0.7	5.81	Lus10004040/RLK, Lus10009107/TNL, Lus10009108/TX, Lus10009109/NBS, Lus10020779/CNL, Lus10020794/TM-CC
Lu5-1554121	5	1554121	T/A	T	−0.67	7.75	*Lus10004719*/TNL, *Lus10004726*/CNL, *Lus10004727*/TN
Lu5-1650980	5	1650980	C/G	C	−0.81	6.61	*Lus10004719*/TNL, *Lus10008486*/RLK, *Lus10008491*/RLK
Lu5-4604607	5	4604607	A/G	A	−0.56	6.58	*Lus10034787*/TM-CC, *Lus10034790*/RLK, *Lus10034795*/RLK
Lu5-13500692	5	13500692	G/A	G	−1.4	11.9	*Lus10029802*/RLK, *Lus10029810*/TX
Lu6-2081466	6	2081466	T/C	C	0.68	8.3	*Lus10017611*/RLK
Lu6-14738507	6	14738507	C/T	C	−2.01	13.34	*Lus10014441*/RLP
Lu6-15455712	6	15455712	A/G	A	−1.42	9.63	*Lus10021003*/RLK, *Lus10021022*/RLK
Lu6-15506450	6	15506450	A/G	A	−1.81	12.62	*Lus10021022*/RLK
Lu7-2452981	7	2452981	C/T	C	−0.53	6.3	*Lus10012159*/RLK
Lu7-2453965	7	2453965	T/C	T	−0.56	7.03	*Lus10012159*/RLK
Lu7-2491132	7	2491132	G/A	G	−0.56	8.05	*Lus10012159*/RLK
Lu8-14317356	8	14317356	A/T	A	−0.98	14.32	*Lus10016620*/RLK, *Lus10016612*/RLP
Lu8-16366918	8	16366918	C/T	C	−1.38	10.9	*Lus10022340*/RLK, *Lus10022345*/RLK, *Lus10022351*/CNL
Lu8-17270785	8	17270785	C/G	C	−1.08	9.59	*Lus10000591*/TM-CC
Lu8-17749357	8	17749357	G/A	G	−1.23	10.16	*Lus10011039*/RLP, *Lus10011064*/RLP

(continued)

Table 8.5 (continued)

Tag QTN	Chr	Position	SNP	Favorable allele	Effect	R^2	Gene/annotation
Lu8-18251174	8	18251174	G/A	G	−1.45	10.38	*Lus10007812*/TNL, *Lus10007813*/TNL, *Lus10007814*/TNL, *Lus10007821*/TNL, *Lus10007822*/TNL, *Lus10007823*/OTHER, *Lus10007825*/TNL, *Lus10007826*/TNL, *Lus10007828*/TNL, *Lus10007829*/OTHER, *Lus10007830*/NL, *Lus10007831*/TNL, *Lus10007836*/TNL, *Lus10007852*/TX
Lu8-18447612	8	18447612	T/C	T	−1.41	11.66	*Lus10007790*/TNL, *Lus10007795*/TM-CC, *Lus10007808*/TNL, *Lus10007809*/NL, *Lus10007810*/TNL, *Lus10007811*/TNL, *Lus10007812*/TNL, *Lus10007813*/TNL, *Lus10008540*/RLK
Lu8-22525597	8	22525597	T/C	T	−0.3	2.74	*Lus10015350*/TNL[a]
Lu8-23104696	8	23104696	C/A	C	−1.8	16.53	*Lus10018470*/TX
Lu8-23142500	8	23142500	T/C	T	−1.56	13.34	*Lus10018459*/RLK, *Lus10018470*/TX
Lu9-1067536	9	1067536	A/C	A	−0.67	5.06	*Lus10028975*/TM-CC[a]
Lu9-1430465	9	1430465	G/C	G	−0.69	10.76	*Lus10004333*/RLK
Lu9-4333365	9	4333365	C/A	C	−2.22	23.39	*Lus10040315*/TM-CC
Lu9-6270376	9	6270376	A/G	A	−0.81	14.34	*Lus10031043*/RLK, *Lus10031058*/TM-CC
Lu9-19857367	9	19857367	G/A	G	−1.7	12.67	*Lus10011917*/RLK
Lu10-8700793	10	8700793	A/G	A	−0.53	12.1	*Lus10039958*/RLP
Lu10-16054459	10	16054459	A/G	G	0.31	1.2	*Lus10022900*/CNL[a]
Lu11-3330783	11	3330783	A/T	A	−1.11	7.09	*Lus10042097*/TM-CC
Lu12-474480	12	474480	C/T	T	0.51	8.33	*Lus10020016*/CNL
Lu12-1621325	12	1621325	T/A	T	−1.9	9.41	*Lus10023391*/RLK
Lu12-1874446	12	1874446	G/A	A	0.34	4.3	*Lus10023329*/TN[a]
Lu12-2719326	12	2719326	C/T	C	−0.62	9.9	*Lus10006971*/TM-CC
Lu12-5795458	12	5795458	A/G	G	0.54	9.67	*Lus10037786*/TM-CC
Lu12-5819991	12	5819991	C/G	G	0.35	6.9	*Lus10037786*/TM-CC

