Shabir Hussain Wani *Editor*

Disease Resistance in Crop Plants

Molecular, Genetic and Genomic Perspectives

Springer

Disease Resistance in Crop Plants

Shabir Hussain Wani
Editor

Disease Resistance in Crop Plants

Molecular, Genetic and Genomic Perspectives

Springer

Editor
Shabir Hussain Wani
Mountain Research Centre for Field Crops
Sher-e-Kashmir University of Agricultural
Sciences and Technology of Kashmir
Srinagar, Jammu and Kashmir, India

ISBN 978-3-030-20727-4 ISBN 978-3-030-20728-1 (eBook)
https://doi.org/10.1007/978-3-030-20728-1

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Dedication

Professor Robert McIntosh is an Australian scientist who has dedicated his life to wheat rusts and to the resistance genetics of wheat. Wheat researchers recognize him for the atlas of wheat rust resistance genes published jointly with Colin R. Wellings and Robert F. Park. Indeed, he is an inspirational figure not only for wheat researchers but also for researchers in other fields.
Prof. McIntosh rooted himself to Australian agriculture from his childhood. Growing up at Gloucester in New South Wales, he spent his early years on a dairy farm.

Prof. McIntosh has been closely associated with the University of Sydney through undergraduate and postgraduate studies (PhD, 1969) and later continuous service within the Plant Breeding Institute (PBI) for more than 60 years. He served as director of Rust Research within the PBI from 1980 to 2000.

Prof. McIntosh made significant contributions to wheat rust research. His pre-molecular era studies on chromosome location and genetic linkage in wheat resulted in the documentation of 7 leaf rust resistance genes, 14 stem rust resistance genes, and 2 stripe rust resistance genes. His research enabled the commercial deployment of white seeded varieties with leaf rust resistance gene Lr24 and stem rust resistance gene Sr24 in Australia where these genes remained effective in agriculture for a much longer period than elsewhere; indeed, Sr24 is still effective after almost 40 years. He led the early Australian research on stripe rust after the pathogen was introduced in 1979. His research explained sequential losses of chromosome 3R resistances in day length-insensitive 2D(2R)-substituted triticale cultivars. He has published more than 175 research papers in international and national journals and has coordinated and published the internationally accepted wheat gene catalogue for wheat from 1973.

Prof. McIntosh retired from his academic position in 2000, but he continues to work as an emeritus. He has been honored with several international fellowships including a Postdoctoral Fellowship at the Department of Genetics, University of Missouri, in

1969–1970; a Royal Society Fellowship at the Plant Breeding Institute, Cambridge, in 1977; and Visiting Professorships at Kansas State University in 1993 and Kyoto University in 2000–2001. He has also given lectures on host-pathogen relationships on multiple occasions at the International Maize and Wheat Improvement Centre (CIMMYT), Mexico (1987), and several institutions in China. He served on the External Advisory Committee of the Bill & Melinda Gates Foundation-supported international project "Durable Rust Resistance in Wheat (DRRW)" administered by Cornell University from 2007 to 2015 and was editor of various proceedings of the Borlaug Global Rust Initiative.

Prof. McIntosh has been recipient of many national and international honors for his work on wheat rust research, including Order of Australia (AO) in 2009. Other notable awards include the Farrer Memorial Medal for services to agriculture in 1976; Daniel McAlpine Memorial Lecture, Australasian Plant Pathology Society in 1985; Medal of the Australian Institute of Agricultural Science in 1987; Fellow of the Australian Institute of Agricultural Science in 1988; a Personal Chair in Cereal Genetics and Cytogenetics in 1993; Fellow of the Australian Academy of Science in 1993; J.C. Walker Memorial Lecture, University of Wisconsin, USA, in 1994; Fellow of the American Phytopathological Society, E.C. Stakman Award, University of Minnesota, St Paul, USA, in 2002; Centenary Medal, awarded by the Australian Government "For Service to Australian

Society and Science in Genetics" in 2003; "Wheat Warrior" Award from the Crawford Fund to mark the occasion of the CIMMYT Board Meeting in Canberra in 2010; Tian Fu Friendship Award, Sichuan Province, China, in 2016; and "The Norman" – awarded by the Borlaug Global Rust Initiative in 2018. He was an instructor for annual BGRI training workshops at Njoro, Kenya, from 2009 to 2018.

Prof. McIntosh is an effective teacher and mentor. Several postgraduate students completed their studies under his mentorship. He supervised or co-supervised nine postgraduate students. This book covers different aspects of disease resistance in crop plants including wheat and is dedicated to the contributions of Professor Robert McIntosh to the world wheat community.

Foreword

I am delighted to know that Dr. Shabir Hussain Wani has edited this volume entitled *Disease Resistance in Crop Plants: Molecular, Genetic and Genomic Perspectives* for the internationally reputed publisher Springer Nature. Recently, in 2016, he has successfully completed 1 year postdoctoral fellowship program at Michigan State University, USA, and worked on dissection of *Pythium* root rot resistance in soybean using molecular genetics approaches utilizing SNP markers. The outcome of this postdoc research came out in the form of a good publication in the journals *Genetics Society of America* and *G3: Genes, Genomes, Genetics*. He had a good experience to work in the area of plant biotechnology particularly molecular breeding approaches for the development of disease resistance in plants. I appreciate his enthusiasm and devotion for science, including research, teaching, and dissemination of scientific knowledge.

Yield losses caused by pathogens, animals, and weeds are altogether responsible for losses ranging between 20% and 40% of the global agricultural productivity. Nevertheless, it is estimated that 30 to 40% of harvests are lost each year throughout the production chain. Disease development in plants continues, having a great impact on these societies. Host plant resistance is largely the most promising control method for environmental, economic, and social reasons. Therefore, genes for

resistance to diseases and pests may fairly be considered most imperative natural resources for global food security. The evolution of a next-generation phase of disease resistance research is proceeding, and both the public and private sectors are moving to exploit the novel tools and prospects offered by genetics and molecular biology. Maximum disease resistance traits are polygenic in nature and controlled by several genes positioned at putative quantitative trait loci (QTLs). Although quantitative disease resistance (QDR) is a durable and broad-spectrum form of resistance in plants, the identification of the genes responsible for QDR is an upcoming area of research. Furthermore, the sources of resistance are generally found in wild relatives or cultivars of less agronomic significance, so introgression of disease resistance traits into commercial crop varieties typically involves many generations of backcrossing to restore the promising genotype. Molecular marker-assisted breeding (MAB), still, facilitates the preselection of traits even prior to their expression. Most of the plant diseases involve a complex network assimilating manifold response pathways prompted by discrete pathogen molecular elements. By digging deep into the portrayal of the molecular signals necessary for pathogen identification and dissection of the cellular phenomenon that describes the utterance of resistance, it has opened new vistas for sustainable crop disease management. This edited volume by Dr. Wani includes recent advances in disease control for major food crops using the novel molecular and genetic techniques.

Dr. Wani has done an outstanding endeavor by editing this volume, including high-quality chapters from the international- as well as national-level experts in various research fields. The chapters included in this book are nicely written by potential scientists and researchers belonging to various developed and developing nations. This book describes the recent advances in plant disease management utilizing genetic and genomic approaches and their application in important agricultural crops like rice, wheat, maize, barley, pulses, etc. Recent techniques, like genome editing and genomic selection, and their importance and application in the development of disease-resistant crops have also been included. I congratulate Dr. Wani for unraveling this edited volume and hope that this will be a useful reference material for the researchers, student, and policy-makers.

G. P. Singh
Director, ICAR-IIWBR
Karnal, India

Contents

Chapter 1
RETRACTED CHAPTER: Impact of Biotic and Abiotic Stresses on Plants, and Their Responses

Bilal Ahmad, Aamir Raina, and Samiullah Khan

1.1 Introduction

In the present era of drastic climate changes such as global warming, erratic rainfall and depletion of arable land and water resources, plants encounter a diverse range of climate-induced biotic and abiotic stresses (Atkinson et al. 2013; Narsai et al. 2013; Prasch and Sonnewald 2013; Suzuki et al. 2014; Mahalingam 2015; Pandey et al. 2015; Ramegowda and Senthil-Kumar 2015). Stress may be defined as an adverse condition for plant growth and development, caused by either environmental or biological factors, or both. Under natural conditions, concurrent occurrence of two or more different types of stresses—such as drought and salinity, drought and heat are more detrimental to global crop production. Concurrent abiotic stresses are more destructive in disrupting plant metabolism and reducing yield than the same stresses occurring separately at different growth stages. Co-occurrence of drought and heat stress or drought and salinity stress during summer are examples of combined abiotic stresses. Biotic stresses also play a central role in regulating outbreaks of pests, pathogens, insects and weeds (Coakley et al. 1999; Scherm and Coakley 2003; McDonald et al. 2009; Ziska et al. 2010; Peters et al. 2014). These

The original version of this chapter was retracted: The retraction note to this chapter is available at https://doi.org/10.1007/978-3-030-20728-1_12

B. Ahmad
Plant Physiology Laboratory, Department of Botany, Aligarh Muslim University, Aligarh, India

A. Raina (✉) · S. Khan
Mutation Breeding Laboratory, Department of Botany, Aligarh Muslim University, Aligarh, India

Botany Section, Women's College, Aligarh Muslim University, Aligarh, India

S. H. Wani (ed.), *Disease Resistance in Crop Plants*,
https://doi.org/10.1007/978-3-030-20728-1_1

stresses also influence plant–pest interactions by altering the physiological and adaptive responses of plants (Scherm and Coakley 2003). Because of their enhanced water use efficiency, weeds outcompete crops under abiotic stress (Patterson 1995; Ziska et al. 2010; Valerio et al. 2013). Abiotic stress has a massive impact on plant growth; consequently, it is responsible for huge losses in yield. The consequential growth reductions can reach upto 50% in most plant species (Wang et al. 2003). Daryanto et al. (2016) reported that the yield of maize is reduced by up to 40% and 21% reduction in the yield of wheat is also noted with a 40% water reduction. The cowpea yield is also decreased, and the extent of the reduction varies between 34% and 68%, depending on the developmental stage and drought stress (Farooq et al. 2017). In case of cowpea, which is an important crop in Africa, and source of food to the millions of farmers, the yield reduction can vary to a great extent depending on the developmental stage and the severity of drought stress. In 2002 it was estimated that soil salinity alone caused losses of more than US$11 billion annually and affected about 10% of the world's arable land, greatly influencing global food production and is considered as the main stress to influence the global crop productivity (Tanji 2002; Ahmad et al. 2019)

In addition to several combinations of abiotic stresses, plants also encounter multiple biotic stresses, commonly through pathogen or herbivore attack simultaneously or sequentially. Biotic stress is an additional threat and puts a great pressure on plant productivity (Mordecai 2011; Maron and Crone 2006; Maron and Kauffman 2006; Strauss and Zangerl 2002; Brown and Hovmoller 2002). A common case of combined biotic stresses is simultaneous attacks by bacterial and fungal pathogens on plants. For example, combined attacks by the bacterium *Xanthomonas arboricola* and fungal pathogens such as *Fusarium* spp., *Alternaria* spp., *Cladosporium* spp., *Colletotrichum* spp., or *Rhodopsis* spp. cause brown apical necrosis of *Juglans regia* (Belisario et al. 2002). Plants are severely damaged by concurrent fungal, bacterial and viral infections, which lead to more severe disease symptoms than separate infections with these pathogens.

The impact of concurrent stresses on plants is determined by the types of interactions between various kinds of stress factors (Prasch and Sonnewald 2013; Choudhary et al. 2016). Therefore, the impact of concurrent stresses can be evaluated by understanding the underlying mechanisms of such interactions between various stress factors. Mittler (2006) and Suzuki et al. (2014) showed that the interactions between various stress factors can have either positive or negative effects on plant growth. Plants act in response to concurrent stresses by inducing the expression of diverse set of genes whose products such as secondary metabolites (phenolics) play critical roles in alleviating a broad range of stresses (Niakoo et al. 2019). Plants alter their responses to concurrent stress factors and reveal numerous distinctive responses, along with other general responses. Improved plant tolerance to concurrent stresses involves recognition of physiomorphological traits that are affected by these concurrent stresses. Bearing in mind the global occurrence and the influence of concurrent stresses on agricultural productivity, this chapter attempts to provide

insights into the current understanding of stress combinations and improvement of physiomorphological traits to mitigate the effects of concurrent stresses. The significance of studies assessing the impact of concurrent stresses on plant growth is documented and additionally, some important and common examples of different, naturally occurring combinations of stresses are described.

1.1.1 Stress Combinations Occurring in Nature

Stresses are categorized as single, multiple individual, concurrent, and repetitive stresses, depending on the number of interacting factors. A single stress involves only one stress factor, whereas multiple individual stresses represent two or more stresses occurring without any overlap and concurrent stresses represent two or more stresses occurring simultaneously with a little overlap. In repetitive stresses, plants face a single stress or multiple stresses followed by recovery periods, which may be of shorter or longer duration. Several spells of hot days or multiple events of drought and heat stress may occur at different developmental stages of plants. The interactions between various stress factors may either enhance the tolerance capacity or predispose the plant toward a wide range of stresses. For example, drought facilitates the growth of *Macrophomina phaseolina* in the roots of *Sorghum bicolor* and results in a severe reduction in its productivity (Goudarzi et al. 2011). Likewise, the productivity of *Vitis vinifera* is reduced by the occurrence of concurrent drought and cold stress in North China (Su et al. 2015). Plants growing in hot and dry regions such as arid and semi-arid areas are often challenged by the onset of concurrent salinity and heat stress. In the Mediterranean region cold and light stresses are most prevalent and affect the growth and development of plants (Loreto and Bongi 1989). The frost durability of *Triticum aestivum* and the production of *Cicer arietinum* are significantly reduced by concurrent cold and ozone stresses and by concurrent salinity and ozone stresses, respectively (Barnes and Davison 1988; Welfare et al. 2002). Likewise, the combination of salinity and ozone stress plays a critical role in decreasing yield of chickpea and rice cultivars. As in the case of diverse concurrent abiotic stresses, plants are faced with the challenge of concurrent biotic stresses and are damaged more severely by the combinations of fungal and bacterial infections than by infections with these pathogens individually. Narasimhane and Venturi (2015) have documented the incidence of different concurrent biotic stresses and their impacts on plant growth and yield. Plants have evolved a perception network that enables them to perceive both biotic and abiotic stressors simultaneously and help them to mitigate the devastating impact of stresses. The effects of abiotic stresses such as drought or salinity may lead to either susceptibility or resistance of plants to biotic stresses such as powdery mildew, rust, and wilt depending on the timing and severity drought and/or salinity stress.

1.1.2 Impact of Stress Combinations on Plant Physiology and Development

The nature of the interactions between the stressors and the duration of stress exposure can lead to a series of effects on the plant growth, development overall yield. The nature of the interactions between stressors also determines the extent of the influence on crop productivity. For instance, abiotic–abiotic stresses such as concurrent drought and heat stress can lead to a greater reduction in the crop yield due to increased soil water evaporation. Mittler (2006) noted that the synergistic effects of drought and heat stress on the physiological aspects of plant growth lead to substantial reduction in crop yield and Stuart et al. (1984) reported that weeds outcompete crops because of their efficient water use ability during concurrent drought and heat stress. These concurrent stresses cause substantial drop in the leaf water potential and transpiration rate, which eventually result in increased leaf and canopy temperature particularly in tropical and subtropical environments (Turner et al. 2001; Simoes-Araujo et al. 2003). Several workers have reported that concurrent stress induced increase in the transpiration rate affects vital physiological processes in plants. Drought and heat stress greatly impact nutrient relations, consequently retarding growth by limiting the nutrient mobility through diffusion, and also lead to reductions in the mass, number and growth of roots (Barber 1995; Wahid et al. 2007; Huang et al. 2012). Drought and heat stress alter photopigments and damage thylakoid membranes, usually leading to either reduced chlorophyll biosynthesis and increased chlorophyll degradation or combined effects of both processes (Anjum et al. 2011; Dutta et al. 2009). The damage due to these concurrent stresses affects light reactions occurring in the thylakoid lumen and light-dependent chemical reactions taking place in the stroma. Camejo et al. (2005) reported that photosystem II is very sensitive to concurrent stresses, and its activity is significantly altered or even reduced to zero under severe heat stress. In the event of concurrent abiotic–biotic stresses such as heat and pathogen stress, heat stress promotes the growth of pathogens and leads to occurrence of a wide range of bacterial and fungal diseases such as wilt in tomato (caused by *Ralstonia solanacearum*), seedling blight and bacterial fruit blotch of cucurbits (caused by *Acidovorax avenae*), and panicle blight in rice (caused by *Burkholderia glumae*) (Kudela 2009). Ladanyi and Horvath (2010) reported that heat stress negatively influences the growth and development of plants but promotes pathogen growth and reproduction. In addition to the promotive effects on pathogen growth, heat stress favors the growth of various vectors, thereby facilitating the occurrence of vector borne diseases. Another example of concurrent biotic–abiotic stresses is salinity and pathogen stress. Salinity influences the virulence of pathogens, the physiology of plants and the activity of microbes in the soil (Triky-Dotan et al. 2005). Daami-Remadi et al. (2009) reported that salinity causes more sporulation in fungi and leads to severe *Fusarium* wilt in tomato.

Concurrent abiotic–abiotic or abiotic–biotic stresses do not necessarily affect plant growth and development negatively, as one stress may enhance plant tolerance to the other stress. Some concurrent stresses counteract the effects of one

another and eventually result in a net neutral or positive effect on plant growth; therefore, the yield is not always reduced. The yield of *Medicago truncatula* (alfalfa) was improved under concurrent drought and ozone stress as compared with individual drought and ozone stress (Puckette et al. 2007) The improved yield was attributed to enhanced tolerance of the alfalfa plants towards this stress combination. Similarly, concurrent salt and heat stresses led to an improved yield of *Solanum lycopersicum* in comparison with individual salt and heat stresses, and attributed this increase in yield to the improved tolerance of tomato plants towards concurrent salt and heat stresses (Rivero et al. 2014).

1.1.3 Complex Interactions in Stress Combinations

Unlike simple interactions in the aforementioned stress combinations, some stress combinations interact in a complex manner and have variable effects on plants. Examples are the effects of concurrent heat–pathogen and concurrent drought–pathogen stress combinations on *T. aestivum* and *Avena sativa* (oats). Coakley et al. (1999) reported that exposure of *T. aestivum* and *A. sativa* to heat stress facilitates growth and reproduction of *Puccinia* spp., thereby increasing their susceptibility to more severe infection. However, in *Cynodon dactylon* (Bermuda grass) the same stress enhances tolerance to a wide range of rust diseases. Heat–pathogen and drought–pathogen interactions are considered agroeconomically important stress combinations. Pautasso et al. (2010) and Garrett et al. (2006) reviewed the influences of concurrent heat and pathogen interactions on plants. Plant interactions with concurrent drought and pathogen stress have been well investigated in cases of abiotic and biotic stress combinations (Carter et al. 2009; Király et al. 2008; Mayek-Perez et al. 2002; McElrone et al. 2003; Ramegowda et al. 2013; Sharma et al. 2007; Wang et al. 2009; Xu et al. 2008). Here we emphasize the effects of abiotic and biotic stress combinations on plants, with special reference to drought and pathogen stress combination.

1.2 Potential Traits for Genotype Screening for Combined Drought and Pathogen Stress Tolerance

1.2.1 Root System Architecture

The spatial configuration of the root system is referred to as the root system architecture (RSA). The genetic control of the RSA and its relationship to increased productivity under stress is well documented in a wide range of crops, especially cereals. Roots play vital roles in crop production by facilitating water and nutrient uptake, forming symbiotic associations with fungi and bacteria, providing

anchorage and serving as storage organs. Additionally, they serve as the main interface for interactions between the plants and various stress factors, and they play a vital role in mitigating the devastating impacts of stress on plant growth and development. The types of interactions that occur between roots and stress factors are determined by the organization and structure of the roots such as their length and density. Resistance to drought stress in rice varieties is linked to increased root length density (RLD) and a wide root diameter. Allah et al. (2010) reported that drought-resistant rice varieties had a greater RLD, which promoted access to the moisture available in the deeper layers of the soil. Under drought stress, maize with a greater RLD and fewer lateral roots showed a higher photosynthetic rate, a more favorable plant water status and greater stomatal conduction than maize with a lesser RLD and more lateral roots. Zhan et al. (2015) reported that the presence of fewer but longer lateral roots led to good use of water available in the deeper layers of the soil by virtue of enhanced rooting, thereby helping the plant to perform better under drought stress (Lynch et al. 2014). The RSA also plays a critical role in reducing pathogen infection in plants. Higginbotham et al. (2004) reported that *T. aestivum* lines with increased root length were less vulnerable to fungal infection with *Pythium debaryanum* and *Pythium ultimum*. Berta et al. (2005) reported that the fungal pathogen *Rhizoctonia solani* decreased root length, root branching and root tips which eventually impaired water absorption from deeper layers of the soil. Hence, it can be concluded that pathogen infection could be reduced to a great extent by increasing the RLD. The RSA plays a key role in crop plant's responses to drought stress and pathogen attack; however, drought and pathogen stress often occur concurrently in field conditions, which leads to greater damage to plants due to complete disruption of the RSA. For instance, in a study of chickpea plants exposed to concurrent drought and infection with the pathogen *Ralstonia solanacearum*, plants that faced progressive drought with 2 and 4 days of *R. solanacearum* infection were categorized as experiencing short-duration (SD) and long-duration (LD) stresses, respectively. The study revealed that SD combined stress reduced the growth and reproduction of the pathogen, but there was no significant change in LD combined stress (Sinha et al. 2017). Dryden and Van Alfen (1984) reported stunted growth of *Phaseolus vulgaris* under concurrent stresses caused by drought and the pathogen *Fusarium solani*. The reduced growth was attributed to root rot caused by the pathogen, thereby limiting acquisition of water from deeper layers of the soil. Concurrent drought and pathogen stress are often reported to decrease plant size, leaf area, hydraulic conductance and photosynthetic and transpiration rates (Pennypacker et al. 1991; Abd El-Rahim et al. 1998; Choi et al. 2013).

The timing of pathogen attacks and the onset of drought affect plant growth in different ways, as seen in *S. lycopersicum* infected with *Phytophthora parasitica*. A pathogen attack during drought stress resulted in greater damage as evidenced by decreased root numbers and root mass, with a greater proportion of brown roots and lower fresh weight than those seen with a pathogen attack followed by drought stress. Schroth and Hildebrand (1964) and Duniway (1977) also reported that root rot disease is more severe in plants exposed to concurrent drought and pathogen

stress. They attributed the severity of infection to drought-induced increased release of root exudates such as alanine, proline, pentose, and glucose, which serve as nutrients for the growth of soilborne pathogens. Apart from increased exudate release, pathogens also induce changes in the composition of root exudates, and this has been reported in tomato roots infected with *Fusarium oxysporum*. The pathogen attack induced greater release of succinic acid and restricted the release of citric acid, whereas in uninfected plants, such a trend in the release of exudates was not found (Kamilova et al. 2006).

Several researchers have reported contradictory findings of no correlation between drought and the severity of pathogen infection. Balota et al. (2005) found that *Gaeumannomyces graminis* infection in *Triticum* had similar effects under low and severe drought stresses. Likewise, infection of *T. aestivum* cultivars with *Pythium irregulare* and *R. solani* did not result in any change in root lesions under drought stress versus well-watered conditions (Aldahadha 2012). The RLD gets affected and that impairs water acquisition under combined drought and pathogen stress. The RLD is high in plants that show tolerance to concurrent drought and pathogen stress. Taking the vital role of the RLD into consideration, these traits offer a basis for screening for varieties with tolerance to combined drought and pathogen stress.

Modern genetic tools have identified quantitative trait loci (QTLs) linked to the RSA under drought stress (Comas et al. 2013). For instance, one QTL known as root-abscisic acid 1 (ABA1) is linked to root branching and root mass (Giuliani et al. 2005). While working on *Arabidopsis thaliana*, Fitz Gerald et al. (2006) and Xiong et al. (2006) reported another QTL that was associated with abscisic acid–stimulated inhibition of lateral root growth. Therefore, to accomplish the development of drought-resistant and pathogen-resistant plants, a broader study is needed to screen QTLs linked to effective and efficient RSA.

1.2.2 Leaf Pubescence

Under drought or normal conditions the transpiration rate plays a central role in the plant response to a stress stimulus. The traits that affect the rate of transpiration include leaf characteristics such as the leaf area, root-to-leaf ratio, leaf orientation, leaf shape, leaf thickness, and distribution of stomata. Among these, the important factors are the leaf surface characteristics (pubescence/glabrousness). The presence and pattern of hairs (trichomes) on the leaf surface and their density are controlled by both the genotype and the habitat of the plants. Trichomes are modified epidermal cells, which may be branched or unbranched, and glandular or nonglandular, depending on the plant species. Plants show wide variations in the density and pattern of trichomes as a response to mitigate the impacts of combined drought and pathogen stress (Ehleringer et al. 1976; Wagner 1991; Wagner et al. 2004). The trichomes facilitate foliar absorption of water and play a vital role in maintaining leaf hydration in plants found in semiarid climates. In *Arabidopsis* a drought tolerance

mutant named cap binding protein 20 (*cbp20*) revealed more trichomes and lower stomatal conductance than control plants (Papp et al. 2004; Jäger et al. 2011). Research on *Phlomis fruticosa* (Jerusalem sage) and *Hedera helix* (ivy) exposed to drought stress revealed that they maintain a low water potential by absorbing dew droplets via their trichomes, unlike plants without trichomes (Grammatikopoulos and Manetas 1994). Additionally, the photosynthetic rate of pubescent leaves was greater than that of glabrous leaves under drought conditions (Grammatikopoulos and Manetas 1994). Roy et al. (1999) reported that *Sinapis arvensis* (wild mustard) subjected to drought stress produced more trichomes than unstressed plants.

Lai et al. (2000) reported that glandular trichomes also resist the spread of pathogen infection by releasing oxidative enzymes, as is evident in *Solanum tuberosum* infected with *Phytophthora infestans*. Furthermore, trichomes reduce the relative humidity of the leaf surface, thereby making the conditions unfavorable for fungal spore germination (Lai et al. 2000). Secretion of T-phylloplanins from the glandular trichomes of tobacco inhibited the growth and reproduction of *Peronospora tabacina* (the causal agent of blue mold disease) in comparison with mock-inoculated plants (Kroumova et al. 2007; Nguyen et al. 2016). It was concluded that trichomes can also prevent the spread of infection by release of antifungal components. Armstrong-Cho and Gossen (2005) reported that trichome exudates in chickpea are capable of preventing the spread of infection with *Ascochyta rabiei* (the causal agent of ascochyta blight). The inhibition of the growth and reproduction of *A. rabiei* was found to be exudate concentration dependent, and a lower concentration promoted the infection. The number of nonglandular trichomes was found to be increased in *Hordeum vulgare* exposed to concurrent drought and pathogen stress, in comparison with control plants (Liu and Liu 2016). Furthermore, it can be concluded that concurrent drought and pathogen stress tolerance is directly correlated with the number and kind of trichomes present all over the leaf surface. Ehleringer et al. (1976) stated that both glandular and nonglandular trichomes release antimicrobial components, which thereby serve as the first line of defense against pathogens. Monier and Lindow (2003) reported contradictory findings and reported that trichomes promoted the growth and reproduction of *Pseudomonas syringae*. They attributed this to the retention of water by the trichomes and suggested that exudates released from the broken cuticle at the base of the trichomes might favor microbial growth. Calo et al. (2006) reported that in *A. thaliana*, a mutant designated as *gl1* (*GLABROUS1*) had lower trichome density and increased resistance to *Botrytis cinerea*, whereas another mutant designated as *try* (*TRYPTYCHON*) had higher trichome density and decreased resistance.

Further studies need to be undertaken to fully understand the role of trichomes in pathogen infection. Under concurrent drought and pathogen stress, the roles of glandular trichomes and their exudates in cases where trichomes enhance pathogen growth need to be studied. Gene-mapping studies have screened and isolated leaf pubescence–linked QTLs in many plants, including *Gossypium hirsutum* and *A. thaliana* (Lacape and Nguyen 2005; Bloomer et al. 2014). It can be assumed that increased numbers of trichomes play a critical role in enhancing the tolerance to concurrent drought and pathogen stress, and trichomes can be considered a

potential morphophysiological trait conferring tolerance to this stress combination. Isolation of QTLs that govern the number, density, and antimicrobial exudates of trichomes can enable plant breeders to create varieties with better tolerance to concurrent abiotic–biotic stresses. Moreover, it is useful to explore the genes and biochemical pathways that regulate the density and secretions of trichomes, which can be suitably modified to confer tolerance to combined stresses.

1.2.3 Leaf Water Potential and Leaf Turgidity

Under concurrent drought and pathogen stress, plants reveal wide variations in their leaf water potential and leaf turgidity which could be attributable to increases in hydraulic resistance and cell turgor loss (Paul and Ayres 1984; Yan et al. 2017). An alteration in the leaf water potential is directly correlated with soil moisture and is also influenced by pathogen stress, which can disrupt or even devastate the plant's vascular system. Concurrent drought and pathogen stress negatively affect the traits that play a role in maintenance of the leaf water potential and leaf turgidity—for instance, stomatal closure in response to drought stress reported by several workers. Some pathogens may decrease the plant water content even under sufficient soil moisture conditions, as seen in *P. vulgaris* infected with *Uromyces phaseoli* (the causal agent of leaf rust), which releases toxins that inhibit stomatal closure and lead to increased water loss. This further reduces the leaf water potential and leaf turgidity of plants under drought stress (Duniway and Durbin 1971), which indicates that pathogen attack can influence drought tolerance. McElrone et al. (2003) reported that the leaf water potential and leaf turgidity can be considered a physiological parameter for evaluation of the plant water status under concurrent stresses. They investigated the influences of separate and concurrent stresses caused by drought and the pathogen *Xylella fastidiosa* (the causal agent of bacterial leaf scorch) on the leaf water potential of Virginia creeper (*Parthenocissus quinquefolia*). A low water potential and less leaf turgidity was found in plants exposed to these stresses concurrently, causing more severe scorch symptoms than those seen in plants that faced separate drought and pathogen stress. The decreased hydraulic conductance and increased embolism in response to infection could be attributable to low water potential less leaf turgidity. Likewise, Burman and Lodha (1996), while studying the impacts of concurrent drought and *M. phaseolina* stress in cowpea (*Vigna unguiculata*), found drastic decreases in the leaf water potential, leaf turgidity, and transpiration rate under combined stress. Similarly, Paul and Ayres (1984) reported a decreased leaf water potential in *Senecio vulgaris* (groundsel) subjected to concurrent drought and infection with *Puccinia lagenophorae* (the causal agent of rust). They attributed the reduced leaf water potential to cuticle breakdown stimulated by the infection and its subsequent sporulation. Similarly, Mayek-Perez et al. (2002) reported a high transpiration rate, reduced water potential and low stomatal resistance in *P. vulgaris* subjected to simultaneous drought and *M. phaseolina* stress. Drought stress caused the plants to synthesize carbohydrates,

which promoted the growth and reproduction of *M. phaseolina*. Moreover, it was found that resistant varieties maintained a higher leaf water potential than susceptible varieties. Contradictory results were reported by Pennypacker et al. (1991) in alfalfa exposed to concurrent drought and *Verticillium albo-atrum* (the causal agent of wilt stress), revealing a high leaf water potential than that seen in drought-stressed plants. Hence, it can be concluded that the impacts of concurrent drought and pathogen stress may have different influences on the leaf water potential and leaf turgidity depending on the type of plant and the type of pathogen.

The QTLs that govern the regulation of the leaf water potential have been identified in several plants. Bernier et al. (2009) and Shamsudin et al. (2016) identified a QTL in rice plants, designated as *qDTY12.1*, that regulates the leaf water potential under drought stress. Identification of QTLs associated with the xylem diameter and xylem pit anatomy can be used to explore molecular pathways and provide greater understanding of the mechanisms that confer tolerance to concurrent drought and pathogen infection. Pouzoulet et al. (2014) reported that xylem vessel dimensions play a vital role in conferring tolerance to vascular pathogen infection. *V. vinifera* genotypes with a smaller xylem diameter were found to be less affected by fungal vascular wilt pathogens. Hence, the plant water potential can be used as a potential morphophysiological trait to screen plants for resistance to concurrent drought and pathogen infection.

1.2.4 Cuticular Wax and Composition of Cuticlar Layer

Cuticular wax and composition of cuticlar layer is of paramount importance in conferring tolerance to concurrent drought stress and pathogen invasion. Kim et al. (2007) reported that *Sesamum indicum* (sesame) exposed to drought stress produced higher-density cuticular wax than unstressed plants. In response to these combined stresses, plants show wide variations in cuticular wax composition (Marcell and Beattie 2002; Kosma et al. 2009). The cuticular layer serves as a physical barrier to pathogen infection as it is hydrophobic in nature and lacks any moisture content (Martin 1964). Several workers have documented the vital role of the cuticular layer in conferring resistance to drought and pathogen stress. Kosma et al. (2009) reported that exposure of *Arabidopsis* plants to drought stress induced an increase in the concentration of the cuticular wax components, resulting in increased wax deposition in stressed plants. Hameed et al. (2002) reported that the thickness of the cuticular layer is determined by drought stress, and it can also determine the resistance to drought stress, as observed in drought-resistant *T. aestivum* plants, which possessed a thicker cuticle than susceptible plants. Marcell and Beattie (2002) subjected control and glossy mutants of *Zea mays* (*gl4*) to *Clavibacter michiganensis* (the causal agent of leaf blight and Goss's wilt in maize). They found that control plants were less affected, with fewer bacterial colonies present on their leaf surfaces than on those of the *gl4* mutants, which exhibited a thin cuticular layer due to a modified wax biosynthetic pathway. The greater sporulation of the pathogen may

have been attributable to increased nutrient and water exudation through the weak cuticular layer, eventually favoring greater pathogen growth in the *gl4* mutants. Jenks et al. (1994), while working on mutants of *S. bicolor*, reported that bloomless (*bm*) mutants exhibited a thin cuticular layer and were more susceptible to infection with *Setosphaeria turcica* (the causal agent of leaf blight) than control plants. Furthermore, the transpiration rate was higher in the *bm* mutant plants than in the control plants. This apparently reflects the fact that the cuticular wax thickness can be employed to identify plants tolerant to *Exserohilum turcicum*. However, the importance of cuticular wax under concurrent stresses is yet to be studied. A detailed study of the pathways that alter the structure and composition of the cuticle layer may be useful in exploring targets that can be manipulated to provide plants with enhanced resistance to concurrent drought and pathogen stress. In rice plants, Srinivasan et al. (2008) have identified a QTL on chromosome 8 for epicuticular wax, the leaf transpiration rate, and the harvest index, colocated with QTLs associated with shoot- and root-related drought tolerance traits. Considering the significance of cuticular wax and composition of cuticular layer in conferring tolerance to pathogen invasion, isolation of QTLs associated with wax content and disease tolerance need to pay a wider attention. Therefore, cuticular wax and composition of cuticular layer may be considered a potential trait that can be used to screen plants for tolerance to concurrent drought and pathogen infection.

1.2.5 Canopy Temperature

Tolerance to drought and pathogen stress can be evaluated by measuring the canopy temperature (Gonzalez-Dugo et al. 2005). In response to concurrent drought and pathogen infection, plants alter their transpiration rate, thereby changing their canopy temperature to sustain growth. Under drought and pathogen stress the canopy temperature varies between leaves, as stress-induced drooping and curling of leaves cause differences in reflection of radiation (Jackson 1986). The canopy temperature plays a major role in plant growth under drought stress, as it has been observed that wheat plants under drought stress have a higher canopy temperature and a lower yield than well-watered plants (Blum et al. 1989). Moreover, it was reported that plants that had a lower canopy temperature were drought resistant, whereas plants with a higher canopy temperature were susceptible to drought stress (Blum et al. 1989). Plants that maintain a high canopy temperature under drought stress conditions have a lower plant water status and thus are less adapted to drought stress (Blum 2009). The significance of the canopy temperature in preventing pathogen infection was also reported by Eyal and Blum (1989). In comparison with control plants, the canopy temperature of wheat plants infected with *Mycosphaerella graminicola* (the causal agent of *Septoria tritici* blotch) was high, and the increase in canopy temperature was directly linked to the severity of the disease. The canopy temperature of *T. aestivum* plants infected with *M. graminicola* could be positively correlated with the occurrence of the disease, as infected plants had a higher

canopy temperature. The rise in canopy temperature could be attributable to cuticular layer damage caused by pathogen invasion. Therefore, assessment of the canopy temperature could be helpful in identifying infected and uninfected plants (Eyal and Blum 1989). Pinter et al. (1979) and Dow et al. (1988) studied alterations in the canopy temperature in *Beta vulgaris* (sugar beet) subjected to concurrent drought and pathogen infection. They reported that sugar beet has a high canopy temperature under concurrent drought and infection with *Pythium aphanidermatum* (the causal agent of root rot). The sudden rise in the canopy temperature could be attributable to pathogen-induced root damage, hampering water uptake and causing a reduction in the plant water potential. Likewise, *Cucumis sativus* (cucumber) infected with the pathogen *Pseudoperonospora cubensis* (the causal agent of downy mildew) showed a higher canopy temperature than control plants (Chaerle et al. 2006). Pinter et al. (1979) reported a raised canopy temperature in *Gossypium* spp. infected with *Phymatotrichum omnivorum* (the causal agent of *Phymatotrichum* root rot) under drought stress. Similarly, under concurrent drought and infection with *M. phaseolina* (the causal agent of charcoal rot infection), a raised leaf temperature and reduced stomatal resistance were noted in *P. vulgaris* (Mayek-Perez et al. 2002). Hence, as the canopy temperature shows significant variations under concurrent drought and pathogen infection, it can be considered a potential trait for evaluation of the concurrent drought and pathogen tolerance of plants. Infrared thermometers can be employed for measurement of the canopy temperature; thereby, screening for plant tolerance to concurrent drought and pathogen infection can be done.

1.3 Role of Genomics in Developing Crops with Combined Drought and Pathogen Stress Tolerance

A few important molecular studies have recently been employed to elucidate the molecular responses of plants to combined drought and pathogen stress. These studies have not only shed light on plant defense mechanisms against combined stresses but also revealed some potential candidates for improvement of plant tolerance to combined stresses. Some of the important candidate genes identified so far are methionine gamma lyase (*AtMGL*, a methionine homeostasis gene), rapid alkalinization factor-like 8 (*AtRALFL8*, involved in cell wall remodeling), and azelaic acid induced 1 (*AZI1*, which functions in systemic plant immunity) (Atkinson et al. 2013). Tolerance to combined drought and pathogen stress is also contributed by genes involved in cross talk between the drought-associated and pathogen infection–associated signaling pathways. The roles of proline and polyamine metabolism in combined drought and pathogen stress tolerance in *A. thaliana* and *V. vinifera* have also been indicated by some studies (Hatmi et al. 2015; Gupta et al. 2016). The identified candidate genes can be suitably modulated to confer enhanced tolerance to these combined stresses. The modification can be done by genome editing using tools such as the CRISPR/Cas9 [clustered regularly interspaced short palindromic

repeats and CRISPR-associated protein 9] system. CRISPR/Cas9 can also be used to modulate the transcription of the genes of interest by guiding catalytically inactive dead Cas9 (dCas9) or dCas9 fused with transcriptional repressors/activators to the promoter of a gene. Further research in this direction using the different functional genomic approaches can thus help to reveal the responses of plants to combined drought and pathogen stress.

1.4 Conclusion and Future Perspectives

Plants grown under field conditions face a combination of different abiotic and biotic stresses and to mitigate the effects plats have evolved complex signalling pathways. The interactions between these stresses and their impacts on plants have been discussed here. The interactions between the two different types of stress conditions may either negatively or positively affect plant growth. For example, a coexisting drought can modulate the interaction of different pathogens and plants differently, leading to either suppression of pathogen growth or an increase in it. Therefore, it becomes very important to study the interaction between the two different types of stresses in order to better understand the net impact of stress combinations on plants. Several important diseases such as dry root rot, powdery mildew, and charcoal rot are significantly affected by concurrent drought conditions, and identification and development of superior cultivars can be done if a mechanistic understanding of the interactions between pathogen and drought stress is attained. Strategies for improving crop performance under combined drought and pathogen stress require deeper understanding. Attempts to understand the interactions have already commenced in the form of transcriptomic studies. Well-designed experiments involving simultaneous drought and pathogen stress on plants have also been undertaken, revealing some aspects of drought–pathogen interactions (Gupta et al. 2016; Sinha et al. 2016). Plant genotypes can be screened for traits such as their root system architecture, leaf water potential, leaf turgidity, leaf pubescence, and leaf cuticular waxes for identification of superior germplasm lines. To vividly assess the effects of different stress combinations on plants, it is imperative to design experiments that can reveal different aspects of interactions between the two different types of stresses. A well-considered stress imposition protocol that is not very different from stresses occurring under field conditions, complemented by relevant physiological assays and the recently evolved genomic tools, can help uncover the responses of plants to stress combinations. Understanding obtained from studies on plant responses to combined drought and pathogen stresses can be utilized by breeders and field pathologists to better analyze the performance of tolerant genotypes. Further development of crop simulation models involving a combination of drought and pathogen stress can help in disease forecasting in places where concurrence of the two stresses is prevalent. Thus, integrative efforts made by crop modeling experts, agronomists, field pathologists, breeders, physiologists, and molecular biologists can efficiently lead to development of combined-stress-tolerant crops that can perform well under field conditions.