(continued)

Table 8.5 (continued)

Tag QTN	Chr	Position	SNP	Favorable allele	Effect	R^2	Gene/annotation
Lu12-16056974	12	16056974	A/C	A	−1.26	11.26	*Lus10043083*/RLK
Lu13-1919638	13	1919638	G/A	G	−1.55	13.67	*Lus10026845*/TX
Lu13-2227366	13	2227366	T/C	C	0.41	1.21	*Lus10026988*/RLK[a]
Lu13-14299019	13	14299019	A/G	G	0.39	8.28	*Lus10034637*/RLK, *Lus10034642*/RLK
Lu15-976617	15	976617	T/A	T	−1.65	16.08	*Lus10011216*/TX, *Lus10011223*/RLK, *Lus10011229*/TM-CC
Lu15-995626	15	995626	T/A	T	−0.44	6.27	*Lus10011216*/TX, *Lus10011223*/RLK, *Lus10011229*/TM-CC
Lu15-14719354	15	14719354	T/C	C	0.33	4.07	*Lus10014810*/RLK[a]

RLK: receptor-like protein kinase; RLP: receptor-like protein; TM-CC: transmembrane coiled-coil protein; NBS: nucleotide-binding site domain; LRR: leucine-rich repeat; TIR: Toll/interleukin-1 receptor-like domain; TNL: TIR-NBS-LRRs; TN, TIR–NBS; TX, TIR–unknown.[a] QTNs in genes

Of note, Chr 8 contains an important genomic region associated with pasmo resistance. A total of 49 QTNs were identified on Chr 8, and nine of them were classified stable and major QTNs with nearby candidate genes (Table 8.5). QTNs Lu8-18251174 ($R^2 = 10.38\%$) and Lu8-18447612 ($R^2 = 11.66\%$) both co-located with TNL gene clusters. Lu8-18251174 had high LD correlations with both *Lus10007830* (NL) and *Lus10007831* (TNL), while Lu8-18447612 was significantly correlated with *Lus10007790* (TNL) (Fig. 8.6a). In addition, QTN Lu8-22525597 ($R^2 = 2.74\%$) is located within TNL gene *Lus10015350* (Table 8.5 and Fig. 8.6b). Besides TNL genes in this genomic region, the RLK gene *Lus10016620* was also found to be significantly correlated with QTN Lu8-14317356 ($R^2 = 14.32\%$) (Fig. 8.6c).