References

Abd El-Rahim MF, Fahmy GM, Fahmy ZM (1998) Alterations in transpiration and stem vascular tissues of two maize cultivars under conditions of water stress and late wilt disease. Plant Pathol 47:216–223. https://doi.org/10.1046/j.1365-3059.1998.00211.x

Ahmad B, Raina A, Naikoo MI, Khan S (2019) Role of methyl jasmonates in salt stress tolerance in crop plants. In: Plant Signalling Molecules (Eds Khan MIR, Reddy PS, Ferrante A, Khan NA). Woodhead Publishing, Elsevier, Duxford, United Kingdom pp. 371–384. https://doi.org/10.1016/B978-0-12-816451-8.00023-X

Aldahadha AMA (2012) Effect of root diseases and drought on water use efficiency of wheat. Doctoral thesis, University of New England, Armidale

Allah AA, Shimaa A, Zayed B, Gohary AE (2010) The role of root system traits in the drought tolerance of rice (*Oryza sativa* L.). Int J Agric Biol Sci 1:83–87

Anjum SA, Wang LC, Farooq M, Hussain M, Xue LL, Zou CM (2011) Brassinolide application improves the drought tolerance in maize through modulation of enzymatic antioxidants and leaf gas exchange. J Agron Crop Sci 197:177–185. https://doi.org/10.1111/j.1439-037X.2010.00459.x

Armstrong-Cho C, Gossen BD (2005) Impact of glandular hair exudates on infection of chickpea by *Ascochyta rabiei*. Can J Bot 83:22–27. https://doi.org/10.1139/b04-147

Atkinson NJ, Lilley CJ, Urwin PE (2013) Identification of genes involved in the response to simultaneous biotic and abiotic stress. Plant Physiol 162:2028–2041. https://doi.org/10.1104/pp.113.222372

Balota M, Rush CM, Payne WA, Lazar MD (2005) The effect of take-all disease on gas-exchange rates and biomass in two winter wheat lines with different drought response. Plant Soil 275:337–348. https://doi.org/10.1007/s11104-005-2680-y

Barber SA (1995) Soil nutrient bioavailability: a mechanistic approach., 2nd edn. Wiley, New York

Barnes JD, Davison AW (1988) The influence of ozone on the winter hardiness of Norway Spruce [*Picea abies* (L) Karst]. New Phytol 108:159–166. https://doi.org/10.1111/j.1469-8137.1988.tb03692.x

Belisario A, Maccaroni M, Corazza L, Balmas V, Valier A (2002) Occurrence and etiology of brown apical necrosis on Persian (English) walnut fruit. Plant Dis 86:599–602. https://doi.org/10.1094/PHYTO-01-15-0029-R

Bernier J, Serraj R, Kumar A, Venuprasad R, Impa S, Veereshgowda RP, Oane R, Spaner D, Atlin G (2009) The large-effect drought-resistance QTL *qtl12.1* increases water uptake in upland rice. Field Crop Res 110(2):139–146

Berta G, Sampo S, Gamalero E, Massa N, Lemanceau P (2005) Suppression of *Rhizoctonia* root-rot of tomato by *Glomus mossae* BEG12 and *Pseudomonas fluorescens* A6RI is associated with their effect on the pathogen growth and on the root morphogenesis. Eur J Plant Pathol 111:279–288. https://doi.org/10.1007/s10658-004-4585-7

Bloomer RH, Lloyd AM, Symonds VV (2014) The genetic architecture of constitutive and induced trichome density in two new recombinant inbred line populations of *Arabidopsis thaliana*: interactive plasticity, epistasis, and bidirectional leaf damage response. BMC Plant Biol 14:119. https://doi.org/10.1186/1471-2229-14-11

Blum A (2009) Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. Field Crop Res 112:119–123. https://doi.org/10.1016/j.fcr.2009.03.009

Blum A, Shpiler L, Golan G, Mayer J (1989) Yield stability and canopy temperature of wheat genotypes under drought-stress. Field Crop Res 22:289–296. https://doi.org/10.1016/0378-4290(89)90028-2

Brown JKM, Hovmoller MS (2002) Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. Science 297:537–541

Burman U, Lodha S (1996) *Macrophomina phaseolina* induced changes in plant water relations of resistant and susceptible cowpea genotypes. Indian Phytopathol 49:254–259

Calo L, García I, Gotor C, Romero LC (2006) Leaf hairs influence phytopathogenic fungus infection and confer an increased resistance when expressing a *Trichoderma* α-1,3-glucanase. J Exp Bot 57:3911–3920. https://doi.org/10.1093/jxb/erl155

Camejo D, Rodriguez P, Morales MA, Dell'amico JM, Torrecillas A, Alarcon JJ (2005) High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. J Plant Physiol 162:281–289. https://doi.org/10.1016/j.jplph.2004.07.014

Carter AH, Chen XM, Garland-Campbell K, Kidwell KK (2009) Identifying QTL for high temperature adult-plant resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in the spring wheat (*Triticum aestivum* L.) cultivar 'Louise'. Theor Appl Genet 119:1119–1128

Choi HK, Alberto I, Francisco GS, Douglas C (2013) Water deficit modulates the response of *Vitis vinifera* to the Pierce's disease pathogen *Xylella fastidiosa*. Mol Plant-Microbe Interact 26:1–46. https://doi.org/10.1094/MPMI-09-12-0217-R

Choudhary A, Pandey P, Senthil-Kumar M (2016) Tailored responses to simultaneous drought stress and pathogen infection in plants. In: Hossain MA, Wani SH, Bhattacharjee S, Burritt DJ, Tran L-SP (eds) Drought stress tolerance in plants, vol Vol 1. Springer, Cham, pp 427–443. https://doi.org/10.1007/978-3-319-28899-4_18

Coakley SM, Scherm H, Chakraborty S (1999) Climate change and plant disease management. Annu Rev Phytopathol 37:399–426. https://doi.org/10.1146/annurev.phyto.37.1.399

Comas LH, Becker SR, Cruz VMV, Byrne PF, Dierig DA (2013) Root traits contributing to plant productivity under drought. Front Plant Sci 4:442. https://doi.org/10.3389/fpls.2013.00442

Daami-Remadi M, Souissi A, Oun HB, Mansour M, Nasraoui B (2009) Salinity effects on *Fusarium* wilt severity and tomato growth. Dyn Soil Dyn Plant 3:61–69

Daryanto S, Wang L, Jacinthe PA (2016) Global synthesis of drought effects on maize and wheat production. PLoS One 11:e0156362. https://doi.org/10.1371/journal.pone.0156362

Dow RL, Powell NL, Porter DM (1988) Effects of modification of the plant canopy environment on *Sclerotinia* blight of peanut. Peanut Sci 15:1–5. https://doi.org/10.3146/i0095-3679-15-1-1

Dryden P, Van Alfen NK (1984) Soil moisture, root system density, and infection of roots of pinto beans by *Fusarium solani* f. sp. *phaseoli* under dryland conditions. Phytopathology 74:132–135. https://doi.org/10.1094/Phyto-74-132

Duniway J, Durbin R (1971) Detrimental effect of rust infection on the water relations of bean. Plant Physiol 48(1):69–72

Duniway JM (1977) Predisposing effect of water stress on the severity of Phytophthora root rot in safflower. Phytopathology 67:884–889. https://doi.org/10.1094/Phyto-67-884

Dutta S, Mohanty S, Tripathy BC (2009) Role of temperature stress on chloroplast biogenesis and protein import in pea. Plant Physiol 150:1050–1061. https://doi.org/10.1104/pp.109.137265

Ehleringer J, Björkman O, Mooney HA (1976) Leaf pubescence: effects on absorptance and photosynthesis in a desert shrub. Science 192(4237):376–377

Eyal Z, Blum A (1969) Canopy temperature as a correlative measure for assessing host response to *Septria tritici* blotch of wheat. Plant Dis 73:468–471. https://doi.org/10.1094/PD-73-0468

Farooq M, Gogoi N, Barthakur S, Baroowa B, Bharadwaj N, Alghamdi SS, Siddique KH (2017) Drought stress in grain legumes during reproduction and grain filling. J Agron Crop Sci 203(2):81–102

Fitz Gerald JN, Lehti-Shiu MD, Ingram PA, Deak KI, Biesiada T, Malamy JE (2006) Identification of quantitative trait loci that regulate *Arabidopsis* root system size and plasticity. Genetics 172:485–498. https://doi.org/10.1534/genetics.105.047555

Garrett KA, Dendy SP, Frank EE, Rouse MN, Travers SE (2006) Climate change effects on plant disease: genomes to ecosystems. Annu Rev Phytopathol 44:489–509. https://doi.org/10.1146/annurev.phyto.44.070505.143420

Giuliani S, Sanguineti MC, Tuberosa R, Bellotti M, Salvi S, Landi P (2005) *Root-ABA1*, a major constitutive QTL, affects maize root architecture and leaf ABA concentration at different water regimes. J Exp Bot 56:3061–3070. https://doi.org/10.1093/jxb/eri303

Gonzalez-Dugo MP, Moran MS, Mateos L, Bryant R (2005) Canopy temperature variability as an indicator of crop water stress severity. Irrig Sci 24:233–240. https://doi.org/10.1007/s00271-005-0022-8

Goudarzi S, Banihashemi Z, Maftoun M (2011) Effect of salt and water stress on root infection by *Macrophomina phaseolina* and ion composition in shoot in sorghum. Iran J Plant Pathol 47:69–83

Grammatikopoulos G, Manetas Y (1994) Direct absorption of water by hairy leaves of *Phlomis fruticosa* and its contribution to drought avoidance. Can J Bot 72(12):1805–1811

Gupta A, Dixit SK, Senthil-Kumar M (2016) Drought stress predominantly endures *Arabidopsis thaliana* to *Pseudomonas syringae* infection. Front Plant Sci 7:808

Hameed M, Mansoor U, Ashraf M, Rao AUR (2002) Variation in leaf anatomy in wheat germplasm from varying drought-hit habitats. Int J Agric Biol 4:12–16

Hatmi S, Gruau C, Trotel-Aziz P, Villaume S, Rabenoelina F, Baillieul F et al (2015) Drought stress tolerance in grapevine involves activation of polyamine oxidation contributing to improved immune response and low susceptibility to *Botrytis cinerea*. J Exp Bot 66:775–787. https://doi.org/10.1093/jxb/eru436

Higginbotham RW, Paulitz TC, Kidwell KK (2004) Virulence of *Pythium* species isolated from wheat fields in eastern Washington. Plant Dis 88:1021–1026. https://doi.org/10.1094/PDIS.2004.88.9.1021

Huang B, Rachmilevitch S, Xu J (2012) Root carbon and protein metabolism associated with heat tolerance. J Exp Bot 63:3455–3465. https://doi.org/10.1093/jxb/ers06

Jackson RD (1986) Remote sensing of biotic and abiotic plant stress. Annu Rev Phytopathol 24:265–287. https://doi.org/10.1146/annurev.py.24.090186.001405

Jäger K, Fábián A, Tompa G, Deák C, Höhn M, Olmedilla A, Barnabás B, Papp I (2011) New phenotypes of the drought-tolerant *cbp20 Arabidopsis thaliana* mutant have changed epidermal morphology. Plant Biol 13(1):78–84

Jenks MA, Joly RJ, Peters PJ, Rich PJ, Axtell JD, Ashworth EN (1994) Chemically induced cuticle mutation affecting epidermal conductance to water vapor and disease susceptibility in *Sorghum bicolor* (L.) Moench. Plant Physiol 105:1239–1245. https://doi.org/10.1104/pp.105.4.1239

Kamilova F, Kravchenko LV, Shaposhnikov AI, Makarova N, Lugtenberg B (2006) Effects of the tomato pathogen *Fusarium oxysporum* f. sp *radicis-lycopersici* and of the biocontrol bacterium *Pseudomonas fluorescens* WCS365 on the composition of organic acids and sugars in tomato root exudate. Mol Plant Microbe Interact 19:1121–1126. https://doi.org/10.1094/MPMI-19-1121

Kim KS, Park SH, Jenks MA (2007) Changes in leaf cuticular waxes of sesame (*Sesamum indicum* L.) plants exposed to water deficit. J Plant Physiol 164(9):1134–1143

Király L, Hafez YM, Fodor J, Király Z (2008) Suppression of tobacco mosaic virus–induced hypersensitive-type necrotization in tobacco at high temperature is associated with downregulation of NADPH oxidase and superoxide and stimulation of dehydroascorbate reductase. J Gen Virol 89:799–808

Kosma DK, Bourdenx B, Bernard A, Parsons EP, Lü S, Joubès J, Jenks MA (2009) The impact of water deficiency on leaf cuticle lipids of *Arabidopsis*. Plant Physiol 151(4):1918–1929

Krouma A, Shepherd RW, Wagner GJ (2007) Impacts of *T-phylloplanin* gene knockdown and of *Helianthus* and *Datura* phylloplanins on *Peronospora tabacina* spore germination and disease potential. Plant Physiol 144(4):1843–1851

Kutasla V (2009) Potential impact of climate change on geographic distribution of plant pathogenic bacteria in Central Europe. Plant Prot Sci 45:S27–S32

Lacape J-M, Nguyen TB (2005) Mapping quantitative trait loci associated with leaf and stem pubescence in cotton. J Hered 96:441–444. https://doi.org/10.1093/jhered/esi052

Ladanyi M, Horvath L (2010) A review of the potential climate change impact on insect populations—general and agricultural aspects. Appl Ecol Environ Res 8:143–152. https://doi.org/10.15666/aeer/0802_143151

Lai A, Cianciolo V, Chiavarini S, Sonnino A (2000) Effects of glandular trichomes on the development of *Phytophthora infestans* infection in potato (*S. tuberosum*). Euphytica 114:165–174. https://doi.org/10.1023/A:1003924318577

Lamichhane JR, Venturi V (2015) Synergisms between microbial pathogens in plant disease complexes: a growing trend. Front Plant Sci 6:385. https://doi.org/10.3389/fpls.2015.00385
Liu X, Liu C (2016) Effects of drought-stress on *Fusarium* crown rot development in barley. PLoS One 11(12):e0167304
Loreto F, Bongi G (1989) Combined low temperature–high light effect on gas-exchange properties of jojoba leaves. Plant Physiol 91:1580–1585. https://doi.org/10.1104/pp.91.4.1580
Lynch JP, Chimungu JG, Brown KM (2014) Root anatomical phenes associated with water acquisition from drying soil: targets for crop improvement. J Exp Bot 65:6155–6166. https://doi.org/10.1093/jxb/eru162
Mahalingam R (2015) Consideration of combined stress: a crucial paradigm for improving multiple stress tolerance in plants. In: Mahalingam R (ed) Combined stresses in plants. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-319-07899-1_1
Marcell LM, Beattie GA (2002) Effect of leaf surface waxes on leaf colonization by *Pantoea agglomerans* and *Clavibacter michiganensis*. Mol Plant-Microbe Interact 15(12):1236–1244
Maron JL, Crone E (2006) Herbivory: effects on plant abundance, distribution and population growth. Proc R Soc B 273:2575–2584
Maron JL, Kauffman M (2006) Habitat-specific consumer impacts on plant population dynamics. Ecology 87:113–124
Martin JT (1964) Role of cuticle in the defense against plant disease. Annu Rev Phytopathol 2:81–100. https://doi.org/10.1146/annurev.py.02.090164.000501
Mayek-Perez N, Garcia-Espinosa R, Lopez-Castaneda C, Acosta-Gallegos JA, Simpson J (2002) Water relations, histopathology and growth of common bean (*Phaseolus vulgaris* L.) during pathogenesis of *Macrophomina phaseolina* under drought stress. Physiol Mol Plant Pathol 60:185–195. https://doi.org/10.1006/pmpp.2001.0318
McDonald A, Riha S, DiTommasob A, DeGaetanoa A (2009) Climate change and the geography of weed damage: analysis of US maize systems suggests the potential for significant range transformations. Agric Ecosyst Environ 130:131–140. https://doi.org/10.1016/j.agee.2008.12.007
McElrone AJ, Sherald JL, Forseth IN (2001) Interactive effects of water stress and xylem-limited bacterial infection on the water relations of a host vine. J Exp Bot 54(381):419–430
Mittler R (2006) Abiotic stress, the field environment and stress combination. Trends Plant Sci 11:15–19. https://doi.org/10.1016/j.tplants.2005.11.002
Monier JM, Lindow SE (2003) Differential survival of solitary and aggregated bacterial cells promotes aggregate formation on leaf surfaces. Proc Natl Acad Sci U S A 100:15977–15982. https://doi.org/10.1073/pnas.2536560100
Mordecai EA (2011) Pathogen impacts on plant communities: unifying theory, concepts, and empirical work. Ecol Monogr 81:429–441
Narsai R, Wang C, Chen J, Wu J, Shou H, Whelan J (2013) Antagonistic, overlapping and distinct responses to biotic stress in rice (*Oryza sativa*) and interactions with abiotic stress. BMC Genomics 14:93. https://doi.org/10.1186/1471-2164-14-93
Niakoo MH, Dar MI, Raghib F, Jaleel H, Ahmad B, Raina A, Khan FA, Naushin F (2019) Role and regulation of plants phenolics in abiotic stress tolerance: an overview. In: Plant Signalling Molecules (Eds Khan MIR, Reddy PS, Ferrante A, Khan NA). Woodhead Publishing, Elsevier, Oxford, United Kingdom pp. 157–168. https://doi.org/10.1016/B978-0-12-816451-8.00009-5
Nguyen TTX, Dehne H-W, Steiner U (2016) Maize leaf trichomes represent an entry point of infection for *Fusarium* species. Fungal Biol 120(8):895–903
Oerke E, Steiner U, Dehne H, Lindenthal M (2006) Thermal imaging of cucumber leaves affected by downy mildew and environmental conditions. J Exp Bot 57(9):2121–2132
Pandey P, Ramegowda V, Senthil-Kumar M (2015) Shared and unique responses of plants to multiple individual stresses and stress combinations: physiological and molecular mechanisms. Front Plant Sci 6:723
Papp I, Mur L, Dalmadi A, Dulai S, Koncz C (2004) A mutation in the cap binding protein 20 gene confers drought. Plant Mol Biol 55(5):679–686
Patterson DT (1995) Effects of environmental stress on weed/crop interaction. Weed Sci 43:483–490

Paul N, Ayres P (1984) Effects of rust and post-infection drought on photosynthesis, growth and water relations in groundsel. Plant Pathol 33(4):561–569

Pautasso M, Döring TF, Garbelotto M, Pellis L, Jeger MJ (2012) Impacts of climate change on plant diseases—opinions and trends. Eur J Plant Pathol 133:295–313. https://doi.org/10.1007/s10658-012-9936-1

Pennypacker B, Leath K, Hill R (1991) Impact of drought stress on the expression of resistance to *Verticillium albo-atrum* in alfalfa. Phytopathology 81(9):1014–1024

Peters K, Breitsameter L, Gerowitt B (2014) Impact of climate change on weeds in agriculture: a review. Agric Sustain Dev 34:707–721. https://doi.org/10.1007/s13593-014-0245-2

Pinter PJ, Stanghellini ME, Reginato RJ, Idso SB, Jenkins AD, Jackson RD (1979) Remote detection of biological stresses in plants with infrared thermometry. Science 205:585–586. https://doi.org/10.1126/science.2054406.585

Pouzoulet J, Pivovaroff AL, Santiago LS, Rolshausen PE (2014) Can vessel dimension explain tolerance toward fungal vascular wilt diseases in woody plants? Lessons from Dutch elm disease and esca disease in grapevine. Front Plant Sci 5:253. https://doi.org/10.3389/fpls.2014.00253

Prasch CM, Sonnewald U (2013) Simultaneous application of heat, drought, and virus to *Arabidopsis* plants reveals significant shifts in signaling networks. Plant Physiol 162(4):1849–1866. https://doi.org/10.1104/pp.113.221044

Puckette MC, Weng H, Mahalingam R (2007) Physiological and biochemical responses to acute ozone-induced oxidative stress in *Medicago truncatula*. Plant Physiol Biochem 45:70–79. https://doi.org/10.1016/j.plaphy.200612.004

Ramegowda V, Senthil-Kumar M (2015) The interactive effects of simultaneous biotic and abiotic stresses on plants: mechanistic understanding from drought and pathogen combination. J Plant Physiol 176:47–54. https://doi.org/10.1016/j.jplph.201411.008

Ramegowda V, Senthil-Kumar M, Ishiga Y, Kaundal A, Udayakumar M, Mysore KS (2013) Drought stress acclimation imparts tolerance to *Sclerotinia sclerotiorum* and *Pseudomonas syringae* in *Nicotiana benthamiana*. Int J Mol Sci 14:9497–9513

Rivero RM, Mestre TC, Mittler R, Rubio F, García-Sanchez F, Martinez V (2014) The combined effect of salinity and heat reveals a specific physiological, biochemical and molecular response in tomato plants. Plant Cell Environ 37:1059–1073. https://doi.org/10.1111/pce.12199

Roy BA, Stanton ML, Eppley SM (1999) Effects of environmental stress on leaf hair density and consequences for selection. J Evol Biol 12:1089–1103. https://doi.org/10.1046/j.1420-9101.1999.00107.x

Scherm H, Coakley SM (2003) Plant pathogens in a changing world. Australas Plant Pathol 32:157–165. https://doi.org/10.1071/AP03015

Schroth MN, Hildebrand DC (1964) Influence of plant exudates on root-infecting fungi. Annu Rev Phytopathol 2:101–132. https://doi.org/10.1146/annurev.py.02.090164.000533

Shamsudin NAA, Swamy BM, Ratnam W, Cruz MTS, Raman A, Kumar A (2016) Marker assisted pyramiding of drought yield QTLs into a popular Malaysian rice cultivar, MR219. BMC Genet 17(1):30

Sharma RC, Duveiller E, Ortiz-Ferrara G (2007) Progress and challenge towards reducing wheat spot blotch threat in the Eastern Gangetic Plains of South Asia: is climate change already taking its toll? Field Crop Res 103:109–118

Sinnes-Araujo JL, Rumjanek NG, and Margis-Pinheiro M (2003) Small heat shock proteins genes are differentially expressed in distinct varieties of common bean. Braz J Plant Physiol 15:33–41. https://doi.org/10.1590/S1677-04202003000100005

Sinha R, Gupta A, Senthil-Kumar M (2016) Understanding the impact of drought on foliar and xylem invading bacterial pathogen stress in chickpea. Front Plant Sci 7:902. https://doi.org/10.3389/fpls.2016.00902

Sinha R, Gupta A, Senthil-Kumar M (2017) "Concurrent drought stress and vascular pathogen infection induce common and distinct transcriptomic responses in chickpea." Frontiers in Plant Science 8(2017):333

Srinivasan S, Gomez SM, Kumar SS, Ganesh SK, Biji KR, Senthil A et al (2008) QTLs linked to leaf epicuticular wax, physio-morphological and plant production traits under drought

stress in rice (*Oryza sativa* L.). Plant Growth Regul 56:245–256. https://doi.org/10.1007/s10725-008-9304-5

Strauss SY, Zangerl AR (2002) Plant–insect interactions in terrestrial ecosystems. In: Herrera CM, Pellmyr O (eds) Plant–animal interactions: an evolutionary approach. Blackwell Science, Oxford, UK, pp 77–106

Stuart BL, Harrison SK, Abernathy JR, Krieg DR, Wendt CW (1984) The response of cotton (*Gossypium hirsutum*) water relations to smooth pigweed (*Amaranthus hybridus*) competition. Weed Sci 32:126–132

Su L, Dai Z, Li S, Xin H (2015) A novel system for evaluating drought–cold tolerance of grapevines using chlorophyll fluorescence. BMC Plant Biol 15:82. https://doi.org/10.1186/s12870-015-0459-8

Suzuki N, Rivero RM, Shulaev V, Blumwald E, Mittler R (2014) Abiotic and biotic stress combinations. New Phytol 203:32–43. https://doi.org/10.1111/nph.12797

Tanji KK (2002) Salinity in the soil environment. In: Läuchli A, Lüttge U (eds) Salinity: environment—plants—molecules. Springer, Dordrecht, pp 21–51. https://doi.org/10.1007/0-306-48155-3_2

Triky-Dotan S, Yermiyahu U, Katan J, Gamliel A (2005) Development of crown and root rot disease of tomato under irrigation with saline water. Phytopathology 95:1438–1447. https://doi.org/10.1094/PHYTO-95-1438

Turner NC, Wright GC, Siddique KHM (2001) Adaptation of grain legumes (pulses) to water-limited environments. Adv Agron 71:193–231. https://doi.org/10.1016/S0065-2113(01)71015-2

Valerio M, Lovelli S, Perniola M, Di Tommaso T, Ziska L (2013) The role of water availability on weed–crop interactions in processing tomato for southern Italy. Acta Agric Scand Sect B 63:62–68. https://doi.org/10.1080/09064710.2012.715184

Wagner GJ (1991) Secreting glandular trichomes: more than just hairs. Plant Physiol 96(3):675–679

Wagner G, Wang E, Shepherd R (2004) New approaches for studying and exploiting an old protuberance, the plant trichome. Ann Bot 93(1):3–11

Wahid A, Gelani S, Ashraf M, Foolad MR (2007) Heat tolerance in plants: an overview. Environ Exp Bot 61:199–223. https://doi.org/10.1016/j.envexpbot.2007.05.011

Wang W, Vinocur B, Altman A (2003) Plant responses to drought; salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218:1–14

Wang Y, Bao Z, Zhu Y, Hua J (2009) Analysis of temperature modulation of plant defense against biotrophic microbes. Mol Plant-Microbe Interact 22:498–506

Welfare K, Yeo AR, Flowers TJ (2002) Effects of salinity and ozone, individually and in combination, on the growth and ion contents of two chickpea (*Cicer arietinum* L.) varieties. Environ Pollut 120:397–403. https://doi.org/10.1016/S0269-7491(02)00109-4

Xiong L, Wang RG, Mao G, Koczan JM (2006) Identification of drought tolerance determinants by genetic analysis of root response to drought stress and abscisic acid. Plant Physiol 142:1065–1074. https://doi.org/10.1104/pp.106.084632

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

Yan H, Wu L, Filardo F, Yang X, Zhao X, Fu D (2017) Chemical and hydraulic signals regulate stomatal behavior and photosynthetic activity in maize during progressive drought. Acta Physiol Plant 39(6):125

Zhan A, Schneider H, Lynch J (2015) Reduced lateral root branching density improves drought tolerance in maize. Plant Physiol 168:1603–1615. https://doi.org/10.1104/pp.15.00187

Ziska LH, Tomecek MB, Gealy DR (2010) Evaluation of competitive ability between cultivated and red weedy rice as a function of recent and projected increases in atmospheric CO_2. Agron J 102:118–123. https://doi.org/10.2134/agronj2009.0205

Chapter 2
RETRACTED CHAPTER: Cloning of Genes Underlying Quantitative Resistance for Plant Disease Control

P. S. Shanmugavadivel, K. Aravind Kumar, K. R. Soren, and Garima Yadav

2.1 Introduction

Plant diseases are accountable for substantial yield losses in most crop species and pose a threat to global food security and sustainability. Plants fight against pathogen invasion via either qualitative (or vertical or complete) resistance mediated by disease resistance (R) genes, or quantitative (or horizontal or partial) resistance governed by multiple genes or quantitative disease resistance (QDR) genes. Improving crop resistance to pathogens through conventional breeding, marker-assisted breeding (MAB), and transgenic development is an option to manage disease incidence and minimize yield losses. Hence, we need to identify the genes responsible for qualitative as well as quantitative disease resistance (Nelson et al. 2018). Qualitative or complete resistance is often based on major resistance genes encoding cytoplasmic proteins carrying nucleotide-binding and leucine-rich repeat domains (NLR proteins). These NLR proteins directly or indirectly detect the presence of pathogen-derived molecules, called effectors, which are introduced into the host cell by a pathogen and thus facilitate infection (Bent and Mackey 2007). An NLR protein–mediated defense response is activated after effector recognition and often includes a hypersensitive response (HR); rapid, localized programmed cell death at the point of pathogen penetration; and other responses, including ion flux, an oxidative burst, lipid peroxidation, and cell wall fortification (Coll et al. 2011). In other way, QDR is controlled by multiple quantitative trait loci (QTLs)/gene(s), which interact with each other and also with the environment. Resistance mediated by QTLs usually has smaller individual effects than that conferred by R genes, but it is broad-spectrum or

The original version of this chapter was retracted: The retraction note to this chapter is available at https://doi.org/10.1007/978-3-030-20728-1_12

P. S. Shanmugavadivel (✉) · K. Aravind Kumar · K. R. Soren · G. Yadav
Division of Plant Biotechnology, ICAR-Indian Institute of Pulses Research, Kanpur, Uttar Pradesh, India

S. H. Wani (ed.), *Disease Resistance in Crop Plants*,
https://doi.org/10.1007/978-3-030-20728-1_2

non-race-specific resistance, and it is considered a promising alternative to less durable race-specific resistance for crop improvement. However, the mechanisms underlying quantitative disease resistance are thought to be more diverse than those responsible for qualitative disease resistance. Numerous race-specific resistance genes (R) have been deployed by breeders, but each one has had limited durability, presumably because of rapid pathogen evolution. Non-race-specific genes generally have broad-spectrum resistance and are more effective at adult plant stages, providing partial and usually more durable resistance than race-specific genes. The durability of the resistance is dependent on many factors, including the biology, genetics, and evolution of the relevant pathogen. The failure of gene-for-gene resistance traits to provide durable and broad-spectrum resistance has paved the way to the search for genes underlying quantitative resistance in plants (Huard-Chauveau et al. 2013). In the recent past, particularly in the last decade, a few genes responsible for QDR to various pathogens have been cloned and validated successfully in different crop plants, and those genes are elaborated in this chapter.

2.2 Plant Immune Systems at a Glance

A plant contains two major innate immune responses: pathogen-associated molecular pattern (PAMP)–triggered immunity (PTI) (Boller and He 2009) and effector-triggered immunity (ETI) (Jones and Dangl 2006). The PTI response includes activation of mitogen-activated protein kinases (MAPKs), induction of reactive oxygen species (ROS), deposition of callose, and induction of pathogenesis-related (PR) genes. A ROS burst constitutes an early response to pathogen attack by strengthening cell walls through cross-linking of glycoproteins and by activating defense-signaling components. The pathogen delivers effector molecules into plant cells to inhibit the host PTI response and/or to create a favorable host cell environment. Plants have developed intracellular sensors encoded by resistance (R) genes containing a nucleotide-binding site and leucine-rich repeats (NBS-LRRs), which perceive pathogen effectors directly or indirectly, leading to ETI. ETI confers strong resistance against particular pathogens, especially for a particular race, and elicit a hypersensitive response; however, this is not durable, because of the rapid evolution of pathogen effectors. PTI is an important factor in nonhost resistance-the phenomenon whereby whole plants are resistant to most microbial pathogens and this contributes to quantitative resistance.

2.3 Model Explaining Quantitative Disease Resistance

Plant defense responses include plant preformed physical or chemical barriers (e.g., rigid cell walls, presence of cuticles or trichomes, production of toxic or repellent compounds) and immune signaling responses. The immune signaling–mediated resistance mechanism corresponds to a zig-zag model (a two-level defense system)

(Jones and Dangl 2006; Dodds and Rathjen 2010). At the first level, pathogen elicitors called pathogen-associated molecular patterns are perceived by the plant cell surface and transmembrane pattern recognition receptors (PRRs), initiating a signaling cascade leading to PTI, which is efficient against a broad spectrum of pathogens. To overcome PTI, pathogens produce virulence factors called effectors, which can promote effector-triggered susceptibility (ETS) by interfering with the host defense response mechanism. In turn, plant intracellular resistance proteins, known as NLR proteins, specifically recognize the effectors and activate the second ETI level of plant defense. ETI is a strong defense response often associated with a hypersensitive response, characterized by rapid and local cell death. However, this type of qualitative response is generally single pathogen species specific or even strain specific. The zig-zag model is limited largely to describing interactions between hosts and biotrophic pathogens, and is less suitable for understanding host–necrotrophic pathogen interactions. It does not account for all of the complexities of host–pathogen interactions, which lead to a wide range of host immune responses (Pritchard and Birch 2014).

The invasion model describes plant immunity as a surveillance system that continually evolves to detect pathogen invasion, which may be more useful for describing the nuanced layers of plant defense (Cook et al. 2015). In this model, plants recognize invasion patterns (IPs) that are derived from pathogens (such as microbe-associated molecular patterns (MAMPS) or effectors) or endogenous elicitors that result from infection, such as damage-associated molecular patterns (DAMPs). An IP is recognized by IP-triggered receptors (IPTRs). PTI and ETI are viewed less as strictly contrasting responses and instead as continuous immune outputs resulting from variation between different IPs and IPTRs. Such a model accounts for QDR. QDR is less well understood than PTI or ETI. QDR is characterized by a reduction of disease rather than an absence of disease, and shows typical polygenic inheritance.

2.3.1 Importance of Quantitative Disease Resistance

1. ETI produces complete resistance primarily with one R protein; hence, pathogen effector proteins can evolve easily to overcome one R protein and ETI-mediated resistance. In contrast, multiple genes underlie QDR; hence, the evolutionary pressure on pathogens is significantly decreased; therefore, QDR may be a good source of durable resistance.
2. ETI is most effective against biotrophic pathogens. It frequently results in a hypersensitive response to limit biotrophic pathogen growth and colonization, and typically leads to full resistance against these pathogens. However, necrotrophic pathogens feed on dead tissues and exploit this cell death to increase their own virulence. On the other hand, QDR provides an effective means of control of both biotrophic and necrotrophic pathogens.

3. QDR can result from quantitative variation in the components of either PTI or ETI, as well as from completely different mechanisms.
4. Many QDR loci are effective against multiple races of a given pathogen, providing broad-spectrum resistance, or are effective against multiple pathogens. However, QDR loci involved in race- or isolate-specific resistance are becoming increasingly common (Poland et al. 2009; Roux et al. 2014). Isolate-specific QTLs may represent evidence for the minor-gene-for-minor-gene model of QDR, originally conceptualized by Parlevliet and Zadoks (1977).
5. QDR is effective against bacteria, fungi, viruses, and nematodes, as well as pathogens that infect different parts or different developmental stages of the plant.

2.4 Quantitative Disease Resistance Dissection

Linkage analysis, a nested association-mapping (NAM) approach, and genome-wide association studies (GWAS) have been used routinely to identify the genomic loci influencing multiple disease resistance loci, otherwise called QDR loci. A locus identified through this analysis encompasses hundreds of genes and many candidate genes, which makes it very difficult to identify the true causal gene(s). In some cases, multiple linked genes (such as groups of functionally related defense genes involved in secretory processes and cell wall reinforcement) have been shown to underlie a single QDR locus. As for most quantitative traits, genetic dissection of QDR is challenging, and the relationship between phenotypes and molecular mechanisms is not as well understood. However, map-based cloning of resistance-conferring QTLs has proved to be extremely difficult, owing to (i) small genetic effects, (ii) variations in disease severity across different geographical locations and years, and (iii) lack of uniformity in the evaluation of disease symptoms. The durability of disease resistance is very difficult to measure in a short period of time; moreover, evaluation of durability is hampered if the QTLs have different genetic backgrounds. For instance, a resistance QTL may have a more significant effect when introgressed or transformed in a highly susceptible background. Although some of these genes have been applied, their deployment in elite cultivars has been limited because of their close linkage with genes controlling undesirable agricultural traits. For instance, wheat Lr34 lines produce less grain than those without Lr34 (Chen et al. 2016); the recessive barley *mlo* mutant causes early senescence-like leaf chlorosis (Piffanelli et al. 2002). Cloning of QDR loci has proved to be challenging because of the small effect of many QDR loci and the difficulty in consistently phenotyping disease traits across environments. Tremendous progress in the last few years has been achieved in narrowing down mapped QDR loci to the individual gene level.

Table 2.1 lists cloned QDR genes and their molecular mechanisms against different host–pathogen systems.

Table 2.1 Cloned quantitative disease resistance (*QDR*) genes and their molecular mechanisms against different host–pathogen systems

S. No.	Mechanism	Gene	Crop/host	Disease; pathogen	References
1	Pathogen recognition	*Pb1*	Rice	Rice blast; *Magnaporthe oryzae*	Hayashi et al. (2010)
		OsWAK14, *OsWAK91*, *OsWAK92*, and *OsWAK112d*	Rice	Rice blast; *Magnaporthe oryzae*	Delteil et al. (2016)
		RCG1	Maize	Anthracnose stalk rot; *Colletotrichum graminicola*	Broglie et al. (2006, 2011)
		Pi35	Rice	Rice blast; *Magnaporthe oryzae*	Fukuoka et al. (2014)
		RRS1 and *RPS4*	*Arabidopsis*	Black rot; *Xanthomonas campestris* pv. *campestris* race 2	Debieu et al. (2016)
		ZmWAK	Maize	Head smut; *Sporisorium reilianum*	Zuo et al. (2015)
		Htn1	Maize	Northern leaf blight; *Exserohilum turcicum*	Hurni et al. (2015)
		RFO3	*Arabidopsis*	Wilt; *Fusarium oxysporum* f. sp. *matthioli*	Cole and Diener (2013); Diener and Ausubel (2005)
2	Transcriptional response	*C3H12*	Rice	Bacterial blight; *Xanthomonas oryzae* pv. *oryzae*	Deng et al. (2012)
		OsWRKY13	Rice	Blast; *Xanthomonas oryzae* pv. *oryzicola* and *Magnaporthe grisea*	Hu et al. (2008)
3	Signal transduction	*OsMPK6*	Rice	Blight and blast; *Xanthomonas oryzae* pv. *oryzicola* and *Magnaporthe grisea*	Hu et al. (2008)
		RKS1	*Arabidopsis*	Black rot; *Xanthomonas campestris* pv. *campestris*	Roux et al. (2014)
		Yr36 (*WKS1*)	Wheat	Stripe rust; *Puccinia striiformis*	Fu et al. (2009)
		pan1	Maize	Northern leaf blight and Stewart's wilt; *Exserohilum turcicum* and *Pantoea stewartii*	Jamann et al. (2014)
4	Defense response	*Pi21*	Rice	Rice blast; *Magnaporthe oryzae*	Fukuoka et al. (2009)
		Lr34	Wheat	Leaf rust, stripe rust, and powdery mildew; *Puccinia triticina*, *Puccinia striiformis*, and *Blumeria graminis*	Krattinger et al. (2009)

(continued)

Table 2.1 (continued)

S. No.	Mechanism	Gene	Crop/host	Disease; pathogen	References
5	Antimicrobial activity	*Fhb1*	Wheat	*Fusarium* head blight; *Fusarium graminearum*	Rawat et al. (2016)
		OsPAL4	Rice	Bacterial blight, sheath blight, and blast; *Xanthomonas oryzae* pv. *oryzae*, *Rhizoctonia solani*, and *Magnaporthe oryzae*	Tonnessen et al. (2015)
		OsGLP	Rice	Sheath blight and blast; *Rhizoctonia solani* and *Magnaporthe oryzae*	Manosalva et al. (2009)
6	Nutritional restriction	*OsDR8*	Rice	Blight and blast; *Xanthomonas oryzae* pv. *oryzicola* and *Magnaporthe oryzae*	Hu et al. (2008)
		Lr67	Wheat	Leaf rust, stripe rust, stem rust, and powdery mildew; *Puccinia triticina*, *Puccinia striiformis* f. sp. *tritici*, *Puccinia graminis* f. sp. *tritici*, and *Blumeria graminis* f. sp. *tritici*	Moore et al. (2015)
		Rhg4	Soybean	Soybean cyst; *Heterodera glycines* Ichinohe	Liu et al. (2012a, b)
		Rhg1	Soybean	Cyst nematode; *Heterodera glycines* Ichinohe	Cook et al. (2012)
7	Microbial movement	*ZmREM6.3*	Maize	Northern leaf blight; *Exserohilum turcicum*	Jamann et al. (2016)
		ZmCCoAOMT2	Maize	Southern leaf blight, gray leaf spot, and northern leaf blight; *Cochliobolus heterostrophus*, *Cercospora zeae-maydis*, and *Exserohilum turcicum*	Yang et al. (2017a, b)
8	Hormones	*GH3-2*	Rice	Blight and blast; *Xanthomonas oryzae* and *Magnaporthe oryzae*	Fu et al. (2011)
		GH3-8	Rice	Blight and blast; *Xanthomonas oryzae* and *Magnaporthe oryzae*	Hu et al. (2008); Ding et al. (2008)
9	Others	*Camalexin*	*Arabidopsis*	*Plasmodiophora brassicae*	Lemarie et al. (2015)
		POQR	*Arabidopsis*	*Sclerotinia sclerotiorum*	Badet et al. (2017)

2.4.1 *Quantitative Disease Resistance Genes in* Arabidopsis

The *Arabidopsis thaliana* locus *RESISTANCE TO POWDERY MILDEW8* (*RPW8*) contains two naturally polymorphic, dominant R genes—*RPW8.1* and *RPW8.2*—which individually control resistance to a broad range of powdery mildew pathogens. The predicted *RPW8.1* and *RPW8.2* proteins are different from the previously characterized R proteins (NBS-LRR proteins); they induce localized, salicylic acid (SA)–dependent defenses similar to those induced by R genes that control specific resistance (Xiao et al. 2001). *RPW8.2* is induced and specifically targeted to the extrahaustorial membrane (EHM), an enigmatic interfacial membrane believed to be derived from the host cell plasma membrane. There, RPW8.2 activates an SA signaling–dependent defense strategy, which concomitantly enhances the encasement of the haustorial complex and on-site accumulation of H_2O_2, presumably for constraining the haustorium while reducing oxidative damage to the host cell, thus leading to broad-spectrum resistance against diverse races of powdery mildew. Natural mutations that impair either defense activation or EHM targeting of RPW8.2 compromise the efficacy of RPW8.2-mediated resistance (Wang et al. 2009).

Huard-Chauveau et al. (2013) identified *RKS1* (*Resistance related KinaSe 1*) from *QRX3* QTL through map-based cloning and functional validation, which confers broad-spectrum resistance to *Xanthomonas campestris* (*Xc*), a bacterial vascular pathogen of crucifers. *RKS1* has been reported to confer QDR in *A. thaliana* to most but not all races of the bacterial pathogen *X. campestris* pathovar (pv.) *campestris* (*Xcc*). *RKS1* encodes an atypical kinase that mediates a quantitative resistance mechanism in plants by restricting bacterial spread from the infection site. In addition to *RKS1*, Debieu et al. (2016) identified genes (*At5g22540* gene confers resistance to *Xcc12824* (race 2); *RRS1/RPS4* confers resistance to *XccCFBP6943* (race 6)) involved in resistance to *Xc* with strikingly different ranges of specificity, suggesting that QDR to *Xc* involves a complex network integrating multiple response pathways triggered by distinct pathogen molecular determinants.

A. thaliana ecotypes differ in their susceptibility to *Fusarium* wilt diseases. The ecotype Tanguilt-0 (Ty-0) is susceptible to *Fusarium oxysporum* forma specialis (f. sp.) *matthioli*, whereas Columbia-0 (Col-0) is resistant. Diener and Ausubel (2005) mapped *RFO1* loci (*Resistance to Fusarium Oxysporum 1*) from Col-0 accession following map-based cloning, which encodes a novel type of dominant disease resistance protein that confers broad-spectrum resistance to *Fusarium* races. *RFO1* is identical to the *Arabidopsis* gene *WAKL22* (WALL-ASSOCIATED KINASE–LIKE KINASE 22), which encodes a receptor-like kinase that does not contain an extracellular leucine-rich repeat domain. A Col-0 *rfo1* loss-of-function mutant was more susceptible to *F. matthioli*, *F. conglutinans*, and *F. raphani*.

EFR, a PRR from *Arabidopsis*, confers responsiveness to bacterial elongation factor Tu in *Nicotiana benthamiana* and tomato (*Solanum lycopersicum*), making them more resistant to a range of phytopathogenic bacteria from different genera. Heterologous expression of PAMP recognition systems (*EFR*) could be used to

engineer broad-spectrum disease resistance to important bacterial pathogens (Lacombe et al. 2010). A receptor kinase in *Arabidopsis*, *flagellin sensing 2* (*FLS2*), confers recognition of bacterial flagellin (*flg22*) and activates a manifold defense response. Vetter et al. (2012) identified extensive variation in *flg22* perception, most of which results from changes in protein abundance. PRRs such as *Arabidopsis EFR* and rice *Xa21* are taxonomically restricted and are absent from most plant genomes. Schwessinger et al. (2015) demonstrated that heterologous expression of the dicotyledonous PRR *efr* in rice leads to ligand-dependent activation of defense responses. Rice plants expressing *EFR* or the chimeric receptor *EFR::XA21*, containing the EFR ectodomain and the XA21 intracellular domain, sense both *Escherichia coli*–derived and *Xanthomonas oryzae* pv. *oryzae* (*Xoo*)–derived elf18 peptides at subnanomolar concentrations. Treatment of *EFR* and *EFR::XA21* transgenic rice leaf tissue with elf18 leads to MAPK activation, ROS production, and defense gene expression (Schwessinger et al. 2015).

Camalexin, a sulfur-containing, tryptophan-derived secondary metabolite, is considered to be the major phytoalexin involved in biotic responses in *A. thaliana*. Lemarie et al. (2015) studied the possible role of camalexin accumulation in two *Arabidopsis* genotypes with different levels of basal resistance to the compatible eH strain of the clubroot agent *Plasmodiophora brassicae* and found that high levels of clubroot-triggered camalexin biosynthesis play a role in the quantitative control of partial resistance of *Arabidopsis* to clubroot. A *POQR* encodes prolyl-oligopeptidase (POP) in *arabidopsis* is reported to exert QDR against *Sclerotinia sclerotiorum*, agent of the white mold disease. Loss of this gene compromised QDR against *S. sclerotiorum* but not against a bacterial pathogen. The same amino acid changes occurred after independent duplications of *POQR* in ancestors of multiple plant species, including *A. thaliana* and tomato (Badet et al. 2017).

2.4.2 Quantitative Disease Resistance Genes in Rice

Although large numbers of QTLs for bacterial blight and blast resistance have been identified, very few have been cloned on the basis of a map-based cloning approach. All blast resistance genes (called *Pi*) encode NBS-LRR proteins, except for the *Pid2* and *Piz* genes. *Pid2* encodes a b-lectin receptor–like kinase (Chen et al. 2006), and *Pi21* encodes a proline-rich protein containing a metal-binding domain. Though *pi21* confers non-race-specific, durable resistance (Fukuoka et al. 2009), it does not affect the yield or grain quality, making *pi21* a good candidate for marker-assisted selection (MAS), but unfortunately its application is limited by the close linkage of *pi21* to a gene (LOC_Os04g32890) that causes inferior grain quality (Fukuoka et al. 2009). Fukuoka et al. (2014) cloned *Pi35* through map-based cloning and identified multiple functional polymorphisms that allow effective control of the disease. *Pi35* is allelic to *Pish*, which mediates race-specific resistance to blast and encodes a protein containing a NBS-LRR domain. Multiple functional polymorphisms cumulatively enhance resistance, and an amino acid residue in an LRR of *Pi35* is strongly

associated with the gene's mediation of quantitative but consistent broad-spectrum resistance to pathogen isolates in Japan, in contrast to *Pish*, which mediates resistance to only a single isolate. The rice *Xa21* gene confers broad and persistent resistance against *X. oryzae* pv. *oryzae*, which was isolated by positional cloning. The protein of this gene carries both a leucine-rich repeat motif and a serine–threonine kinase–like domain, which suggests a role in cell surface recognition of a pathogen ligand and subsequent activation of an intracellular defense response by phosphorylation of downstream genes (Song et al. 1995). Through phosphorylation and cleavage of its intracellular kinase domain, *Xa21* perceives the presence of *Xoo* and relays the signal to the nucleus through multistep signal cascades involving some key proteins such as *XA21* binding protein 3 (XB3), MAPK5, MAPK12, and transcription factors (TFs) including OsWRKY62 and OsWRKY76 in the nucleus (Peng et al. 2015).

Hu et al. (2008) and Kou et al. (2010) followed a candidate gene strategy that integrates linkage map, expression profile, and functional complementation analyses to identify the genes underlying minor resistance QTLs in rice–*Xoo* and rice–*Magnaporthe grisea*, and to ascertain whether defense-responsive genes are important resources of resistance QTLs in rice systems. Seven such genes—*WRKY13*, *GH3-1*, *GH3-2*, *GH3-8*, *OsDR8*, *NRR* and *MAPK6*—were identified as candidate genes, using this strategy, and are also present in already mapped QTL regions. *OsWRKY13* is a transcription regulator which positively regulates rice resistance to bacterial blight and blast diseases. The *OsWRKY13*-associated disease resistance pathway synergistically interacts via *OsWRKY13* with the glutathione/glutaredoxin system and the flavonoid biosynthesis pathway to monitor redox homeostasis and to putatively enhance the biosynthesis of antimicrobial flavonoid phytoalexins, respectively (Qiu et al. 2008). Overexpression of *OsWRKY13* in a susceptible rice line enhanced rice resistance to *Xoo*, with the lesion area ranging from 24% to 49%, compared with 62% for the susceptible wild type; *OsWRKY13*-overexpressing plants also showed enhanced resistance to *M. grisea*, with the lesion degree ranging from 0 to 3, compared with 4–5 for the susceptible wild type (Qiu et al. 2007). *OsDR8* gene functions upstream of the signal transduction pathway located on chromosome 7, and encodes an enzyme-like protein involved in thiamine biosynthesis, which positively regulates rice resistance to bacterial blight and blast. The expression of *OsDR8* was induced in resistance reactions against different *Xoo* strains and *M. grisea* isolates. *OsDR8*-suppressing plants showed reduced resistance or susceptibility to *Xoo* and *M. grisea*. The exogenous application of thiamine complements the compromised defense of the *OsDR8*-silenced plants (Wang et al. 2006).

Indole-3-acetic acid (IAA), the major form of auxin in rice, helps invaders into plant cells by IAA-induced loosening of the cell wall, a natural protective barrier of plant cells against invaders. *X. oryzae* pv. *oryzae*, *X. oryzae* pv. *oryzicola*, and *M. grisea* secrete IAA, in turn inducing rice to synthesize its own IAA at the infection site. Then IAA induces the production of expansins (cell wall–loosening proteins) and makes rice vulnerable to pathogen entry and colonization. *GH3-2*, a minor resistance QTL, has been shown to be associated with variation in quantitative

resistance to bacterial blight, bacterial streak, and blast in rice. The *GH3-2* gene in rice encodes an IAA–amido synthetase, and GH3-2 positively regulates rice disease resistance by suppressing pathogen-induced accumulation of IAA in rice. Activation of *GH3-2* inactivates IAA by catalyzing the formation of an IAA–amino acid conjugate, which results in the suppression of expansin genes and results in broad-spectrum and partial resistance against *Xoo*, *Xoc*, and *M. grisea* (Fu et al. 2011). Thus, *GH3-2* mediates basal resistance by suppressing pathogen-induced IAA accumulation. Similarly, the *GH3-8* gene contributes to the minor resistance QTLs located on chromosome 7, encoding IAA–amido synthetase, involved in resistance reactions for bacterial blight and blast diseases, and functioning in auxin-dependent development, as well as activating disease resistance in an SA signaling– and jasmonic acid (JA) signaling–independent pathway. This gene activates basal resistance by inhibiting auxin activity/free IAA accumulation, which functions as a virulence factor in pathogen infection. *GH3-8*-overexpressing plants showed enhanced resistance to *Xoo*, with the lesion area ranging from 24% to 54%, compared with 78% in the susceptible wild type (Ding et al. 2008).

OsMPK6 encodes an MAPK, and suppressing or knocking out *OsMPK6* enhanced rice resistance to different races of *Xoo*, with the lesion area ranging from 5% to 37%, compared with 71% measured for the susceptible wild type (Yuan et al. 2007). Characterization of these genes indicates that their products do not directly interact with pathogen effectors in disease resistance, and their roles in defense responses will not be changed by the mutation of pathogens. Modulation of their expression pattern can enhance rice resistance to both bacterial blight and blast, suggesting that the resistance mediated by these genes is non–race specific, durable, and broad spectrum. *OsWRKY13*, *OsDR8*, *GH3-8*, and *OsMPK6* gene products do not directly interact with pathogen effectors in disease resistance. Modulation of the expression of *OsWRKY13*, *OsDR8*, and *OsMPK6* can enhance rice resistance, in a non-race-specific manner to both bacterial blight and blast (Hu et al. 2008). *OsWRKY45* encodes a WRKY-type TF and has at least two alleles—*OsWRKY45-1* and *OsWRKY45-2*—which have ten amino acid differences. *OsWRKY45-1* is a negative regulator of resistance against *Xoo* and *Xoc* (*X. oryzae* pv. *oryzicola*), whereas *OsWRKY45-2* is a positive regulator in rice resistance against the two types of pathogenic bacteria. Nevertheless, both alleles are positive regulators in rice resistance against *M. grisea* (Kou et al. 2010).

The wall-associated kinases (WAKs) function as a positive regulator of fungal disease resistance in several plant species, and *WAK* genes are reportedly often transcriptionally regulated during infection. *OsWAK14*, *OsWAK91*, and *OsWAK92* positively regulate quantitative resistance against blast disease, and *OsWAK112d* is a negative regulator of blast resistance. The transcriptional regulation of the *OsWAK* genes is triggered by chitin and is partially under the control of the chitin receptor *CEBiP*. *OsWAK91* is required for H_2O_2 production and is sufficient to enhance defense gene expression during infection (Delteil et al. 2016). The zinc-finger proteins harboring the motif with three conserved cysteine residues and one histidine residue (CCCH) belong to a large family. One of the rice CCCH-type zinc-finger proteins, C3H12,

containing five typical CX(8)–CX(5)–CX(3)–H zinc-finger motifs, is involved in the rice–*Xoo* interaction. Activation of C3H12 partially enhanced resistance to *Xoo*, accompanied by accumulation of JA, and induced expression of JA signaling genes in rice. In contrast, knockout or suppression of C3H12 resulted in partially increased susceptibility to *Xoo*, accompanied by decreased levels of JA and expression of JA signaling genes in rice (Deng et al. 2012).

Resistance against *M. oryzae* is controlled by both monogenically and polygenically in rice. The resistance to blast disease caused by *M. oryzae* conferred by QTLs in rice lacks a hypersensitive response, yet it restricts the development of lesions. Durable disease resistance (DR) against *M. oryzae* is found in the durably resistant cultivar Owarihatamochi (OW) and is controlled by four QTLs: *pi21*, *Pi34-qBR4-2*, and *qBR12-1* (Fukuoka and Okuno 2001). *Pi21* encodes a proline-rich protein with a putative heavy metal–binding domain, and a putative protein–protein interaction motif confers non-race-specific resistance to blast disease. Wild-type *Pi21* slows the plant's defense responses, which may support optimization of defense mechanisms. The response in resistant *pi21* plants after pathogen attack is not as fast or as strong as the R gene response. This slower induction of defense may be another type of incompleteness that may contribute to the durability of a plant's resistance. Deletions in its proline-rich motif inhibit this slowing and cause susceptibility to disease (Fukuoka et al. 2009). The resistant *pi21* allele is present only in *japonica* rice, which can be transferred to other genotypes through MAS. The candidate genes for *Pi34* encode previously uncharacterized proteins with significantly different amino acid sequences between resistance and susceptible cultivars (Zenbayashi-Sawata et al. 2007). *qBR4-2* is a complex genetic locus including three tightly linked loci: *qBR4-2a*, *qBR4-2b*, and *qBR4-2c*. *qBR4-2a* and *qBR4-2b* appear to encode proteins with a putative nucleotide-binding site and leucine-rich repeats. The effect of *qBR4-2c* was smallest among the three, but its combination with the donor alleles of *qBR4-2a* and *qBR4-2b* significantly enhanced blast resistance (Fukuoka et al. 2012). *Pi63* is allelic to *qBR4-2b*, which encodes an NBS-LRR protein whose transcript expression level is associated with the level of resistance (Xu et al. 2014). A novel allele, *bsr-d1*, is involved in broad-spectrum, durable resistance to *M. oryzae* in the Digu rice variety, with high resistance to a broad spectrum of *M. oryzae* races. *Bsr-d1* encodes a C_2H_2-type TF which is directly regulated by an *MYB* family TF. *M. oryzae* induces *Bsr-d1* expression in susceptible rice cultivars to suppress host immunity, facilitating its pathogenesis, but not in resistant cultivars. These two TFs regulate expression of H_2O_2-degradation enzyme–coding genes (specifically, the two peroxidase genes Os07g04470 and Os10g39170) to accomplish resistance to *M. oryzae*, constituting a novel mechanism employed in rice blast resistance. Thus, *bsr-d1* likely confers durable, broad-spectrum resistance in rice by regulating peroxide accumulation (Li et al. 2017). The rice *bsr-k1* (broad-spectrum resistance Kitaake-1) mutant, which confers broad-spectrum resistance against *M. oryzae* and *X. oryzae* pv. *oryzae* with no major penalty in terms of key agronomic traits, has been identified through map-based cloning (Zhou et al. 2018). *Bsr-k1* encodes a tetratricopeptide repeat (TPR)–containing protein, which regulates immunity-related genes. This protein

especially binds to messenger RNAs (mRNAs) of multiple *OsPAL* (*OsPAL1–7*) genes and suppresses *OsPAL1* mRNA accumulation, thereby promoting *OsPAL* turnover. Loss of function of the *Bsr-k1* gene leads to accumulation of *OsPAL1–7* mRNAs in the bsr-k1 mutant and confers enhanced resistance against diverse races of *M. oryzae* and *Xoo*. Furthermore, overexpression of *OsPAL1* in wild-type rice TP309 confers resistance to *M. oryzae*, supporting the role of *OsPAL1* in disease resistance (Zhou et al. 2018). Deng et al. (2017) mapped the rice *Pigm* locus, which contains a cluster of 13 genes, including three genes encoding NLR receptors (R4, R6, and R8) that confer durable resistance to the fungus *M. oryzae* without a yield penalty. Among these NLR receptors, *PigmR* (Pigm R6) confers broad-spectrum resistance against a worldwide collection of *M. oryzae* isolates, whereas *PigmS* (*PigmR8*) competitively attenuates PigmR homodimerization to suppress resistance. The increased expression of *PigmS* suppresses *PigmR*-mediated resistance. *PigmR* was constitutively expressed at a low level in all tissues, whereas *PigmS* was highly expressed in pollen and panicles, with only trace expression in other organs. The epigenetic regulation of *PigmS* fine-tunes disease resistance and the trade-off between defense and yield by high expression of *PigmS* in pollen, which might facilitate fertilization through an unrecognized mechanism (Deng et al. 2017).

Bacterial streak is an important disease of rice in Asia, and no simply inherited sources of resistance have been identified in rice. A maize R gene recognizes a rice pathogen, *X. oryzae* pv. *oryzicola*, which causes bacterial streak disease in rice but does not cause disease in maize. *Rxo1* conditions a resistance reaction to a diverse collection of pathogen strains. Surprisingly, *Rxo1* also controls resistance to the unrelated pathogen *Burkholderia andropogonis*, which causes bacterial stripe of sorghum and maize. *Rxo1* has a nucleotide-binding site–leucine-rich repeat structure, similar to those of many previously identified R genes (Zhao et al. 2005). Panicle blast 1 (Pb1) is a blast resistance gene derived from the *indica* cultivar "Modan" and Pb1-mediated resistance is characterized by durability and quantitative resistance. The rice *Pb1* gene encodes a coiled-coil–nucleotide-binding site–leucine-rich repeat (CC-NBS-LRR) protein with an atypical structure. *Pb1* transcript levels are increased in *Pb1*+ cultivars, and this expression pattern accounts for the developmentally changing pattern of blast resistance in *Pb1*+ cultivars (Hayashi et al. 2010). Rice cultivars containing *Pb1* have not experienced breakdown after almost 30 years of cultivation. The atypical protein structure of *Pb1* compared with R proteins, such as the absence of the P-loop and the degenerated functional motifs, suggests a unique mechanism for its activation and/or downstream signaling, and could be a key to durable resistance (Hayashi et al. 2010). A germin-like protein gene (*OsGLP*) family member governs broad-spectrum disease resistance in rice (blast and sheath blight disease) and barley (powdery mildew) (Zimmermann et al. 2006; Manosalva et al. 2009). On chromosome 8 of rice, a cluster of 12 germin-like protein (*OsGLP*) gene members exhibited resistance to rice blast disease and, of the 12 *OsGLPs*, one clustered subfamily (*OsGER4*), identified by a RNA interference (RNAi) approach, contributed most to blast and sheath blight disease resistance (Manosalva et al. 2009).

A *qBlsr5a* QTL mapped on chromosome 5 confers resistance to bacterial leaf streak. Xie et al. (2014) narrowed down the QTL and identified a gene (LOC_Os05g01710) that encodes the gamma chain of transcription initiation factor IIA (*TFIIAc*), which has a nucleotide variation that cause amino acid change and further disease reaction to pathogens. The nucleotide substitutions resulted in a change of the 39th amino acid from valine (in the susceptible parent) to glutamic acid (in the resistant parent). *OsPAL4*, a member of the phenylalanine ammonia lyase gene family located on rice chromosome 2, confers bacterial blight and sheath blight disease resistance. Mutation of *OsPAL4* increased expression of the *OsPAL2* gene and decreased expression of the unlinked *OsPAL6* gene (Tonnessen et al. 2015). Rice stripe virus (RSV) causes one of the most serious viral diseases of rice. Five *indica*-derived major RSV resistance QTLs—*Stv-bi*, $qSTV11^{IR24}$, $qSTV11^{TQ}$, $qSTV11^{KAS}$, and $qSTV11^{SG}$—have been mapped to the long arm of rice chromosome 11 (Hayano-Saito et al. 2000), but none of them were cloned. A major QTL derived from Kasalath (highly resistant to RSV), $qSTV11^{KAS}$, was fine-mapped (Zhang et al. 2011) and then cloned (Wang et al. 2014b). The resistant allele of rice STV11 (STV11-R) encodes a sulfotransferase (*OsSOT1*) catalyzing the conversion of SA into sulfonated SA (SSA), whereas the gene product encoded by the susceptible allele STV11-S loses this activity. Introgression of the STV11-R allele in susceptible cultivar or heterologous transfer of STV11-R into tobacco plants confers effective resistance against RSV and thus confers durable resistance to RSV (Wang et al. 2014b). Similarly, Kwon et al. (2012) fine-mapped another RSV resistance QTL, $qSTV11^{SG}$, and identified three candidate genes—LOC_Os11g31430 (Expressed protein), LOC_Os11g31450 (Expressed protein with kinase domain), and LOC_Os11g31470 (Expressed protein)—which are exclusively expressed in the susceptible variety but not in the resistant varieties. The expression profiles of these three genes were consistent with their quantitative nature along with incomplete dominance.

2.4.3 Quantitative Disease Resistance Genes in Wheat and Barley

Lr34, *Lr46* (not cloned), and *Lr67* provide partial resistance to all races of leaf rust (*Puccinia triticina*; Pt), stripe rust (*Puccinia striiformis* f. sp. *tritici*; Pst), stem rust (*Puccinia graminis* f. sp. *tritici*; Pgt), and powdery mildew (*Blumeria graminis* f. sp. *tritici*; Bgt) in adult wheat plants. *Lr34* confers durable resistance to rusts and powdery mildew disease. It has been used for over 100 years and has proved to be durable. It is expressed in adult plants during the critical grain-filling stage. It is most effective in the flag leaf and stimulates senescence-like processes in the flag leaf tips and edges. Initially, it was mapped on the short arm of chromosome 7D between the two markers gwm1220 and SWM10 (Bossolini et al. 2006; Spielmeyer et al. 2008). The *Lr34* gene codes for a protein that resembles adenosine triphosphate–binding cassette transporters (a putative ABC transporter) and confers durable resistance to

multiple fungal pathogens in wheat. Alleles of *Lr34* conferring resistance or susceptibility differ by three genetic polymorphisms. One single-nucleotide polymorphism was located in the large intron 4, and the other two sequence differences were located in exons. Deletion of three base pairs (ttc) found in exon 11 resulted in the deletion of a phenylalanine residue, whereas a second single-nucleotide polymorphism in exon 12 converted a tyrosine to a histidine in the resistant cultivar. Both sequence differences located in exons affect the first transmembrane domain connecting the two nucleotide-binding domains, and may alter the structure and substrate specificity of the transporter (Krattinger et al. 2009).

Similarly, *Lr67* confers quantitative resistance to rust diseases, including powdery mildew, and encodes a predicted hexose transporter. The resistant form (LR67res) differs from the susceptible form of the same protein (LR67sus) by two amino acids, which are conserved in orthologous hexose transporters (Moore et al. 2015). LR67res may cause reduced hexose transport through a dominant-negative interference mechanism by forming inactive heteromultimeric protein complexes. The partial resistance conferred by LR67res to different biotrophic pathogens of wheat and barley could be due to the blocking of apoplastic hexose retrieval by host cells, thereby increasing the hexose-to-sucrose ratio in the leaf apoplasm, which in turn induces a sugar-mediated signaling response that results in a more hostile environment for pathogen growth. The LR67res inhibition of hexose retrieval may mimic the ubiquitous plant response to pathogen invasion of elevated cell wall invertase activity, which alters the extracellular apoplastic hexose-to-sucrose ratio and elicits a hexose-mediated defense response (Sonnewald et al. 2012; Proels and Hückelhoven 2014). The resistance allele (LR67res) is present in older, tall wheat varieties that predate "Green Revolution" semidwarf wheat, and no yield penalty is associated with Lr67res (Hiebert et al. 2010), suggesting that intensive selection for the *RhtD1b* semidwarf gene has simultaneously fixed the Lr67sus allele in the modern wheat germplasm.

Yr36 provides high temperature–dependent quantitative resistance to diverse stripe rust races. The gene *Yr36* (*WKS1*), which is present in wild wheat but absent in modern pasta and bread wheat varieties, confers non-race-specific resistance to stripe rust at relatively high temperatures (25–35 °C). This gene was first discovered in wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides* accession FA15-3) and encodes a kinase-START protein. *Yr36* resistance, originally discovered in adult plants, has some effectiveness in seedlings at high temperatures. A kinase and putative START lipid-binding domains of this gene are necessary to confer temperature-dependent resistance to stripe rust disease (Fu et al. 2009). The phosphorylation of thylakoid-associated ascorbate peroxidase (tAPX) by *WKS1.1* reduces the ability of the cells to detoxify ROS and contributes to cell death. This response takes several days longer than typical hypersensitive cell death responses, thus allowing the limited pathogen growth and restricted sporulation that is characteristic of the *WKS1* partial resistance response to stripe rust (Gou et al. 2015).

Wheat powdery mildew is caused by *B. graminis* f. sp. *tritici* (*Bgt*). The *Pm21* gene, originating from *Dasypyrum villosum*, confers high resistance to all known *Bgt* races. Recently, the *Pm21* gene was cloned by following integrated approaches

of resistance gene analog (RGA)–based cloning via comparative genomics, physical and genetic mapping, barley stripe mosaic virus–induced gene silencing (BSMV-VIGS), large-scale mutagenesis, and genetic transformation. *Pm21* encodes a typical CC-NBS-LRR protein and confers broad-spectrum resistance to wheat powdery mildew (He et al. 2017).

Natural and induced loss-of-function mutations of the *Mildew resistance locus o* (*Mlo*) gene confer broad-spectrum resistance against most *B. graminis* f. sp. *hordei* (*Bgh*) isolates in barley. *Mlo* is a member of an ancient eukaryotic gene family that is conserved throughout the plant kingdom (Kusch et al. 2016), and its role in powdery mildew resistance has been well studied in various species. On susceptible host plants, once sporelings of the pathogen land on the leaf or stem surface, these sporelings germinate and form an appressorium within 2 hours. The appressorium attempts to penetrate the epidermal layer by generating a penetration peg. If the pathogen successfully enters the host cell in the following hours of infection, the penetration peg enlarges to develop a feeding structure known as a haustorium. Thereafter, the pathogen will complete its asexual life cycle on the leaf surface with development of epiphytic hyphae, production of conidiophores, and release of new spores (Glawe 2008). In the case of resistant *mlo* plants, a near-complete arrest of pathogen growth occurs at the penetration stage when the germinating spore is not able to develop a haustorium.

A novel wheat ortholog of the *DIR1* gene—*TaDIR1-2*, isolated from Suwon11, a Chinese cultivar of wheat—contributes to negative regulation of wheat resistance against *P. striiformis* f. sp. *tritici* by modulating ROS- and/or SA-induced signaling. A *TaDIR1-2* transcript was significantly induced during compatible interaction of wheat with the stripe rust pathogen, *P. striiformis* f. sp. *tritici* (*Pst*). However, treatments with SA and low temperature significantly upregulated the expression of *TaDIR1-2* (Ahmed et al. 2017).

Similarly, Wang et al. (2017) identified and cloned three wheat homeologous genes that are highly similar to barley *HvRar1*, designated as *TaRar1-2A*, *TaRar1-2B*, and *TaRar1-2D*. These genes confer defense against infection with the stripe rust pathogen mediated by *YrSu*, a stripe rust resistance gene, and the defense occurred through SA to influence ROS accumulation and a hypersensitive response. The three TaRAR1 proteins all contain two conserved cysteine- and histidine-rich domains (CHORD-I and -II) shared by known RAR1-like proteins. The expression of *TaRar1* is tissue specific and upregulated during stripe rust infection.

2.4.4 Quantitative Disease Resistance Genes in Maize

A QTL (*qNLB1.02B73*) on the short arm of chromosome 1, conditioning resistance to northern leaf blight (NLB), has been identified as a pleiotropic locus in maize. This locus confers resistance not only to NLB (caused by the fungus *Setosphaeria turcica*) but also to Stewart's wilt (caused by the bacterium *Pantoea stewartia*) and common rust (caused by the fungus *Puccinia sorghi*). A maize remorin

(*ZmREM6.3*)—a chaperonin gene present in this QTL interval, reported to be involved in quantitative resistance against NLB—has been identified following high-resolution fine-mapping, expression analysis, and mutants in maize. Expression of *ZmREM6.3* was higher in the resistant line and was downregulated upon infection with an *S. turcica* race 1 isolate in both the susceptible and resistant near-isogenic lines (NILs). The downregulation of *ZmREM6.3* may indicate that it is an important part of the defense response and thus is targeted by the pathogen (Jamann et al. 2016). A quantitative trait locus, *qMdr9.02* on chromosome 9 of maize, is associated with resistance to three important foliar maize diseases: southern leaf blight (SLB), gray leaf spot, and NLB. These QTLs were further narrow downed to the gene level through fine-mapping, association analysis, expression analysis, insertional mutagenesis, and a transgenic approach, and identified a gene, *ZmCCoAOMT2*, which encodes a caffeoyl-CoA *O*-methyltransferase. This gene is associated with the phenylpropanoid pathway and lignin production, and confers quantitative resistance to both SLB and gray leaf spot. The resistance is governed by allelic variation at the level of both gene expression and the amino acid sequence, thus resulting in differences in levels of lignin and other metabolites of the phenylpropanoid pathway, and regulation of programmed cell death (Yang et al. 2017a). The maize inbred line "Tx303" conditions quantitative resistance to NLB and qualitative resistance to Stewart's wilt. A receptor-like kinase gene, *pan1*, has been implicated as a quantitative susceptibility gene for NLB and Stewart's wilt. The structural variation plays an important role in resistance conditioned by this region, and *pan1*, a gene conditioning susceptibility for NLB (Jamann et al. 2014). PAN1 has been shown to play a role in promoting features of actin organization that support asymmetric cell division.

Northern corn leaf blight (NCLB) is caused by the hemibiotrophic fungus *Exserohilum turcicum*. The resistance is controlled by a quantitative trait locus named *Htn1*, which confers partial NCLB resistance by delaying the onset of lesion formation. The *Htn1* locus represents an important source of genetic resistance against NCLB, which was originally introduced from a Mexican landrace into modern maize breeding lines. The locus contains three candidate genes encoding two wall-associated receptor–like kinases (*ZmWAK-RLK1* and *ZmWAK-RLK2*) and one wall-associated receptor–like protein coding gene (*ZmWAK-RLP1*). *ZmWAK-RLK1* contains a nonarginine aspartate (non-RD) kinase domain, typically found in plant innate immune receptors. The quantitative *Htn1* disease resistance in maize is conferred by an unusual innate immune receptor with an extracellular WAK domain (Hurni et al. 2015). Head smut is a systemic disease in maize caused by the soil-borne fungus *Sporisorium reilianum*, and the resistance is controlled by the quantitative resistance locus *qHSR1*, which has a *ZmWAK* gene. *ZmWAK* spans the plasma membrane, potentially serving as a receptor-like kinase to perceive and transduce extracellular signals. *ZmWAK* was highly expressed in the mesocotyl of seedlings, where it arrested biotrophic growth of the endophytic *S. reilianum*. *ZmWAK*-mediated resistance occurs mainly in the mesocotyl of maize seedlings, rather than in the ear or tassel, where typical symptoms occur; hence, impaired expression in the mesocotyl compromised *ZmWAK*-mediated resistance (Zuo et al. 2015). This resistance

mode implies that *ZmWAK* has evolved to form a spatiotemporally optimized resistance strategy against maize head smut.

Sugarcane mosaic virus (SCMV) causes substantial losses of grain yield and forage biomass in susceptible maize worldwide, and it is controlled by a QTL. Two major resistance QTLs—*Scmv1* and *Scmv2*—were identified. *Scmv1* confers strong early resistance to SCMV, and *Scmv2* mainly functions at later infection stages. No hypersensitive response has yet been found to be associated with maize resistance to SCMV, implying that neither *Scmv1* nor *Scmv2* is likely to be a typical NBS-LRR resistance gene that activates a hypersensitive response. The *ZmTrxh* gene, encoding an atypical h-type thioredoxin, is the causal gene at the *Scmv1* locus, and its transcript abundance due to variation in the upstream regulatory region is correlated strongly with maize resistance to SCMV (Liu et al. 2017). Rawat et al. (2016) identified the *Fhb1* gene—which encodes a chimeric lectin with agglutinin domains and a pore-forming toxin–like (PFT) domain in wheat—through mutation analysis, gene silencing, and transgenic overexpression, and found that a PFT gene at *Fhb1* confers *Fusarium* head blight (FHB) resistance. Kage et al. (2017) identified the *TaWRKY70* TF gene, present in wheat *QTL-2DL*, which regulates downstream metabolite biosynthetic genes to impart resistance against *Fusarium graminearum* (*Fg*), which causes FHB in wheat. The expression of *TaWRKY70* is higher in NIL-R lines as compared with NIL-S lines after *Fg* inoculation. *RCG1* confers resistance to the plant pathogen *Colletotrichum*, which causes anthracnose stalk rot, leaf blight, and top dieback in corn and other cereals (Broglie et al. 2006).

2.4.5 *Quantitative Disease Resistance Genes in Soybean and Potato*

Soybean cyst nematode (SCN; *Heterodera glycines*) is a microscopic roundworm that feeds on the roots of soybean and is a major constraint of soybean production. Soybean *Rhg1* (*Resistance* to *Heterodera glycines*), a quantitative trait locus on chromosome 18, imparts SCN resistance. *Rhg1* disrupts the formation and/or maintenance of most potential nematode feeding sites. The *rhg1-b* allele of soybean is widely used for resistance against SCN and encodes an amino acid transporter (namely, an α-SNAP protein) and a WI12 (wound inducible domain) protein, each contributing to resistance. *Rhg1*-mediated SCN resistance is conferred by a copy number variation that increases the expression of a set of dissimilar genes in a repeated multigene segment (Cook et al. 2012). Liu et al. (2012a) cloned a gene at the *Rhg4* (*Resistance* to *Heterodera glycines* 4) locus, a major quantitative trait locus contributing to cyst nematode resistance. *Rhg4* encodes a serine hydroxymethyl transferase enzyme that is responsible for interconversion of serine and glycine, and is essential for cellular one-carbon folate metabolism. This enzyme is ubiquitous in nature and is structurally conserved across kingdoms. Alleles of *Rhg4* conferring resistance or susceptibility differ by two genetic polymorphisms that alter a key regulatory property of the enzyme and nematode resistance of soybean.

The potato *Rar1* and *Sgt1* genes have been implicated in mediating disease resistance responses against various plant pathogens and pests. The *Rar1* and *Sgt1* genes of an *RB*-containing potato clone were silenced using an RNAi-based approach, and all of the silenced potato plants displayed phenotypically normal growth. The late blight resistance of the *Rar1*-silenced plants was not affected, but silencing of the *Sgt1* gene abolished the *RB*-mediated resistance (Bhaskar et al. 2008).

2.5 New Technologies and Tools for Identifying More Genes Involved in Quantitative Disease Resistance

Innovation in DNA, RNA, and protein sequencing technologies and bioinformatic analysis of sequencing data in recent times have enabled fast detection of QTLs and identification of candidate genes in many crops for many traits, including disease resistance. QTL-seq, targeted sequencing (to narrow down the mapped QTL region), gene mapping via bulked segregant RNA-seq (BSR-seq) (Liu et al. 2012b), MutMap (Abe et al. 2012), target-enriched trait QTL (TEX-QTL) (Guo et al. 2015), genotyping by sequencing (GBS) (Fukuta et al. 2017), indel-seq (Singh et al. 2017), and exome QTL-seq (Hisano et al. 2017) approaches are routinely utilized in mapping and narrowing down the casual QTLs to identify candidate genes in rice. Apart from these, new genome-editing techniques—include zinc-finger nucleases (ZFNs), TAL effector nucleases (TALENs), and the CRISPR–Cas9 [clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9] system—have been shown to be promising in simplifying the process of gene deletion, editing, and insertion in plants, thus helping to validate the identified candidate genes for traits of interest (Wang et al. 2014a; Li et al. 2012). The CRISPR–Cas9 system is currently considered as a method of choice for improving many crops for various traits, as well for identifying genes of interest (Chandrasekaran et al. 2016).

2.6 Conclusion

Though quantitative disease resistance (QDR) is predicted to be highly effective against a broad spectrum of pathogens and long lasting, very few genes/quantitative trait loci (QTLs) have been utilized in crop improvement programs, because of recombination between QTL linked markers and traits, low phenotypic variation, and desired QTL linkage with unwanted genes (linkage drags). Hence, the already mapped QTLs governing QDR have to be further narrowed down to smaller genomic regions, using large segregating immortal populations such as recombinant inbred lines, backcross inbred lines, near-isogenic lines, nested association-mapping populations and doubled haploid populations, and use of high-density maps with simple

sequence repeat markers and single-nucleotide polymorphism markers. Employment of these not only will help to identify closely linked markers for the trait of interest but also will pave the way for narrowing down the QTL region and thus will help in map-based cloning of genes.

References

Abe A, Kosugi S, Yoshida K, Natsume S, Takagi H, Kanzaki H, Matsumura H, Yoshida K, Mitsuoka C, Tamiru M, Innan H, Cano L, Kamoun S, Terauchi R (2012) Genome sequencing reveals agronomically important loci in rice using MutMap. Nat Biotechnol 30:174–178

Ahmed SM, Liu P, Xue Q, Ji C, Qi T, Guo J, Guo J, Kang Z (2017) TaDIR1-2, a wheat ortholog of lipid transfer protein AtDIR1 contributes to negative regulation of wheat resistance against *Puccinia striiformis* f. sp. *tritici*. Front Plant Sci 8:521. https://doi.org/10.3389/fpls.2017.00521

Badet T, Voisin D, Mbengue M, Barascud M, Sucher J, Sadon P, Balague C, Roby D, Raffaele S (2017) Parallel evolution of the POQR prolyl oligo peptidase gene conferring plant quantitative disease resistance. PLoS Genet 13(12):e1007143

Bent AF, Mackey D (2007) Elicitors, effectors, and R genes: the new paradigm and a lifetime supply of questions. Annu Rev Phytopathol 45:399–436

Bhaskar PB, Raasch JA, Kramer LC, Neumann P, Wielgus SM, Austin-Phillips S, Jiang J (2008) Sgt1, but not Rar1, is essential for the RB-mediated broad-spectrum resistance to potato late blight. BMC Plant Biol 8:8. https://doi.org/10.1186/1471-2229-8-8

Boller T, He SY (2009) Innate immunity in plants: an arms race between pattern receptors in plants and effectors in microbial pathogens. Science 324(5928):742–744. https://doi.org/10.1126/science.1171647

Bossolini E, Krattinger SG, Keller B (2006) Development of simple sequence repeat markers specific for the Lr34 resistance region of wheat using sequence information from rice and *Aegilops tauschii*. Theor Appl Genet 113:1049–1062

Broglie K et al (2006) Polynucleotides and methods for making plants resistant to fungal pathogens. US patent 20080016595 A1

Broglie KE et al (2011) Methods for identifying maize plants with RCG1 gene conferring resistance to *Colletotrichum* infection. US patent 8,062,847

Chandrasekaran J, Brumin M, Wolf D, Leibman D, Klap C, Pearlsman M, Sherman A, Arazi T, Gal-On A (2016) Development of broad virus resistance in non-transgenic cucumber using CRISPR/Cas9 technology. Mol Plant Pathol 17:1140–1153

Chen X, Shang J, Chen D, Lei C, Zou Y, Zhai W, Liu G, Xu J, Ling Z, Cao G et al (2006) A B-lectin receptor kinase gene conferring rice blast resistance. Plant J 46:794–804

Chen J, Lubal M, Yang RC, Spaner D (2016) Effect of Lr34/Yr18 on agronomic and quality traits in a spring wheat mapping population and implications for breeding. Mol Breed 36:53

Fokkens L, Diener AC (2013) Diversity in receptor-like kinase genes is a major determinant of quantitative resistance to *Fusarium oxysporum* f. sp. *matthioli*. New Phytol 200:172–184

Coll NS, Epple P, Dangl JL (2011) Programmed cell death in the plant immune system. Cell Death Differ 18:1247–1256

Cook DE, Leem TG, Guom X, Melito S, Wang K, Bayless AM, Wang J, Hughes TJ, Willis DK, Clemente TE, Diers BW, Jiang J, Hudson ME, Bent AF (2012) Copy number variation of multiple genes at Rhg1 mediates nematode resistance in soybean. Science 338:1206–1209

Cook DE, Mesarich CH, Thomma BP (2015) Understanding plant immunity as a surveillance system to detect invasion. Annu Rev Phytopathol 53:541–563

Debieu M, Huard-Chauveau C, Genissel A, Roux F, Roby D (2016) Quantitative disease resistance to the bacterial pathogen *Xanthomonas campestris* involves an *Arabidopsis* immune receptor pair and a gene of unknown function. Mol Plant Pathol 17:510–520

Delteil A, Gobbato E, Cayrol B, Estevan J, Michel-Romiti C, Dievart A, Kroj T, Morel JB (2016) Several wall-associated kinases participate positively and negatively in basal defense against rice blast fungus. BMC Plant Biol 16:1–10

Deng H, Liu H, Li X, Xiao J, Wang S (2012) A CCCH-type zinc finger nucleic acid–binding protein quantitatively confers resistance against rice bacterial blight disease. Plant Physiol 158:876–889

Deng Y, Zhai K, Xie Z, Yang D, Zhu X, Liu J, Wang X, Qin P, Yang Y, Zhang G, Li Q, Zhang J, Wu S, Milazzo J, Mao B, Wang E, Xie H, Tharreau D, He Z (2017) Epigenetic regulation of antagonistic receptors confers rice blast resistance with yield balance. Science 355(6328):962–965

Diener AC, Ausubel FM (2005) RESISTANCE TO FUSARIUM OXYSPORUM 1, a dominant *Arabidopsis* disease-resistance gene, is not race specific. Genetics 171:305–321

Ding X, Cao Y, Huang L, Zhao J, Xu C, Li X, Wang S (2008) Activation of the indole-3-acetic acid–amido synthetase GH3-8 suppresses expansin expression and promotes salicylate- and jasmonate-independent basal immunity in rice. Plant Cell 20(1):228–240

Dodds PN, Rathjen JP (2010) Plant immunity: towards an integrated view of plant–pathogen interactions. Nat Rev Genet 11:539–548. https://doi.org/10.1038/nrg2812

Fu D, Uauy C, Distelfeld A, Blechl A, Epstein L, Chen X, Sela H, Fahima T, Dubcovsky J (2009) A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. Science 323:1357–1360

Fu J, Liu H, Li Y, Yu H, Li X, Xiao J, Wang S (2011) Manipulating broad-spectrum disease resistance by suppressing pathogen-induced auxin accumulation in rice. Plant Physiol 155:589–602

Fukuoka S, Okuno K (2001) QTL analysis and mapping of pi21, a recessive gene for field resistance to rice blast in Japanese upland rice. Theor Appl Genet 103:185–190

Fukuoka S, Saka N, Koga H, Ono K, Shimizu T, Ebana K, Hayashi N, Takahashi A, Hirochika H, Okuno K, Yano M (2009) Loss of function of a proline-containing protein confers durable disease resistance in rice. Science 325:998–1001

Fukuoka S, Mizobuchi R, Saka N, Supr[illegible] J, Matsumoto T, Okuno K, Yano M (2012) A multiple gene complex on rice chromosome 4 is involved in durable resistance to rice blast. Theor Appl Genet 125:551–559

Fukuoka S, Yamanouchi U, Mizobuchi R, Yamanouchi U, Ono K, Kitazawa N, Yasuda N, Fujita Y, Nguyen TTT, Koizumi S, Sugimoto K, Matsumoto T, Yano M (2014) Multiple functional polymorphisms in a single disease resistance gene in rice enhance durable resistance to blast. Sci Rep 4:4550

Furuta T, Ashikari M, Jena KK, Doi K, Reuscher S (2017) Adapting genotyping-by-sequencing for rice F2 population. G3 (Bethesda) 7(3):881–893

Glawe DA (2008) The powdery mildews: a review of the world's most familiar (yet poorly known) plant pathogens. Annu Rev Phytopathol 46:27–51

Gou JY, Li K, Wu K, Wang X, Lin H, Cantu D, Uauy C, Dobon-Alonso A, Midorikawa T, Inoue K, Sanchez J, Fu D, Blechl A, Wallington E, Fahima T, Meeta M, Epstein L, Dubcovsky J (2015) Wheat stripe rust resistance protein WKS1 reduces the ability of the thylakoid-associated ascorbate peroxidase to detoxify reactive oxygen species. Plant Cell 27(6):1755–1770

Gu L, Fan J, Hauser BA, Rhee SY (2015) Target enrichment improves mapping of complex traits by deep sequencing. G3 (Bethesda) 6(1):67–77

Hayano-Saito Y, Saito K, Nakamura S, Kawasaki S, Iwasaki M (2000) Fine physical mapping of the rice stripe resistance gene locus, Stvb-i. Theor Appl Genet 101:59–63

Hayashi N, Inoue H, Kato T, Funao T, Shirota M, Shimizu T, Kanamori H, Yamane H, Hayano-Saito Y, Matsumoto T, Yano M, Takatsuj IH (2010) Durable panicle blast–resistance gene Pb1 encodes an atypical CC-NBS-LRR protein and was generated by acquiring a promoter through local genome duplication. Plant J 64:498–510

He H, Zhu S, Ji Y, Jiang Z, Zhao R, Bie T (2017) Map-based cloning of the gene Pm21 that confers broad spectrum resistance to wheat powdery mildew. bioRxiv:177857. https://doi.org/10.1101/177857

Hiebert CW, Thomas JB, McCallum BD, Humphreys DG, DePauw RM, Hayden MJ, Mago R, Schnippenkoetter W, Spielmeyer W (2010) An introgression on wheat chromosome 4DL in RL6077 (Thatcher∗6/PI 250413) confers adult plant resistance to stripe rust and leaf rust (Lr67). Theor Appl Genet 121:1083–1091

Hisano H, Sakamoto K, Takagi H, Terauchi R, Sato K (2017) Exome QTL-seq maps monogenic locus and QTLs in barley. BMC Genomics 18(1):125

Hu KM, Qiu DY, Shen XL, Li XH, Wang SP (2008) Isolation and manipulation of quantitative trait loci for disease resistance in rice using a candidate gene approach. Mol Plant 1(5):786–793

Huard-Chauveau C, Perchepied L, Debieu M, Rivas S, Kroj T, Kars I, Bergelson J, Roux F, Roby D (2013) An atypical kinase under balancing selection confers broad-spectrum disease resistance in *Arabidopsis*. PLoS Genet 9:e1003766

Hurni S, Scheuermann D, Krattinger SG, Kessel B, Wicker T, Herren G, Fitze MN, Breen J, Presterl T, Ouzunova M, Keller B (2015) The maize disease resistance gene Htn1 against northern corn leaf blight encodes a wall-associated receptor–like kinase. Proc Natl Acad Sci U S A 112(28):8780–8785

Jamann TM, Poland JA, Kolkman JM, Smith LG, Nelson RJ (2014) Unraveling genomic complexity at a quantitative disease resistance locus in maize. Genetics 198:333–344

Jamann TM, Luo X, Morales L, Kolkman JM, Chung CL, Nelson RJ (2016) A remorin gene is implicated in quantitative disease resistance in maize. Theor Appl Genet 129(3):591–602. https://doi.org/10.1007/s00122-015-2650-6

Jones JD, Dangl JL (2006) The plant immune system. Nature 444:323–329

Kage U, Yogendra KN, Kushalappa AC (2017) TaWRKY70 transcription factor in wheat QTL-2DL regulates downstream metabolite biosynthetic genes to resist *Fusarium graminearum* infection spread within spike. Sci Rep 7:42596

Kou Y, Li X, Xiao J, Wang S (2010) Identification of genes contributing to quantitative disease resistance in rice. Sci China Life Sci 53:1263–1273

Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J, McFadden H, Bossolini E, Selter LL, Keller B (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science 323:1360–1363

Kusch S, Pesch L, Panstruga R (2016) Comprehensive phylogenetic analysis sheds light on the diversity and origin of the MLO family of integral membrane proteins. Genome Biol Evol 8:878–895

Kwon T, Lee JH, Park SK, Hwang UH, Cho JH, Kwak DY, Youn YN, Yeo US, Song YC, Nam J, Kang HW, Nam MH, Park JC (2012) Fine mapping and identification of candidate rice genes associated with qSTV11SG, a major QTL for rice stripe disease resistance. Theor Appl Genet 125:1033–1046

Lacombe S, Rougon-Cardoso A, Sherwood E, Peeters N, Dahlbeck D, van Esse HP, Smoker M, Rallapalli G, Thomma BP, Staskawicz B, Jones JD, Zipfel C (2010) Interfamily transfer of a plant pattern-recognition receptor confers broad-spectrum bacterial resistance. Nat Biotechnol 28:365–369

Lemarié S, Robert-Seilaniantz A, Lariagon C, Lemoine J, Marnet N, Levrel A, Jubault M, Manzanares-Dauleux MJ, Gravot A (2015) Camalexin contributes to the partial resistance of *Arabidopsis thaliana* to the biotrophic soil borne protist *Plasmodiophora brassicae*. Front Plant Sci 6:539

Li T, Liu B, Spalding MH, Weeks DP, Yang B (2012) High-efficiency TALEN-based gene editing produces disease-resistant rice. Nat Biotechnol 30:390–392

Li W, Zhu Z, Chern M, Yin J, Yang C, Ran L, Cheng M, He M, Wang K, Wang J, Zhou X, Zhu X, Chen Z, Wang J, Zhao W, Ma B, Qin P, Chen W, Wang Y, Liu J, Wang W, Wu X, Li P, Wang J, Zhu L, Li S, Chen X (2017) A natural allele of a transcription factor in rice confers broad-spectrum blast resistance. Cell 170(1):114–126.e15

Liu S, Kandoth PK, Warren SD, Yeckel G, Heinz R, Alden J, Yang C, Jamai A, El-Mellouki T, Juvale PS, Hill J, Baum TJ, Cianzio S, Whitham SA, Korkin D, Mitchum MG, Meksem K (2012a) A soybean cyst nematode resistance gene points to a new mechanism of plant resistance to pathogens. Nature 492:256–260

Liu S, Yeh CT, Tang HM, Nettleton D, Schnable PS (2012b) Gene mapping via bulked segregant RNA-Seq (BSR-Seq). PLoS One 7(5):e36406

Liu Q, Liu H, Gong Y, Tao Y, Jiang L, Zuo W, Yang Q, Ye J, Lai J, Wu J, Lübberstedt T, Xu M (2017) An atypical thioredoxin imparts early resistance to sugarcane mosaic virus in maize. Mol Plant 10:483–497

Manosalva PM, Davidsonm RM, Liu B, Zhu X, Hulbert SH, Leung H, Leach JE (2009) A germin-like protein gene family functions as a complex quantitative trait locus conferring broad-spectrum disease resistance in rice. Plant Physiol 149:286–296

Moore JW, Herrera-Foessel S, Lan C, Schnippenkoetter W, Ayliffe M, Huerta-Espino J, Lillemo M, Viccars L, Milne R, Periyannan S, Kong X, Spielmeyer W, Talbot M, Bariana H, Patrick JW, Doddsm P, Singhm R, Lagudah E (2015) A recently evolved hexose transporter variant confers resistance to multiple pathogens in wheat. Nat Genet 47:1494–1498

Nelson R, Wiesner-Hanks T, Wisser R, Balint-Kurti P (2018) Navigating complexity to breed disease-resistant crops. Nat Rev Genet 19(1):21–33

Parlevliet JE, Zadoks JC (1977) Integrated concept of disease resistance—new view including horizontal and vertical resistance in plants. Euphytica 26:5–21

Peng H, Chen Z, Fang Z, Zhou J, Xia Z, Gao L, Chen L, Li L, Li T, Zhai W, Zhang L (2015) Rice Xa21 primed genes and pathways that are critical for combating bacterial blight infection. Sci Rep 5:12165

Piffanelli P, Zhou F, Casais C, Orme J, Jarosch B, Schaffrath U, Collins N, Panstruga R, Schulze-Lefert P (2002) The barley MLO modulator of defense and cell death is responsive to biotic and abiotic stress stimuli. Plant Physiol 129:1076–1085