Lus10031043 (RLK) and *Lus10020016* (CNL) are two candidate genes which co-locate with the major QTNs Lu9-6270375 and Lu12-474480, respectively. These two genes are orthologous to *Arabidopsis* resistance genes *AT5G20480.1* and *AT3G07040.1* (*RPM1*), respectively (Xiang et al. 2008; Saijo et al. 2009). *AT5G20480.1* encodes a leucine-rich repeat receptor kinase (LRR-RLK) and behaves as the receptor for bacterial pathogen-associated molecular patterns (PAMPs) EF-Tu (EFR). The LRR-RLK EFR can recognize the bacterial epitopes elf18 that is derived from elongation factor-Tu, and then activates the plant immune response (Saijo et al. 2009). The *Pseudomonas syringae* effector *AvrPto* has been shown to bind receptor kinases, including *Arabidopsis* LRR-RLK EFR, inhibit plant PAMP-triggered immunity and elicit strong immune responses (Xiang et al. 2008). *RPM1* has a tripartite nucleotide binding site at the N-terminal and a tandem array of leucine-rich repeats at the C-terminal, and it conveys resistance to *P. syringae* strains that carry the avirulence genes *avrB* and *avrRpm1*. The *RPM1* gene confers dual pathogen specificity that expresses either of the two unrelated *P. syringae* avirulence genes (Grant et al.

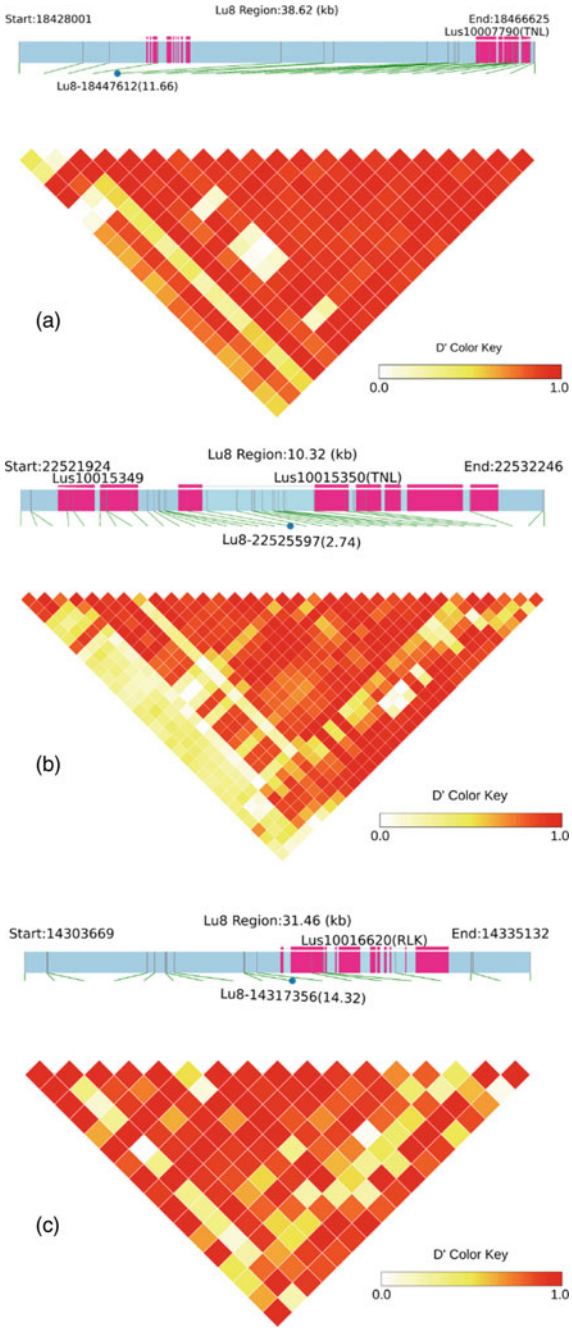

Fig. 8.6 Linkage disequilibrium plots for three QTNs associated with pasmo resistance (**a**). QTN Lu8-18447612 (R^2 = 11.66%) co-located with the gene *Lus10007790* (TNL); (**b**). QTN Lu8-22525597 (R^2 = 2.74%) located within the gene *Lus10015350* (TNL); (**c**). QTN Lu8-14317356 (R^2 = 14.32%) co-located with the gene *Lus10016620* (RLK). The values in parentheses after QTN names are R^2 values

1995). Therefore, *Lus10031043* and *Lus10020016* are two additional candidate genes deserving further functional analyses.