Poland JA, Balint-Kurti PJ, Wisser RJ, Pratt RC, Nelson RJ (2009) Shades of gray: the world of quantitative disease resistance. Trends Plant Sci 14:21–29

Pritchard L, Birch PR (2014) The zigzag model of plant–microbe interactions: is it time to move on? Mol Plant Pathol 15:865–870

Proels RK, Hückelhoven R (2014) Cell-wall invertases, key enzymes in the modulation of plant metabolism during defence responses. Mol Plant Pathol 15:858–864

Qiu D, Xiao J, Ding X, Xiong M, Cai M, Cao Y, Li X, Xu C, Wang S (2007) OsWRKY13 mediates rice disease resistance by regulating defense-related genes in salicylate- and jasmonate-dependent signaling. Mol Plant-Microbe Interact 20:492–499

Qiu D, Xiao J, Xie W, Liu H, Li H, Xiong L, Wang S (2008) Rice gene network inferred from expression profiling of plants overexpressing OsWRKY13, a positive regulator of disease resistance. Mol Plant 1:538–551

Rawat N, Pumphrey MO, Liu S, Zhang X, Tiwari VK, Ando K, Trick HN, Bockus WW, Akhunov E, Anderson JA, Gill BS (2016) Wheat Fhb1 encodes a chimeric lectin with agglutinin domains and a pore-forming toxin–like domain conferring resistance to *Fusarium* head blight. Nat Genet 48:1576–1580

Roux F, Voisin D, Badet T, Balague C, Barlet X, Huard-Chauveau C, Roby D, Raffaele S (2014) Resistance to phytopathogens *e tutti quanti*: placing plant quantitative disease resistance on the map. Mol Plant Pathol 15:427–432

Schwessinger B, Bahar O, Thomas N, Holton N, Nekrasov V, Ruan D, Canlas PE, Daudi A, Petzold CJ, Singan VR, Kuo R, Chovatia M, Daum C, Heazlewood JL, Zipfel C, Ronald PC (2015) Transgenic expression of the dicotyledonous pattern recognition receptor EFR in rice leads to ligand dependent activation of defense responses. PLoS Pathog 11:e1004809

Singh VK, Khan AW, Saxena RK, Sinha P, Kale SM, Parupalli S, Kumar V, Chitikineni A, Vechalapu S, Kumar CVS, Sharma M, Ghanta A, Yamini KN, Muniswamy S, Varshney RK (2017) Indel-seq: a fast-forward genetics approach for identification of trait-associated putative candidate genomic regions and its application in pigeonpea (*Cajanus cajan*). Plant Biotechnol J 15(7):906–914

Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, Fauquet C, Ronald P (1995) A receptor kinase–like protein encoded by the rice disease resistance gene, Xa21. Science 270:1804–1806

Sonnewald S, Priller JP, Schuster J, Glickmann E, Hajirezaei MR, Siebig S, Mudgett MB, Sonnewald U (2012) Regulation of cell wall–bound invertase in pepper leaves by *Xanthomonas campestris* pv. *vesicatoria* type three effectors. PLoS One 7:e51763

Spielmeyer W, Singh RP, McFadden H, Wellings CR, Huerta-Espino J, Kong X, Appels R, Lagudah ES (2008) Fine scale genetic and physical mapping using interstitial deletion mutants of Lr34/Yr18: a disease resistance locus effective against multiple pathogens in wheat. Theor Appl Genet 116:481–490

Tonnessen BW, Manosalva P, Lang JM, Baraoidan M, Bordeos A, Mauleon R, Oard J, Hulbert S, Leung H, Leach JE (2015) Rice phenylalanine ammonia-lyase gene OsPAL4 is associated with broad spectrum disease resistance. Plant Mol Biol 87(3):273–286

Vetter MM, Kronholm I, He F, Haweker H, Reymond M, Bergelson J, Robatzek S, de Meaux J (2012) Flagellin perception varies quantitatively in *Arabidopsis thaliana* and its relatives. Mol Biol Evol 29:1655–1667

Wang G, Ding X, Yuan M, Qiu D, Li X, Xu C, Wang S (2006) Dual function of rice OsDR8 gene in disease resistance and thiamine accumulation. Plant Mol Biol 60:437–449

Wang W, Wen Y, Berkey R, Xiao S (2009) Specific targeting of the *Arabidopsis* resistance protein RPW8.2 to the interfacial membrane encasing the fungal haustorium renders broad-spectrum resistance to powdery mildew. Plant Cell 21:2898–2913

Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, Qiu JL (2014a) Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat Biotechnol 32:947–951

Wang Q, Liu Y, He J, Zheng X, Hu J, Liu Y, Dai H, Zhang Y, Wang B, Wu W, Gao H, Zhang Y, Tao X, Deng H, Yuan D, Jiang L, Zhang X, Guo X, Cheng Z, Wu C, Wangm H, Yuan L, Wan J (2014b) STV11 encodes a sulphotransferase and confers durable resistance to rice stripe virus. Nat Commun 5:4768

Wang X, Wang Y, Liu P, Ding Y, Mu X, Liu X, Wang X, Zhao M, Huai B, Huang L, Kang Z (2017) TaRar1 is involved in wheat defense against stripe rust pathogen mediated by YrSu. Front Plant Sci 8:156. https://doi.org/10.3389/fpls.2017.00156

Xiao S, Ellwood S, Calis O, Patrick E, Li T, Coleman M, Turner JG (2001) Broad-spectrum mildew resistance in *Arabidopsis thaliana* mediated by RPW8. Science 291:118–120

Xie X, Chen Z, Cao J, Guan H, Liu D, Li C, Lan T, Duan Y, Mao D, Wu W (2014) Toward the positional cloning of qBlsr5a, a QTL underlying resistance to bacterial leaf streak, using overlapping sub-CSSLs in rice. PLoS One 9(4):e95751. https://doi.org/10.1371/journal.pone.0095751

Xu X, Hayashi N, Wang CT, Kobayashi N, Kawasaki S, Takatsuji H, Jiang CJ (2014) Rice blast resistance gene Pikahei-1(t), a member of a resistance gene cluster on chromosome 4, encodes a nucleotide-binding site and leucine-rich repeat protein. Mol Breed 34:691

Yang Q, Balint-Kurti P, Xu M (2017a) Quantitative disease resistance: dissection and adoption in maize. Mol Plant 10:402–413

Yang Q, He Y, Kabahuma M, Chaya T, Kelly A, Borrego E, Bian Y, El Kasmi F, Yang L, Teixeira P, Kolkman J, Nelson R, Kolomiets M, Dangl JL, Wisser R, Caplan J, Li X, Lauter N, Balint-Kurti P (2017b) A gene encoding maize caffeoyl-CoA *O*-methyltransferase confers quantitative resistance to multiple pathogens. Nat Genet 49:1364–1372

Yuan B, Shen X, Li X, Xu C, Wang S (2007) Mitogen-activated protein kinase OsMPK6 negatively regulates rice disease resistance to bacterial pathogens. Planta 226:953–960

Zenbayashi-Sawata K, Fukuoka S, Katagiri S, Fujisawa M, Matsumoto T, Ashizawa T, Koizumi S (2007) Genetic and physical mapping of the partial resistance gene, pi34, to blast in rice. Phytopathology 97:598–602

Zhang YX, Wang Q, Jiang L, Liu LL, Wang BX, Shen YY, Cheng XN, Wan JM (2011) Fine mapping of qSTV11KAS, a major QTL for rice stripe disease resistance. Theor Appl Genet 122:1591–1604

Zhao B, Lin X, Poland J, Trick H, Leach J, Hulbert S (2005) A maize resistance gene functions against bacterial streak disease in rice. Proc Natl Acad Sci U S A 102:15383–15388

Zhou X, Liao H, Chern M et al (2018) Loss of function of a rice TPR-domain RNA-binding protein confers broad-spectrum disease resistance. Proc Natl Acad Sci U S A 115:3174–3179

Zimmermann G, Baumlein H, Mock H, Himmelbach A, Schweizer P (2006) The multigene family encoding germin-like proteins of barley: regulation and function in basal host resistance. Plant Physiol 142:181–192

Zuo W, Chao Q, Zhang N, Ye J, Tan G, Li B, Xing Y, Zhang B, Liu H, Fengler KA, Zhao J, Zhao X, Chen Y, Lai J, Yan J, Xu M (2015) A maize wall-associated kinase confers quantitative resistance to head smut. Nat Genet 47:151–157

Chapter 3
CRISPR-Based Tools for Crop Improvement: Understanding the Plant–Pathogen Interaction

Shazia Mukhtar, Meenakshi Raina, Jebi Sudan, Aejaz Ahmad Dar, Ananda Mustafiz, and Sumita Kumari

3.1 Introduction

The concept of sustainable agricultural production was adopted to address the challenges arising from the rapid increase in the world's human population by increasing the production and productivity of crop plants while minimizing the adverse effects on the environment. Environmental vagaries, both biotic and abiotic, are the major bottleneck in achieving the full potential of improved genotypes. Among the various biotic stresses, plant diseases constitute a major threat to sustainable crop production over a longer period of time. Various approaches—including the use of pesticides, better agronomic practices, conventional molecular plant breeding, and genetic modification approaches have been continuously combined to achieve durable resistance against disease-causing pathogens. However, enhancement of the resistance of crop plants has been shown to be most effective, sustainable, and economical strategy to deal with pathogens (Boyd et al. 2013).

Over the course of evolution, plants have also evolved various intricate mechanisms to strengthen their own defensive mechanisms against these pathogens. The response to a pathogen attack starts with the recognition of pathogen-associated molecular patterns (PAMPs) by surface-localized pattern recognition receptors (PRRs), triggering a cascade of reactions for the elimination of the pathogen (Zipfel 2014). Thus, PAMP-triggered immunity (PTI) is considered the first and main line of defense in the war against pathogens (Andolfo et al. 2016). As large numbers of genes are involved in PTI, this complicates the identification of genes that are involved in the pathways responsible for plant–pathogen interactions. Hence, efforts

S. Mukhtar · M. Raina · J. Sudan · A. A. Dar · S. Kumari (✉)
School of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu and Kashmir, India

A. Mustafiz
Faculty of Life Sciences and Biotechnology, South Asian University, New Delhi, India

S. H. Wani (ed.), *Disease Resistance in Crop Plants*,
https://doi.org/10.1007/978-3-030-20728-1_3

are being made to identify key genes that can be transferred to elite varieties to provide durable resistance. Once the candidate genes are known, they need to be introgressed into elite germplasms through either conventional or molecular breeding approaches. Modern omics technology has made the identification of susceptibility/resistance genes feasible in any species, thus providing a large number of potential targets for crop protection. However, attempts to validate these candidate genes have been hindered by the unavailability of a rapid, precise, and efficient gene-targeting system in plants.

Over the decades, various techniques have also been employed to transfer genes from wild relatives to domesticated varieties. However, conventional breeding takes approximately 8–10 years to pyramid multiple disease resistance genes into a variety. This long duration sometimes causes rapid breakdown of resistant cultivars because of high pathogenic variability and fast mutation rates. RNA interference (RNAi)–based approaches have been found to be a good alternative in regulating the expression of various disease-related genes through silencing of transcription factor genes (Liu et al. 2012; Koch et al. 2016; Rawat et al. 2016; Panwar et al. 2017). However, transgenics from RNAi suffer from some major drawbacks. The expression level of transgenes varies in different transgenic lines; thus, huge populations of plants need to be examined to correctly identify the set of plants in which the transgene is highly expressed over various generations. The insertion of transgenes into nontarget sites in the genome and introduction of undesirable traits are also other concerns. Moreover, plants developed through RNAi-based approaches must undergo rigorous regulatory processes before their commercialization, as they are placed under the category of 'transgenics'; therefore, there is a need to use more novel biotechnological strategies that provide crop plants with enhanced plant immunity and permanent broad-spectrum resistance against pathogens with minimum loss.

In recent years, developments in sequence-specific nucleases (SSNs) to introduce double-strand breaks at the target loci of interest have resulted in highly precise genome-editing tools, thereby initiating a new era of targeted genome engineering. The gene-specific DNA double-strand breaks (DSBs) caused by the SSNs are repaired primarily by high-fidelity homologous recombination (HR) or error-prone nonhomologous end-joining (NHEJ) pathways. Moreover, in comparison with RNAi, SSN-based genome editing can achieve complete knockout without incorporating exogenous DNA. These SSNs commonly include zinc-finger nucleases (ZFNs), transcription activator–like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated proteins (Cas). Among these tools, CRISPR/Cas9 has been found to be the most effective SSN. CRISPR was first described by Ishino et al. 1987, who discovered a group of 29 nucleotide repeats divided by nonrepetitive short sequences in *Escherichia coli*. CRISPR/Cas systems are part of the adaptive immune system of bacteria and archaea that protects them against invading nucleic acids such as viruses by cleaving the foreign DNA in a sequence-dependent manner. CRISPR/Cas targets either the DNA or the RNA of the invading pathogen (Sander and Joung 2014). CRISPRs are tandem series of short repeat sequences, which are separated

by a spacer sequence that has the same homology as that of the foreign sequence. Cas9 is a DNA endonuclease that is guided by RNA to target foreign DNA for inhibition (Kumar et al. 2016). The CRISPR/Cas9 system (also known as third-generation programmable nuclease) has been highly valued as the most efficient, easy, and specific technique for targeting the desired DNA fragment by using engineered nucleases. As this mechanism edits the genome rather than inserting some transgenes, the crops obtained from this technique are not classified as 'transgenics' and thus are likely to be more easily commercialized. This technique has found applications in many areas for crop improvement and has been used for providing resistance against diseases in various crops, including rice (Wang et al. 2015, 2016), wheat (Wang et al. 2014), maize (Svitashev et al. 2015), sorghum and tobacco (Jiang et al. 2013), tomato (Brooks et al. 2014), soybean (Jacobs et al. 2015), and potato (Wang et al. 2015).

The CRISPR/Cas9 system provides certain advantages over earlier editing techniques:

1. A wide range of viable targets: CRISPR/Cas requires a 20-bp target sequence preceding 5′-NGGPAM and thus can be used in large variety of genomes.
2. Easy delivery into cells: A shorter length of guide RNA (gRNA) makes delivery into cells easier than with ZFN/TALEN, which require longer sequences.
3. Single-mode engineering: When targeting a single site, two different ZFNs and TALENs must be engineered, consisting of many repetitive sequences. However, in the case of the CRISPR/Cas9 system, the Cas9 protein does not require re-engineering for each new target site. Once a target site is selected, only one cloning step is required to generate the final constructs carrying single-guide RNAs (sgRNAs).
4. Multiplexing: The target specificity of the CRISPR/Cas system is dependent only on sgRNAs, which are encoded by short sequences of ~100 bp, so it is possible to achieve simultaneous multiplex gene editing of plant loci by cotransforming multiple sgRNAs.

Recent scientific studies have led to development of efficient variations of this powerful tool wherein single base modifications are possible in both DNA and RNA. Base editors utilize CRISPR components such as gRNAs and Cas9/Cas13 or other nucleases, but do not cut the double helix. Instead, they chemically alter single bases with deaminase enzymes such as TadA and ADAR (Gaudelli et al. 2017; Cox et al. 2017). These modifications allow both transient changes in only the expressed part of the genome and extremely high sensitivity when DNA is targeted.

3.2 CRISPR-Engineered R Genes as Candidates for Resistance Against Pathogens

Plants have devised their own defensive mechanisms to suppress diseases and to eliminate damage caused by pathogens. Plants respond to pathogens by recognizing them at a cellular level, thereby triggering complex signaling pathways.

Thus, the plant–pathogen interaction is a multifarious process influenced by the pathogen and the molecules derived by plants, which include mainly sugars, lipopolysaccharides, and proteins (Boyd et al. 2013). The interaction involves three different stages: interaction, activation/modulation, and effective resistance/immunity (Andolfo et al. 2014). In the initial stage of the plant–pathogen interaction, the primary plant metabolism is altered and conformational changes occur in the targets of virulence factors. The second stage involves induction of PRR/Nibbler–triggered signaling following conformational changes in virulence factors. The third stage involves induction of hormone-tempered resistance, which includes primary metabolism feedback regulation through modifications in plant metabolic pathways. Plant–pathogen interactions encompass two responses: PTI and effector-triggered immunity (ETI). Upon pathogen attack, the PTI response is incited primarily by the recognition of PAMPs, which are evolutionarily conserved microbial elicitors and are ubiquitously present in all types of pathogens. There are certain receptors (such as PRRs) with high affinity, which are located on the surface. They are recognized as plasma membrane proteins and can be either receptor-like proteins (RLPs) or receptor-like kinases (RLKs) (Macho and Zipfel 2014; Sreekanta et al. 2015). ETI is another robust response that is characteristic of the innate immune system in plants, which is activated by R genes upon release of effectors by pathogens into plants, and this mechanism is in accordance with the gene-for-gene hypothesis (Flor 1971; Boyd et al. 2013). PTI offers basic resistance against pathogens through an arrangement of various cellular responses, including production of reactive oxygen species, ion flux across the membrane, mitogen-activated protein kinase (MAPK) cascade phosphorylation, overexpression of defense-related resistance genes, and long-term responses, which include deposition of callose (Zhang et al. 2008; Wu et al. 2014; Azizi et al. 2015). Plants are reservoirs of diverse resistance genes conferring protection against pathogen attacks. It has also been found that the majority of resistance genes in plants contain a short stretch coding for nucleotide-binding leucine-rich repeat (NB-LRR) protein (McHale et al. 2006). As such, pattern triggered immunity (PTI) and effector-triggered immunity (ETI) both act as a basal defense mechanism, which represses further action of the pathogen and disease spread.

With the increasing requirement for crop yield stability, breeding research is focused on designing plants that respond to pathogen attacks and elicit resistance responses. Conventional resistance breeding has been directed at introgression of resistance traits from wild cultivars into domesticated cultivars (e.g., NB-LRR genes) (Ercolano et al. 2012; Andolfo et al. 2014). The breeding efforts are supplemented with transgenesis, insertional mutagenesis, and genome-editing technologies, which speed up the breeding strategies to develop novel improved varieties. Within plants, allelic variations in the receptor-coding genes often explain genetic variation for disease resistance. It has been observed that resistance mediated by R genes involves identification of a single elicitor, which leads to resistance breakdown at an alarming pace. However, R gene sources are limited, which has restricted positioning of new resistance genes through traditional and transgenic approaches. Thus, there is a need to synthesize novel resistance genes with specific useful responses in

breeding programs. Current transgenic programs allow effective R gene transfer among species of diverse plants (Faino et al. 2010; Horvath and Barrangou 2010; Narusaka et al. 2013). Genome-editing tools have proved to be advantageous for engineering and designing R genes with new characteristics and their subsequent transfer into their homologs. Polymorphic amino acids in the coiled-coil (CC) and nucleotide-binding domains, which are known to be involved in recognition specificity, are targeted by genome editing (Ashikawa 2012). Studies have also shown that a paired amino acid mutation increases the caliber of the resistance protein, thereby triggering cell death (Stirnweis et al. 2014). In a recent study, artificial receptors of immunity were engineered, conferring resistance to pathogens that were evolutionary diverse (Giannakopoulou et al. 2015). Genome-editing tools have also been harnessed well to combine various pathogen recognition sites (PRSs) into a novel engineered R gene to impart resistance against various conserved pathogen effectors and PAMPs. Various studies have demonstrated that some motifs are enough for determining resistance in the plant host. For example, the EDVID motif of the CC domain is a consensus motif that has been demonstrated to be indispensable for R protein function (Rairdan et al. 2008). Further investigations have also shown that a hypersensitive reaction is triggered by overexpression of the isolated toll/interleukin-1 receptor domains of several Nibbler proteins (Zhang et al. 2014; Swiderski et al. 2009; Bernoux et al. 2011; Collier et al. 2011; Maekawa et al. 2011). Other studies have revealed that subdomain modular assembly from various pathogen recognition receptors is harnessed to form receptors that are functional. To provide resistance against divergent pathogens, a study was conducted where extracellular leucine-rich repeat receptor kinases (eLRRs) of the *EFR* receptor were exchanged by related parts from divergent species (e.g., from *XA21* or *FLS2*) (Albert et al. 2010; De Lorenzo et al. 2011; Schwessinger et al. 2015). This is an efficient approach wherein disease resistance is improved by stacking engineered R genes in one cultivar, imparting resistance to diverse pathogens (Piquerez et al. 2014).

3.3 Tackling Viral Pathogens Through CRISPR/Cas9–Based Genome Editing

3.3.1 Viruses as Potential Targets of CRISPR

Phytopathogens—including bacteria, fungi, viruses, and nematodes—are ubiquitously present in natural ecosystems and threaten agricultural production globally, which eventually increases the challenge of food security. Of all phytopathogens, viruses are the most important players, causing significant global yield losses accounting for about 10–15% of losses annually (Mahy and Van Regenmortel 2009). To reach the goal of food security, control of viruses is essential for crop protection. Various conventional strategies have been devised to manage plant viruses, which include management of vectors through pesticide use, activation of

natural predators, and usage of physical obstacles such as ultraviolet (UV) light–absorbing sheets and reflective mulches (Legg et al. 2017). In addition to this, certain cultural practices have been adopted for control of viral diseases, viz., weed management, early sowing, virus-free planting material, removal of infected plants, and crop-free periods. Moreover, it is very difficult to accomplish the goal of permanent management of viral diseases through chemical and cultural approaches, as problematic epidemiological factors (such as rapid evolution of viruses, unforeseeable expansion of virus host ranges, vector migration dynamics, local climatic conditions, plant senescence, and crop varieties) are associated with outbreaks of viral disease (Loebenstein and Katis 2014). Above all, a traditional strategy alone is ineffective, environmentally unsafe, expensive, and labor intensive. Thus, the most efficient way to accomplish the goal of combating viral diseases is to enhance cellular immunity in plants by improvising plant genotypes that are resistant to viruses or virus vectors, and utilizing them in conjunction with other conventional strategies.

Over the past few decades, improvement of crops for virus resistance has relied on utilization of viral resistance genes through breeding approaches, but these approaches are time consuming, labor intensive, and more complex (Gómez et al. 2009). To attain durable viral resistance, certain alternative strategies have been devised to engineer resistant plants. These strategies are categorized into two groups on the basis of their functional molecules, viz., RNA-mediated resistance and protein-mediated resistance. The mechanism of protein-mediated resistance has not been elucidated clearly, but the RNA-mediated mechanism (also known as the RNA-silencing pathway) has proved to be an efficient technique for engineering resistant plants. Other strategies include genetic engineering technology through ballistic bombardment and *Agrobacterium*-mediated transformation (Ye 2015).

Despite being powerful techniques, they suffer from the main limitation of having potential off-target effects. Earlier genetic engineering technologies harnessed for improving crops against viruses were ZFNs and TALENs, which were effective, but altering DNA-binding proteins to target a gene of interest can be time consuming and expensive (Ceasar et al. 2016). The limitations of these technologies were surpassed with the advent of the futuristic genome technology known as CRISPR/Cas9.

CRISPR/Cas9 technology has been utilized as a novel tool for viral resistance in plants against a host of viruses (Table 3.1). This novel technology is applicable to both DNA- and RNA-based viruses. Development of virus resistance in plants with the help of the CRISPR/Cas9 approach is based on two extensive procedures. In the first strategy, viral factors are targeted for genome manipulation in viruses. The second strategy involves targeting factors of the host plant that are accountable for the viral cycle by editing the plant genome.

3.3.1.1 Targeting Viral Genes: Potential Approach for Combating Geminiviruses or DNA Viruses with CRISPR/Cas9

Novel resistance has been demonstrated through use of the CRISPR/Cas9 system in various crops against geminivirus, which is known to cause serious damage in many dicotyledonous crops. In this system, specific double-stranded DNA of

Table 3.1 Recent studies undertaken to combat plant viruses by use of the CRISPR/Cas9 [clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9] system

Targeted viruses	Plant species	Genes targeted	Reference
Bean yellow dwarf virus (BeYDV)	*Nicotiana benthamiana*	Rep A/Rep and LIR	Baltes et al. (2015)
Beet severe curly top virus (BSCTV)	Arabidopsis thaliana and *N. benthamiana*	Rep, IR, and CP	Ji et al. (2015)
Beet curly top virus (BCTV), *Merremia* mosaic virus (MeMV), and tomato yellow leaf curl virus (TYLCV)	*N. benthamiana*	Rep, IR, and CP	Ali et al. (2015)
Cotton leaf curl Kokhran virus (CLCuKoV), tomato yellow leaf curl Sardinian virus (TYLCSV), TYLCV, MeMV, BCTV-Logan, and BCTV-Worland	Cotton and *N. benthamiana*	DNA satellite sequences	Ali et al. (2016)
Cucumber vein yellowing virus (CVYV), papaya ring spot mosaic virus-W (PRSV-W), and zucchini yellow mosaic virus (ZYMV)	*Cucumis sativa*	eIF4E host factor	Chandrasekaran et al. (2016)
Turnip mosaic virus (TuMV)	*A. thaliana*	eIF(iso)4E host factor	Pyott et al. (2016)

CP coat protein, *eIF4E* eukaryotic initiation factor 4E, *eIF(iso)4E* eukaryotic initiation factor, *IR* intergenic region, *LIR* long intergenic region, *Rep* replication associated protein

geminiviruses is targeted by gRNAs to disrupt important replication genes, which leads to suppression of viral replication. For example, in transgenic *Nicotiana benthamiana*, the Rep gene (also known as the replication initiator protein of bean yellow dwarf virus (BeYDV)) was targeted and mutations were introduced by utilizing the CRISPR/Cas9 system, which ultimately resulted in resistance against the viruses (Ali et al. 2015; Baltes et al. 2015). Further, it was also found that one sgRNA that targets the bean yellow dwarf virus genome confers plant resistance by using Cas9, which is devoid of catalytic activity. Additionally, this property aids in eliminating potential off-target effects in the plant genome (Baltes et al. 2015). Another study (Ji et al. 2015) utilizing CRISPR/Cas9 machinery in *N. benthamiana* introduced mutations at target sequences of the virus and demonstrated plant resistance against beet severe curly top virus (BSCTV). In a recent study, Cas9/gRNA machinery for virus interference was developed in plants where coat proteins, viral Rep genes, and a conserved intergenic region (IR) were targeted in *N. benthamiana* plants, providing broad-spectrum resistance against beet curly top virus (BCTV), *Merremia* mosaic virus (MeMV), and tomato yellow leaf curl virus (TYLCV) (Ali et al. 2015). Thus, effective obstruction of multiple monopartite and bipartite geminiviruses in one host was achieved by a single gRNA targeting a conserved sequence in the origin of replication. Following this, another study by Ali et al. 2016 suggested that CRISPR/Cas9 technology can be utilized against cotton leaf curl Kokhran virus (CLCuKoV) in cotton plants by in silico design of multiple gRNAs that target DNA satellite sequences, providing simultaneous broad-spectrum resistance against various

begomoviruses (CLCuKoV, tomato yellow leaf curl Sardinian virus (TYLCSV), TYLCV, MeMV, BCTV-Logan, and BCTV-Worland). In all of these studies, the viral genome was mutated in *N. benthamiana* plants by expression of the CRISPR/Cas9 system, which impeded replication of the virus and resulted in diminishment of contagious symptoms of viral infection. Further knowledge is required to assess the evolution of this resistance over generations and in more adverse environments (Chaparro-Garcia et al. 2015).

Viruses can evade CRISPR/Cas9 machinery by targeting coding regions, which leads to production of viral variants, resulting in their efficient replication and their subsequent movement in plant systems. Conversely, if conserved regions are targeted, viral variants are not generated and thus the CRISPR/Cas9 machinery is not evaded, which leads to durable and broad-spectrum resistance in plants against viruses. The aforementioned studies are summarized in Table 3.1.

3.3.1.2 Targeting Plant Genomes for Virus Resistance: A Remedy for Combating RNA Viruses

Targeting the RNA genome of the virus directly has not been successfully so far, as Cas9/gRNA machinery can only be used to target DNA viruses. In the future, this drawback will be overcome, as RNA can be cleaved by programming Cas9, which is mediated by the type III-B CRISPR/Cas system (O'Connell et al. 2014; Hale et al. 2009). These Cas9 mutants can target and cleave RNA viruses in plants. However, the efficiency of this system in combating RNA viruses in comparison with DNA viruses is yet to be established. To overcome this limitation, another strategy has been developed to combat RNA viruses, where, instead of the viral genome, plant genes that are accountable for causing viral infection are targeted directly by the CRISPR/Cas9 system, thus imparting resistance to the plants (Zaidi et al. 2016; Pyott et al. 2016; Chandrasekaran et al. 2016). It has been postulated that certain host factors (eIFs), also known as eukaryotic translation factors, are required by RNA viruses to perpetuate inside the host plants. Various plant genes or transcription-like initiation factors, such as eIF(iso)4E and eIF4E, are known to be involved directly in causing RNA viral infection (Sanfacon 2015). Also, these genes have been recognized as recessive resistance alleles, which impart resistance to potyviruses in diverse plants (Pyott et al. 2016; Chandrasekaran et al. 2016). In *Cucumis sativa* (cucumber) the eIF4E gene was mutated by targeted genome editing, which resulted in resistance against three pivotal cucumber viruses belonging to the Potyviridae family, which includes cucumber vein yellowing virus (CVYV), papaya ring spot mosaic virus-W (PRSV-W), and zucchini yellow mosaic virus (ZYMV) (Chandrasekaran et al. 2016). In another study carried out in *Arabidopsis thaliana*, eIF(iso)4E, a host factor essential for the life cycle of potyvirus TuMV (turnip mosaic virus), was deleted with utilization of CRISPR/Cas9 machinery (Pyott et al. 2016). Thus, host translation initiation factors are fundamental candidates which can be targeted for resistance against viruses (Sanfacon 2015).

3.3.2 CRISPR/Cas9–Mediated Resistance Against Fungal Pathogens

Fungal pathogens in plants pose a serious threat to global crop production, leading to crop losses estimated at 15% annually (Oerke 2006; LoPresti et al. 2015; Schwessinger et al. 2015). To combat fungal pathogens, farmers relied on fungicides for a long time. Because of their adverse effects on health, other strategies were devised, which included crop rotation, development of resistant cultivars, and transgenic approaches. As these practices have certain limitations, this necessitated the development of other novel tools for overcoming pathogens. A wide range of fungi have been successfully targeted using CRISPR/Cas9 (Table 3.2).

A study was carried out in *Phytopthora sojae*, an oomycete responsible for causing damping-off disease in soybean. Using the RXLR effector gene Avr4/6 as a target, it was observed that this gene was successfully knocked out by employing CRISPR/Cas9 machinery and was subsequently replaced by the selectable marker *npt*II, unraveling extraordinary roles for the two interrelated R gene loci *RPS4* and *RPS6*. It was also observed that short indels in each mutation were present particularly at the cleavage site of Cas9 (Fang and Tyler 2016). Another investigation was carried out in rice by Wang et al. 2016, in which they reported the improvement of rice blast resistance by engineering a CRISPR/Cas9 SSN (C-ERF922) targeting the OsERF922 gene in rice. The results showed that the number of fungal blast lesions was significantly decreased in mutant lines as compared with wild-type plants at both the seedling and tillering stages. Thus, gene modification via CRISPR/Cas9 is a useful approach for enhancing blast resistance in rice.

Additionally, various susceptibility genes have been manipulated using CRISPR/Cas9 technology to confer resistance against fungal diseases in plants. For example, in hexaploid wheat, the TaMLO-1 locus (also known as the mildew resistance locus) was targeted, conferring resistance against powdery mildew disease caused by

Table 3.2 Fungal diseases targeted by employing CRISPR/Cas9 [clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9] system

Fungus targeted	Disease	Crop	Gene targeted	Reference
Phytopthora sojae	Damping-off	Soybean	*Avr4/6* (RXLR effector gene)	Fang and Tyler (2016)
Magnaporthe oryzae	Rice blast	Rice	*OsERF922* (ethylene response transcription factor)	Wang et al. (2016)
Sporisorium scitamineum	Smut	Sugarcane	*mfa2* (matting gene)	Lu et al. (2017)
Oidium neolycopersici	Powdery mildew	Tomato	*SlMlo* (susceptibility gene for powdery mildew)	Nekrasov et al. (2017)
Erysiphe graminis	Powdery mildew	Wheat	*TaMLO-1* (susceptibility gene)	Wang et al. (2014)

mfa2 matting factor 2, *OsERF922 Oryza sativa* ethylene response factor 922, *SlMlo Solanum lycopersicum* mildew locus, *TaMLO Triticum aestivum* mildew locus

Erysiphe graminis (Wang et al. 2014). In another study performed in tomato (*Solanum lycopersicum*), a 48-bp deletion was carried out at the SlMlo1 locus, which gave rise to a transgene-free tomato (tomelo) with resistance against powdery mildew disease caused by *Oidium neolycopersici* (Nekrasov et al. 2017).

Lu et al. (2017) reported the development of a CRISPR/Cas9 and transfer DNA (T-DNA)–based dual vector system that allowed efficient knockout or knock-in of a gene of interest in *Sporisorium scitamineum* in a site-specific manner. The dual system that was developed greatly facilitates gene function study in *S. scitamineum* and could potentially be used for other basidiomycete fungi.

The most interesting fact about the CRISPR/Cas9 system is that it can target various independent genes by employing several sgRNAs, as observed in filamentous fungus (*Trichoderma reesei*), yeast (*Saccharomyces cerevisiae*), and two plant systems (*Arabidopsis* and *Oryza sativa*) (Liu et al. 2014; Cong et al. 2013; Zhang et al. 2015; Endo et al. 2015).

3.3.3 Bacteria and Nematodes as Targets of Genome Editing

The emergence of antimicrobial-resistant bacteria poses a serious threat to a wide range of plants and animals. CRISPR-based antibacterials are a novel method for producing an arsenal of antibacterials capable of targeting any pathogenic bacteria. Because it has high sequence specificity, CRISPR/Cas can easily distinguish between pathogenic or commensal bacterial species. To alter CRISPR/Cas machinery to attack rather than defend bacteria, CRISPR guide RNAs are designed to target either virulent or essential genes that are specific to pathogens (Greene 2018). However, the delivery of CRISPR/Cas9 antibacterials (160-kDa protein–RNA complexes) poses a big challenge to ensure they are effective when crossing through the bacterial membrane. Phages are also used to package vectors encoding CRISPR/Cas9, and such phages result in speedy killing of specific bacteria within complex bacterial populations (Gomaa et al. 2014). Methods are now being developed to engineer phage scaffolds to change species specificity (Ando et al. 2015), and new genome-editing strategies are being explored to resensitize bacteria to antibiotics (Yosef et al. 2015). Peng et al. (2017) have recently engineered canker-resistant citrus plants through CRISPR/Cas9–targeted editing of the susceptibility gene CsLOB1 promoter. Plants resistant to bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* have been obtained by similar genome-editing tools targeting transcriptional regulation of S genes by the effector (Li et al. 2015).

Plant pathogenic nematodes mainly include root knot nematodes and cyst nematodes. Besides being extremely damaging to their host plants, they facilitate secondary infection by other plant pathogens such as bacteria and fungi. The control most commonly used against nematodes consists of nematicides, many of which have been known to detrimentally affect the environment, consequently leading to bans on their use in many countries across the globe. Genomics-assisted advances in understanding of pathogenicity have led to identification of a range of candidate

genes that can be targeted through RNAi/knockdown to prevent host–pathogen interaction. However, off-target effects and regulatory issues associated with RNAi-based methods limit their use (Rual et al. 2007; Banerjee et al. 2017).The development of CRISPR/Cas9 genome-editing technology is very helpful for understanding of the biology of nematode parasites and for its ability to treat the infestation. The CRISPR/Cas system could be instrumental in gaining insights into host–parasite and parasite–vector interactions, and the genetic basis of parasitism. A large number of CRISPR/Cas9 genome-editing techniques have been reported in *Caenorhabditis elegans*, which included co-CRISPR (Kim et al. 2014), co-conversion (Arribere et al. 2014), bacterial feeding (Liu et al. 2014), SapTrap (Schwartz and Jorgensen 2016), and self-excising cassettes (Dickinson et al. 2015). CRISPR/Cas9 technology has been successfully translated from the model nematode *C. elegans* to several pathogenic nematodes, including Strongyloides spp., Ascaris suum, Brugia malayi, and Haemonchus contortus (Ward 2015; Britton et al. 2016; Zamanian and Andersen 2016) thus opening up a new direction to address nematode pathogens in plants.

3.4 Conclusions

Plant resistance against pathogens is the most viable environmentally friendly option to reduce pathogen-induced crop losses. Conventional strategies are still being successfully used to transfer desired traits into susceptible genotypes; however, genome-editing tools offer a lucrative solution to keep pace with the rapidly evolving nature of phytopathogens. Among the various genome-editing variants, CRISPR/Cas9 has become a method of choice because of its relative ease and specificity. Over the last 5 years, several successful attempts have been made, through the use of CRISPR/Cas9, to develop plant resistance against viruses, fungi, and bacteria. Further modifications in CRISPR-based genome editing to target specific bases in DNA, as well as RNA, tremendously enhance the potential of these tools to engineer plant resistance against biotic factors.

References

Albert M, Jehle AK, Mueller K, Eisele C, Lipschis M, Felix G (2010) *Arabidopsis thaliana* pattern recognition receptors for bacterial elongation factor Tu and flagellin can be combined to form functional chimeric receptors. J Biol Chem 285:19035–19042

Ali Z, Abulfaraj A, Idris A, Ali S, Tashkandi M, Mahfouz MM (2015) CRISPR/Cas9–mediated viral interference in plants. Genome Biol 16:238

Ali Z, Ali S, Tashkandi M, Zaidi SS-E-A, Mahfouz MM (2016) CRISPR/Cas9–mediated immunity to geminiviruses: differential interference and evasion. Sci Rep 6:26912

Ando H, Lemire S, Pires DP, Lu TK (2015) Engineering modular viral scaffolds for targeted bacterial population editing. Cell Syst 1:187–196

Andolfo G, Jupe F, Witek K, Etherington GJ, Ercolano MR, Jones JD (2014) Defining the full tomato NB-LRR resistance gene repertoire using genomic and cDNA RenSeq. BMC Plant Biol 14:120

Andolfo G, Iovieno P, Frusciante L, Ercolano MR (2016) Genome-editing technologies for enhancing plant disease resistance. Front Plnt Sci 7:1813

Arribere JA, Bell RT, Fu BXH, Artiles KL, Hartman PS, Fire AZ (2014) Efficient marker-free recovery of custom genetic modifications with CRISPR/Cas9 in *Caenorhabditis elegans*. Genetics 198:837–846

Ashikawa I (2012) Regions outside the leucine-rich repeat domain determine the distinct resistance specificities of the rice blast resistance genes Pik and Pik-m. Mol Breed 30:1531–1535

Azizi G, Yazdani R, Magaji Hamid K, Razavi A, Mirshafiey A (2015) IL-22 produced by T helper cell 22 as a new player in the pathogenesis of immune thrombocytopenia. Endocr Metab Immune Disord Drug Targets 15(3):242–250

Baltes NJ, Hummel AW, Konecna E, Cegan R, Bruns AN, Bisaro DM (2015) Conferring resistance to geminiviruses with the CRISPR–Cas prokaryotic immune system. Nat Plants 1:15145

Banerjee S, Banerjee A, Gill SS, Gupta OP, Dahuja A, Jain PK, Sirohi A (2017) RNA interference: a novel source of resistance to combat plant parasitic nematodes. Front Plant Sci 8:834

Bernoux MVT, Williams S, Warren C, Hatters D, Valkov E (2011) Structural and functional analysis of a plant resistance protein TIR domain reveals interfaces for self-association, signaling, and auto regulation. Cell Host Microbe 9:200–211

Boyd LA, Ridout C, O'Sullivan DM, Leach JE, Leung H (2013) Plant–pathogen interactions: disease resistance in modern agriculture. Trends Genet 29(4):233–240

Britton C, Roberts B, Marks ND (2016) Functional genomics tools for *Haemonchus contortus* and lessons from other helminths. Adv Parasitol 93):599–623

Brooks C, Nekrasov V, Lippman ZB, Van Eck J (2014) Efficient gene editing in tomato in the first generation using the clustered regularly interspaced short palindromic repeats/CRISPR-associated9 system. Plant Physiol 166(3):1292–1297

Ceasar SA, Rajan V, Prykhozhij SV, Berman JN, Ignacimuthu S (2016) Insert, remove or replace: a highly advanced genome editing system using CRISPR/Cas9. Biochim Biophys Acta 1863:2333–2344

Chandrasekaran J, Brumin M, Wolf D, Leibman D, Klap C, Pearlsman M (2016) Development of broad virus resistance in non-transgenic cucumber using CRISPR/Cas9 technology. Mol Plant Pathol 17:7

Chaparro-Garcia A, Kamoun S, Nekrasov V (2015) Boosting plant immunity with CRISPR/Cas. Genome Biol 16:254

Collier SM, Hamel LP, Moffett P (2011) Cell death mediated by the N terminal domains of a unique and highly conserved class of NB-LRR protein. Mol Plant-Microbe Interact 24:918–931

Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N (2013) Multiplex genome engineering using CRISPR/Cas systems. Sci 339:819–823

Cox DB, Gootenberg JS, Abudayyeh OO, Franklin B, Kellner MJ, Joung J, Zhang F (2017) RNA editing with CRISPR–Cas13. Science 358(6366):1019–1027

De Lorenzo G, Brutus A, Savatin DV, Sicilia F, Cervone F (2011) Engineering plant resistance by constructing chimeric receptors that recognize damage associated molecular patterns (DAMPs). FEBS Lett 585:1521–1528

Dickinson DJ, Pani AM, Heppert JK, Higgins CD, Goldstein B (2015) Streamlined genome engineering with a self-excising drug selection cassette. Genetics 200:1035–1049

Endo M, Mikami M, Toki S (2015) Multigene knock out utilizing off-target mutations of the CRISPR/Cas9 system in rice. Plant Cell Physiol 56:41–47

Ercolano MR, Sanseverino W, Carli P, Ferriello F, Frusciante L (2012) Genetic and genomic approaches for R-gene mediated disease resistance in tomato: retrospects and prospects. Plant Cell Rep 31:973–985

Faino L, Carli P, Testa A, Cristinzio G, Frusciante L, Ercolano MR (2010) PotatoR1resistance gene confers resistance against *Phytophthora infestans* in transgenic tomato plants. Eur J Plant Pathol 128:233

Fang Y, Tyler BM (2016) Efficient disruption and replacement of an effector gene in the oomycete *Phytophthora sojae* using CRISPR/Cas9. Mol Plant Pathol 17:127–139

Flor HH (1971) Current status of the gene-for-gene concept. Annu Rev Phytopathol 9(1): 275–296

Gaudelli NM, Komor AC, Rees HA, Packer MS, Badran AH, Bryson DI, Liu DR (2017) Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. Nature 551(7681):464

Giannakopoulou A, Steele JFC, Segretin ME, Bozkurt TO, Zhou J, Robatzek S (2015) Tomato I2 immune receptor can be engineered to confer partial resistance to the oomycete *Phytophthora infestans* in addition to the fungus *Fusarium oxysporum*. Mol Plant-Microbe Interact 28:1316–1329

Gomaa AA, Klumpe HE, Luo ML, Selle K, Barrangou R, Beisel CL (2014) Programmable removal of bacterial strains by use of genome-targeting CRISPR–Cas systems. Mol Bio 5:e00928–e00913

Gómez P, Rodríguez-Hernández AM, Moury B, Aranda MA (2009) Genetic resistance for the sustainable control of plant virus disease: breeding, mechanisms and durability. Eur J Plant Pathol 125:1–22

Greene AC (2018) CRISPR-based antibacterials: transforming bacterial defense into offense. Trends Biotech 36:127–130

Hale CR, Zhao P, Olson S, Duff MO, Graveley BR, Wells L (2009) RNA-guided RNA cleavage by a CRISPR RNA–Cas protein complex. Cell 139:945–956

Horvath P, Barrangou R (2010) CRISPR/Cas, the immune system of bacteria and archaea. Science 327:167–170

Ishino Y, Shinagawa H, Makino K, Amemura M, Nakata A (1987) Nucleotide sequence of the *iap* gene, responsible for alkaline phosphatase isozyme conversion in *Escherichia coli*, and identification of the gene product. J Bact 169:5429–5433

Jacobs TB, LaFayette PR, Schmitz RJ, Parrott WA (2015) Targeted genome modifications in soybean with CRISPR/Cas9. BMC Biotechnol 15:16

Ji X, Zhang H, Zhang Y, Wang Y, Gao C (2015) Establishing a CRISPR-Cas-like immune system conferring DNA virus resistance in plants. Nat Plants 1:15144

Jiang W, Zhou H, Bi H, Fromm M, Yang B, Weeks DP (2013) Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in *Arabidopsis*, tobacco, sorghum and rice. Nucleic Acids Res 41(20):e188

Kim H, Ishidate T, Ghanta KS, Seth M, Conte D, Shirayama M, Mello CC (2014) A co-CRISPR strategy for efficient genome editing in *Caenorhabditis elegans*. Genetics 197:1069–1080

Koch A, Biedenkopf D, Furch A, Weber L, Rossbach O, Abdellatef E, Linicus L, Johannsmeier J, Jelonek L, Goesmann A, Cardoza V (2016) An RNAi-based control of *Fusarium graminearum* infections through spraying of long dsRNAs involves a plant passage and is controlled by the fungal silencing machinery. PLoS Pathog 12(10):e1005901

Kumar V, Baweja M, Singh PK, Shukla P (2016) Recent developments in systems biology and metabolic engineering of plant–microbe interactions. Front Plant Sci 7:1421

Legg JP, Shirima R, Tajebe LS, Guastella D, Boniface S, Jeremiah S (2017) Biology and management of *Bemisia* white fly vectors of cassava virus pandemics in Africa. Pest Manag Sci 70:1446–1453

Li L, He ZY, Wei XW, Gao GP, Wei YQ (2015) Challenges in CRISPR/Cas9 delivery: potential roles of nonviral vectors. Hum Gene Ther 26:452–462

Liu DF, Chen XJ, Liu JQ, Ye JC, Guo ZJ (2012) The rice ERF transcription factor OsERF922 negatively regulates resistance to *Magnaporthe oryzae* and salt tolerance. J Exp Bot 63(10):3899–3912

Liu P, Long L, Xiong K, Yu B, Chang N, Xiong J-W, Zhu Z, Liu D (2014) Heritable/conditional genome editing in *C. elegans* using a CRISPR–Cas9 feeding system. Cell Res 24:886–889

Loebenstein G, Katis N (2014) Control of plant virus diseases: seed-propagated crops. In: Gad L, Nikolaos K (eds) Advance virus research. Academic, Cambridge, p 11

LoPresti L, Lanver D, Schweizer G, Tanaka S, Liang L, Tollot M (2015) Fungal effectors and plant susceptibility. Annu Rev Plant Biol 66:513–545

Lu S, Shen X, Chen B (2017) Development of an efficient vector system for gene knock-out and near in-*cis* gene complementation in the sugarcane smut fungus. Sci Rep 7:3113
Macho AP, Zipfel C (2014) Plant PRRs and the activation of innate immune signaling. Mol Cell 54(2):263–272
Maekawa T, Cheng W, Spiridon LN, Toller A, Lukasik E, Saijo Y (2011) Coiled-coil domain-dependent homodimerization of intracellular barley immune receptors define saminimal functional module for triggering cell death. Cell Host Microbe 9:187–199
Mahy BWJ, Van Regenmortel MHV (2009) Desk encyclopedia of plant and fungal virology. Academic, Cambridge, MA
McHale L, Tan X, Koehl P, Michelmore RW (2006) Plant NBS-LRR proteins: adaptable guards. Genome Biol 7(4):212
Narusaka M, Kubo Y, Hatakeyama K, Imamura J, Ezura H, Nanasato Y (2013) Interfamily transfer of dual NB-LRR genes confers resistance to multiple pathogens. PLoS One 8:e55954
Nekrasov V, Wang C, Win J, Lanz C, Weigel D, Kamoun S (2017) Rapid generation of a transgene free powdery mildew resistant tomato by genome deletion. Sci Rep 7:482
O'Connell MR, Oakes BL, Sternberg SH, East-Seletsky A, Kaplan M, Doudna JA (2014) Programmable RNA recognition and cleavage by CRISPR/Cas9. Nature 516:263–266
Oerke EC (2006) Crop losses to pests. J Agric Sci 144:31–43
Panwar V, Jordan M, McCallum B, Bakkeren G (2017) Host-induced silencing of essential genes in *Puccinia triticina* through transgenic expression of RNAi sequences reduces severity of leaf rust infection in wheat. Plant Biotech J 23:1–11
Peng A, Chen S, Lei T, Xu L, He Y et al (2017) Engineering canker-resistant plants through CRISPR/Cas9-targeted editing of the susceptibility gene CsLOB1 promoter in citrus. Plant Biotech J 15:1509–1519
Piquerez SJM, Harvey SE, Beynon JL, Ntoukakis V (2014) Improving crop disease resistance: lessons from research on *Arabidopsis* and tomato. Front Plant Sci 5:671
Pyott DE, Sheehan E, Molnar A (2016) Engineering of CRISPR/Cas9–mediated poty virus resistance in transgene free *Arabidopsis* plants. Mol Plant Pathol 4:1–13
Rairdan GJ, Collier SM, Sacco MA, Baldwin TT, Boettrich T, Moffett P (2008) The coiled-coil and nucleotide binding domains of the potato Rx disease resistance protein function in pathogen recognition and signaling. Plant Cell 20:739–751
Rawat N, Pumphrey MO, Liu S, Zhang X, Tiwari VK, Ando K, Trick HN, Bockus WW, Akhunov E, Anderson JA, Gill BS (2016) Wheat Fhb1 encodes a chimeric lectin with agglutinin domains and a pore-forming toxin-like domain conferring resistance to *Fusarium* head blight. Nat Gen 48(12):1576
Rual JF, Klitgord N, Achaz G (2007) Novel insights into RNAi off-target effects using *C. elegans* paralogs. BMC Genomics 8(1):106
Sander JD, Joung JK (2014) CRISPR–Cas systems for editing, regulating and targeting genomes. Nat Biotech 32(4):347
Sanfacon H (2015) Plant translation factors and virus resistance. Viruses 7:3392–3419
Schwartz ML, Jorgensen EM (2016) SapTrap, a toolkit for high-throughput CRISPR/Cas9 gene modification in *Caenorhabditis elegans*. Genetics 202:1277–1288
Schwessinger B, Bahar O, Thomas N, Holton N, Nekrasov V, Ruan D (2015) Transgenic expression of the dicotyledonous pattern recognition receptor EFR in rice leads to ligand-dependent activation of defense responses. PLoS Pathog 11:e1004809
Sreekanta S, Bethke G, Hatsugai N, Tsuda K, Thao A, Wang L, Glazebrook J (2015) The receptor-like cytoplasmic kinase PCRK1 contributes to pattern-triggered immunity against *Pseudomonas syringae* in *Arabidopsis thaliana*. New Phytol 207(1):78–90
Stirnweis D, Milani SD, Jordan T, Keller B, Brunner S (2014) Substitutions of two amino acids in the nucleotide-binding site domain of a resistance protein enhance the hypersensitive response and enlarge the PM3F resistance spectrum in wheat. Mol Plant-Microbe Interact 27:265–276
Svitashev S, Young JK, Schwartz C, Gao H, Falco SC, Cigan AM (2015) Targeted mutagenesis, precise gene editing, and site-specific gene insertion in maize using Cas9 and guide RNA. Plant Physiol 169(2):931–945

Swiderski MR, Birker D, Jones JDG (2009) The TIR domain of TIR-NB-LRR resistance proteins is a signaling domain involved in cell death induction. Mol Plant-Microbe Interact 22:157–165
Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C et al (2014) Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat Biotechnol 32(9):947–995
Wang S, Zhang S, Wang W, Xiong X, Meng F, Cui X (2015) Efficient targeted mutagenesis in potato by the CRISPR/Cas9 system. Plant Cell Rep 34(9):1473–1476
Wang F, Wang C, Liu P, Lei C, Hao W, Gao Y et al (2016) Enhanced rice blast resistance by CRISPR/Cas9–targeted mutagenesis of the ERF transcription factor gene OsERF922. PLoS One 11:e0154027
Ward JD (2015) Rapid and precise engineering of the *Caenorhabditis elegans* genome with lethal mutation co-conversion and inactivation of NHEJ repair. Genetics 199(2):363–377
Wu L, Chen H, Curtis C, Fu ZQ (2014) Go in for the kill: how plants deploy effector-triggered immunity to combat pathogens. Virulence 5(7):710–721
Ye X (2015) Development and application of plant transformation techniques. J Integr Agric 14:411–413
Yosef I, Manor M, Kiro R, Qimron U (2015) Temperate and lytic bacteriophages programmed to sensitize and kill antibiotic-resistant bacteria. Proc Natl Acad Sci USA 112:7267–7272
Zaidi SS, Tashkandi M, Mansoor S, Mahfouz MM (2016) Engineering plant immunity: using CRISPR/Cas9 to generate virus resistance. Front Plant Sci 7:1–10
Zamanian M, Andersen EC (2016) Prospects and challenges of CRISPR/Cas genome editing for the study and control of neglected vector-borne nematode diseases. FEBS J 283(17):3204–3221
Zhang W, He SY, Assmann SM (2008) The plant innate immunity response in stomatal guard cells invokes G-protein-dependent ion channel regulation. Plant J 56(6):984–996
Zhang F, Wen Y, Guo X (2014) CRISPR/Cas9 for genome editing: progress, implications and challenges. Human Mol Gen 23(1):40–46
Zhang D, Li Z, Li J-F (2015) Genome editing: new antiviral weapon for plants. Nat Plan 1:15146
Zipfel C (2014) Plant pattern-recognition receptors. Trends in Imm 35(7):345–351

Chapter 4
Disease Resistance in Wheat: Present Status and Future Prospects

Mandeep S. Randhawa, Sridhar Bhavani, Pawan K. Singh, Julio Huerta-Espino, and Ravi P. Singh

4.1 Introduction

Wheat is the world's second most important cereal crop after either rice or maize and is the major source of calories and protein in human food, particularly in developing nations (Curtis et al. 2002). Annually, over 700 million tonnes of wheat is harvested from approximately 215 million hectares globally, which is more than the production of any other crop (WHEAT 2013). Wheat has adapted itself to diverse climatic conditions and, as such, is grown over a range of altitudes and latitudes under irrigated, severe drought, and wet conditions. The global demand for wheat is projected to rise by 60% by 2050 because of the increase in the world's human population and changing livelihoods. Therefore, the average global wheat yields on a per-hectare basis will need to increase to approximately 5 tonnes per hectare from the current 3 tonnes per hectare (WHEAT 2013; Singh et al. 2016).

Efforts to continually increase yield and quality are not without challenges. Wheat production has been threatened by unexpected abiotic and biotic stresses due to abrupt environmental changes or movement of pathogens. In addition, urbanization

M. S. Randhawa (✉)
International Maize and Wheat Improvement Center (CIMMYT), ICRAF Campus, Nairobi, Kenya
e-mail: m.randhawa@cgiar.org

S. Bhavani · P. K. Singh · R. P. Singh
International Maize and Wheat Improvement Center (CIMMYT), Texcoco, Estado de México, Mexico

J. Huerta-Espino
International Maize and Wheat Improvement Center (CIMMYT), Texcoco, Estado de México, Mexico

Campo Experimental Valle de México, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Chapingo, Estado de México, Mexico

S. H. Wani (ed.), *Disease Resistance in Crop Plants*,
https://doi.org/10.1007/978-3-030-20728-1_4

has resulted in reduced availability of suitable farmland for wheat cultivation. The monoculture of modern wheat cultivars with low genetic diversity has resulted in pathogen resurgences, which threaten wheat supplies (Figueroa et al. 2017).

Of the nearly 200 diseases and pests that have been documented, 50 are considered economically important because of their potential to damage crops and affect farmers' incomes (Weise 1987). Potential grain yield losses of 18% due to diseases and actual losses of 13% under current disease control have been estimated (Oerke 2006). Among biotic stresses, pathogenic fungi represent a significant challenge to wheat production. This chapter consolidates information on the present status of key diseases that affect wheat production, their causal pathogenic fungi, and future prospects for breeding for disease resistance. Generally, pathogenic fungi can be broadly classified into two categories—biotrophic and necrotrophic fungi—on the basis of their lifestyle.

4.2 Diseases Caused by Biotrophic Fungi

Biotrophic fungi are obligate parasites that survive on living plants to obtain the nutrients they require for survival, thus weakening the plant system and resulting in poor plant health and reduced grain fill. The causal pathogens have distinct strains or physiological races, which can be determined by testing the host response to infection in a set of tester lines carrying different resistance genes or their combinations (Singh et al. 2016). Among biotrophic fungi, rust pathogens continue to affect and threaten the world's wheat production (Roelfs et al. 1992), although powdery mildew has also emerged as an economically important disease.

4.2.1 Stem Rust

Stem rust (SR), caused by *Puccinia graminis* f. sp. *tritici* (Pgt), is one of the most damaging fungal diseases of wheat (Roelfs et al. 1992) and is found in warmer and humid wheat-growing regions. Masses of dark-red urediniospores on the leaf sheaths, stems, glumes, and awns of susceptible plants are typical symptoms of SR infection (Kolmer 2005). SR causes yield losses through a reduction in grain size and lodging of the plant (Leonard and Szabo 2005). In severe epidemics, farmers can loose their total crop harvest if susceptible cultivars are grown in rust hot spot areas (Saari and Prescott 1985; Dean et al. 2012). Breeding for resistance against SR was the foundation of the Green Revolution in the mid- to late twentieth century (Peterson 2001). Thus, SR was under control for over 3–4 decades because of deployment of SR resistance genes, including the 1BL.1RS translocation–carrying gene *Sr31*, until the emergence of the notorious Ug99 race.

SR has become a significant disease in recent years, since a highly aggressive Pgt race known as Ug99 or TTKSK was detected in 1998 in Uganda, with combined

virulence against *Sr31* and various other commonly deployed resistance genes (Pretorius et al. 2000; Jin et al. 2007, 2008). Upon testing, 90% of the wheat cultivars across the globe were found to be susceptible to this strain (Singh et al. 2011). In the following years, new races in the Ug99 family with additional virulence against *Sr24* (Jin et al. 2008; Pretorius et al. 2010; Visser et al. 2011), *Sr36* (Jin et al. 2009), and *SrTmp* (Newcomb et al. 2016) caused susceptible reactions in a number of wheat varieties carrying the respective genes. Urediniospores of races in the Ug99 lineage have dispersed to such a large extent that these races have been identified in 13 East African countries, Southern Africa, Yemen, Egypt, and Iran, and it is anticipated that wheat-growing areas in Asia and beyond are largely at risk of SR (Singh et al. 2015b). Consequently, there has been a drastic reduction in the number of effective resistance genes that can be deployed in wheat cultivars (Randhawa et al. 2018). Moreover, other races not related to Ug99 have appeared in various parts of the world. In 2014, a Digalu race caused a highly devastating epidemic of SR in Ethiopia, and a similar race was reported in Germany (Olivera Firpo et al. 2015, 2017) and more recently in the UK (Lewis et al. 2018). In addition, another highly aggressive race not belonging to Ug99 was detected in Sicily in 2016 and caused losses in both bread and durum wheat (Bhattacharya 2017). However, combination of genes that are effective against the Ug99 lineage, including *Sr47*, could provide a way to control the Sicily variant of SR.

4.2.2 Stripe Rust

Stripe rust (YR), caused by *Puccinia striiformis* f. sp. *tritici* (Pst), is prevalent in cooler wheat-growing regions with wet weather (Chen et al. 2014). YR is considered the most significant disease because of its potential to cause total yield losses in susceptible cultivars, and because of its frequent and wide occurrence (Chen 2005). According to recent estimates, the global annual losses caused by YR are US$1 billion (Beddow et al. 2015). Average annual economic losses of AU$127 million attributable to YR have been estimated in Australia (Murray and Brennan 2009).

Traditionally, YR has manifested in regions with cooler temperatures; however, since 2000, new aggressive races adapted to warmer climates have dispersed to other parts of the world with previously low YR incidence rates and caused damage to wheat crop in many countries (Ali et al. 2014). Genetic diversity studies have indicated a clonal nature of Pst populations in Europe, Australia, and North America, whereas significant levels of diversity prevail within some pathogen populations (Chen et al. 2014). The Pst populations in western China and Central Asia are diverse, consistent with those in the Himalayan and nearby regions as the center of pathogen diversity, where sexual recombination appears to be common (Ali et al. 2014; Hovmøller et al. 2011). Recent studies have traced the origin of newly emerged race groups in Europe to Himalayan regions, indicating the role of incursions in the population structure (Hovmøller et al. 2015; Hubbard et al. 2015)

4.2.3 Leaf Rust

Leaf rust (LR), caused by *Puccinia triticina* (Pt), is considered the most common and widely distributed of the three rust diseases (Bolton et al. 2008; Huerta-Espino et al. 2011). The pathogen is primarily present in wheat-growing areas with modest temperatures and moist conditions. Reductions in kernel size, kernel weight, and numbers of grains per spike are the main factors that result in yield losses. Between 2000 and 2004, total economic losses of US$350 million attributable to LR in the USA were estimated (Huerta-Espino et al. 2011). In Australia, yield losses of AU$12 million ascribed to LR were calculated (Murray and Brennan 2009). Previously, LR was a more devastating disease in Mexico and South Asia; recently, it has not been so important, because of the use of cultivars with durable resistance provided by race non-specific minor genes (Huerta-Espino et al. 2011).

4.2.4 Powdery Mildew

The pathogenic fungus *Blumeria graminis* f. sp. *tritici* is the causal agent of powdery mildew (PM), which is considered an important disease of wheat with wider distribution in regions with dry and cool climatic conditions. This disease is of great significance in China, Europe, and South America (Dubin and Duveiller 2011). Like YR, this disease has adapted to warmer and drier regions because of intensive agriculture practices with use of nitrogen fertilizers and high irrigation rates (Cowger et al. 2012). Therefore, wheat grown in South Asia is highly prone to PM if susceptible varieties are grown in conducive environments. In comparison with rusts, PM conidia do not disseminate to distant locations but do evolve at a fast rate (Duveiller et al. 2007). Yield losses attributable to PM vary with the seasons and locations: 5–17% in North Carolina, below 10% in Western Europe, a record high of 20% in the UK, 10–15% (but sometimes reaching 30–35%) in Russia, up to 62% in Brazil, and 30–40% in China during heavy epidemics (Mehta 2014).

4.3 Diseases Caused by Necrotrophic Fungi

Necrotrophic fungi are facultative parasites that can survive on dead or dying tissues when they do not have access to living wheat plants. The wheat diseases *Fusarium* head blight (FHB), *Septoria tritici* blotch (STB), tan spot (TS), spot blotch (SB), and *Stagonospora nodorum* blotch (SNB)—which are caused by different necrotrophs—are of utmost significance, as they result in greater yield reductions. More recently, wheat blast (WB) in Bangladesh, previously reported in South America, has attracted significant attention from wheat researchers.

4.3.1 Fusarium *Head Blight*

Fusarium head blight (FHB) is the most damaging floral disease of wheat and is the most frequent and widespread one in wheat-growing regions of North America, East Asia, and Europe. FHB disease is caused predominantly by the ascomycete fungus *Fusarium graminearum*, although more than 18 species are known to cause FHB. FHB disease leads to early senescence of the wheat head, with shriveled grains. Combinations of the pathogen *F. graminearum* with other cereal-infecting *Fusarium* species can cause severe FHB epidemics (Brown and Proctor 2013). Recurrence of FHB with severe epidemics in the 1990s threatened several major wheat producers, including Argentina, China, Canada, the USA, and parts of Europe (Buerstmayr et al. 2012). In the USA, economic losses of US$3 billion attributable to FHB were estimated between the early 1990s and 2008 (Schumann and D'Arcy 2009).

Warm and moist weather conditions prior to and during anthesis enhance FHB growth in wheat. FHB results in significant yield losses, and quality deterioration through accrual of mycotoxin in the grain is the most damaging manifestation. Early infections around anthesis cause greater yield losses (due to floret sterility or poor grain filling) than later infections, which mainly lead to a higher mycotoxin buildup (Buerstmayr et al. 2012). The mycotoxin deoxynivalenol (DON), produced by *F. graminearum* and *F. culmorum*, is not only a virulence factor for wheat; it is also a major food safety risk to humans, animals, and natural ecosystems (Buerstmayr et al. 2012; Figueroa et al. 2017).

Many countries have placed legal limits on acceptable mycotoxin levels to regulate marketing of wheat and its products for the benefit of various end users. Wheat grain may have no market value in the case of heavy mycotoxin contamination, and sharp reductions in the market price of wheat grain may occur in cases where mycotoxin levels exceed the permitted thresholds (McMullen et al. 2012). In the USA, economic losses of US$2.59 billion (29.9%) caused by FHB in wheat and barley from 1998 to 2001, out of total losses of US$7.67 billion from 1993 to 2001, were estimated (McMullen et al. 2012).

In China, 5–10% yield losses are usually caused by FHB, but the damage can increase to 20–40% during severe epidemics and can even cause complete loss. With an occurrence rate of 46%, a total of 29 FHB epidemics (12 severe and 17 moderate) between 1950 and 2012 were recorded in China (Buerstmayr et al. 2012; Cheng et al. 2012). Yield losses of 50–60% and 70% in Europe and South America, respectively, have been reported (Buerstmayr et al. 2012; Kohli and Diaz de Ackermann 2013; Mehta 2014).

4.3.2 Leaf-Spotting Diseases

The wheat diseases *S. tritici* blotch, tan spot, spot blotch, and *S. nodorum* blotch constitute the leaf-spotting disease (LSD) complex. On some occasions, several LSDs can concurrently infect wheat plants, but the components differ from one region to other despite similar symptoms (Singh et al. 2016). Under favorable conditions, more than 50% yield losses may be caused by LSDs (Duveiller and Sharma 2012; Goodwin 2012; Singh et al. 2010). Usually, in susceptible wheat germplasms, LSDs result in yield reduction through poor grain filling, lower test weights, smaller numbers of grains per spike, and quality deterioration due to shriveled grains, red smudge, salmon-pink or red discoloration, and black point (May et al. 2014; Singh et al. 2010). Stubble retention on the soil surface under conservation agriculture practices is the main reason for the increasing occurrence of LSD epidemics (Singh et al. 2016). Moreover, several other practices such as shorter crop rotations, monoculture (wheat-after-wheat crop sequences), and growth of susceptible cultivars have contributed significantly to the rise in LSD epidemics globally. Frequent rains and longer durations of moist conditions also play an important role in LSD infection.

4.3.2.1 *Septoria tritici* Blotch

The fungus *Zymoseptoria tritici* (synonym: *Mycosphaerella graminicola*) is the causal pathogen of STB, which is a primary leaf disease in most of the wheat-growing regions of world. The pathogen shows a high level of genetic diversity because of its heterothallic (two mating types) nature. During the nonparasitic phase, the pathogen survives on dead or dying host tissues, producing many windborne ascospores for long-distance transmission. In Europe, annual economic losses of €0.28–1.2 billion, including chemical control costs, were estimated (Fones and Gurr 2015). In Australia, annual economic losses of AU$20 million were attributed to STB (Murray and Brennan 2009). However, in recent years this disease has become more prevalent and virulent in many wheat production areas (Milgate et al. 2014). The development of fungicide resistance in Europe is of major concern for STB management.

4.3.2.2 Tan Spot

Tan spot (TS), also known as yellow spot or yellow leaf blotch, is caused by *Pyrenophora tritici-repentis* (anamorph: *Drechslera tritici-repentis*). It decreases the kernel weight and the numbers of kernel per spike (Shabeer and Bockus 1988). Usually, leaf infections are characterized by oval or diamond-shaped to elongated spots, with a yellow border and a small dark brown spot in the center, producing an "eyespot" type of symptom. Occasionally, kernel infection can occur. Infected

kernels can develop a reddish discoloration on the seed coat, which is commonly is called "red smudge." Although TS fungus is a hemibiotroph, it is also considered to be a necrotroph because it causes extensive tissue damage to the host in its parasitic phase and survives on dead or dying host tissues in its nonparasitic phase. Globally, eight races of TS fungus have been established on the basis of their ability to induce necrosis and/or chlorosis symptoms in a set of wheat differentials (Singh et al. 2010). TS occurs in all wheat-growing regions of the world, including Europe, North America, South America, South Asia, and Australia. In Australia it is considered the primary source of yield loss, as it causes average annual economic losses in excess of AU$200 million (Murray and Brennan 2009). As reported by Duveiller et al. (2007), TS fungus is a component of the *Helminthosporium* leaf blight complex in Asia. The use of minimum-tillage or zero-tillage practices has contributed to the rise in TS as a significant disease in affected areas (Bockus and Claasen 1992; Rees and Platz 1979). However, the worldwide impact of the disease is difficult to assess, because of a lack of available data.

4.3.2.3 Spot Blotch

Spot blotch (SB), also called *Helminthosporium* leaf blight or foliar blight, is caused by *Cochliobolus sativus* (anamorph: *Bipolaris sorokiniana*). It is one of the most devastating foliar diseases. The fungus is heterothallic, as it requires opposite mating types for sexual reproduction, and it is a hemibiotroph, with a biotrophic phase during the initial infection, followed by a necrotrophic growth phase. Globally, 25 million hectares of wheat-growing areas are affected by SB. It is prevalent in wheat-growing regions of Bangladesh, Nepal, Bolivia, eastern India, Brazil, southeast China, southeast Australia, northeast Argentina, Paraguay, Zambia, northern Kazakhstan, and the Great Plains of the USA and Canada (Duveiller et al. 2005). Under favorable conditions, SB can cause up to 50% yield reductions (Sharma and Duveiller 2004; Singh et al. 2004).

4.3.2.4 *Stagonospora nodorum* Blotch

The fungal pathogen *Parastagonospora nodorum* (anamorph: *Stagonospora nodorum*) causes SNB, also called *Septoria* glume blotch. It is a necrotrophic fungus and infects both glumes and leaves, causing glume and leaf blotch. SNB is more predominant in wheat-producing areas with wet and moist conditions, and it is more destructive when those conditions persist until the heading stage (Singh et al. 2016). Significant yield reductions are caused by SNB in wheat-growing regions of Europe, North America, and Australia. In Australia, annual economic losses of AU$108 million attributable to SNB have been reported (Murray and Brennan 2009). Besides Australia, reports indicate prevalence of this disease throughout parts of France and the Scandinavian countries. By the 1980s, STB had fully replaced SNB in the UK

(Bearchell et al. 2005), but in recent years we have observed occurrence of SNB in southern Europe and STB in northern Europe.

4.3.3 Wheat Blast

Wheat blast (WB) is one of the most devastating diseases and is caused by *Magnaporthe oryzae* pathotype *triticum* (MoT). It was first identified in Parana State in Brazil in 1985 and subsequently dispersed to Argentina, Bolivia, and Paraguay (Igarashi et al. 1986). The disease was limited to South America until its detection in Bangladesh in 2016 (Islam et al. 2016). The occurrence of the pathogen in South Asia is due to an incursion of the South American pathogen rather than an independent evolution event, as indicated through phylogenomics and population genetics studies (Islam et al. 2016). As WB primarily affects the spikes, it is a head disease. Losses can be huge when the pathogen attacks the rachis at the base of the spike, causing whole or fractional spike death, which affects grain filling, depending upon the time of infection (Duveiller et al. 2011). Warm and moist conditions increase the chances of WB development. In Brazil, yield reductions of 11–55% in the highly susceptible cultivar Anahuac were documented from 1988 to 1992. The Anahuac cultivar was withdrawn in the mid-1990s; however, constant yield reductions happened even with the use of cultivars with better resistance (Urashima et al. 2009). In Brazil, even two rounds of fungicide applications could not control the losses; 14–32% losses in two widely grown cultivars were observed during the 2005 blast epidemics (Urashima et al. 2009).

4.4 Resistance to Fungal Diseases

4.4.1 Present Status

Genetic resistance to rusts can be classified into two categories: race-specific resistance (also known as seedling or qualitative resistance) and race non-specific resistance (Bariana 2003; Periyannan et al. 2017). To date, more than 200 rust resistance genes have been characterized and formally designated in wheat or wild relatives; most of these confer race-specific resistance (McIntosh et al. 1995, 2016, 2017). At least 60 of these genes are designated as SR resistance genes (McIntosh et al. 1995, 2016, 2017). *Sr31* was one of the most widely utilized race-specific SR resistance genes (Singh et al. 2006); however, its presence at the International Maize and Wheat Improvement Center (CIMMYT) has been drastically reduced following testing against Ug99 races in Kenya. Evolution of virulence against *Sr31* with the emergence of Ug99 led to susceptibility to SR in most of the wheats grown around the globe. New races in the Ug99 lineage and the Digalu race overcame several

genes such as *Sr21*, *Sr24*, *Sr36*, *Sr38*, and *SrTmp* (Jin et al. 2008, 2009; Olivera Firpo et al. 2015; Pretorius et al. 2010). At present, the genes *Sr2*, *Sr23*, *Sr25*, *Sr33*, *Sr35*, *Sr45*, *Sr47*, and *Sr50* are the most valuable ones for protection against newly evolved races (Singh et al. 2015b).

Seventy-eight YR resistance genes have been characterized and formally named (McIntosh et al. 1995, 2016, 2017). However, most of these genes have been rendered ineffective with emergence of virulent races in many parts of the world, except for a few combinations, such as the combination of *Yr5* and *Yr15*, that remain effective worldwide. Likewise, 77 LR resistance genes have been genetically characterized and documented (McIntosh et al. 1995, 2016, 2017). Out of these, *Lr1*, *Lr3*, *Lr10*, and *Lr20* have been commonly deployed in wheat cultivars (Dakouri et al. 2013).In general, race-specific resistance genes are rendered ineffective with continual emergence of new virulent races of rust pathogens through mutation and recombination (Randhawa et al. 2018). It has been well documented through cloning of 11 race-specific genes in wheat (*Sr22*, *Sr33*, *Sr35*, *Sr45*, *Sr50*, *Yr5*, *Yr10*, *Lr1*, *Lr10*, *Lr21*, and *Lr22*) that these genes encode nucleotide-binding site–leucine-rich repeat (NBS-LRR) proteins (Ellis et al. 2014; Mago et al. 2015; Steuernagel et al. 2016; Thind et al. 2017; Marchal et al. 2018).

Markers linked to several SR resistance genes—*Sr2* (Hayden et al. 2004; Mago et al. 2011), *Sr22* (Periyannan et al. 2011), *Sr24* and *Sr26* (Mago et al. 2005), *Sr32* (Mago et al. 2013), *Sr38* (Helguera et al. 2003), *Sr43* (Niu et al. 2014), *Sr45* (Periyannan et al. 2014), *Sr55* (Moore et al. 2015), *Sr56* (Bansal et al. 2014), and *Sr57* (Lagudah et al. 2006; Krattinger et al. 2009)—have been published.

For YR resistance, several gene marker associations have been reported: *Yr1* (Randhawa 2015), *Yr5* (Marchal et al. 2018), *Yr10* (Wang et al. 2002), *Yr15* (Peng et al. 2000), *Yr17* (Helguera et al. 2003), *Yr18* (Lagudah et al. 2006), *Yr26* (Zhang et al. 2013), *Yr28* (Singh et al. 2000), *Yr46* (Moore et al. 2015), *Yr50* (Liu et al. 2013), *Yr51* (Randhawa et al. 2014), *Yr57* (Randhawa et al. 2015), *Yr59* (Zhou et al. 2014a), *Yr61* (Zhou et al. 2014b), *Yr64*, and *Yr65* (Cheng et al. 2014). Similarly, gene marker associations reported for LR resistance are *Lr1* (Feuillet et al. 1995), *Lr9*, *Lr10* (Schachermayr et al. 1994, 1997), *Lr19* (Prins et al. 2001; Cherukuri et al. 2003), *Lr24* (Schachermayr et al. 1995; Dedryver et al. 1996), *Lr26* (Mago et al. 2002), *Lr28* (Sohail et al. 2014), *Lr34* (Lagudah et al. 2006; Krattinger et al. 2009), *Lr35* (Gold et al. 1999; Seyfarth et al. 1999), *Lr37* (Helguera et al. 2003), *Lr51* (Helguera et al. 2005), *Lr67* (Hiebert et al. 2010; Moore et al. 2015), and *Lr68* (Herrera-Foessel et al. 2012). These markers can be strategically used for selection of desirable gene combinations along with phenotypic assays. Protocols for reported markers need to be optimized and validated before use in selection of plants carrying the respective genes. The DNA of the donor parent should be included in the PCR reactions of markers to avoid any error.

Alternatively, race non-specific rust resistance genes offer durable resistance, as the pathogen cannot easily overcome them (Johnson 1988), and this is often referred to as adult plant resistance (APR). Hence, identification and strategic deployment of new sources of race non-specific APR to rusts are essential. To date, only seven race non-specific APR genes have been genetically characterized and formally designated

in wheat: *Sr2*/*Yr30*, *Lr34*/*Yr18*/*Sr57*/*Pm38*, *Lr46*/*Yr29*/*Sr58*/*Pm39*, *Lr67*/*Yr46*/*Sr55*/*Pm46*, *Lr68*, *Sr56*, and *Yr36* (Bansal et al. 2014; Dyck 1987, 1991; Hare and McIntosh 1979; Herrera-Foessel et al. 2011, 2012; Singh et al. 1998; Uauy et al. 2005). Cloning of the APR genes *Yr36*, *Lr34*/*Yr18*/*Sr57*/*Pm38*, and *Lr67*/*Yr46*/*Sr55*/*Pm46* has revealed the roles of cytoplasmic protein kinase, adenosine triphosphate (ATP)–binding cassette transporter, and hexose transporter, respectively, in mediating resistance (Fu et al. 2009;Krattinger et al. 2009; Moore et al. 2015).

Growing resistant cultivars is the most cost-effective and ecologically safe control method for PM. To date, 58 PM resistance genes have been formally cataloged; most of these provide race-specific resistance in wheat (McIntosh et al. 2016, 2017). It is desirable to know the virulence pattern of isolates to generate effective combinations of race-specific resistance genes (Wang et al. 2005). However, this approach is not a preferred method to control PM, as it does not result in longer-lasting effects, because of emergence of new races of the pathogen with matching virulence. Alternatively, deployment of combinations of race non-specific resistance genes is a promising method. As discussed above in the section for rust resistance, only three race non-specific resistance genes have been identified, out of which two pleiotropic genes (*Lr34*/*Yr18*/*Sr57*/*Pm38* and *Lr67*/*Yr46*/*Sr55*/*Pm46*) have been cloned (Krattinger et al. 2009; Moore et al. 2015).

Genetic resistance to FHB is mainly quantitative and is controlled by multiple moderate- to minor-effect genes (Singh et al. 2016). Although genetic resistance is the most cost-effective method, it is hard to accomplish in commercial cultivars, because of its complex behavior. This complexity is further enhanced by various resistance mechanisms, e.g., invasion (type I), fungal spread (type II), toxin accumulation (type III), kernel infection (type IV), and yield reduction (type V) (Mesterhazy et al. 2005). FHB resistance also displays significant correlations with heading, plant height, and anther extrusion of the wheat plant (Buerstmayr et al. 2012). To date, seven genetic loci—*Fhb1*, *Fhb2*, *Fhb4*, and *Fhb5* from wheat, and *Fhb3*, *Fhb6*, and *Fhb7* from wild relatives—have been formally named as FHB resistance genes (Guo et al. 2015). The cultivars Sumai 3 from China and Frontana from Brazil have been identified as sources of moderate resistance to FHB.

STB can also be effectively managed through deployment of qualitative or quantitative resistance. To date, 21 major genes conferring qualitative resistance have been identified and tagged using molecular markers (Brown et al. 2015). Since variation in the pathogen population does not occur more rapidly, the pathogen remains unchanged; therefore, resistance provided by major genes behaves as durable, however, in reality it cannot be durable as it is provided by major gene (Singh et al. 2016). On the other hand, quantitative resistance is more durable in field conditions and is often broad-spectrum resistance. Brown et al. (2015) provided a comprehensive review of 167 quantitative trait loci (QTLs) identified in wheat; some QTLs were mapped at or near *Stb6*. Genetic control of STB is supported through the availability of high-yielding wheat lines with combined resistance to rusts and STB under high disease pressures in wheat-growing areas globally (Singh et al. 2016).

Resistance to TS is controlled by both major and minor genes. The pathogenicity of Ptr is largely attributed to three necrotrophic effectors—ToxA, ToxB, and ToxC—which interact directly or indirectly with the products of the dominant host genes *Tsn1*, *Tsc2*, and *Tsc1*, respectively (Ciuffetti et al. 2010). Therefore, host resistance is highly correlated with toxin insensitivity, and eight race-specific genes have been identified. The wheat–*P. tritici-repentis* interaction mainly follows the toxin model of the gene-for-gene hypothesis. Several broad-spectrum QTLs and recessively inherited resistance genes are also known (Singh et al. 2016).

SB resistance in wheat is mostly quantitatively inherited (Singh et al. 2016). CIMMYT has developed high-yielding wheat lines with moderate to high levels of SB resistance (Singh et al. 2015a). A list of SB-resistant genotypes has been compiled by Duveiller and Sharma (2012). Several studies have been conducted on the association of SB resistance with agronomic and morphological traits (e.g., plant height, leaf angle, maturity, and stay-green) in association with leaf tip necrosis (Singh et al. 2016). The pleiotropic rust resistance gene *Lr34/Yr18/Sr57/Pm38/Ltn1* was shown to confer partial resistance to SB and was designated as *Sb1* (Singh et al. 2015a, b). Recently, the *Sb2* and *Sb3* genes were mapped on chromosomes 5B and 3B by Kumar et al. (2015) and Lu et al. (2015), respectively.

Host resistance play an important role in controlling SNB, and several independently inherited loci have been reported to confer both quantitative and qualitative resistance (Francki 2013). The responses of these genes depend upon environmental and pleiotropic effects, including plant height and heading time (Tommasini et al. 2007). In general, quantitative resistance is observed in field studies, whereas single-gene resistance has also been observed in some studies conducted in greenhouse environments (Feng et al. 2004; Singh et al. 2009). Several toxins have been identified as playing an important role in host–pathogen interactions in SNB disease.

The use of host resistance is the most preferred method to control WB. A dearth of resistant sources has reduced the effective utilization of genetic resistance in breeding programs, further hindered by the need for field tests and the localized nature of disease. However, knowledge on WB resistance has significantly increased. Its emergence in Bangladesh in 2016 greatly contributed to this knowledge through the setting-up of a platform in Bangladesh/Bolivia that performs testing of wheat lines from South Asia and other parts of the world. Cultivars such as BH1146, BR18, IPR85, CD113, and CNT8 showed moderate levels of resistance in comparison with the higher levels of resistance observed in derivatives of the CIMMYT line Milan (Kohli et al. 2011). Resistance to WB has been assumed to be both qualitative and quantitative; the former has been validated at the seedling stage (Maciel et al. 2014). To date, eight resistance genes have been identified and designated as *Rmg1–Rmg8* in wheat (Anh et al. 2015; Nga et al. 2009). Recently, Cruz et al. (2016) assessed the effect of the 2NS/2AS translocation from *Aegilops ventricosa* on WB resistance and noted a 50.4–80.5% reduction in disease. However, unpublished reports from Paraguay have indicated that a proportion of the new isolates that were tested appeared to have overcome this resistance (Singh et al. 2016).

4.4.2 Future Prospects

Use of advanced technologies, marker-assisted selection (MAS), genomic selection, transgenics, and gene editing will help to increase the efficiency of breeding programs around the world. However, each technology has its own limitations. In high-income countries, MAS has been used frequently in selection of disease resistance in wheat (e.g., rust resistance in Australia). Globally, breeding programs depend on phenotypic selection because of the high cost of genotyping, lack of diagnostic and reliable markers, and high phenotypic selection accuracy (Singh et al. 2016). In addition, it is always desirable to use MAS in parallel with phenotypic assays in order to avoid use of false positives and poor, agronomically weak plants. MAS is helpful in selection of race-specific resistance genes in wheat.

Gene pyramiding is the transfer of two or more disease resistance genes in wheat using MAS and conventional backcrossing methods. This approach is time consuming and is a slow way to transfer resistance genes; it largely depends upon the availability of reliable, breeder-friendly markers. Transfer of gene cassettes or gene stacks (also known as gene stacking) is another emerging technology being used in wheat. Desirable combinations of effective resistance genes can be combined and transformed into wheat as gene cassettes or gene stacks. This can result in faster improvements in disease resistance of current high-yielding cultivars. However, it is still debatable whether the resultant gene-stacked wheat is a cisgenic or transgenic product. Since the genes used to prepare gene cassettes or gene stacks originate or are derived from wheat or from its relatives (in a few cases), gene-stacked wheat should be considered cisgenic rather than transgenic.

Genomic selection is considered the best strategy for selection of multiple minor-effect loci in comparison with MAS. With genomic selection, a training population that is both phenotyped and genotyped is used to standardize a prediction model, which is further used to predict breeding values, thus enabling selection of candidates prior to phenotyping (Lorenz et al. 2011). Recent studies have reported that greater genetic gains can be obtained by using genomic selection than by using MAS (Rutkoski et al. 2012, 2014) and phenotypic selection (Mirdita et al. 2015). However, Rutkoski et al. (2015) observed equal genetic gains per unit of time from genomic and phenotypic selection for quantitative SR resistance. It is recommended to regularly update the prediction models to maintain prediction accuracy for genomic selection of polygenic traits (Singh et al. 2016).

Recently, genome editing has emerged as a prominent new plant breeding technique, which involves targeted modification of a native DNA sequence. The technique was shown to be effective for improving PM resistance in wheat (Wang et al. 2014) and producing low-gluten wheat (Sánchez-León et al. 2018), and thus exemplifies the potential of gene editing for improving crops for human consumption where conventional breeding could not succeed. The US Department of Agriculture considers that genome-edited products are not genetically modified organisms, but the European Commission has yet to confirm its stance (Jones 2015).

4.5 Conclusion

Since the onset of the Green Revolution, significant progress in wheat improvement has been made through identification and use of new sources of disease resistance, resulting in development of disease-resistant wheat varieties with high yield potential. However, diseases and pests continue to pose significant threats to global wheat production even now. These could be attributable to emergence of new virulent pathogen races, fungicide resistance due to excessive use, and intensive agriculture. Strategic utilization of molecular approaches in conjunction with extensive phenotypic evaluation provide opportunities to harness genetic diversity for disease resistance and increased wheat yields. CIMMYT's network of phenotypic platforms play very important roles in evaluation of wheat germplasms for various diseases and development of high-yielding disease-resistant wheat cultivars with package of desirable traits, thus contributing to enhancement of world wheat production and reducing global food insecurity.