8.3.5 Genomic Evaluation of the Resistance Germplasm

Flax has two morphotypes: seed and fiber. Pasmo resistance correlates with these morphotypes. Significant correlations between morphotype and pasmo ratings ($r = 0.49$, $p < 0.00001$) as well as between morphotype and NFAs ($r = -0.65$, $p < 0.00001$) were observed in the diversity panel which comprised 80 fiber and 290 linseed accessions (Fig. 8.7). Fiber accessions generally appeared to be more resistant to pasmo than linseed accessions. This is likely an indication that fiber flax breeders have expended greater effort into breeding for pasmo resistance than linseed breeders because fiber flax quality can be greatly affected by high pasmo incidence. Aside from artificial selection by breeders, long term natural selection and probably independent domestication of the fiber flax may also account for the differential in pasmo resistance between the morphotypes (Fu et al. 2012).

A variety of pasmo resistance was observed in the core collection (You et al. 2017a), allowing further investigations on a genomic scale. Making use of the QTN information of the genotypes, we identified 14 accessions with resistant phenotypes and high numbers of favorable alleles (Table 8.6). For instance, the fiber accession CN19001 from the Netherlands and the linseed accession CN101367from Georgia, have average pasmo ratings of 2.0 and 1.8 and 354 and 351 favorable alleles, respectively. Netherlands's accessions CN40081 and CN33390 had the most favorable alleles but slightly higher pasmo ratings than the previous two. It is also notable that ten of the 14 resistant accessions are fibers. These fiber and linseed accessions are good parents to further improve flax resistance to pasmo through direct cross breeding through the pyramiding of favorable alleles into elite varieties.

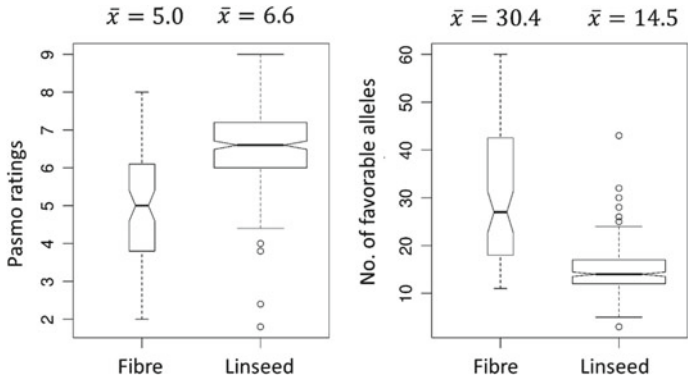

Fig. 8.7 Boxplots of flax morphotypes in terms of flax pasmo ratings and number of favorable alleles in the accessions. *Source* Modified from He et al. (2019b)

Table 8.6 Genetic resources resistant to pasmo disease identified by genomic and phenotypic evaluation

Accession	Country	Morphotype	Pasmo rating	No. of favorable alleles[a]
CN40081	Netherlands	Fiber	3.4	382
CN33390	Netherlands	Fiber	3	381
CN101053	China	Fiber	3	359
CN100929	Netherlands	Fiber	2.6	356
CN18982	France	Fiber	3.2	356
CN19001	Netherlands	Fiber	2	354
CN101367	Georgia	Linseed	1.8	351
CN18983	Netherlands	Fiber	2.8	350
CN18988	France	Fiber	3.2	346
CN101298	Russian	Linseed	2.8	342
CN100939	Russian	Linseed	2.4	328
CN101419	China	Fiber	2.8	328
CN101230	China	Fiber	3	310
CN101299	Russian	Linseed	3	297

[a] Out of 500 QTNs

8.3.6 Evaluation of Genomic Selection (GS)

For complex quantitatively heritable traits, the major purpose of genomewide QTL identification is to provide molecular markers for breeding selection. Some large-effect QTLs such as Lu9-4333365, Lu4-14213405, Lu5-14838893, Lu4-13813266 and Lu9-1896658, have R^2 values exceeding 17%, which could be useful for MAS, but most of the QTNs identified have small allele effects, which would not be considered for MAS but could be valuable for GS. To explore the values of these QTNs in GS, we first assessed the efficiency of various GS models to ascertain the best model for GS of pasmo resistance. The GS models RR-BLUP, GBLUP, BL, BRR, BayesA, BayesB, BayesC, RFR, RKHS and SVR were evaluated using the 500 QTN subset as marker input and the five-year average pasmo rating dataset as the phenotype. The five-fold cross-validation results revealed the same prediction ability (r) of 0.92 for 9/10 models, exception being RFR which had a prediction ability of 0.79 (Fig. 8.8).

We further evaluated GS models with different marker sets to determine the best marker set in the development of GS model for pasmo resistance. Six different marker sets were tested with the six pasmo phenotype datasets using the random five-fold cross-validation scheme. The marker sets were three SNPdata sets (SNP-66723, SNP-9415 and SNP-3057) and three QTL data sets (QTL-500, QTL-134 and QTL-67). SNP-66723 was selected from the 258,873 SNP data set by a Pearson's $\chi 2$ test with Yate's continuity correction to identify all SNPs related to pasmo ratings. SNP-9415 and SNP-3057 are two subsets of SNP-65723 that were selected with probability value thresholds of 0.01 and 0.001, respectively. QTL-67, QTL-134 and QTL-500

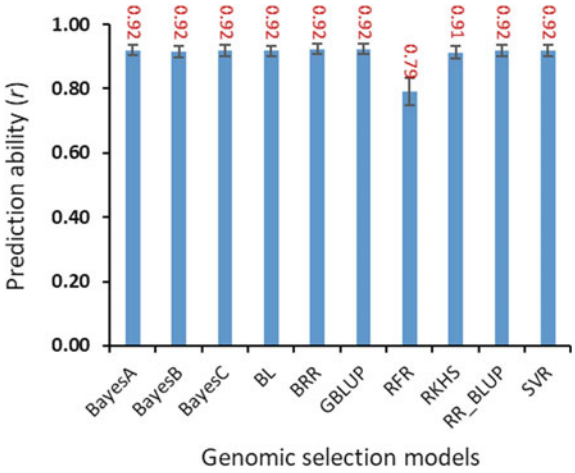

Fig. 8.8 Comparison of prediction ability (*r*) of ten genomic selection (GS) models. The GWAS-derived 500 QTN subset (QTL-500) with the five-year average pasmo rating dataset were used for GS model construction

represent the 500 GWAS-derived unique QTLs, the 134 statistically stable QTLs and 67 non-redundant and stable QTL subsets, respectively. QTL-67 is contained in QTL-134, which is in turn contained in QTL-500. RR-BLUP was used to construct the GS models. Results showed that the GS models with QTL markers consistently outperformed those with SNP markers for all pasmo phenotypic datasets (Fig. 8.9), similarly to our previous results on seven breeding target traits (Lan et al. 2020).

In the three QTL marker based GS models, GS models built from QTL-500 significantly outperformed those from QTL-134 and QTL-67, indicating that at least a portion of the minor-effect QTNs contribute positively to the development of the GS models. The similar prediction ability of the two smaller marker sets was anticipated since QTL-67 is fundamentally a non-redundant set of QTL-134. These GS prediction results indirectly serve as a validation of the QTL identified via GWAS. In addition, a prediction ability as high as 0.92, seen in the GS models clearly illustrates the effectiveness of genomic prediction for pasmo resistance by employing a comprehensive range of stable or environment-specific QTLs with large- and small-effect QTLs.