References

Ali S, Gladieux P, Leconte M, Gautier A, Justesen AF, Hovmøller MS, Enjalbert J, Vallavieille-Pope CD (2014) Origin, migration routes and worldwide population genetic structure of the wheat yellow rust pathogen *Puccinia striiformis* f. sp. *tritici*. PLoS Pathol 10:e1003903. https://doi.org/10.1371/journal.ppat.1003903

Anh VL, Anh NT, Tagle AG et al (2015) *Rmg8*, a new gene for resistance to *triticum* isolates of *Pyricularia oryzae* in hexaploid wheat. Phytopathology 105:1568–1572

Bansal U, Bariana H, Wong D, Randhawa M, Wicker T, Hayden M, Keller B (2014) Molecular mapping of an adult plant stem rust resistance gene *Sr56* in winter wheat cultivar Arina. Theor Appl Genet 127:1441–1448. https://doi.org/10.1007/s00122-014-2311-1

Bariana HS (2003) Breeding for disease resistance. In: Thomas B, Murphy DJ, Murray BG (eds) Encyclopedia of applied plant sciences. Academic, Harcourt, Amsterdam, pp 244–253

Bearchell SJ, Fraaije BA, Shaw MW, Fitt BDL (2005) Wheat archive links long-term fungal pathogen population dynamics to air pollution. Proc Natl Acad Sci U S A 102:5438–5442. https://doi.org/10.1073/pnas.0501596102

Beddow JM, Pardey PG, Chai Y, Hurley TM, Kriticos DJ, Braun HJ, Park RF, Cuddy WS, Yonow T (2015) Research investment implications of shifts in the global geography of wheat stripe rust. Nat Plants 1:15132. https://doi.org/10.1038/nplants.2015.132

Bhattacharya S (2017) Deadly new wheat disease threatens Europe's crops. Nature 542:145–146

Bockus WW, Claasen MM (1992) Effects of crop rotation and residue management practices on severity of tan spot of winter wheat. Plant Dis 76:633–636

Bolton MD, Kolmer JA, Garvin DF (2008) Wheat leaf rust caused by *Puccinia triticina*. Mol Plant Pathol 9:563–575

Brown DW, Proctor RH (eds) (2013) *Fusarium*: genomics, molecular and cellular biology. Caister Academic, Norfolk

Brown JKM, Chartrain L, Lasserre-Zuber P, Saintenac C (2015) Genetics of resistance to *Zymoseptoria tritici* and applications to wheat breeding. Fungal Genet Biol 79:33–41

Buerstmayr H, Adam G, Lemmens M (2012) Resistance to head blight caused by *Fusarium* spp. in wheat. In: Sharma I (ed) Disease resistance in wheat. CABI, Wallingford, pp 236–276

Chen X (2005) Epidemiology and control of stripe rust [*Puccinia striiformis* f. sp. *tritici*] on wheat. Can J Plant Pathol 27:314–337

Chen W, Wellings C, Chen X, Kang Z, Liu T (2014) Wheat stripe (yellow) rust caused by *Puccinia striiformis* f. sp. *tritici*. Mol Plant Pathol 15:433–446

Cheng S, Zhang Y, Bie T, Gao D, Zhang B (2012) Strategy of wheat breeding for scab resistance in China. In: Proceedings of the 4th International Symposium on *Fusarium* Head Blight, Nanjing, 23–26 Aug 2012

Cheng P, Xu LS, Wang MN, See DR, Chen XM (2014) Molecular mapping of genes *Yr64* and *Yr65* for stripe rust resistance in hexaploid derivatives of durum wheat accessions PI 331260 and PI 480016. Theor Appl Genet 127:2267–2277

Cherukuri DP, Gupta SK, Charpe A, Koul S, Prabhu KV, Singh RB, Haq QMR, Chauhan SVS (2003) Identification of a molecular marker linked to an *Agropyrone longatum*–derived gene *Lr19* for leaf rust resistance in wheat. Plant Breed 122:204–208

Ciuffetti LM, Manning VA, Pandelova I, Betts MF, Martinez JP (2010) Host-selective toxins, PtrToxA and PtrToxB, as necrotrophic effectors in the *Pyrenophora tritici-repentis*–wheat interaction. New Phytol 187:911–919. https://doi.org/10.1111/j.1469-8137.2010.03362.x

Cowger C, Miranda L, Griffey C, Hall M, Murphy JP, Maxwell J (2012) Wheat powdery mildew. In: Sharma I (ed) Disease resistance in wheat. CABI, Wallingford, pp 84–119

Cruz CD, Peterson GL, Bockus WW, Kankanala P, Dubcovsky J et al (2016) The 2NS translocation from *Aegilops ventricosa* confers resistance to the *triticum* pathotype of *Magnaporthe oryzae*. Crop Sci 56:990–1000

Curtis BC, Rajaram S, Gómez Macpherson H (eds) (2002) Bread wheat: improvement and production, FAO Plant Production and Protection Series, No. 30. FAO, Rome

Dakouri A, McCallum BD, Radovanovic N, Cloutier S (2013) Molecular and phenotypic characterization of seedling and adult plant leaf rust resistance in a world wheat collection. Mol Breed 32:663–677

Dean R, Van Kan JA, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, Rudd JJ, Dickman M, Kahmann R, Ellis J, Foster GD (2012) The top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol 13:414–430. https://doi.org/10.1111/j.1364-3703.2011.00783.x

Dedryver F, Jubier MF, Thouvenin J, Goyeau H (1996) Molecular markers linked to the leaf rust resistance gene *Lr24* in different wheat cultivars. Genome 39:830–835

Dubin HJ, Duveiller E (2011) Fungal, bacterial and nematode diseases of wheat: breeding for resistance and other control measures. In: Bonjean AP, Angus WJ, Van Ginkel M (eds) The world wheat book: a history of wheat breeding, vol 2. Lavoisier, Paris, pp 1131–1181

Duveiller E, Sharma RC (2012) Wheat resistance to spot blotch or foliar blight. In: Sharma I (ed) Disease resistance in wheat. CABI, Wallingford, pp 120–135

Duveiller E, Kandel YR, Sharma RC, Shrestha SM (2005) Epidemiology of foliar blights (spot blotch and tan spot) of wheat in the plains bordering the Himalayas. Phytopathology 95:248–256

Duveiller E, Singh RP, Nicol JM (2007) The challenges of maintaining wheat productivity: pests, diseases, and potential epidemics. Euphytica 157:417–430

Duveiller E, Hodson D, Sonder K, von Tiedemann A (2011) An international perspective on wheat blast. Phytopathology 101:S220

Dyck PL (1987) The association of a gene for leaf rust resistance with the chromosome 7D suppressor of stem rust resistance in common wheat. Genome 29:467–469

Dyck PL (1991) Genetics of adult-plant leaf rust resistance in 'Chinese Spring' and 'Sturdy' wheats. Crop Sci 31:309–311

Ellis JG, Lagudah ES, Spielmeyer W, Dodds PN (2014) The past, present and future of breeding rust resistant wheat. Front Plant Sci 5:641. https://doi.org/10.3389/fpls.2014.00641

Feng J, Ma H, Hughes GR (2004) Genetics of resistance to *Stagonospora nodorum* blotch of hexaploid wheat. Crop Sci 44(6):2043–2048

Feuillet C, Messmer M, Schachermayr G, Keller B (1995) Genetic and physical characterization of the *Lr1* leaf rust resistance locus in wheat (*Triticum aestivum* L.). Mol Gen Genet 248:553–562

Figueroa M, Hammond-Kosack KE, Solomon PS (2017) A review of plant diseases—a field perspective. Mol Plant Pathol 19:1523–1536. https://doi.org/10.1111/mpp.12618

Fones H, Gurr S (2015) The impact of *Septoria tritici* blotch disease on wheat: an EU perspective. Fungal Genet Biol 79:3–7

Francki MG (2013) Improving *Stagonospora nodorum* resistance in wheat: a review. Crop Sci 53:355–365

Fu D, Uauy C, Distelfeld A, Blechl A, Epstein L, Chen X, Sela H, Fahima T, Dubcovsky J (2009) A kinase-START gene confers temperature dependent resistance to wheat stripe rust. Science 323:1357–1360

Gold J, Harder D, Townley-Smith F, Aung T, Procunier J (1999) Development of a molecular marker for rust resistance genes *Sr39* and *Lr35* in wheat breeding lines. Electron J Biotechnol 2:1

Goodwin SB (2012) Resistance in wheat to *Septoria* diseases caused by *Mycosphaerella graminicola* (*Septoria tritici*) and *Phaeosphaeria* (*Stagonospora*) *nodorum*. In: Sharma I (ed) Disease resistance in wheat. CABI, Wallingford, pp 151–159

Guo J, Zhang X, Hou Y, Cai J, Shen X, Zhou T, Xu H, Ohm HW, Wang H, Li A, Han F, Wang H, Kong L (2015) High-density mapping of the major FHB resistance gene *Fhb7* derived from *Thinopyrum ponticum* and its pyramiding with *Fhb1* by marker-assisted selection. Theor Appl Genet 128:2301–2316

Hare RA, McIntosh RA (1979) Genetic and cytogenetic studies of durable adult-plant resistances in 'Hope' and related cultivars to wheat rusts. Z Planzenzuchtung 83:350–367

Hayden MJ, Kuchel H, Chalmers KJ (2004) Sequence tagged microsatellites for the *Xgwm533* locus provide new diagnostic markers to select for the presence of stem rust resistance gene *Sr2* in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1641–1647

Helguera M, Khan IA, Kolmer J, Lijavetzky D, Zhong-qi L (2003) PCR assays for the *Lr37-Yr17-Sr38* cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. Crop Sci 43:1839–1847

Helguera M, Vanzetti L, Soria M, Khan IA, Kolmer J (2005) PCR markers for *Triticum speltoides* leaf rust resistance gene *Lr51* and their use to develop isogenic hard red spring wheat lines. Crop Sci 45:728–734

Herrera-Foessel SA, Lagudah ES, Huerta-Espino J, Hayden MJ, Bariana HS, Singh D, Singh RP (2011) New slow-rusting leaf rust and stripe rust resistance genes *Lr67* and *Yr46* in wheat are pleiotropic or closely linked. Theor Appl Genet 122:239–249

Herrera-Foessel SA, Singh RP, Huerta-Espino J, Rosewarne GM, Periyannan SK, Viccars L, Calvo-Salazar V, Lan CX, Lagudah ES (2012) *Lr68*: a new gene conferring slow rusting resistance to leaf rust in wheat. Theor Appl Genet 124:1475–1486

Hiebert CW, Thomas JB, McCallum BD, Humphreys DG, DePauw RM, Hayden MJ, Mago R, Schnippenkoetter W, Spielmeyer W (2010) An introgression on wheat chromosome 4DL in RL6077 (Thatcher∗6/PI250413) confers adult plant resistance to stripe rust and leaf rust (*Lr67*). Theor Appl Genet 121:1083–1091

Hovmøller MS, Sørensen CK, Walter S, Justesen AF (2011) Diversity of *Puccinia striiformis* on cereals and grasses. Annu Rev Phytopathol 49:197–217

Hovmøller MS, Walter S, Bayles RA, Hubbard A, Flath K, Sommerfeldt N, Leconte M, Czembor P, Rodriguez-Algaba J, Thach T, Hansen JG, Lassen P, Justesen AF, Ali S, de Vallavieille-Pope C (2015) Replacement of the European wheat yellow rust population by new races from the centre of diversity in the near-Himalayan region. Plant Pathol 65:402–411

Hubbard A, Lewis CM, Yoshida K, Ramirez-Gonzalez RH, de Vallavieille-Pope C, Thomas J, Kamoun S, Bayles R, Uauy C, Saunders DGO (2015) Field pathogenomics reveals the emergence of a diverse wheat yellow rust population. Genome Biol 16:23. https://doi.org/10.1186/s13059-015-0590-8

Huerta-Espino J, Singh RP, German S, McCallum B, Park R, Chen WQ, Bhardwaj S, Goyeau H (2011) Global status of wheat leaf rust caused by *Puccinia triticina*. Euphytica 179:143–160

Igarashi S, Utimada C, Igarashi L, Kazuma A, Lopes R (1986) *Pyricularia* sp. em trigo. I. Ocorrencia de *Pyricularia* sp. no Estado do Parana. Fitopatol Bras 11:351–352

Islam MT, Croll D, Gladieux P, Soanes DM, Persoons A, Bhattacharjee P, Hossain MS, Gupta DR, Rahman MM, Mahboob MG, Cook N, Salam MU, Surovy MZ, Sancho VB, Maciel JLN, Nhani Junior A, Castroagudin VL, Reges JTA, Ceresini PC, Ravel S, Kellner R, Fournier E, Tharreau D, Lebrun MH, McDonald BA, Stitt T, Swan D, Talbot NJ, Saunders DGO, Win J, Kamoun S (2016) Emergence of wheat blast in Bangladesh was caused by a South American lineage of *Magnaporthe oryzae*. BMC Biol 14:84. https://doi.org/10.1186/s12915-016-0309-7

Jin Y, Singh RP, Ward RW, Wanyera R, Kinyua M, Njau P, Fetch T, Pretorius ZA, Yahyaoui A (2007) Characterization of seedling infection types and adult plant infection responses of monogenic *Sr* gene lines to race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis 91:1096–1099. https://doi.org/10.1094/PDIS-91-9-1096

Jin Y, Szabo LJ, Pretorius ZA, Singh RP, Ward R, Fetch T (2008) Detection of virulence to resistance gene *Sr24* with race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis 92:923–926. https://doi.org/10.1094/PDIS-92-6-0923

Jin Y, Szabo LJ, Rouse MN, Fetch T Jr, Pretorus ZA, Wanyera R, Njau P (2009) Detection of virulence to resistance gene *Sr36* within the TTKS race lineage of *Puccinia graminis* f. sp. *tritici*. Plant Dis 93:367–370. https://doi.org/10.1094/PDIS-93-4-0367

Johnson R (1988) Durable resistance to yellow (stripe) rust in wheat and its implications in plant breeding. In: Simmonds NW, Rajaram S (eds) Breeding strategies for resistance to the rusts of wheat. CIMMYT, Mexico DF, pp 63–75

Jones HD (2015) Regulatory uncertainty over genome editing. Nat Plants 1:14011. https://doi.org/10.1038/nplants.2014.11

Kohli MM, Diaz de Ackermann M (2013) Resistance to *Fusarium* head blight in South American wheat germplasm. In: Alconada Magliano TM, Chulze SN (eds) *Fusarium* head blight in Latin America. Springer, New York, pp 263–297

Kohli MM, Mehta YR, Guzman E, De Viedma L, Cubilla LE (2011) *Pyricularia* blast: a threat to wheat cultivation. Czech J Genet Plant Breed 47:S130–S134

Kolmer JA (2005) Tracking wheat rust on a continental scale. Curr Opin Plant Biol 8:441–449

Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J, McFadden H, Bossolini E, Selter LL, Keller B (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science 323:1360–1363. https://doi.org/10.1126/science.1166453

Kumar S, Roder MS, Tripathi SB, Kumar S, Chand R, Joshi AK, Kumar U (2015) Mendelization and fine mapping of a bread wheat spot blotch disease resistance QTL. Mol Breed 35:218. https://doi.org/10.1007/s11032-015-0411-5

Lagudah ES, McFadden H, Singh RP, Huerta-Espino J, Bariana HS, Spielmeyer W (2006) Molecular genetic characterization of the *Lr34*/*Yr18* slow rusting resistance gene region in wheat. Theor Appl Genet 114:21–30

Leonard KJ, Szabo LJ (2005) Stem rust of small grains and grasses caused by *Puccinia graminis*. Mol Plant Pathol 6:99–111

Lewis CM, Persoons A, Bebber DP, Kigathi RN, Maintz J, Findlay K, Bueno-Sancho V, Corredor-Moreno P, Harrington SA, Kangara N, Berlin A, García R, Germán SE, Hanzalová A, Hodson DP, Hovmøller MS, Huerta-Espino J, Imtiaz M, Mirza JI, Justesen AF, Niks RE, Omrani A, Patpour M, Pretorius ZA, Roohparvar R, Sela H, Singh RP, Steffenson B, Visser B, Fenwick PM, Thomas J, Wulff BBH, Saunders DGO (2018) Potential for re-emergence of wheat stem rust in the United Kingdom. Commun Biol 1:13. https://doi.org/10.1038/s42003-018-0013-y

Liu J, Chang Z, Zhang X, Yang Z, Li X, Jia J, Zhan H, Guo H, Wang J (2013) Putative *Thinopyrum intermedium*–derived stripe rust resistance gene *Yr50* maps on wheat chromosome arm 4BL. Theor Appl Genet 126:265–274

Lorenz AJ, Chao S, Asoro FG, Heffner EL, Hayashi T, Iwata H, Smith KP, Sorrells ME, Jannink JC (2011) Genomic selection in plant breeding: knowledge and prospects. Adv Agron 110:77–123

Lu P, Liang Y, Li D, Wang Z, Li W, Wang G, Wang Y, Zhou S, Wu Q, Xie J, Zhang D, Chen Y, Li M, Zhang Y, Sun Q, Han C, Liu Z (2015) Fine genetic mapping of spot blotch resistance gene *Sb3* in wheat (*Triticum aestivum*). Theor Appl Genet 129:577–589

Maciel JLN, Ceresini PC, Castroagudin VL, Zala M, Kema GHJ, McDonald BA (2014) Population structure and pathotype diversity of the wheat blast pathogen *Magnaporthe oryzae* 25 years after its emergence in Brazil. Phytopathology 104:95–107

Mago R, Spielmeyer W, Lawrence GL, Lagudah ES, Ellis GJ (2002) Identification and mapping of molecular markers linked to rust resistance genes located on chromosome 1RS of rye using wheat-rye translocation lines. Theor Appl Genet 104:1317–1324

Mago R, Bariana HS, Dundas IS, Spielmeyer W, Lawrence GJ, Pryor AJ, Ellis G (2005) Development of PCR markers for the selection of wheat stem rust resistance genes *Sr24* and *Sr26* in diverse wheat germplasm. Theor Appl Genet 111:496–504

Mago R, Brown-Guedira G, Dreisigacker S, Breen J, Jin Y, Singh R, Appels R, Lagudah ES, Ellis J, Spielmeyer W (2011) An accurate DNA marker assay for stem rust resistance gene *Sr2* in wheat. Theor Appl Genet 122:735–744

Mago R, Verlin D, Zhang P, Bansal U, Bariana H, Jin Y, Ellis J, Hoxha S, Dundas I (2013) Development of wheat–*Aegilops speltoides* recombinants and simple PCR-based markers for *Sr32* and a new stem rust resistance gene on the 2S#1 chromosome. Theor Appl Genet 126:2943–2955

Mago R, Zhang P, Vautrin S, Simkova H, Bansal U, Luo M-C, Rouse M, Karaoglu H, Periyannan S, Kolmer J, Jin Y, Ayliffe MA, Bariana H, Park RF, McIntosh R, Dolezel J, Berges H, Spielmeyer W, Lagudah ES, Ellis JG, Dodds PN (2015) The wheat *Sr50* reveals a rich diversity at a cereal disease resistance locus. Nat Plants 1:15186. https://doi.org/10.1038/nplants.2015.186

Marchal C, Zhang J, Zhang P, Fenwick P, Steuernagel B, Adamski NM, Boyd L, McIntosh R, Wulff BBH, Berry S, Lagudah E, Uauy C (2018) BED-domain containing immune receptors confer 2 diverse resistance spectra to yellow rust. Nat Plants 4:662. https://doi.org/10.1038/s41477-018-0236-4

May WE, Fernandez MR, Selles F, Lafond GP (2014) Agronomic practices to reduce leaf spotting and *Fusarium* kernel infections in durum wheat on the Canadian prairies. Can J Plant Sci 94:141–152

McIntosh RA, Wellings CR, Park RF (eds) (1995) Wheat rusts: an atlas of resistance genes. CSIRO, Melbourne

McIntosh RA, Dubcovsky J, Rogers J, Morris C, Appels R, Xia XC (2016) Catalogue of gene symbols for wheat: 2015–16 supplement. https://shigen.nig.ac.jp/wheat/komugi/genes/macgene/supplement2015.pdf

McIntosh RA, Dubcovsky J, Rogers J, Morris C, Xia XC (2017) Catalogue of gene symbols for wheat: 2017 supplement. https://shigen.nig.ac.jp/wheat/komugi/genes/macgene/supplement2017.pdf

McMullen M, Bergstrom G, De Wolf E, Dill-Macky R, Hershman D, Shaner G, Van Stanford D (2012) A unified effort to fight an enemy of wheat and barley: *Fusarium* head blight. Plant Dis 96:1712–1728

Mehta YR (2014) Wheat diseases and their management. Springer, New York, p 256

Mesterhazy A, Bartok T, Kaszonyi G, Varga M, Toth B, Varga J (2005) Common resistance to different *Fusarium* spp. causing *Fusarium* head blight in wheat. Eur J Plant Pathol 112:267–281

Milgate A, Hollaway G, Wallwork H, Thomas G (2014) *Septoria tritici* blotch fact sheet. GRDC, Canberra

Mirdita V, He S, Zhao Y, Korzun V, Bothe R, Ebmeyer E, Reif JC, Jiang Y (2015) Potential and limits of whole genome prediction of resistance to *Fusarium* head blight and *Septoria tritici* blotch in a vast central European elite winter wheat population. Theor Appl Genet 128:2471–2481

Moore JW, Herrera-Foessel S, Lan C, Schnippenkoetter W, Ayliffe M, Huerta-Espino J, Lillemo M, Viccars L, Milne R, Periyannan S, Kong X, Spielmeyer W, Talbot M, Bariana H, Patrick JW, Dodds P, Singh R, Lagudah E (2015) A recently evolved hexose transporter variant confers

resistance to multiple pathogens in wheat. Nat Genet 47:1494–1498. https://doi.org/10.1038/ng.3439

Murray GM, Brennan JP (2009) Estimating disease losses to the Australian wheat industry. Australas Plant Pathol 38:558–570

Newcomb M, Olivera PD, Rouse MN, Szabo LJ, Johnson J, Gale S, Luster DG, Wanyera R, Macharia G, Bhavani S, Hodson D, Patpour M, Hovmoller MS, Fetch TG Jr, Jin Y (2016) Kenyan isolates of *Puccinia graminis* f. sp. *tritici* from 2008 to 2014: virulence to *SrTmp* in the Ug99 race group and implications for breeding programs. Phytopathology 106:729–736. https://doi.org/10.1094/PHYTO-11-14-0302-FI

Nga NTT, Hau VTB, Tosa Y (2009) Identification of genes for resistance to a *Digitaria* isolate of *Magnaporthe grisea* in common wheat cultivars. Genome 52:801–809

Niu Z, Klindworth DL, Yu G, Friesen TL, Chao S, Jin Y, Cai X, Ohm JB, Rasmussen JB, Xu S (2014) Development and characterization of wheat lines carrying stem rust resistance gene *Sr43* derived from *Thinopyrum ponticum*. Theor Appl Genet 127:969–980

Oerke E-C (2006) Crop losses to pests. J Agric Sci 144:31–43

Olivera Firpo P, Newcomb M, Szabo L, Rouse MN, Johnson JL, Gale SW, Luster D, Hodson D, Cox JA, Burgin L (2015) Phenotypic and genotypic characterization of race TKTTF of *Puccinia graminis* f. sp. *tritici* that caused a wheat stem rust epidemic in southern Ethiopia in 2013/14. Phytopathology 105:917–928

Olivera Firpo P, Newcomb M, Flath K, Sommerfeldt-Impe N, Szabo L, Carter M, Luster D, Jin Y (2017) Characterization of *Puccinia graminis* f. sp. *tritici* isolates derived from an unusual wheat stem rust outbreak in Germany in 2013. Plant Pathol 66:1258–1266. https://doi.org/10.1111/ppa.12674

Peng JH, Fahima T, Roeder MS, Huang QY, Dahan A, Li YC, Grama A, Nevo E (2000) High-density molecular map of chromosome region harboring stripe-rust resistance genes *YrH52* and *Yr15* derived from wild emmer wheat, *Triticum dicoccoides*. Genetica 109:199–210

Periyannan SK, Bansal UK, Bariana HS, Pumphrey M, Lagudah ES (2011) A robust molecular marker for the detection of shortened introgressed segment carrying the stem rust resistance gene *Sr22* in common wheat. Theor Appl Genet 122:1–7

Periyannan S, Bansal U, Bariana H, Deal K, Luo MC, Dvorak J, Lagudah E (2014) Identification of a robust molecular marker for the detection of the stem rust resistance gene *Sr45* in common wheat. Theor Appl Genet 127:947–955

Periyannan S, Milne RJ, Figueroa M, Lagudah ES, Dodds PN (2017) An overview of genetic rust resistance: from broad to specific mechanisms. PLoS Pathog 13:e1006380. https://doi.org/10.1371/journal.ppat.1006380

Peterson PD (2001) Stem rust of wheat: from ancient enemy to modern foe. American Phytopathology Society (APS) Press, St. Paul

Pretorius ZA, Singh RP, Wagoire WW, Payne TS (2000) Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis*. f. sp. *tritici* in Uganda. Plant Dis 84:203. https://doi.org/10.1094/PDIS.2000.84.2.203B

Pretorius ZA, Bender CM, Visser B, Terefe T (2010) First report of a *Puccinia graminis* f. sp. *tritici* race virulent to the *Sr24* and *Sr31* wheat stem rust resistance genes in South Africa. Plant Dis 94:784. https://doi.org/10.1094/PDIS-94-6-0784C

Prins R, Groenewald JZ, Marais GF, Snape JW (2001) AFLP and STS tagging of *Lr19*, a gene conferring resistance to leaf rust in wheat. Theor Appl Genet 103:618–624

Randhawa M (2015) Molecular mapping of rust resistance in wheat: discovery to deployment. PhD dissertation, University of Sydney

Randhawa M, Bansal U, Valárik M, Klocova B, Dolezel J, Bariana H (2014) Molecular mapping of stripe rust resistance gene *Yr51* in chromosome 4AL of wheat. Theor Appl Genet 127:317–324

Randhawa MS, Bariana HS, Mago R, Bansal UK (2015) Mapping of a new stripe rust resistance locus *Yr57* on chromosome 3BS of wheat. Mol Breed 35:65

Randhawa MS, Singh RP, Lan C, Basnet BR, Bhavani S, Huerta-Espino J, Forrest KL, Hayden M (2018) Interactions among genes *Sr2*/*Yr30*, *Lr34*/*Yr18*/*Sr57* and *Lr68* confer enhanced adult

plant resistance to rust diseases in common wheat (*Triticum aestivum* L.) line 'Arula. Aust J Crop Sci 12:1023–1033
Rees RG, Platz GJ (1979) The occurrence and control of yellow spot of wheat in north-eastern Australia. Aust J Exp Agric 19:362–372
Roelfs AP, Singh RP, Saari EE (1992) Rust diseases of wheat: concepts and methods of disease management. CIMMYT, Mexico DF
Rutkoski J, Benson J, Jia Y, Brown-Guedira G, Jannink J-L, Sorrells M (2012) Evaluation of genomic prediction methods for *Fusarium* head blight resistance in wheat. Plant Genome 5:51–61
Rutkoski J, Poland JA, Singh RP, Huerta-Espino J, Bhavani S, Barbier H, Rouse MN, Jannik J-L, Sorrells M (2014) Genomic selection for quantitative adult plant stem rust resistance in wheat. Plant Genome 7(3):1–10
Rutkoski J, Singh RP, Huerta-Espino J, Bhavani S, Poland J, Jannik J-L, Sorrells M (2015) Genetic gain from phenotypic and genomic selection for quantitative resistance to stem rust of wheat. Plant Genome 8. https://doi.org/10.3835/plantgenome2014.10.0074
Saari EE, Prescott JM (1985) World distribution in relation to economic losses. In: Roelfs AP, Bushnell WR (eds) The cereal rusts. Diseases, distribution, epidemiology and control, vol 2. Academic, Orlando, pp 259–298
Sánchez-León S, Gil-Humanes J, Ozuna CV, Giménez MJ, Sousa C, Voytas DF, Barro F (2018) Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9. Plant Biotechnol J 16:902–910. https://doi.org/10.1111/pbi.12837
Schachermayr GM, Siedler H, Gale MD, Winzeler H, Winzeler M, Keller B (1994) Identification and localization of molecular markers linked to the *Lr9* leaf rust resistance gene of wheat. Theor Appl Genet 88:110–115
Schachermayr GM, Messmer MM, Feuillet C, Winzeler H, Winzeler M, Keller B (1995) Identification of molecular markers linked to the *Agropyrone longatum*–derived leaf rust resistance gene *Lr24* in wheat. Theor Appl Genet 90:982–990
Schachermayr GM, Feuillet C, Keller B (1997) Molecular markers for the detection of the wheat leaf rust resistance gene *Lr10* in diverse genetic backgrounds. Mol Breed 3:65–74
Schumann GL, D'Arcy CJ (2009) Essential plant pathology. American Phytopathology Society, St. Paul
Seyfarth R, Feuillet C, Schachermayr G, Winzeler M, Keller B (1999) Development of a molecular marker for the adult plant leaf rust resistance gene *Lr35* in wheat. Theor Appl Genet 99:554–560
Shabeer A, Bockus WW (1988) Tan spot effects on yield and yield components relative to growth stage in winter wheat. Plant Dis 72:599–602
Sharma RC, Duveiller E (2004) Effect of *Helminthosporium* leaf blight on performance of timely and late-seeded wheat under optimal and stressed levels of soil fertility and moisture. Field Crops Res 89:205–218
Singh RP, Mujeeb-Kazi A, Huerta-Espino J (1998) *Lr46*: a gene conferring slow rusting resistance to leaf rust in wheat. Phytopathology 88:890–894
Singh RP, Nelson JC, Sorrels ME (2000) Mapping *Yr28* and other genes for resistance to stripe rust in wheat. Crop Sci 40:1148–1155
Singh DP, Sharma AK, Tewari AN, Singh KP, Singh AK, Singh RN, Singh SP, Kalappanawar IK, Dodan DS, Singh VK (2004) Assessment of losses due to leaf blight in popular varieties of wheat (*Triticum aestivum*) under different sowing conditions and agroclimatic zones in India. Indian J Agric Sci 74:110–113
Singh RP, Hodson DP, Jin Y, Huerta EJ, Kinyua M, Wanyera R, Njau P, Ward RW (2006) Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. CAB Rev 1:54
Singh PK, Feng J, Mergoum M, McCartney CA, Hughes GR (2009) Genetic analysis of seedling resistance to *Stagonospora nodorum* blotch in selected tetraploid and hexaploid wheat genotypes. Plant Breed 128:118–123

Singh PK, Singh RP, Duveiller E, Mergoum M, Adhikari TB, Elias EM (2010) Genetics of wheat–*Pyrenophora tritici-repentis* interactions. Euphytica 171:1–13

Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Bhavani S, Njau P, Herrera-Foessel S, Singh PK, Singh S, Govindan V (2011) The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. Annu Rev Phytopathol 49:465–481. https://doi.org/10.1146/annurev-phyto-072910-095423

Singh PK, Zhang Y, He X, Singh RP, Chand R et al (2015a) Development and characterization of the 4th CSISA-spot blotch nursery of bread wheat. Eur J Plant Pathol 143:595–605

Singh RP, Hodson DP, Jin Y, Lagudah ES, Ayliffe MA, Bhavani S, Rouse MN, Pretorius ZA, Szabo LJ, Huerta-Espino J, Basnet BR, Lan C, Hovmøller MS (2015b) Emergence and spread of new races of wheat stem rust fungus: continued threat to food security and prospects of genetic control. Phytopathology 105:872–884

Singh RP, Singh PK, Rutkoski J, Hodson DP, He X, Jørgensen LN, Hovmøller MS, Huerta-Espino J (2016) Disease impact on wheat yield potential and prospects of genetic control. Annu Rev Phytopathol 54:303–322

Sohail Y, Bansal U, Bariana H, Chhuneja P, Mumtaz A, Rattu A, Trethowan R (2014) Identification of a co-dominant eSTS marker linked with leaf rust resistancegene *Lr28* in wheat (*Triticum aestivum* L.). Australian J Crop Sci 8(8):1210–1215

Steuernagel B, Periyannan SK, Hernandez-Pinzon I, Witek K, Rouse MN, Yu G, Hatta A, Ayliffe M, Bariana H, Jones JD (2016) Rapid cloning of disease-resistance genes in plants using mutagenesis and sequence capture. Nat Biotechnol 34:652–655

Thind AK, Wicker T, Simkova H, Fossati D, Moullet O, Brabant C, Vrana J, Dolezel J, Krattinger SG (2017) Rapid cloning of genes in hexaploidy wheat using cultivar-specific long-range chromosome assembly. Nat Biotechnol 35:793–796. https://doi.org/10.1038/nbt.3877

Tommasini L, Schnurbusch T, Fossati D, Mascher F, Keller B (2007) Association mapping of *Stagonospora nodorum* blotch resistance in modern European winter wheat varieties. Theor Appl Genet 115:697–708

Uauy C, Brevis JC, Chen X, Khan I, Jackson L, Chicaiza O, Distelfeld A, Fahima T, Dubcovsky J (2005) High-temperature adult-plant (HTAP) stripe rust resistance gene *Yr36* from *Triticum turgidum* ssp. *dicoccoides* is closely linked to the grain protein content locus *Gpc-B1*. Theor Appl Genet 112:97–105

Urashima AS, Grosso CRF, Stabili A, Freitas EG, Silva CP, Netto DCS, Franco I, Merola Bottan JHM (2009) Effect of *Magnaporthe grisea* on seed germination, yield and quality of wheat. In: Wang GL, Valent B (eds) Advances in genetics, genomics and control of rice blast disease. Springer, New York, pp 267–277. https://doi.org/10.1007/978-1-4020-9500-9_27

Visser B, Herselman L, Park RF, Karaoglu H, Bender CM, Pretorius ZA (2011) Characterization of two new *Puccinia graminis* f. sp. *tritici* races within the Ug99 lineage in South Africa. Euphytica 179:119–127. https://doi.org/10.1007/s10681-010-0269-x

Wang L, Ma J, Zhou R, Wang X, Jia J (2002) Molecular tagging of the yellow rust resistance gene *Yr10* in common wheat, PI178383 (*Triticum aestivum* L). Euphytica 124:71–73

Wang ZL, Li LH, He ZH, Duan XY, Zhou YL, Chen XM, Lillemo M, Singh RP, Wang H, Xia XC (2005) Seedling and adult plant resistance to powdery mildew in Chinese bread wheat cultivars and lines. Plant Dis 89:457–463

Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, Qiu JL (2014) Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat Biotechnol 32:947–951

Weise MV (1987) Compendium of wheat diseases, 2nd edn. American Phytopathology Society, St. Paul

WHEAT (2013) Wheat: the vital grain of civilization and food security. CGIAR Research Program wheat 2013 annual report. CGIAR, Mexico DF

Zhang X, Han D, Zeng Q, Duan Y, Yuan F, Shi J, Wang Q, Wu J, Huang L, Kang Z (2013) Fine mapping of wheat stripe rust resistance gene *Yr26* based on collinearity of wheat with *Brachypodium distachyon* and rice. PLoS ONE 8:e57885

Zhou XL, Wang MN, Chen XM, Lu Y, Kang ZS, Jing JX (2014a) Identification of *Yr59* conferring high temperature adult plant resistance to stripe rust in wheat germplasm PI178759. Theor Appl Genet 127:935–945

Zhou XL, Han DJ, Chen XM, Gou HL, Guo SJ, Rong L, Wang QL, Huang LL, Kang ZS (2014b) Characterization and molecular mapping of stripe rust resistance gene *Yr61* in winter wheat cultivar Pindong34. Theor Appl Genet 127:2349–2358

Chapter 5
Rice, Marker-Assisted Breeding, and Disease Resistance

Sahil Mehta, Baljinder Singh, Priyanka Dhakate, Mehzabin Rahman, and Md Aminul Islam

5.1 Introduction

According to the Population Reference Bureau (https://www.prb.org/data/), the human world population is increasing exponentially and will definitely cross the 10 billion figures by 2053 (PRB 2016). As a result, it will become very difficult to feed this enormous global population as the population always grows geometrically while our food productivity increases arithmetically. Moreover, the current global population has already overexploited various nonrenewable and renewable resources of the earth. This overexploitation has even worsened the climate quality by addition of multiple pollutants to the environment (http://www.preservearticles.com). Furthermore, there has been cutting down of trees at a tremendous scale, the decline in soil fertility, shrinkage of cultivable lands, huge depletion of water resources, desertification, global warming, and destruction of the ozone layer (Duke 2018; Oerke 2005). All these factors have affected the total food productivity negatively leading to food scarcity, hunger, and famine. This is also highlighted by the Intergovernmental Panel on Climate Change (IPCC) reports (http://www.ipcc.ch). According to their reports, the whole scenario of food production will get worse in the upcoming three decades.

Hence, this exponentially growing population will certainly put pressure on the total food production from the limited cultivable land in the future (Oerke 2005; Wallace et al. 2003). Presently, there is a wide gap between the global food productivity, demand, and supply. In order to bridge this gap, we need to reassess

S. Mehta
International Centre for Genetic Engineering and Biotechnology, New Delhi, India

B. Singh · P. Dhakate · M. A. Islam (✉)
National Institute of Plant Genome Research, New Delhi, India

M. Rahman
Bimala Prasad Chaliha College, Nagarbera, Assam, India

S. H. Wani (ed.), *Disease Resistance in Crop Plants*,
https://doi.org/10.1007/978-3-030-20728-1_5

the policies related to food production and security. In addition, the refinements must be done in the agricultural practices along grain storage facilities and management skills to boost up our production efficiency by multiple folds (Rezbova and Skubna 2012).

5.2 Factors Influencing Production Efficiency

Globally, the humans either consume or utilize various cereals, pseudocereals, pulses, oil-yielding plants, fiber-yielding plants, spices, and medicinal plants. All these different classes of plants are cultivated at either a larger or smaller scale in the fields. In the fields, these plants are continuously exposed to many environmental stresses which affect growth, development, survival, and its subsequent yield (Atkinson and Urwin 2012; Bellard et al. 2012). These environmental stresses have a major negative impact on crop production worldwide and can be categorized into two groups: (i) biotic factors and (ii) abiotic factors. The biotic factor encompasses living organisms which have either predatory or symbiotic relationships with the host plant. This class includes fungi, bacteria, nematodes, weeds, insects, parasites, rodents, birds, and viruses present in the environment (Singh et al. 2018).

Among biotic stresses, the phytopathogens cause a variety of diseases in nonresistant plants by misbalancing the plant's metabolism at cellular, molecular, hormonal, and physiological levels. This huge plethora of plant diseases is the most serious biological constraint which affects the food utilization component. This is also evident from fact that the plant diseases account for global agricultural losses ranging between 20% and 45% which is supplemented by another 5–10% during postharvest storage (Bellard et al. 2012; Oerke 2005; Pathak and Khan 1994; Savary et al. 2012) with both direct and indirect consequences. The phrase "losses between 20 and 45%" reflects the true costs of crop losses to mainly farmers, consumers, economies, societies, and environments. Therefore, it is essential to develop and improve the previously existing high-yield, disease-resistant crop varieties in the fields for higher food production. Furthermore, the breeders have already improved multiple crops globally for better yields with higher tolerance to multiple stresses including common bean, sorghum, wheat, barley, sugarcane, and rice (Ashkani et al. 2015; Jena and Mackill 2008; Miedaner and Korzun 2012; Mohamed et al. 2014; Mundt 2014; Nelson et al. 2018; Shakoor et al. 2017; Stenberg et al. 2015; Wiesner-Hanks and Nelson 2016).

5.3 Rice (*Oryza sativa* L.): A High-Valued Cash Crop

Following corn, rice is the second most important high-valued cash crop in the whole world (Rice - Statistics & Facts 2018). It is established as a part of food diet for about 50% of the whole human population (Khush 2005). In 2017, nearly 488.6

million metric tons of rice was harvested globally in about 11% of the world's total land (Rice - Statistics & Facts 2018). Traditionally, many Asian countries including China, India, Japan, Thailand, Indonesia, Pakistan, etc. produce around 90% of the total rice globally. According to the recent official data, China produced over 210 million metric tons of rice.

In addition, it is an established model plant for monocotyledons (Garg and Jaiswal 2016). Due to the availability of the rice genome sequence (Goff et al. 2002; Yu et al. 2002), sequence maps (Sasaki 2005) and multiple databases like Oryzabase (Kurata and Yamazaki 2006), QlicRice (Smita et al. 2011), RiceSRTFDB (Priya and Jain 2013), RiceVarMap (Zhao et al. 2014), RiTE (Copetti et al. 2015), IsomiR Bank (Zhang et al. 2016), and ARMOUR (Sanan-Mishra et al. 2018), this cereal crop is at the focal point of functional genomics, comparative genomics, epigenomics, transcriptomics, proteomics, and metabolomics (Crossa et al. 2017; Helmy et al. 2011; Mosa et al. 2017; Muthuramalingam et al. 2018; Parida et al. 2018; Smita et al. 2011). It is also evident from the fact that "rice" fetched around 451,000 publications in the Google scholar. Furthermore, this has opened a new series of rice breeding programs (Dnyaneshwar et al. 2018; Lee et al. 2015; Reinke et al. 2018; Singh et al. 2012a; Telebanco-Yanoria et al. 2010; Xiao et al. 2017; Yap et al. 2016) aimed to better the diverse traits including tiller architecture, disease resistance, grain size, grain quality, grain content, etc.

Multiple rice diseases act as major constraints to the rice production by altering the normal physiological activity of a rice plant grown all over the world. There are multiple pathogens which cause an enormous plethora of diseases such as (1) rice blast (leaf and collar), (2) rice blast (node and neck), (3) brown spot, (4) false smut, (5) rice sheath blight, (6) leaf scald, (7) bakanae disease, (8) narrow brown spot, (9) sheath rot, (10) stem rot, (11) grassy stunt disease of rice, (12) rice ragged stunt, (13) tungro disease of rice, (14) rice stripe virus disease, (15) yellow mottle disease of rice, (16) bacterial blight, (17) bacterial leaf streak, (18) red stripe of rice, and (19) sheath brown rot. The data have been adapted from Rice Knowledge Bank (http://www.knowledgebank.irri.org/), Rice Knowledge Management Portal (http://www.rkmp.co.in/), and the American Phytopathological Society (https://www.apsnet.org/Pages/default.aspx).

5.4 Insights into the Marker-Assisted Breeding

For many centuries, the breeders utilize conventional breeding strategies to introduce beneficial traits from related species to high-yielding varieties. However, there are few limitations of conventional breeding methods including (i) time consuming, (ii) based on phenotypic evaluation and selection, and (iii) difficult to transfer traits with polygenic inheritance (Crossa et al. 2017; Jiang 2013). To overcome the limitations of traditional breeding strategies, an alternate, more fast, and accurate approach is being utilized by agricultural scientists from the last few decades – marker-assisted breeding (MAB) (Fig. 5.1) (Balachiranjeevi et al. 2018; Jairin et al.

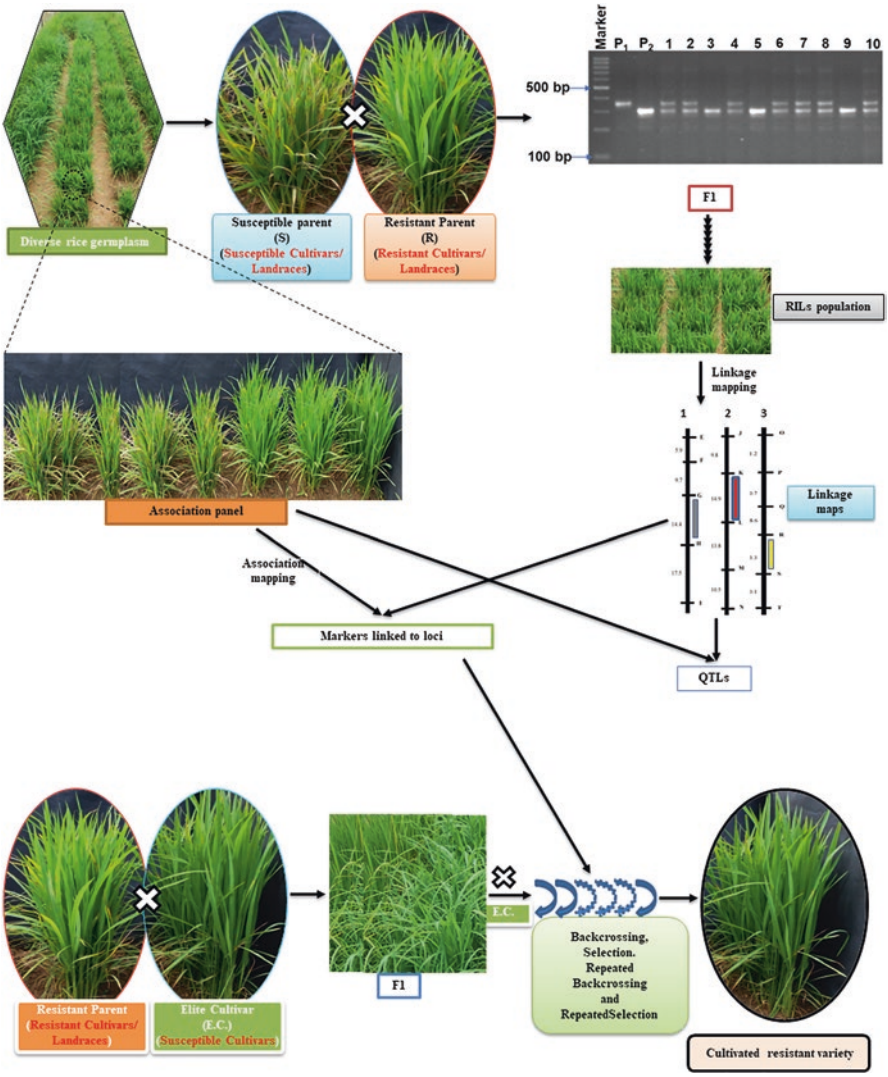

Fig. 5.1 An overview of marker-assisted breeding (MAB)

2017; Jiang 2013; Xu and Crouch 2008). This MAB approach is boosted by advances in genomic techniques and sequences, availability of genome sequences, online databases, and many bioinformatic tools. Over the years, the MAB approach has emerged as an irreplaceable tool as it primarily focuses on improving overall performance, yield stability, traits including tolerances to multiple stresses, and acceptance by farmers (Balachiranjeevi et al. 2018; Gur and Zamir 2004; Jairin et al. 2017; Jiang 2013; Nelson et al. 2018; Shakoor et al. 2017). In addition, this is supported by the fact that the term "marker-assisted breeding" fetched around 17,500 publications in the Google Scholar (https://scholar.google.co.in/) (Fig. 5.2).

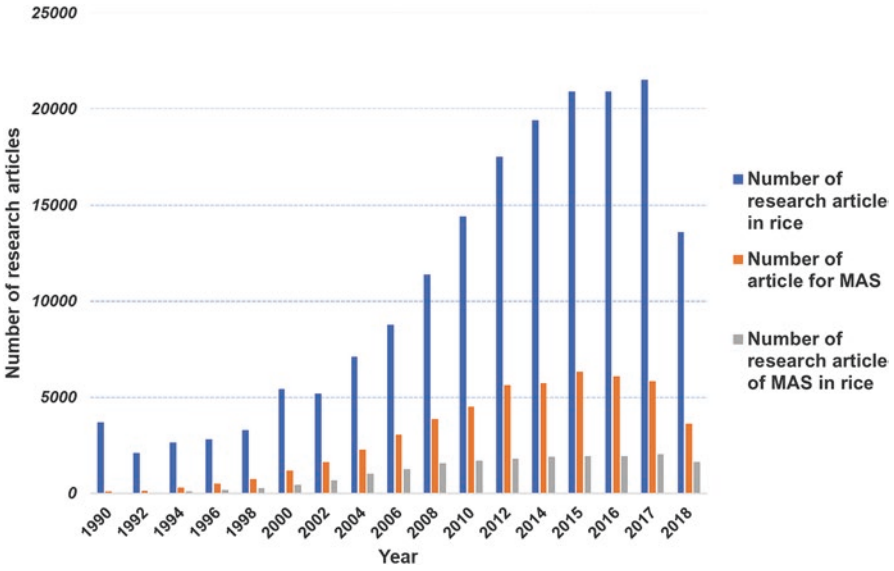

Fig. 5.2 The numbers of articles with the terms *rice, marker-assisted selection* (MAS), and *MAS in rice* by years (1990–2018) from Google Scholar (https://scholar.google.co.in/). (Accessed at 25 September, 2018)

This breeding approach utilizes DNA-based molecular markers which are tightly linked to the trait of interest for efficient screening of phenotype (Gur and Zamir 2004; Holland 2004; Jiang 2013; Salvi and Tuberosa 2005; Wang et al. 2007). These molecular markers used are simple, cost-effective, polymorphic PCR markers which display recombination frequency less than 2% between the marker and the target gene (Jiang 2013; Mohler and Singrün 2004; Peng et al. 2000). In the early 1990s, Beckmann and Soller (1983) popularized the first use of markers in crop improvement and focused on the marker-assisted backcrossing (MABC) issues. A few years later, Lande and Thompson (1990) studied the quantitative traits using the marker-assisted selection approach. This opened up the way for application of MAB in breeding studies which triggered a series of publications (Dwivedi et al. 2007; Frisch and Melchinger 2001; Gimelfarb and Lande 1995; Guimarães 2007; Gur and Zamir 2004; Whittaker et al. 1997; Zhang and Smith 1993).