8.4 Future Perspectives

Resistance to diseases such as pasmo, Fusarium wilt and powdery mildew is a complex quantitative trait in flax. The conventional approach to flax genetic improvement still involves cross breeding through hybridization of two parents followed by offspring segregation and phenotypic selection. In such conventional approach,

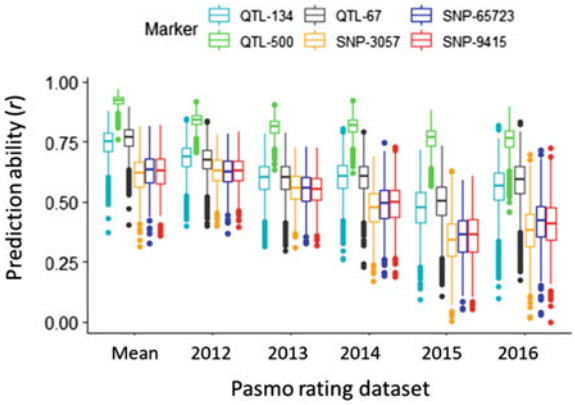

Fig. 8.9 Comparison of prediction ability (r) of RR-BLUP prediction models constructed using six different marker sets and the five-year average pasmo rating dataset using a random five-fold cross-validation scheme. SNP-66723 is a SNP subset selected from 258,873 SNPs by a Pearson's $\chi 2$ test with Yate's continuity correction to identify all SNPs statistically correlated with pasmo ratings. SNP-9415 and SNP-3057 are two subsets of SNP-65723 that were selected at different probability thresholds. QTL-67, QTL-134 and QTL-500 represent the 500 unique QTL, the 135 stable QTL and the 67 non-redundant QTL subsets identified by GWAS, respectively. QTL-67 is comprised within QTL-134, which is in turn comprised within QTL-500

the quantitative inheritance nature of these disease resistances impedes the rapid pyramiding of desirable or resistant alleles/genes from donor parents into a single plant, resulting in slow advance in resistance breeding for these biotic stresses in flax. To date, the majority of registered flax varieties are moderately resistant to pasmo, Fusarium wilt and powdery mildew. However, large-scale QTL identification through linkage-based QTL mapping and GWAS has already identified a large number of QTLs associated with biotic stresses in flax, including large-and minor effect QTLs. QTL markers identified from the flax core collection offer the potential to enhance selection accuracy and efficiency of cross breeding through GS. In addition, QTL markers of parents can be combined with genetic simulation to generate virtual crosses and their offspring populations (Khan et al. 2022). Then GS can be applied to predict GCA of parents and SCA of the virtual crosses, which facilitate parent selection and cross making to make best crosses.

The "breeding by design" was proposed by Peleman and Voort (2003), aiming to gather favorable alleles or QTLs associated with breeding target traits from potential genetic resources to develop superior varieties. We have identified an array of QTNs related to the traits of interest, including biotic stresses, and deciphered the distribution of the favorable alleles on the genetic resources. We also found that the identified QTNs were primarily additive. Therefore, this offers a genomic approach to evaluate all genetic resources based on their genomewide QTN content. Furthermore, based on complementarity of favorable alleles among parents, suitable parents can be selected to "design" potential superior varieties. Such varieties may contain

all favorable alleles in one variety and can be implemented through conventional breeding, MAS and GS.

Some candidate genes have been predicted for some of the significant QTNs, but validation and characterization of these candidate genes via functional genomic approaches remain challenging. Once their functions are validated and functional markers are developed, precision breeding through gene editing technologies is expected to be a revolutionary strategy towards rapid and accurate pyramiding of multiple resistant genes into elite flax varieties. The impending first successful application of GE in flax has the potential to accelerate the deployment of precision breeding technologies in flax genetic improvement.

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Correction to: Genomic Designing for Biotic Stress Resistant Peanut

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The original version of the book was published with incorrect affiliations for the authors in the chapter 4. The chapter and book have been updated with the changes.

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