The MAB approach is usually used in plant breeding programs and studies when (i) the traits have either complex inheritance or low penetrance; (ii) the traits are expressed in either developmental stages or specific environmental condition; (iii) to speed up the backcrossing and minimize the time required for phenotyping while breeding; (iv) to allow effective selection of recessive alleles and (v) to pyramid the several monogenic traits; (vi) to assemble desired traits more precisely in the same genotype within fewer selection cycles as compared to the conventional breeding approaches; and (vii) in addition to minimize the unintentional genetic background losses (Ashkani et al. 2015; Crossa et al. 2017; Jiang 2013; Xu and Crouch 2008). Furthermore, this MAB approach also considers the

value of carefully directed choice of parental lines, large-scale genotyping for desired traits, as well as the genetic background.

Due to the huge work and advantages, these approach has been widely implied by the breeders to breed the rice against various pathogens (Chen et al. 2008; Dnyaneshwar et al. 2018; Huang et al. 1997; Lee et al. 2015; Narayanan et al. 2002; Reinke et al. 2018; Sugiura et al. 2004; Telebanco-Yanoria et al. 2010; Xiao et al. 2017; Yap et al. 2016). To support this fact, the term "rice and marker-assisted breeding" have fetched about 10,600 publications in the Google Scholar (Fig. 5.2).

5.4.1 Rice Blast Disease

In the world today, rice blast is considered by far the most serious, studied rice disease due to its worldwide distribution (over 75 countries) and associated huge economic losses (Miah et al. 2013). Many researchers considered this disease as a model disease because of epidemiology, molecular pathology, genetics, and available genome sequence (Dean et al. 2005; Kumar and Rao 2018). This fact is supported by the fact that "rice blast" fetched around 47,200 publications in the Google Scholar (https://scholar.google.co.in/). The disease is caused by *Pyricularia grisea (Magnaporthe grisea*, teleomorph) of the family *Magnaporthaceae* which attack from seedling to late-tillering stages. It even causes rice blast epidemics which lead up to 60% yield losses (Fig. 5.3) in rice-growing countries. It causes white, bluish, or grayish lesions in every part of a plant such as grain, neck, collar, leaf, nodes, and panicles (Table 5.1).

Recent studies reveal about how these diseases work and the genes related to resistance against rice blast (Li et al. 2007; Miah et al. 2013; Singh et al. 2011). The challenge for the research community is to produce biofortified rice with higher resistance to abiotic stresses and diseases (Kumar and Rao 2018) at a lower cost. Furthermore, the plant disease management strategies are focused primarily to bear against the blast disease of rice. One of the primary strategies is to breed the rice for blast resistance (Kumar and Rao 2018; Singh et al. 2011). In the breeding context, the molecular markers have been applied unambiguously over the decades (Hari et al. 2013; Hittalmani et al. 2000; Jena and Mackill 2008; Kwon et al. 2008; Luo et al. 2017; Man et al. 2016; Reinke et al. 2018; Tanweer et al. 2015; Telebanco-Yanoria et al. 2010). There are many types of markers which have been used in marker-assisted breeding such as RFLP (Hittalmani et al. 2000), STS (Kwon et al. 2008; Narayanan et al. 2002), SNP (Kwon et al. 2008; Reinke et al. 2018; Xiao et al. 2017), CAPS (Man et al. 2016), and SSR (Chen et al. 2004; Gouda et al. 2013; Khan et al. 2018; Khanna et al. 2015; Miah et al. 2017). It is also evident from the fact that "markers and rice blast" fetched around 16,000 publications in the Google Scholar (https://scholar.google.co.in/). In the literature, there are many successful examples of the application of marker-assisted breeding approach to enhance resistance against blast (Chen et al. 2008; Chen et al. 2004; Hari et al. 2013; Khan et al. 2018; Khanna et al. 2015; Narayanan et al. 2002; Reinke et al. 2018; Telebanco-Yanoria et al. 2010; Wen and Gao 2012) (Table 5.2).

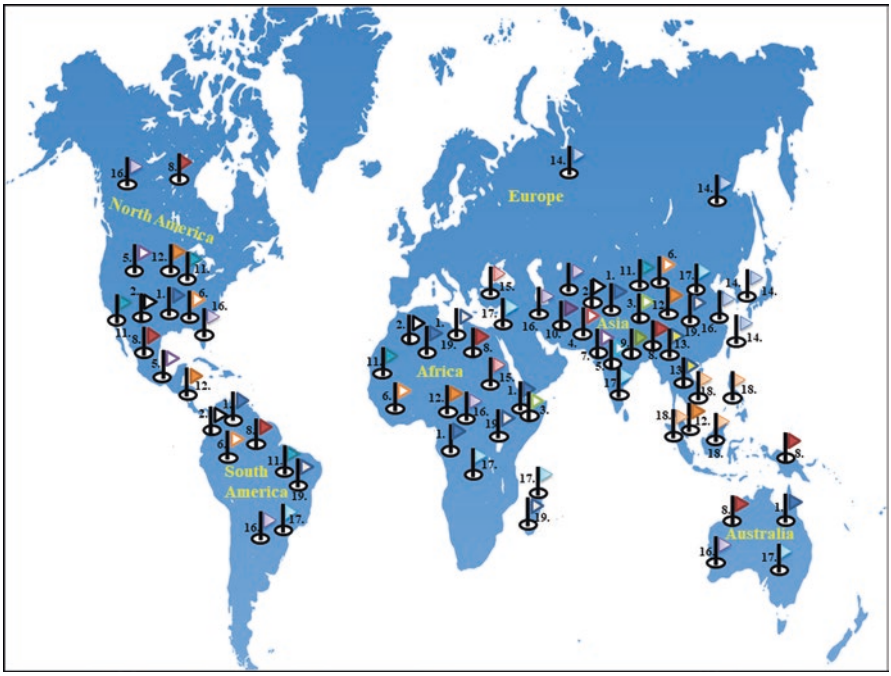

Fig. 5.3 Schematic representation of the major rice diseases in the world

At the beginning of twenty-first century, Hittalmani et al. (2000) combined three different R genes, namely, *Pi1* (using STMS marker, RM224), *Pita* (using STMS marker, RM247), and *Piz-5* (using STMS marker, RM208) from three different parents into the recurrent CO39 background to enhance tolerance against blast disease. Narayanan et al. (2002) reported the combination of *Piz-5* gene in the IR50 background from the donor parent CO39-NIL using an RG64 marker. Chen et al. (2004) developed the introgressed lines of three different R genes Pi-d(t), Pi-ta2, and Pi-b in the rice cultivar G46B. The addition of these genes enhanced the tolerance against the blast disease; however, there were no unexpected changes in the morphology of the introgressed lines. Similarly, the Pi-33, Pi-2, and Pi-1 genes pyramiding lead to enhanced resistance against blast disease (Chen et al. 2008). In another instance, Wongsaprom et al. (2010) transferred two quantitative trait loci (QTLs), namely, qBI11 and qBI1, from the donor JHN parent into the susceptible parent RD6 using markers such as RM212, RM144, RM319, and RM224. Similarly, Koide et al. (2010) introgressed the R gene *Pish* from the donor IRBLsh-S[CO] into the genetic background of CO39 using markers RM7419, RM1268, RM6648, and RM5811. In addition, they also introgressed the *Pib gene* into the CO39 background from the donor parent IRBLb-W[CO]. Four QTLs, namely, QTL1, QTL2, QTL11, and QTL12, were transferred from JaoHom Nin into the IR64 cultivar to suppress the activity of the blast pathogen (Sreewongchai et al. 2010). As another example, the transfer of R genes such as *Piz-54* and *Pi5* from the parents Tetep and C101A51 into the PRR78 background also enhanced the yield of the recurrent parent up to

Table 5.1 List of all major diseases of rice and the respective causing pathogens. The data have been adapted from Rice Knowledge Bank (http://www.knowledgebank.irri.org/), Rice Knowledge Management Portal (http://www.rkmp.co.in/), and the American Phytopathological Society (https://www.apsnet.org/Pages/default.aspx)

Sl. No.	Disease name	Pathogen	Parts affected	Symptoms	Transmission	Environment	Yield loss
Fungal diseases							
1.	Rice blast (leaf, panicle, and collar)	*Magnaporthe grisea/Pyricularia oryzae*	Leaf, leaf sheath, seedling, collar, neck, panicle	Spindle-shaped white to gray-green spots with dark red or green or necrotic borders	Wind	Low soil moisture, low temperature around 18–22 °C, and prolonged rain showers	30–75%
2.	Rice blast (node and neck)	*Magnaporthe oryzae*	Grains, leaf, leaf sheath, collar, node, neck, panicle	Banded pattern of infection on nodes, blackish or light brown lesions, grayish-brown lesions on the neck, panicle breaking	Wind	Low temperature around 16–24 °C and soil with low moisture content, frequent and prolonged rain shower periods along with dew formation on leaves	20–50%
3.	Brown spot of rice	*Bipolaris oryzae*	Leaf, leaf sheath, glumes, spikelet, seeds	Small, circular, yellow-brown lesions girdle around the coleoptile in seedlings Small, circular or oval, dark brown to the gray lesion with light reddish-brown margins in leaves	Wind	Temperature around 16–35 °C, high relative humidity (85–100%), and unflooded and nutrient-deficient soil	5–45%
4.	False smut	*Ustilaginoidea virens*	Spikelet, grains	Mass of velvety yellow fruiting bodies on grains	Wind	Temperature ranging between 25 and 35 °C, relative humidity of more than 92%, and soil with high nitrogen content	35–45%
5.	Rice sheath blight	*Rhizoctonia solani*	Leaf, tillers	Oval or ellipsoidal, irregular greenish or gray to white lesions with brown margins on the leaves	Wind, water	High temperature with 28–32 °C, relative humidity about 85–100%, and soils with high nitrogen fertilization	10–35%

6.	Leaf scald	*Microdochium albescens*	Coleoptiles, leaf, flower, grains	Dark brown, oblong lesions on leaf tips and edges, translucent leaf tips, flower deformation, glume discoloration	Seeds, stubbles	Low temperature with 18–22 °C, relative humidity about 85–100%, and soils with high nitrogen fertilization	15–20%
7.	Bakanae disease	*Fusarium fujikuroi*	Roots, leaf, tillers, grains	Pale, thin leaves, abnormally tall growth, reduction in tillers, and completely filled grains	Seed, wind, water	Temperature ranging between 25 and 35 °C, relative humidity of more than 75%	3.7–50%
8.	Narrow brown spot	*Sphaerulina oryzina*	Leaf, leaf sheaths, panicle, glumes	Dark brown, linear lesions on leaves and a net blotch on leaf sheath, plant lodging, premature ripening of grains	Wind	Temperature with 25–28 °C, humidity of at least 96%, and potassium-deficient soils	1–3%
9.	Sheath rot	*Sarocladium oryzae*	Grains, panicle	Irregular spots with dark reddish-brown margins; sterile, dark brown rot panicles; unfilled, discolored seeds	Wind, wounds by insects	Temperature with 20–28 °C, humidity of above 85%, and soils with high amounts of nitrogen fertilizer application	3–20%
10.	Stem rot	*Nakataea oryzae*	Panicle, culms	Small, irregular black lesions, chalky grains production, unfilled panicles, lodging	Water, wounds by insects	Temperature with 20–25 °C, humidity of above 75%, and soils with high amounts of nitrogen fertilizer application	30–80%
Viral diseases							
11.	Grassy stunt disease of rice	*Rice grassy stunt virus*	Tillers, panicle	Plant stunting, grassy growth, rosette appearance with excessive tillering, short, narrow yellowish-green leaves with small rusty blotches	Brown planthopper	Temperature ranging between 25 and 28 °C, with relative humidity above 75%	10–35%
12.	Rice ragged stunt	*Rice ragged stunt virus*	Leaf, panicle, grains	Stunting, spirally twisted, yellow-brown leaves with serrated uneven edges	Brown planthopper	Temperature range between 25 and 28 °C, with relative humidity above 65%	35–80%
13.	Tungro disease of rice	*Rice tungro bacilliform virus, Rice tungro spherical virus*	Leaf, tiller, grains	Stunting, leaf discoloration, reduced tillers, partly filled grains, stubble formation	Green leafhopper	Temperatures range between 25 and 30 °C, with relative humidity above 65%	20–95%

(continued)

Table 5.1 (continued)

Sl. No.	Disease name	Pathogen	Parts affected	Symptoms	Transmission	Environment	Yield loss
14.	Rice stripe virus disease	*Rice stripe virus*	Leaf, tiller	Stunting/mottling; chlorosis; yellowish-white stripes; necrotic streaks on the leaves; folded, wilted, and droopy leaves; fewer tillers with many whitish to brown and deformed, premature panicles	Brown planthoppers	Temperatures at 25–30 °C, with relative humidity above 75%	30–100%
15.	Yellow mottle disease of rice	*Rice yellow mottle virus*	Seedling, leaf, tillers	Stunting, mottled and twisted leaves, yellow-green or orange linear spots or streaks on leaf veins, fewer tillers, discoloration and poor panicle exsertion	Beetles, grasshoppers, cows, rats, donkeys, mechanically transmitted	Temperatures at 24–32 °C, with relative humidity above 75%	10–100%
Bacterial diseases							
16.	Bacterial blight disease of rice	*Xanthomonas oryzae* pv. *Oryzae*	Seedling, leaf	Wilting of seedlings, water-soaked or yellow-orange stripes, straw-colored leaves	Wind, water	Temperature range between 25 and 34 °C, relative humidity above 75% with high nitrogen fertilization on the soil	20–70%
17.	Red stripe of rice	*Xanthomonas rubrilineans*	Leaf, leaf sheath	Dark orange, pin-sized, necrotic and coalescing lesions on leaf and leaf sheath	Wind	High temperature at 30–35 °C, high relative humidity about 95% and soil with high nitrogen supply	2–5%
18.	Sheath brown rot	*Pseudomonas fuscovaginae*	Leaf sheath, grains, seedling	Irregular dark green, water-soaked lesions, yellow to brown discoloration in leaves and infected seedlings, discolored, deformed, or empty grains	Seed	Temperatures at 20–22 °C, high humidity of around 85%, and high altitude around 1200–1700 m above sea level	72–98%
19.	Bacterial leaf streak	*Xanthomonas oryzae* pv. *oryzicola*	Leaf	Tiny, brown to yellowish-gray, water-soaked lesions within leaf veins, browning, and drying of leaves	Seeds, stubbles	High temperature at 30–35 °C and relative humidity above 80%	3–17%

Table 5.2 Successful examples of marker-assisted breeding (MAB) for blast resistance breeding in rice

Sl. No.	Gene/QTL name	Marker used	Marker type	Donor parent	Recurrent parent	References
1	*Pi1*	Npb 181	RFLP	Lac23	CO39	Hittalmani et al. (2000)
	Pita	RZ397		Pai-kan-tao		
	Piz-5	RG64		A5173		
2	*Piz-5*	RG64	STS	CO39-NIL (C101A51)	IR50	Narayanan et al. (2002)
3	Pi-d(t)	–	SSR	Digu	G46B	Chen et al. (2004)
	Pi-b			BL-1		
	Pi-ta2			Pi-4		
4	*Pia, Pib,*	Pca72, NSb,	SNP, SCAR, and STS	Seolak, Daeseong, and Bongkwang	Chucheong	Kwon et al. (2008)
	Pii, Pi5,	JJ817,				
	Pita, Pita-2,	YL100/YL10, YL155/YL87, YL183/YL87,				
		z4792, zt4792, z60510, zt6057, pBA14, NBS2-O/NBS2-U				
	Piz, Piz-t,	K6415				
	Pi9	K6441				
	Pik	K39575				
	Pik-m	T256				
	Pik-p					
	Pit					
5	Pi-1, Pi-2 and Pi-33	–	SSR	C101LAC and C101A51	Jin 23B	Chen et al. (2008)
6	*Pib, Piz-5, Pi9, Pi3, Pia, Pik-s, Pik, Pik-h, Pi7(t), Pita* and *Pita-2*	–	SSR	BL1, C101A51 (5173), WHD-1S-75-1-127, C104PKT, Zenith, Shin2, Kanto 51, K3, RIL29, C105TTP2L9, and Pi No. 4	Lijiangxintuanheigu (LTH)	Telebanco-Yanoria et al. (2010)

(continued)

Table 5.2 (continued)

Sl. No.	Gene/QTL name	Marker used	Marker type	Donor parent	Recurrent parent	References
7	qBI1	RM212 and RM319	SSR	JaoHom Nin (JHN)	RD6	Wongsaprom et al. (2010)
	qBI11	RM144 and RM224				
8	*Pish*	RM7419, RM1268, RM6648, and RM5811	SSR	IRBLsh-S[CO]	CO 39	Koide et al. (2010)
		Pibdom and RM208				
	Pib			IRBLb-W[CO]		
9	QTL1, QTL2, QTL11, and QTL12	RM212, RM319, RM208, RM139, and RM179	SSR	IR64 X JaoHom Nin (JHN)	P55, P187, and P280 (F2)	Sreewongchai et al. (2010)
10	*Pi54*	RM206	SSR	Tetep	Improved Pusa Basmati 1	Singh et al. (2012b)
11	*Piz-5* and *Pi54*	AP5930 and RM206	SSR	C101A51 and Tetep	PRR78	Singh et al. (2012a)
12	*Pi-9(t)*	pB8	–	P2	Luhui 17	Wen and Gao (2012)
13	*Pi1* and *Pi2*	MRG4766 and AP22	SSR	BL122 and CBB23	Rongfeng B	Fu et al. (2012)
14	*Pi1* and *Piz-5*	RM5926 and AP5659-5	SSR	CO39	PRR78	Gouda et al. (2013)
15	*Pi54*	RM206	SSR	Samba Mahsuri (SM2154)	IR 58025A	Hari et al. (2013)
16	*Pi39*	39SM	Indel	Q15	Yuexiangzhan Yueyinsiniao	Hua et al. (2015)
17	*Pita, Pi1, Pi54, Pi5, Pib, Pi9,* and *Pi2*	YL155/YL87, RM224, RM206, C1454, Pibdom/RM208, Nbs2Pi9/AP5659–5 and AP4007	SSR	DHMASQ164-2a, IRBLz5-CA, IRBLb-B, IRBL5-M, and IRBL9-W	PB1	Khanna et al. (2015)
18	*Pi-b* *Pi-kh*	RM208 RM206	SSR	Pongsu Seribu 2	MR219	Tanweer et al. (2015)

19	Pizt	–	SSR	Toride 1	Yangdao 6 (93–11)	Wu et al. (2016)
	Pi2			C101A51		
	Pigm			Gumei 4		
	Pi40			IR65482		
	Pi9			75-1-127		
	Piz			Fukunishiki		
20	*Pi2*	AP5659-5	SSR	RPBio Patho-1	RPHR-1005	Kumar et al. (2016)
21	*Pi40*	9871.T7E2b	CAPS DNA	IR83260-1-1-1-5-B-3-1-2-B	Osmancik-97 and Halilbey	Man et al. (2016)
22	*Pi1*	RM224 and RM1233	SSR	BPT5204	Intan	Hegde and Prashanthi (2016)
	Pi2	AP-5930 and AP-5659-5				
23	*Pi46*	RM224	SSR	H4	Hang-Hui-179 (HH179)	Xiao et al. (2016)
	Pita	YL155/YL87and YL183/YL87				
24	*Pi2*	RM527	SSR	C101-A-51	Buyarin and Kuboyar	Usatov et al. (2016)
	Pi1 and *Pi33*	RM224 and RM310		C101-LAC		
25	*Pi2* and *Pi54*	AP5659-5 and RM206	SSR	PUSA 1602 and PUSA 1603	PB1121 and PB6	Ellur et al. (2016a)
26	*Pi9*	–	SNP	75-1-127	07GY31	Xiao et al. (2017)
	Pizt			Toride 1		
	Pi54			K3		
27	*Piz, Pi2,* and *Pi9*	RM6836 and RM8225	SSR	Pongsu Seribu 1	MR219	Miah et al. (2017)
28	*Pi2 and Pi9*	–	SNP	C101A51 and IRBL9-W	R179	Luo et al. (2017)
29	*Pik* and *Pib*	K6415 and NSb	SNP	IR65482-7-126-1-2	Junam	Reinke et al. (2018)
30	*Pi54, Pi1,* and *Pita*	Pi54MAS, RM224, and YL155/YL87	Indel, SSR, and gene-based marker	DHMAS 70Q 164-1b	Mushk Budji	Khan et al. (2018)

25% (Singh et al. 2012b). Furthermore, the *Pi1*, *Pi2*, *Pi-9(t),* and *Pi54* introgressed lines demonstrated resistance against blast disease without any fitness cost incurred (Fu et al. 2012; Hari et al. 2013; Wen and Gao 2012). Their results were also supported by the findings of other researchers (Hua et al. 2015; Kumar et al. 2016; Tanweer et al. 2015; Usatov et al. 2016; Wu et al. 2016). Similarly, Xiao et al. (2016) transferred the *Pi46* and *Pita* genes from the rice cultivar H4 to the Hang-Hui-179 (HH179) background using MAS approach. They reported the pyramiding of these R genes leads to enhancement in molecular resistance against the blast pathogen *Pyricularia grisea.* In a further study, Man et al. (2016) reported the introgression of *Pi40* in the two elite rice cultivars Halilbey and Osmancik-97. The developed *Pi40* lines showed broad-spectrum resistance against many blast races and enhancement in total. Similarly, the *Pi33* (Usatov et al. 2016) *and Pi54* (Ellur et al. 2016b) introgression showed the enhancement in blast disease resistance in many high-yielding rice cultivars such as Buyarin, PB1121, and Kuboyar. In a further study, Xiao et al. (2017) reported the transfer of three genes (*Pi9, Pi54,* and *Pizt*) into the elite cultivar 07GY31 leads to the higher yields due to the enhanced resistance against blast. In another instance, the pyramiding of other R genes such as *Piz* (Miah et al. 2017)*, Pi2* (Khanna et al. 2015; Luo et al. 2017; Miah et al. 2017), and *Pi9* (Luo et al. 2017; Miah et al. 2017) in different rice cultivars enhanced the disease resistance over the world.

Recently, Reinke et al. (2018) reported the transfer of multiple R genes from the resistant IR65482-7-126-1-2 variety to the susceptible Korean variety Junam using marker-assisted backcrossing (MABC); in addition, they evaluated the multiple parameters such as agronomic performance, stress tolerance, quality of rice grain, and total yield. The *Pib* + *Pik* developed introgressed lines showed enhancement against the rice blast disease; however, there were no negative effects on the overall morphology, tiller architecture, grain quality, and total yield. More recently, Khan et al. (2018) introgressed three R genes *Pi1, Pi54,* and *Pita* in the aromatic rice landrace Mushk Budji using marker-assisted breeding. The triple gene introgressed line showed enhanced resistance against the blast disease under field conditions.

5.4.2 Bacterial Blight Disease of Rice

Next to rice blast disease, rice bacterial blight is a major biotic stress for the rice farmers worldwide (Balachiranjeevi et al. 2018; Jena and Mackill 2008). In several cases, it causes crop loss up to 70% annually. This is supported by the fact that "bacterial blight of rice" fetched around 20,700 publications in the Google Scholar (https://scholar.google.co.in/). This devastating disease is caused by *Xanthomonas oryzae* pv. *oryzae,* a bacterial pathovar (family: *Xanthomonadaceae*). The disease spreads from the infected to healthy plants via water and wind. It is usually observed in humid irrigated lowland areas of Asia, Africa, and Australia where strong winds and continuous heavy rainfall occur. The symptoms are

straw-colored leaves, water-soaked stripes on leaf tips, and wilted seedlings (Gopalakrishnan et al. 2008; Nguyen et al. 2018). In order to reduce the pathogen infection and huge losses, many plant researchers and breeders have identified, mapped, cloned (Gazal et al. 2016; Jena and Mackill 2008), and pyramided several DNA markers in addition to the resistance genes such as *Xa1, Xa4, xa5*, *Xa21, Xa26, Xa27,* etc. into the many susceptible rice cultivars and landraces (Chen et al. 2000; Chen et al. 2001; Dnyaneshwar et al. 2018; Huang et al. 1997; Joseph et al. 2004; Pandey et al. 2013; Sundaram et al. 2008; Toenniessen et al. 2003; Yap et al. 2016) (Table 5.3). This is supported by the fact that the term "markers and bacterial blight" fetched around 14,500 publications in the Google Scholar (https://scholar.google.co.in/).

In the early 1990s, a series of two publications together pioneered the transfer of resistance genes in rice for bacterial blight tolerance (Ronald et al. 1992; Yoshimura et al. 1995). Their work opened up a gateway to the series of continuous improvement of various cultivars over the globe. In another case, Huang et al. (1997) transferred four different R genes, namely, *Xa4* (using Npb181 and Npb78 marker), *xa-5* (using RG556 marker), *xa-13* (using RG136 marker), and *xa-21* (using pTA248 marker) from four different resistant parents to the recurrent IR24 background to enhance tolerance against bacterial blight. In another instance, the introgression of *Xa21* gene in the elite cultivars, namely, Minghui 63 (Chen et al. 2000) and 6078 (Chen et al. 2001), improved the bacterial blight resistance. In addition, they evaluated the agronomic performance in the fields under pathogen attack. Due to multiple resistance genes, many researchers pyramided more than one gene to enhance the resistance of several folds. Using marker-assisted pyramiding (MAP), Singh and colleagues transferred the R genes (*Xa21, xa5,* and *xa13*) to the rice cultivar PR-106 (Singh et al. 2001). Similarly, Toenniessen and coworkers (2003) pyramided the R genes such as *xa5* to the Philippines cultivar IR64 to enhance the bacterial blight resistance. This resulted in the enhancement of the overall yield. Similar results had been reported with the R genes such as *Xa21* and *Xa4* (Jena and Mackill 2008). Using sequence-tagged sites (STS) markers, two genes, namely, *Xa21* and *xa13*, were pyramided into the well-known rice cultivar PB1 from the resistant IRBB55 cultivar to enhance the resistance in the well-known cultivar (Joseph et al. 2004). The same set of work was also used to improve the Indian basmati varieties Taraori Basmati and Basmati 386 (Pandey et al. 2013). Similarly, other workers have improved the resistance of Indian rice cultivars such as IPB (Gopalakrishnan et al. 2008), Samba Mahsuri (Sundaram et al. 2008), Pusa 6B (Basavaraj et al. 2010), PRR 78 (Basavaraj et al. 2010), Pusa Basmati-1 (Singh et al. 2012b), Tapaswini (Dokku et al. 2013), and RD6 (Pinta et al. 2013) against the devastating pathogen *Xanthomonas oryzae* pv. *oryzae* using MABC approach.

Due to the discovery of multiple genes controlling the resistance against bacterial blight, the gene pyramiding has been the choice of breeders in MAB, for example, *xa5* + *Xa21* + *xa33* (Win et al. 2013), *Xa4* + *xa5* + *xa13* + *Xa21* (Guvvala et al. 2013), *Xa4*+ *Xa21* (Luo et al. 2014), *xa13* + *xa21* (Ellur et al. 2016a), *Xa21*+ *Xa27* (Luo et al. 2017; Luo and Yin 2013), and *xa13* + *Xa21* (Arunakumari et al. 2016).

Table 5.3 Successful examples of marker-assisted breeding (MAB) for blight disease resistance breeding in rice

S.No.	Gene/ QTL	Marker(s) used	Marker(s) type	Donor parent	Recurrent parent	Reference(s)
1.	Xa21	pTA248	STS	–	IR 24	Ronald et al. (1992)
2.	*Xa3* *Xa4* *xa5* Xa10	–	RFLP and RAPD markers	IR-BB3 IR-BB4 IR-BB5 IR-BB10	IR 24	Yoshimura et al. (1995)
3.	Xa4 xa5 xa13 Xa21	Npb181 and Npb 78 RG556 RG136 pTA248	RFLP	IRBB4 IRBB5 IRBB13 IRBB21	IR 24	Huang et al. (1997)
4.	Xa21	248, 21, C189, and AB9	RFLP	IRBB21	Minghui 63	Chen et al. (2000)
5.	*xa5* *xa13* *Xa21*	RG556 and RG207 RG136 248	STS	IRBB59	IR65598–112 IR65600–42 IR65600–96	Sanchez et al. (2000)
6.	*Xa21*	21, C189 and AB9	AFLP	IRBB21	6078	Chen et al. (2001)
7.	*xa5* *xa13* *Xa21*	RG556 RG136 pTA248	STS	IRBB 5 IRBB 13 IRBB 21 IRBB 62	PR106	Singh et al. (2001)
8.	*xa5* *xa13* *Xa21*	RG 556 and RM 122 RG 136 pTA 248	STS	IRBB 5 IRBB 13 IRBB 21	IR 24	Ramalingam et al. (2002)
9.	xa-5	RG556	CAPS	IRBB5	IR 64	Toenniessen et al. (2003)
10.	*xa13* *Xa21*	RG136 pTA248	STS	IRBB55	Pusa Basmati 1 (PB1)	Joseph et al. (2004)
11.	*Xa7* *Xa21*	M1, M2, M3, M4, and M5 248	Gene linked RAPD	DV 85 IRBB21	Minghui 63	Zhang et al. (2006)
12.	*xa5* *xa13* *Xa21*	RG556 RG136 pTA248	STS	SS1113	Samba Mahsuri (BPT5204)	Sundaram et al. (2008)
13.	*xa13* *Xa21*	RG136 pTA248	STS	IRBB55	Pusa Basmati 1 (PB1)	Gopalakrishnan et al. (2008)
14.	*Xa4* *xa5* *xa13* *Xa21*	G181 RG556 RG136 pTA248	RFLP STS STS STS	NH56	Jyothi and IR50	Bharathkumar et al. (2008)
15.	*xa5* *xa13* *Xa21*	RG556 RG136 pTA248	STS	SS1113	Triguna	Sundaram et al. (2008)

(continued)

Table 5.3 (continued)

S.No.	Gene/ QTL	Marker(s) used	Marker(s) type	Donor parent	Recurrent parent	Reference(s)
16.	*Xa23*	RM206	SSR	CBB23–2	Lu-You-Zhan	Zhou et al. (2009)
17.	*xa13* *Xa21*	RG136pTA248	STS	Pusa1460	Pusa RH10	Basavaraj et al. (2010)
18.	*xa5* *xa13* *Xa21*	RM122 RG136 pTA248	STS	IRBB60	ADT43 and ADT47	Bharani et al. (2010)
19.	*xa13* *Xa21*	RG136pTA248	STS	PR106-P2	Dehraduni Basmati	Rajpurohit et al. (2011)
20.	*Xa21*	pTA248	STS	Improved Samba Mahsuri	KMR-3R	Hari et al. (2011)
21.	*xa13* *Xa21*	RG136pTA248	STS	IET 17948	Basmati 370 and Basmati 386	Bhatia et al. (2011)
22.	*Xa4* *Xa21* *Xa27*	RM224 21 and pTA248 5198	SSR STS RFLP	IR64 IRBB21 IRBB27	MH725	Luo et al. (2012)
23.	*Xa23*	RM206	SSR	CBB23	Rongfeng B	Fu et al. (2012)
24.	*Xa7* *Xa21* *Xa22* *Xa23*	RM20593 pTA248 RM224 03STS1	Gene linked	Huahui20 Huahui20 96L011 CBB23	Huahui 1035	Huang et al. (2012)
25.	*Xa21*	PB7-PB8	Gene specific	IR1188	Khao Dawk Mali 105 (KDML105)	Win et al. (2013)
26.	*Xa5* *xa13* *Xa21*	RG 556 RG 136 pTA248	STS	IRBB 60	Tapaswini	Dokku et al. (2013)
27.	*Xa21* *Xa27*	21 RMXa27	STS SSR	WH421 IRBB27	Khao Dawk Mali 105	Luo and Yin (2013)
28.	*Xa21* *xa13*	pTA 248 and xa13-prom	Gene based	Improved Samba Mahsuri	Taraori Basmati and Basmati 386	Pandey et al. (2013)
29.	*xa5*	RM122/ RM159	SSR	IR62266	RD6	Pinta et al. (2013)
30.	*Xa21*	pTA248	STS	Samba Mahsuri (i.e., SM2154)	IR 58025A	Hari et al. (2013)
31.	Xa4 xa5 xa13 Xa21	Npb181 RM122 RG136 pTA248	STS and SSR	IRBB60	Mahsuri PRR78 KMR3	Guvvala et al. (2013)
32.	xa5 Xa21 xa33	PAxa5 PB7–PB8 RM7243 and RM5509	Gene specific Gene specific SSR	RGDU-07097-1-8M-9 (RG-9)	'Yn 3248-2-128-76-4-3-75' (MK-75)	Win et al. (2013)

(continued)

Table 5.3 (continued)

S.No.	Gene/ QTL	Marker(s) used	Marker(s) type	Donor parent	Recurrent parent	Reference(s)
33.	Xa4 Xa21	RM224 21	SSR STS	WH421	Siputeh	Luo et al. (2014)
34.	*Xa21*	pTA248	STS	RP-Bio-Patho-2	DRR17B	Balachiranjeevi et al. (2018)
35.	Xa23	C189	EST	CBB23	Guangzhan63S (GZ63S) Liangyou6326	Ni et al. (2015)
36.	Xa23	M-Xa23	Indel	HBQ810	Guangzhan63-4S (GZ63-4S)	Jiang et al. (2015)
37.	*xa13* *Xa21*	xa13prom PTA248	Gene based	SPS97 and Pusa1460	PB1121 and PB6	Ellur et al. (2016b)
38.	*xa13* *Xa21*	xa13prom PTA248	Gene based	Improved Samba Mahsuri (ISM)	MTU1010	Arunakumari et al. (2016)
39.	*Xa38*	Os04g53050-1	Gene linked	PR114-*Xa38*	PB1121	Ellur et al. (2016a)
40.	Xa4 xa5 Xa7 xa13 Xa21	Xa4F/4R RM604F/604R Xa7F/7-1R/7-2R Xa13F/13R Xa21F/21R	Gene linked	IRBB66	Tainung82 (TNG82) Tainung84 (TNG84) Tainung71 (TNG71) Tai-kang2 (TK2) Tai-kang9 (TK9) Tai-kang16 (TK16) Taoyuan3 (TY3) Tainan110 (TN11)	Yap et al. (2016)
41.	*xa5* *xa13* *Xa21*	*Xa5S and xa5SR/R* *RG136* *pTA248*	STS	CRMAS 2232-85	Jalmagna	Pradhan et al. (2016)
42.	*Xa21* *Xa33*	pTA248 RMWR7.6	Gene linked	RPBio Patho-1 FBR1-15	RPHR-1005	Kumar et al. (2016)
43.	*Xa21* *Xa27*	21 RMXa27	STS SSR	Wan Hui 21 and IRBB27	9311	Luo et al. (2017)
44.	Xa21 xa13 xa5	pTA248 RG136 RG556	STS	IRBB-60	CSR-30	Baliyan et al. (2018)
45.	Xa21 xa13 xa5	Xa 21 and PT248-1 xa13Pro xa5R, xa5S, and RM13	SSR	RP-Bio-226	Dubraj and Safri 17	Dnyaneshwar et al. (2018)

(continued)

Table 5.3 (continued)

S.No.	Gene/ QTL	Marker(s) used	Marker(s) type	Donor parent	Recurrent parent	Reference(s)
46.	*Xa21*	pTA248	RFLP	IRBB21	LT2	Nguyen et al. (2018)
47.	Xa21 Xa33	PTA248 RMWR7.6	Gene-specific	Improved Samba Mahsuri (ISM) Samba Mahsuri (FBR1-15EM)	DRR17B	Balachiranjeevi et al. (2018)
48.	*Xa40*	ID55.WA3 and RM1233	STS and SSR	IR65482-7-126-1-2	Junam	Reinke et al. (2018)
49.	*Xa7*	RM20582	SSR	YR7029–39	Guangzhan63-4S	Mi et al. (2018)
50.	*xa13 Xa21*	xa13 prom and pTA248	Gene based	–	CO 43	Krishnakumar and Kumaravadiv et al. (2018)
51.	*Xa38*	Os04g53050-1	Gene specific	PR 114	Improved Samba Mahsuri (ISM)	Yugander et al. (2018)

In other instance, Ni et al. (2015) also reported the role of *Xa23* in resistance against *Xanthomonas oryzae*. In addition, they transferred the *Xa23* to susceptible Chinese cultivars (Guangzhan63S and Liangyou6326). Their finding confirmed the results of Jiang and group (2015). In the year 2016, Yap and workers reported the transfer of more than four genes (using Xa7F/7–1R/7-2R, RM604F/604R, Xa13F/13R, Xa21F/21R, and Xa4F/4R markers) from the donor IRBB66 cultivar to eight Chinese rice cultivars (Yap et al. 2016). In a further study, Dnyaneshwar et al. (2018) pyramided three R genes (*xa5, xa13,* and *Xa21*) to the two elite rice cultivars Safri 17 and Dubraj to enhance the bacterial blight tolerance. The donor parent used in their study was the well-known RP-Bio-226 rice cultivar. Similarly, the same gene combination of *xa5*+ *xa13*+ *Xa21* was expressed in the Jalmagna (Pradhan et al. 2016) and CSR-30 background (Baliyan et al. 2018). Similarly, the introgression of other R genes such as *Xa21* (Balachiranjeevi et al. 2018; Chen et al. 2001; Hari et al. 2013; Nguyen et al. 2018), Xa33 (Balachiranjeevi et al. 2018; Kumar et al. 2016), and *Xa7* (Mi et al. 2018) enhanced the bacterial blight disease resistance in different rice cultivars around the globe. In another study, Yugander and workers (2018) reported the role of the unidentified gene (*Os*04g53050-1) in bacterial blight resistance. In addition, they transferred the Os04g53050-1 to the susceptible recurrent Improved Samba Mahsuri cultivar from the well-known PR114 rice cultivar. Recently, Reinke and colleagues (2018) transferred the *Xa40* gene (using ID55.WA3 and RM1233 markers) to the susceptible Korean variety Junam using marker-assisted backcrossing; the *Xa40* introgressed lines showed enhancement against the bacterial blight with no negative effects on the grain quality, tiller architecture, and total yield.

5.4.3 Other Diseases

In addition to rice blast and bacterial blight disease, there are more diseases worldwide which are considered as devastating for the rice fields worldwide. It includes rice sheath blight (pathogen: *Rhizoctonia solani)*, rice stripe disease (pathogen: *Rice stripe virus*), brown spot (pathogen: *Bipolaris oryzae*), bakanae disease (pathogen: *Fusarium fujikuroi*), bacterial leaf streak (pathogen: *Xanthomonas oryzae* pv. *oryzicola*), and many more (Table 5.1). These different pathogens spread by either wind/water or both and causes about 5–35% annual losses in total rice production. However, there are very few successful reports in the literature regarding MAB use for resistance breeding in rice (Table 5.4).

The devastating rice sheath blight is caused by the soil saprotroph *Rhizoctonia solani* (teleomorph: *Thanatephorus cucumeris*) all over the world (Yellareddygari et al. 2014). It survives in unfavorable conditions up to 2 years by sclerotia formation. The symptoms include ellipsoidal, irregular greenish lesions on leaf sheaths during flowering and empty grains. The pathogen spreads by either wind or water and causes about 10–35% annual losses in rice production. Various research groups have transferred either QTLs or pyramided genes to enhance rice sheath blast tolerance. Zuo et al. (2008) and colleagues reported the transfer of QTL (qSB-9Tq) from TeQing into the different japonica rice cultivars enhanced the tolerance against the rice sheath blight. As another example, Wang et al. (2012) transferred two QTLs, namely, *qSB12–1* and *qSB9–2* (using markers RM215, RM245, and RM277), into the recurrent parent Lemont. Similarly, Chen et al. (2014) reported the transfer of *qSB-7* and *qSB-9* into the elite rice variety WLJ1 increased the disease tolerance in the susceptible variety WLJ1. As another study, Singh and coworkers improved the rice cultivars, namely, Improved Pusa Basmati and Pusa 6B, by transferring QTLs (*qSBR11–1, qSBR11–1qSBR11–2,* and *qSBR7–1*) (Singh et al. 2015, 2012b).

In Asian and African countries, the panicle blast disease has emerged as the most potent disease according to many breeders and plant pathologists (Fig. 5.3). As a result, various breeders have successfully reported the enhanced resistance in susceptible rice cultivars such as Koshihikari and Ilmi (Lee et al. 2015; Sugiura et al. 2004) using markers (B4 and RM206). Similarly, there are reports related to enhanced tolerance in susceptible rice cultivars against the rice stripe disease (Sugiura et al. 2004; Xu et al. 2013). Table 5.4 summarizes about few successful reports of MAB for enhanced resistance for other rice diseases.

5.5 Conclusion and Future Prospects

Oryza sativa is one of the highly important valued cash crops around the globe (Rice - Statistics & Facts 2018). In the last few decades, rapid progress in plant breeding and agricultural technology has significantly enhanced the overall rice production, quality, and total yield. One of the main reasons is the marker-assisted breeding (MAB) programs. These promising programs involve (i) identification of

Table 5.4 Successful examples of marker-assisted breeding (MAB) for other diseases resistance breeding in rice

S. No.	Disease	Gene/ QTL	Marker(s) used	Marker(s) type	Donor parent	Recurrent parent	References
Bacterial diseases							
1.	Sheath blight	qSB-9Tq	Y747, Y84, and Y935	Indel	TeQing	9 *japonica* cultivars	Zuo et al. (2008)
		qSB9–2 qSB12–1	RM215 RM245 RM277	SSR	TeQing-into-Lemont backcross introgression lines (TILs) – TIL:567, TIL:615, and TIL:642	Lemont	Wang et al. (2012)
		qSBR11–1	*RM224* RM7443	SSR	Tetep	Improved Pusa Basmati 1	Singh et al. (2012b)
		qSB-7 qSB-9	RM11 and RM346 Y74.7, Y83–2, Y90.2 and Y93.5	SSR Indel	TeQing	WLJ1	Chen et al. (2014)
		qSBR11–1 qSBR11–2 qSBR7–1	RM224 and RM7332 RM209 RM336	SSR	Tetep	Pusa 6B	Singh et al. (2015)
2.	Panicle blast	*Pb1*	B4	CAPS	StNo.1	Koshihikari	Sugiura et al. (2004)
		Pb1	RM206	SSR	Hwayeong	Ilmi	Lee et al. (2015)
Viral diseases							
3.	Rice stripe disease	*Stvbi*	ST10	Gene linked	StNo.1	Koshihikari	Sugiura et al. (2004)
		Stvbi	S1	STS	B5	Shengdao 15, Shengdao 16, and Xudao 3	Xu et al. (2013)

molecular markers tightly linked to QTLs and disease resistance genes; (ii) introgression of these resistance genes into the elite, susceptible cultivars; and (iii) assessment of the progeny on various agronomic parameters. These programs target traits like tiller architecture, grain quality, seed size, fragrance, mineral content, abiotic stress tolerance, and biotic stress tolerance in a more cost-effective,

convenient, and precise manner with little accidental harms. As discussed above, MAB have been successfully used for the improvement of many rice cultivars and cultivars tolerant to blast (Chen et al. 2008; Hua et al. 2015; Narayanan et al. 2002; Reinke et al. 2018), bacterial blight (Dnyaneshwar et al. 2018; Luo et al. 2017; Yap et al. 2016), and other diseases (Lee et al. 2015; Singh et al. 2012b). However, the cost of using DNA markers is expensive but a worthy investment as it fastens up the breeding programs with little accidental harms. In future, the cost-effective DNA markers, gene stacking, MAS strategies, and breeding efforts will provide the high-yielding, biotic stress-resistant, abiotic stress-tolerant, aromatic, and biofortified "super rice 10" variety.

References

Arunakumari K, Durgarani C, Satturu V, Sarikonda K, Chittoor P, Vutukuri B, Laha G, Nelli A, Gattu S, Jamal M (2016) Marker-assisted pyramiding of genes conferring resistance against bacterial blight and blast diseases into Indian rice variety MTU1010. Rice Sci 23:306–316

Ashkani S, Rafii MY, Shabanimofrad M, Miah G, Sahebi M, Azizi P, Tanweer FA, Akhtar MS, Nasehi A (2015) Molecular breeding strategy and challenges towards improvement of blast disease resistance in rice crop. Front Plant Sci 6:886

Atkinson NJ, Urwin PE (2012) The interaction of plant biotic and abiotic stresses: from genes to the field. J Exp Bot 63:3523–3543

Balachiranjeevi C, Naik BS, Kumar AV, Harika G, Swamy MH, Masood HS, Kumar DT, Miriyala A, Kale R, Yugender A (2018) Marker-assisted pyramiding of two major broad-spectrum bacterial blight resistance genes, Xa21 and Xa33 into an elite maintainer line of rice, DRR17B. bioRxiv:368712

Baliyan N, Malik R, Rani R, Mehta K, Vashisth U, Dhillon S, Boora KS (2018) Integrating marker-assisted background analysis with foreground selection for pyramiding bacterial blight resistance genes into Basmati rice. C R Biol 341:1–8

Basavaraj SH, Singh VK, Singh A, Singh A, Singh A, Anand D, Yadav S, Ellur RK, Singh D, Gopala Krishnan S, Nagarajan M, Mohapatra T, Prabhu KV, Singh AK (2010) Marker-assisted improvement of bacterial blight resistance in parental lines of Pusa RH10, a superfine grain aromatic rice hybrid. Mol Breed 26:293–305

Beckmann JS, Soller M (1983) Restriction fragment length polymorphisms in genetic improvement: methodologies, mapping and costs. Theor Appl Genet Theor Appl Genet:33–43

Bellard C, Bertelsmeier C, Leadley P, Thuiller W, Courchamp F (2012) Impacts of climate change on the future of biodiversity. Ecol Lett 15:365–377

Bharani M, Nagarajan P, Rabindran R, Saraswathi R, Balasubramanian P, Ramalingam J (2010) Bacterial leaf blight resistance genes (Xa21, xa13 and xa5) pyramiding through molecular marker assisted selection into rice cultivars. Arch Phytopathol Plant Protect 43:1032–1043

Bharathkumar S, Paulraj RD, Brindha P, Kavitha S, Gnanamanickam S (2008) Improvement of bacterial blight resistance in rice cultivars Jyothi and IR50 via marker-assisted backcross breeding. J Crop Improv 21:101–116

Bhatia D, Sharma R, Vikal Y, Mangat G, Mahajan R, Sharma N, Lore JS, Singh N, Bharaj TS, Singh K (2011) Marker-assisted development of bacterial blight resistant, dwarf, and high yielding versions of two traditional basmati rice cultivars. Crop Sci 51:759–770

Chen S, Lin X, Xu C, Zhang Q (2000) Improvement of bacterial blight resistance of Minghui 63, an elite restorer line of hybrid rice, by molecular marker-assisted selection, vol 40, p 239

Chen S, Xu C, Lin X, Zhang Q (2001) Improving bacterial blight resistance of '6078′, an elite restorer line of hybrid rice, by molecular marker-assisted selection. Plant Breed 120:133–137

Chen X, Li S, Ma Y, Li H, Zhou K, Zhu L (2004) Marker-assisted selection and pyramiding for three blast resistance genes, Pi-d (t) 1, Pi-b, Pi-ta2, in rice. Chin J Biotechnol 20:708–714

Chen H, Chen Z, Ni S, Zuo S-M, Pan X-B, Zhu X (2008) Pyramiding three genes with resistance to blast by marker assisted selection to improve rice blast resistance of Jin 23B. Chin J Rice Sci 1:5

Chen Z, Zhang Y, Feng F, Feng M, Jiang W, Ma Y, Pan C, Hua H, Li G, Pan X (2014) Improvement of japonica rice resistance to sheath blight by pyramiding qSB-9TQ and qSB-7TQ. Field Crop Res 161:118–127

Copetti D, Zhang J, El Baidouri M, Gao D, Wang J, Barghini E, Cossu RM, Angelova A, Roffler S, Ohyanagi H (2015) RiTE database: a resource database for genus-wide rice genomics and evolutionary biology. BMC Genomics 16:538

Crossa J, Pérez-Rodríguez P, Cuevas J, Montesinos-López O, Jarquín D, de los Campos G, Burgueño J, Camacho-González JM, Pérez-Elizalde S, Beyene Y (2017) Genomic selection in plant breeding: methods, models, and perspectives. Trends Plant Sci 22:961–975

Dean RA, Talbot NJ, Ebbole DJ, Farman ML, Mitchell TK, Orbach MJ, Thon M, Kulkarni R, Xu J-R, Pan H (2005) The genome sequence of the rice blast fungus *Magnaporthe grisea*. Nature 434:980

Dnyaneshwar SU, Agrawal T, Kadu T, Pradhan A, Verulkar ASKSB (2018) Improvement of Dubraj and Safri-17 varieties for conferring resistance against bacterial leaf blight through marker assisted selection approach. IJCS 6:1785–1790

Dokku P, Das K, Rao G (2013) Pyramiding of four resistance genes of bacterial blight in Tapaswini, an elite rice cultivar, through marker-assisted selection. Euphytica 192:87–96

Duke SO (2018) The history and current status of glyphosate. Pest Manag Sci 74:1027–1034

Dwivedi SL, Crouch JH, Mackill DJ, Xu Y, Blair MW, Ragot M, Upadhyaya HD, Ortiz R (2007) The molecularization of public sector crop breeding: progress, problems, and prospects. Adv Agron 95:163–318

Ellur RK, Khanna A, Bhowmick PK, Vinod K, Nagarajan M, Mondal KK, Singh NK, Singh K, Prabhu KV, Singh AK (2016a) Marker-aided incorporation of Xa38, a novel bacterial blight resistance gene, in PB1121 and comparison of its resistance spectrum with xa13+ Xa21. Sci Rep 6:29188

Ellur RK, Khanna A, Yadav A, Pathania S, Rajashekara H, Singh VK, Krishnan SG, Bhowmick PK, Nagarajan M, Vinod K (2016b) Improvement of basmati rice varieties for resistance to blast and bacterial blight diseases using marker assisted backcross breeding. Plant Sci 242:330–341

Frisch M, Melchinger AE (2001) Marker-assisted backcrossing for simultaneous introgression of two genes. Crop Sci 41:1716–1725

Fu C, Wu T, Liu W, Wang F, Li J, Zhu X, Huang H, Liu ZR, Liao Y, Zhu M (2012) Genetic improvement of resistance to blast and bacterial blight of the elite maintainer line Rongfeng B in hybrid rice (*Oryza sativa* L.) by using marker-assisted selection. Afr J Biotechnol 11:13104–13114

Garg P, Jaiswal P (2016) Databases and bioinformatics tools for rice research. Curr Plant Biol 7-8:39–52

Gazal A, Dar Z, Wani S, Lone A, Shikari A, Ali G, Abidi I (2016) Molecular breeding for enhancing resilience against biotic and abiotic stress in major cereals. SABRAO J Breed Genet 48:1–32

Gimelfarb A, Lande R (1995) Marker-assisted selection and marker-QTL associations in hybrid populations. Theor Appl Genet 91:522–528

Goff SA, Ricke D, Lan T-H, Presting G, Wang R, Dunn M, Glazebrook J, Sessions A, Oeller P, Varma H (2002) A draft sequence of the rice genome (*Oryza sativa* L ssp japonica). Science 296:92–100

Gopalakrishnan S, Sharma R, Anand Rajkumar K, Joseph M, Singh V, Singh A, Bhat K, Singh N, Mohapatra T (2008) Integrating marker assisted background analysis with foreground selection for identification of superior bacterial blight resistant recombinants in Basmati rice. Plant Breed 127:131–139

Gouda PK, Saikumar S, Varma CM, Nagesh K, Thippeswamy S, Shenoy V, Ramesha MS, Shashidhar HE (2013) Marker-assisted breeding of Pi-1 and Piz-5 genes imparting resistance to rice blast in PRR 78, restorer line of Pusa RH-10 B asmati rice hybrid. Plant Breed 132:61–69

Guimarães EP (2007) Marker-assisted selection: current status and future perspectives in crops, livestock, forestry and fish. Food & Agriculture Org, Rome

Gur A, Zamir D (2004) Unused natural variation can lift yield barriers in plant breeding. PLoS Biol 2:e245

Guvvala LD, Koradi P, Shenoy V, Marella LS (2013) Improvement of resistance to bacterial blight through marker assisted backcross breeding and field validation in rice (*Oryza sativa*). Res J Biol 1:52–66

Hari Y, Srinivasarao K, Viraktamath BC, Hariprasad AS, Laha GS, Ahmed MI, Natarajkumar P, Ramesha MS, Neeraja CN, Balachandran SM (2011) Marker-assisted improvement of a stable restorer line, KMR-3R and its derived hybrid KRH2 for bacterial blight resistance and grain quality. Plant Breed 130:608–616

Hari Y, Srinivasarao K, Viraktamath BC, Hari Prasad AS, Laha GS, Ahmed MI, Natarajkumar P, Sujatha K, Srinivas Prasad M, Pandey M (2013) Marker-assisted introgression of bacterial blight and blast resistance into IR 58025B, an elite maintainer line of rice. Plant Breed 132:586–594

Hegde SS, Prashanthi SK (2016) Identification of polymorphic markers and introgression of Pi1 and Pi2 genes for blast resistance in rice. J Farm Sci 29:327–331

Helmy M, Tomita M, Ishihama Y (2011) OryzaPG-DB: rice proteome database based on shotgun proteogenomics. BMC Plant Biol 11:63

Hittalmani S, Parco A, Mew T, Zeigler R, Huang N (2000) Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. Theor Appl Genet 100:1121–1128

Holland JB (2004) Implementation of molecular markers for quantitative traits in breeding programs—challenges and opportunities. New Directions for a Diverse Planet: Proceedings for the 4th International Crop Science Congress Regional Institute, Gosford, Australia, www.cropscience.org.au/icsc2004

Hua L-X, Liang L-Q, He X-Y, Wang L, Zhang W-S, Liu W, Liu X-Q, Lin F (2015) Development of a marker specific for the rice blast resistance gene Pi39 in the Chinese cultivar Q15 and its use in genetic improvement. Biotechnol Biotechnol Equip 29:448–456

Huang N, Angeles E, Domingo J, Magpantay G, Singh S, Zhang G, Kumaravadivel N, Bennett J, Khush G (1997) Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. Theor Appl Genet 95:313–320

Huang B, Xu J, Hou M, Ali J, Mou T (2012) Introgression of bacterial blight resistance genes Xa7, Xa21, Xa22 and Xa23 into hybrid rice restorer lines by molecular marker-assisted selection. Euphytica 187:449–459

Jairin J, Kotchasatit U, Saleeto S, Jearakongman S, Srivilai K, Chamarerk V, Kothcharerk J, Pattawatang P, Korinsak S, Wongsaprom C (2017) Application of marker-assisted breeding to improve biotic stress resistance for rainfed lowland rice in Northeastern Thailand. SABRAO J Breed Genet 49:168–178

Jena KK, Mackill DJ (2008) Molecular markers and their use in marker-assisted selection in rice. Crop Sci 48:1266–1276

Jiang GL (2013) Molecular markers and marker-assisted breeding in plants. In: Plant breeding from laboratories to fields. IntechOpen. Available at https://www.intechopen.com/books/plant-breeding-from-laboratories-to-fields/molecular-markers-and-marker-assisted-breeding-in-plants

Jiang J, Yang D, Ali J, Mou T (2015) Molecular marker-assisted pyramiding of broad-spectrum disease resistance genes, Pi2 and Xa23, into GZ63-4S, an elite thermo-sensitive genic male-sterile line in rice. Mol Breed 35:83

Joseph M, Gopalakrishnan S, Sharma R, Singh V, Singh A, Singh N, Mohapatra T (2004) Combining bacterial blight resistance and Basmati quality characteristics by phenotypic and molecular marker-assisted selection in rice. Mol Breed 13:377–387

Khan GH, Shikari AB, Vaishnavi R, Najeeb S, Padder BA, Bhat ZA, Parray GA, Bhat MA, Kumar R, Singh NK (2018) Marker-assisted introgression of three dominant blast resistance genes into an aromatic rice cultivar Mushk Budji. Sci Rep 8:4091

Khanna A, Sharma V, Ellur RK, Shikari AB, Gopala Krishnan S, Singh UD, Prakash G, Sharma TR, Rathour R, Variar M, Prashanthi SK, Nagarajan M, Vinod KK, Bhowmick PK, Singh NK, Prabhu KV, Singh BD, Singh AK (2015) Development and evaluation of near-isogenic lines for major blast resistance gene(s) in Basmati rice. Theor Appl Genet 128:1243–1259

Khush GS (2005) What it will take to feed 5.0 billion rice consumers in 2030. Plant Mol Biol 59:1–6

Koide Y, Kawasaki A, Telebanco-Yanoria MJ, Hairmansis A, Nguyet NTM, Bigirimana J, Fujita D, Kobayashi N, Fukuta Y (2010) Development of pyramided lines with two resistance genes, Pish and Pib, for blast disease (*Magnaporthe oryzae* B. Couch) in rice (*Oryza sativa* L.). Plant Breed 129:670–675

Krishnakumar R, Kumaravadivel N (2018) Marker-assisted selection for biotic stress (bacterial leaf blight and gall midge) tolerance in Bc4F4 generation of rice (*Oryza sativa* L.). Electron J Plant Breed 9:275–282

Kumar S, Rao M (2018) Conventional and molecular breeding for bacterial leaf blight and blast resistance in rice. J Ecol 3:1–3

Kumar VA, Balachiranjeevi CH, Naik SB, Rambabu R, Rekha G, Harika G, Hajira SK, Pranathi K, Vijay S, Anila M (2016) Marker-assisted improvement of the elite restorer line of rice, RPHR-1005 for resistance against bacterial blight and blast diseases. J Genet 95:895–903

Kurata N, Yamazaki Y (2006) Oryzabase. An integrated biological and genome information database for rice. Plant Physiol 140:12–17

Kwon S-W, Cho Y-C, Kim Y-G, Suh J-P, Jeung J-U, Roh J-H, Lee S-K, Jeon J-S, Yang S-J, Lee Y-T (2008) Development of near-isogenic Japonica rice lines with enhanced resistance to *Magnaporthe grisea*. Mol Cells 25:407–416

Lande R, Thompson R (1990) Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics 124:743–756

Lee J-H, Lee J-Y, Yoon Y-N, Kim S-Y, Hur Y-J, Yeo U-S, Sohn Y-B, Song Y-C, Park D-S, Nam M-H (2015) Enhancement of panicle blast resistance in Korean rice cultivar 'Saeilmi' by marker assisted backcross breeding. Plant Breed Biotechnol 3:1–10

Li Y, Wu C, Jiang G, Wang L, He Y (2007) Dynamic analyses of rice blast resistance for the assessment of genetic and environmental effects. Plant Breed 126:541–547

Luo Y, Yin Z (2013) Marker-assisted breeding of Thai fragrance rice for semi-dwarf phenotype, submergence tolerance and disease resistance to rice blast and bacterial blight. Mol Breed 32:709–721

Luo Y, Sangha JS, Wang S, Li Z, Yang J, Yin Z (2012) Marker-assisted breeding of Xa4, Xa21 and Xa27 in the restorer lines of hybrid rice for broad-spectrum and enhanced disease resistance to bacterial blight. Mol Breed 30:1601–1610

Luo Y, Zakaria S, Basyah B, Ma T, Li Z, Yang J, Yin Z (2014) Marker-assisted breeding of Indonesia local rice variety Siputeh for semi-dwarf phenotype, good grain quality and disease resistance to bacterial blight. Rice 7:33

Luo W, Huang M, Guo T, Xiao W, Wang J, Yang G, Liu Y, Wang H, Chen Z, Zhuang C (2017) Marker-assisted selection for rice blast resistance genes Pi2 and Pi9 through high-resolution melting of a gene-targeted amplicon. Plant Breed 136:67–73

Man S, Vinarao RB, Surek H, Jena KK (2016) Marker-assisted introgression of a broad-spectrum resistance gene, Pi40 improved blast resistance of two elite rice (*Oryza sativa* L.) cultivars of Turkey. Mol Plant Breed 7:1–15

Mi J, Yang D, Chen Y, Jiang J, Mou H, Huang J, Ouyang Y, Mou T (2018) Accelerated molecular breeding of a novel P/TGMS line with broad-spectrum resistance to rice blast and bacterial blight in two-line hybrid rice. Rice 11:11

Miah G, Rafii MY, Ismail MR, Puteh A, Rahim HA, Asfaliza R, Latif MA (2013) Blast resistance in rice: a review of conventional breeding to molecular approaches. Mol Biol Rep 40:2369–2388

Miah G, Rafii MY, Ismail MR, Puteh AB, Rahim HA, Latif MA (2017) Marker-assisted introgression of broad-spectrum blast resistance genes into the cultivated MR219 rice variety. J Sci Food Agric 97:2810–2818

Miedaner T, Korzun V (2012) Marker-assisted selection for disease resistance in wheat and barley breeding. Phytopathology 102:560–566

Mohamed A, Ali R, Elhassan O, Suliman E, Mugoya C, Masiga CW, Elhusien A, Hash CT (2014) First products of DNA marker-assisted selection in sorghum released for cultivation by farmers in sub-Saharan Africa. J Plant Sci Mol Breed 3:1–10

Mohler V, Singrün C (2004) General considerations: marker-assisted selection. Molecular marker systems in plant breeding and crop improvement. Springer, Berlin/Heidelberg, pp 305–317

Mosa KA, Ismail A, Helmy M (2017) Omics and system biology approaches in plant stress research. Plant Stress Tolerance. Springer, Cham, pp 21–34

Mundt CC (2014) Durable resistance: a key to sustainable management of pathogens and pests. Infect Genet Evol 27:446–455

Muthuramalingam P, Krishnan SR, Pandian S, Mareeswaran N, Aruni W, Pandian SK, Ramesh M (2018) Global analysis of threonine metabolism genes unravel key players in rice to improve the abiotic stress tolerance. Sci Rep 8:9270

Narayanan NN, Baisakh N, Cruz V, Gnanamanickam SS, Datta K, Datta SK (2002) Molecular breeding for the development of blast and bacterial blight resistance in rice cv. IR50. Crop Sci 42:2072–2079

Nelson R, Wiesner-Hanks T, Wisser R, Balint-Kurti P (2018) Navigating complexity to breed disease-resistant crops. Nat Rev Genet 19:21

Nguyen HT, Vu QH, Van Mai T, Nguyen TT, Vu LD, Nguyen TT, Nguyen LV, Vu HTT, Nong HT, Dinh TN (2018) Marker-assisted selection of XA21 conferring resistance to bacterial leaf blight in indica rice cultivar LT2. Rice Sci 25:52–56

Ni D, Song F, Ni J, Zhang A, Wang C, Zhao K, Yang Y, Wei P, Yang J, Li L (2015) Marker-assisted selection of two-line hybrid rice for disease resistance to rice blast and bacterial blight. Field Crop Res 184:1–8

Oerke EC (2005) Crop losses to pests. J Agric Sci 144:31–43

Pandey MK, Rani NS, Sundaram RM, Laha GS, Madhav MS, Rao KS, Sudharshan I, Hari Y, Varaprasad GS, Rao LVS (2013) Improvement of two traditional Basmati rice varieties for bacterial blight resistance and plant stature through morphological and marker-assisted selection. Mol Breed 31:239–246

Parida AK, Panda A, Rangani J (2018) Metabolomics-guided elucidation of abiotic stress tolerance mechanisms in plants. In: Plant metabolites and regulation under environmental stress. Academic Press, pp 89–131. Available at https://www.sciencedirect.com/science/article/pii/B9780128126899000054

Pathak MD, Khan ZR (1994) Insect pests of rice. International Rice Research Institute. Available at https://books.google.co.in/books?hl=en&lr=&id=7MuyJh0FLj4C&oi=fnd&pg=PP2&dq=Pathak+MD,+Khan+ZR+(1994)+Insect+pests+of+rice.+Int+Rice+Res+Inst&ots=WcnQJsrAU&sig=2zXw2NM8w5HAwbNG9HSHkDb1Qyk#v=onepage&q=Pathak%20MD%2C%20Khan%20ZR%20(1994)%20Insect%20pests%20of%20rice.%20Int%20Rice%20Res%20Inst&f=false

Peng JH, Fahima T, Röder M, Li Y, Grama A, Nevo E (2000) Microsatellite high-density mapping of the stripe rust resistance gene YrH52 region on chromosome 1B and evaluation of its marker-assisted selection in the F_2 generation in wild emmer wheat. New Phytol 146:141–154

Pinta W, Toojinda T, Thummabenjapone P, Sanitchon J (2013) Pyramiding of blast and bacterial leaf blight resistance genes into rice cultivar RD6 using marker assisted selection. Afr J Biotechnol 12:4432–4438

Population Reference Bureau (PRB) (2016) World population data sheet, Washington, DC. Retrieved 11 July 2019 from https://www.prb.org/2016-worldpopulation-data-sheet/

Pradhan SK, Barik SR, Sahoo A, Mohapatra S, Nayak DK, Mahender A, Meher J, Anandan A, Pandit E (2016) Population structure, genetic diversity and molecular marker-trait association analysis for high temperature stress tolerance in rice. PLoS One 11:e0160027

Priya P, Jain M (2013) RiceSRTFDB: a database of rice transcription factors containing comprehensive expression, cis-regulatory element and mutant information to facilitate gene function analysis. Database 2013:bat027. https://doi.org/10.1093/database/bat1027

Rajpurohit D, Kumar R, Kumar M, Paul P, Awasthi A, Basha PO, Puri A, Jhang T, Singh K, Dhaliwal HS (2011) Pyramiding of two bacterial blight resistance and a semidwarfing gene in type 3 Basmati using marker-assisted selection. Euphytica 178:111–126

Ramalingam J, Basharat HS, Zhang G (2002) STS and microsatellite marker-assisted selection for bacterial blight resistance and waxy genes in rice, *Oryza sativa* L. Euphytica 127:255–260

Reinke R, Kim SM, Kim BK (2018) Developing japonica rice introgression lines with multiple resistance genes for brown planthopper, bacterial blight, rice blast, and rice stripe virus using molecular breeding. Mol Gen Genomics 293(6):1565–1575

Rezbova H, Skubna O (2012) The role of transgenic crops in the future of global food and feed. AGRIS on-line. Papers Econ Informat 4:49

Rice - Statistics & Facts (2018) Statista. Hamburg, Germany. Retrieved 11 July 2019 from https://www.statista.com/topics/1443/rice/

Ronald PC, Albano B, Tabien R, Abenes L, Wu K-s, McCouch S, Tanksley SD (1992) Genetic and physical analysis of the rice bacterial blight disease resistance locus, Xa21. Mol Gen Genet 236:113–120

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

Sanan-Mishra N, Tripathi A, Goswami K, Shukla RN, Vasudevan M, Goswami H (2018) ARMOUR–A Rice miRNA: mRNA interaction resource. Front Plant Sci 9:602

Sanchez AC, Brar DS, Huang N, Li Z, Khush GS (2000) Sequence tagged site marker-assisted selection for three bacterial blight resistance genes in rice. Crop Sci 40:792–797

Sasaki T (2005) The map-based sequence of the rice genome. Nature 436:793

Savary S, Ficke A, Aubertot J-N, Hollier C (2012) Crop losses due to diseases and their implications for global food production losses and food security. Food Security 4(2):519–537

Shakoor N, Lee S, Mockler TC (2017) High throughput phenotyping to accelerate crop breeding and monitoring of diseases in the field. Curr Opin Plant Biol 38:184–192

Singh S, Sidhu JS, Huang N, Vikal Y, Li Z, Brar DS, Dhaliwal HS, Khush GS (2001) Pyramiding three bacterial blight resistance genes (xa5, xa13 and Xa21) using marker-assisted selection into indica rice cultivar PR106. Theor Appl Genet 102:1011–1015

Singh AK, Gopalakrishnan S, Singh VP, Prabhu KV, Mohapatra T, Singh NK, Sharma TR, Nagarajan M, Vinod KK, Singh D (2011) Marker assisted selection: a paradigm shift in Basmati breeding. Indian J Genet Plant Breed 71:120–128

Singh A, Singh VK, Singh SP, Pandian RTP, Ellur RK, Singh D, Bhowmick PK, Gopala Krishnan S, Nagarajan M, Vinod KK, Singh UD, Prabhu KV, Sharma TR, Mohapatra T, Singh AK (2012a) Molecular breeding for the development of multiple disease resistance in Basmati rice. AoB Plants 2012:pls029

Singh VK, Singh A, Singh S, Ellur RK, Choudhary V, Sarkel S, Singh D, Krishnan SG, Nagarajan M, Vinod K (2012b) Incorporation of blast resistance into "PRR78", an elite Basmati rice restorer line, through marker assisted backcross breeding. Field Crop Res 128:8–16

Singh AK, Singh VK, Singh A, Ellur RK, Pandian RTP, Krishnan SG, Singh UD, Nagarajan M, Vinod KK, Prabhu KV (2015) Introgression of multiple disease resistance into a maintainer of Basmati rice CMS line by marker assisted backcross breeding. Euphytica 203:97–107

Singh B, Mehta S, Tiwari M, Bhatia S (2018) Legume breeding for fungal resistance: a lesson to learn. In: Islam MA, Dhakate P (eds) Molecular approaches for plant improvement, vol 1. Kalpaz Publication, New Delhi, pp 159–180

Smita S, Lenka SK, Katiyar A, Jaiswal P, Preece J, Bansal KC (2011) QlicRice: a web interface for abiotic stress responsive QTL and loci interaction channels in rice. Database 2011:bar037

Sreewongchai T, Toojinda T, Thanintorn N, Kosawang C, Vanavichit A, Tharreau D, Sirithunya P (2010) Development of elite indica rice lines with wide spectrum of resistance to Thai blast isolates by pyramiding multiple resistance QTLs. Plant Breed 129:176–180

Stenberg JA, Heil M, Åhman I, Björkman C (2015) Optimizing crops for biocontrol of pests and disease. Trends Plant Sci 20:698–712

Sugiura N, Tsuji T, Fujii K, Kato T, Saka N, Touyama T, Hayano Saito Y, Izawa T (2004) Molecular marker-assisted selection in a recurrent backcross breeding for the incorporation of resistance to rice stripe virus and panicle blast in rice (Oryza sativa L.). Breed Res (Japan) 6:143–148

Sundaram RM, Vishnupriya MR, Biradar SK, Laha GS, Reddy GA, Rani NS, Sarma NP, Sonti RV (2008) Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. Euphytica 160:411–422

Tanweer FA, Rafii MY, Sijam K, Rahim HA, Ahmed F, Ashkani S, Latif MA (2015) Introgression of blast resistance genes (putative Pi-b and Pi-kh) into elite rice cultivar MR219 through marker-assisted selection. Front Plant Sci 6:1002

Telebanco-Yanoria MJ, Koide Y, Fukuta Y, Imbe T, Kato H, Tsunematsu H, Kobayashi N (2010) Development of near-isogenic lines of Japonica-type rice variety Lijiangxintuanheigu as differentials for blast resistance. Breed Sci 60:629–638

Toenniessen GH, O'Toole JC, DeVries J (2003) Advances in plant biotechnology and its adoption in developing countries. Curr Opin Plant Biol 6:191–198

Usatov A, Kostylev P, Azarin K, Markin N, Makarenko M, Khachumova V, Bibov MY (2016) Introgression of the rice blast resistance genes Pi1, Pi2 and Pi33 into Russian rice varieties by marker-assisted selection. Indian J Genet Plant Breed 76:18–23

Wallace JS, Acreman MC, Sullivan CA (2003) The sharing of water between society and ecosystems: from conflict to catchment-based co-management. Philoso Trans R Soc Lond B Biol Sci 358:2011–2026

Wang J, Chapman SC, Bonnett DG, Rebetzke GJ, Crouch J (2007) Application of population genetic theory and simulation models to efficiently pyramid multiple genes via marker-assisted selection. Crop Sci 47:582–588

Wang Y, Pinson S, Fjellstrom R, Tabien R (2012) Phenotypic gain from introgression of two QTL, qSB9-2 and qSB12-1, for rice sheath blight resistance. Mol Breed 30:293–303

Wen S, Gao B (2012) Introgressing blast resistant gene Pi-9 (t) into elite rice restorer Luhui17 by marker-assisted selection. Rice Genom Genet 2:31–36

Whittaker JC, Haley CS, Thompson R (1997) Optimal weighting of information in marker-assisted selection. Genet Res 69:137–144

Wiesner-Hanks T, Nelson R (2016) Multiple disease resistance in plants. Annu Rev Phytopathol 54:229–252

Win KM, Korinsak S, Sirithunya P, Lanceras-Siangliw J, Jamboonsri W, Da T, Patarapuwadol S, Toojinda T (2013) Marker assisted introgression of multiple genes for bacterial blight resistance into aromatic Myanmar rice MK-75. Field Crop Res 154:164–171

Wongsaprom C, Sirithunya P, Vanavichit A, Pantuwan G, Jongdee B, Sidhiwong N, Lanceras-Siangliw J, Toojinda T (2010) Two introgressed quantitative trait loci confer a broad-spectrum resistance to blast disease in the genetic background of the cultivar RD6 a Thai glutinous jasmine rice. Field Crop Res 119:245–251

Wu Y, Yu L, Pan C, Dai Z, Li Y, Xiao N, Zhang X, Ji H, Huang N, Zhao B (2016) Development of near-isogenic lines with different alleles of Piz locus and analysis of their breeding effect under Yangdao 6 background. Mol Breed 36:12

Xiao W-M, Luo L-X, Hui W, Tao G, Liu Y-Z, Zhou J-Y, Zhu X-Y, Yang Q-Y, Chen Z-Q (2016) Pyramiding of Pi46 and Pita to improve blast resistance and to evaluate the resistance effect of the two R genes. J Integr Agric 15:2290–2298

Xiao N, Wu Y, Pan C, Yu L, Chen Y, Liu G, Li Y, Zhang X, Wang Z, Dai Z (2017) Improving of rice blast resistances in japonica by pyramiding major R genes. Front Plant Sci 7:1918

Xu Y, Crouch JH (2008) Marker-assisted selection in plant breeding: from publications to practice. Crop Sci 48:391–407

Xu Q, Ni H, Chen Q, Sun F, Zhou T, Lan Y, Zhou Y (2013) Comparative proteomic analysis reveals the cross-talk between the responses induced by H_2O_2 and by long-term rice black-streaked dwarf virus infection in rice. PLoS One 8:e81640

Yap R, Hsu YC, Wu YP, Lin YR, Kuo CW (2016) Multiplex PCR genotyping for five bacterial blight resistance genes applied to marker-assisted selection in rice (*Oryza sativa*). Plant Breed 135:309–317

Yellareddygari SKR, Reddy MS, Kloepper JW, Lawrence KS, Fadamiro H (2014) Rice sheath blight: a review of disease and pathogen management approaches. J Plant Pathol Microbiol 5:241

Yoshimura S, Yoshimura A, Iwata N, McCouch SR, Abenes ML, Baraoidan MR, Mew TW, Nelson RJ (1995) Tagging and combining bacterial blight resistance genes in rice using RAPD and RFLP markers. Mol Breed 1:375–387

Yu J, Hu S, Wang J, Wong GK-S, Li S, Liu B, Deng Y, Dai L, Zhou Y, Zhang X (2002) A draft sequence of the rice genome (*Oryza sativa* L ssp indica). Science 296:79–92

Yugander A, Sundaram RM, Singh K, Ladhalakshmi D, Rao LVS, Madhav MS, Badri J, Prasad MS, Laha GS (2018) Incorporation of the novel bacterial blight resistance gene Xa38 into the genetic background of elite rice variety improved Samba Mahsuri. PLoS One 13:e0198260

Zhang W, Smith C (1993) Simulation of marker-assisted selection utilizing linkage disequilibrium: the effects of several additional factors. Theor Appl Genet 86:492–496

Zhang J, Li X, Jiang G, Xu Y, He Y (2006) Pyramiding of Xa7 and Xa21 for the improvement of disease resistance to bacterial blight in hybrid rice. Plant Breed 125:600–605

Zhang Y, Zang Q, Xu B, Zheng W, Ban R, Zhang H, Yang Y, Hao Q, Iqbal F, Li A (2016) IsomiR Bank: a research resource for tracking IsomiRs. Bioinformatics 32:2069–2071

Zhao H, Yao W, Ouyang Y, Yang W, Wang G, Lian X, Xing Y, Chen L, Xie W (2014) RiceVarMap: a comprehensive database of rice genomic variations. Nucleic Acids Res 43:D1018–D1022

Zhou Y-L, Xu J-L, Zhou S-C, Yu J, Xie X-W, Xu M-R, Sun Y, Zhu L-H, Fu B-Y, Gao Y-M (2009) Pyramiding Xa23 and Rxo1 for resistance to two bacterial diseases into an elite indica rice variety using molecular approaches. Mol Breed 23:279–287

Zuo S, Zhang L, Wang H, Yin Y, Zhang Y, Chen Z, Ma Y, Pan X (2008) Prospect of the QTL-qSB-9 Tq utilized in molecular breeding program of japonica rice against sheath blight. J Genet Genomics 35:499–505

Chapter 6
Genome Wide Association Study (GWAS) on Disease Resistance in Maize

Vivek Shrestha, Mani Awale, and Avinash Karn

6.1 Introduction

Crop losses due to disease infestation account for a major loss to farmers worldwide. Scientists and farmers have long adopted many traditional and chemical methods to control diseases in crop plants. Intensive use of chemicals, mostly fungicides and pesticides, has been able to control the disease infestation to some extent, but they come at a price. The use of chemicals does not only increase the cost of production, but it also negatively impacts the environment and health of both farmers and the consumers. Often times, the pathogen develops resistivity toward the fungicides being applied on them, which are no longer effective to control the damage that affect the crop yield and quality. Therefore, one sustainable way to reduce the impact of crop yield and quality loss due to plant diseases is to develop disease-resistant crops. Disease resistance breeding has been a major source of disease control (Hammond-Kosack and Jones 1997; Balint-Kurti and Johal 2009; Poland et al. 2009). The simplest way to define disease resistance breeding is the introgression of the disease resistance genes in the plants infected with the disease. The source of the resistance genes is either natural or induced. Disease resistance is generally categorized as qualitative and quantitative resistance. Qualitative resistance is based on a single dominant or recessive gene, race-specific and usually confers a high level of resistance, whereas the quantitative resistance is based on the oligogenic or polygenic inheritance and governed by additive or partial dominant

V. Shrestha (✉)
Division of Biological Sciences, University of Missouri, Columbia, MO, USA
e-mail: vs6d9@mail.missouri.edu

M. Awale
Division of Plant Sciences, University of Missouri, Columbia, MO, USA

A. Karn
School of Integrative Plant Science, Cornell AgriTech, Geneva, NY, USA

S. H. Wani (ed.), *Disease Resistance in Crop Plants*,
https://doi.org/10.1007/978-3-030-20728-1_6

genes and generally race-nonspecific (Wisser et al. 2006). Quantitative disease resistance is given more importance by the plant breeders as it is more durable and has broader specificity (Parlevliet 2002; Poland et al. 2009).

One of the widely known theories regarding the disease resistance/susceptibility is H.H. Flor's gene-for-gene interaction theory (Flor 1971). Gene-for-gene interaction theory originated using flax (*Linumusitatissimum*) as host plant and fungal rust pathogen, *Melampsora lini*. The theory states that, a host plant requires a dominant or semi-dominant resistance R gene with a corresponding avirulence *(Avr)* gene in the pathogen to be disease resistant. R genes are responsible in the plants to detect the *Avr* gene-specific pathogen molecules, resulting in the downstream signal cascades to produce defensins, which trigger defense (Hammond-Kosack and Jones 1997). Hypersensitivity response is commonly seen as a defense response triggering the incompatibility reaction between host and pathogen. Modification or complete loss of the R gene or the *Avr* gene results in disease susceptibility, which is commonly seen in biotrophic pathogens, such as fungi, bacteria, viruses, and nematodes. R gene generally encodes proteins that recognize the pathogen effectors or modification of plant proteins that are the targets of the effectors (Nimchuk et al. 2003). Among the six known classes of R-genes, the most known class is the nucleotide binding, leucine-rich repeat (NB-LRR) amino acids sequence motifs, which are involved in the pathogen recognition and related functions. Unraveling the structural and functional roles of these R genes will be beneficial to improve disease resistance in plants. There were several R genes being identified, isolated, and cloned. The first R gene to be isolated was *Hm1* from maize, which is responsible for resistance against the leaf spot fungus *Cochliobolus carbonum* (Johal and Briggs 1992). *Hm1* encodes for a reductase enzyme that detoxifies the *C. carbonum* HC-toxin. On invasion by the biotrophic pathogens, R-genes confers an effective defense response, usually, by involving in a hypersensitive response, where the tissue immediately adjacent to the site of the pathogen undergoes rapid programmed cell death (Poland et al. 2009). Some other early cloned R genes were *Pto* gene (Martin et al. 1993) that encodes for serine threonine kinase, in tomato against the *Pseudomonas syringae*. Other cloned genes for *Pseudomonas syringae* are *RPS2* in Arabidopsis (Bent et al. 1994), a NBS/LRR protein family. *Xa-21* (Song et al. 1995) gene in rice for *Xanthomonas oryzae* pv. *oryzae*. The list and details of several other important cloned genes can be found in Hammond-Kosack and Jones (1997).

Although the R genes are found to be effective in biotrophic fungus, studies show that the R-genes might not work in a similar fashion against the necrotrophic pathogens. For instance, the hypersensitive response can increase the susceptibility to necrotrophic pathogens, instead of increasing resistance. Although there is immense potential of disease resistance by R genes, observation of the performance of the crop cultivars with different types of resistance have led to the conclusion that quantitative disease resistance is more durable than the typical R-gene mediated resistance (Parlevliet 2002; Poland et al. 2009).

Molecular mechanism of gene-for-gene interaction theory or the host-pathogen interaction were not well known until recent work by Jones and Dangl (2006),

where they studied the molecular mechanism of host-pathogen interaction mainly pertaining to the biotrophic pathogen (Jones and Dangl 2006). They elaborated on the complex multiphase host-pathogen interplay. Briefly, they explained that the plant (host), when invaded by a pathogen, initially recognizes some common feature of those pathogen, which are called microbial associated molecular patterns (MAMPs), using pattern recognizing receptors. For instance, flagellin in bacteria and chitin in fungus are the MAMPs. This recognition event triggers the innate immune response in host plant, also known as the first line of defense or host basal defense, which defends further pathogen invasion or development. In response to that MAMP-triggered immunity of the host, virulent pathogen fight back, releasing effector proteins, which destroy the host basal defense. This triggers the host plant to further use its second line of defense, using resistance genes such as NB-LRR; these recognize the pathogen secreted effector proteins and finally destroy them. This immunity is known as the effector-triggered immunity. The molecular understanding of the host pathogen interaction will definitely be beneficial for the development of disease resistance in plants; however, there are still a lot of unknowns in the field of host pathogen interaction.

Maize has been a model plant for many plant scientists. It has a long history of research in disease resistance. Maize southern leaf blight is one of the biggest epidemics ever known in history, causing loss of a billion dollars to the US economy. Hybrid seed production using the maize carrying Texas cytoplasm for male sterility (cms-T) was popular in the era of 1950s. A race of the southern corn leaf blight called race T was found to be very pathogenic on cms-T maize, causing epidemic in 1970 and 1971 (Ullstrup 1972). It was found later that the pathogen race T produces T-toxin (Pring and Lonsdale 1989) (a family of linear long chain polyketides) that binds specifically to the URF13. URF13 is a peptide of 13 kDa that resides in the inner membrane of mitochondria and acts as a ligand-gated channel (Levings and Siedow 1992). The interaction between the T-toxin and the URF13 transforms the channel to a large pore, causing the membrane to be leaky, and ultimately leading to the cell death. Since then cms-T were eliminated from the elite germplasm, and then polygenic disease resistance studies were introduced (Balint-Kurti and Carson 2006; Balint-Kurti and Johal 2009). The first gene to be cloned in maize is the *Hm1* genes, which confer specific resistance against a leaf blight and ear mold disease of corn caused by *C. carbonum* race 1 (CCR1).The pathogen produces a toxin called HC-toxin. This gene was cloned using transposon tagging and was found to be an NADPH-dependent HC-toxin reductase, which inactivates the HC-toxin by reducing the key carbonyl group on HC-toxin (Johal and Briggs 1992). Studies have shown that 228 R gene analogs have been identified in maize, using the partial sequence data derived from several different maize lines (Xiao et al. 2007). Wisser et al. (2006) studied 50 publications regarding the disease resistance gene in maize, which included 437 QTL and 17 major genes (Wisser et al. 2006). For a more comprehensive review of maize disease management, it is suggested to the readers to look in the following reviews (Pratt and Gordon 2006; Wisser et al. 2006; Balint-Kurti and Johal 2009).

6.2 Association Mapping Versus QTL Mapping

Quantitative or metric traits are those traits which can be measured and possess continuous variation. The loci that govern the genetics of these traits are called quantitative trait loci (QTL).The continuous variation is due to the polygenic inheritance of genes with mostly small additive effects, and these genes are influenced by the environment. Mendelian methods of genetic analysis are not suitable to dissect these quantitative traits and hence different quantitative methods are used to study and understand them. Sax (1923) reported the linkage between seed coat color and seed size in common bean (*Phaseolus vulgaris*) that started the physical localization/mapping of the polygenes (Sax 1923). Development of the concept of the linkage along with the ability to construct the genomic map of the given species leads to the development of the QTL mapping. The first QTL mapping was done by Paterson et al. in 1988, using the restriction fragment length polymorphism in tomato (Paterson et al. 1988). Several reviews on QTL mapping in disease resistance in maize have been published (Wisser et al. 2006; Balint-Kurti and Johal 2009). The general methods of QTL mapping involve the utilization of a mapping population, usually a bi-parental population, derived from the cross between two genetically diverse parents, a dense marker linkage map for a particular species and genotypic data (SNPs, SSRs), standard phenotypic measurement and suitable software program (Singh and Singh 2015), such as R/QTL (Broman et al. 2003), QTL Cartographer (Wang et al. 2007), and so on.

The QTL mapping has been widely used in several crops, such as rice (Ray et al. 1996; Tan et al. 2001; Tian et al. 2015), maize (Lübberstedt et al. 1999; Balint-Kurti and Carson 2006; Li et al. 2008; Park et al. 2014), wheat (Quarrie et al. 1994; Castro et al. 2008; Acuna et al. 2014), tomato (Paterson et al. 1988; Foolad 1999; Causse et al. 2002; Causse et al. 2004), and others. QTL mapping provides the QTL effect size, the additive and dominance effect that are helpful for the trait introgression and improve breeding scheme. QTL mapping, also known as linkage mapping, possesses high power to detect the QTL and has the potential to identify or map the rare functional alleles of genes compared to the association mapping. With all these merits, QTL mapping also possesses multiple demerits, such as genetic variation is limited in the bi-parental mapping, as the mapping population is initiated with just two parents with limited recombination events. Low resolution power is another most challenging issue of QTL mapping. A QTL location may span from a few to tens of centimorgan; usually from 5 to 20 cM, encompassing several hundred genes, which will be time-consuming and difficult to analyze and further validate the identified QTLs (Doerge 2002). Hence, there are only a limited number of known QTLs that has been cloned or tagged at the gene level (Price 2006).

Association mapping, also known as linkage disequilibrium (LD), has emerged as a popular tool to dissect the complex traits at the sequence level. Initially, association mapping had been used extensively in medical genetics, but was limited in plant genetics due to the structured population often found in plants, which may lead to nonfunctional associations. In 2001, Thornsberry et al. introduced association mapping in plants (maize) by using statistical methods to account for the

variation due to population structure (Thornsberry et al. 2001). Since its introduction, association mapping has gained wide popularity in dissecting the complex traits in plants because of the advances in high-throughput genomic technologies, interests in identifying novel and superior allele, and improvements in statistical methods (Zhu et al. 2008).

Association mapping is different to QTL mapping in the following aspects: association mapping usually involves the use of unstructured or natural populations, consisting of diverse sets of individual or taxa. For instance, the Goodman association panel of maize consists of 282 diverse sets of maize inbreds, which collectively include tropical, subtropical, temperate, popcorn, and sweet corn lines, drawn from different environments and different locations (Flint-Garcia et al. 2005). The merit of using such natural population is that it exploits the linkage disequilibrium (LD) from the ancestral recombination present between or among them (Nordborg and Tavaré 2002), unlike linkage in the QTL mapping, which is only from the hybridization between the bi-parental lines. Association mapping utilizes the LD (nonrandom association of the alleles, which is a property of a population unlike linkage, which is a property of individual) between the SNPs and the associated genes or QTLs for detecting the marker-traits association. One of the important aspects of using the association mapping over QTL mapping is its high resolution that can detect the causative variants or causal genes.

Nested Association Mapping (NAM) population is another most widely used population both for the QTL mapping and association studies in Maize (McMullen et al. 2009). NAM is designed in such a way that it can harbor the advantage of both QTL and association mapping. The population was constructed to enable both high power and high resolution through the joint-linkage association analysis. Briefly, the population was designed by crossing a common parent, B73, with the other 24 diverse founder parents, and 200 RILs per family were created using the subsequent selfing for 5 generations of the resulting F1s. The diverse lines consist of mostly the tropical lines, a few temperate, sweet corn, and a popcorn inbred line. The NAM genetic map is a composite map created using 4699 RILs combined across the 25 families representing 1106 loci, with the average marker density of one marker on every 1.3 cM (McMullen et al. 2009).

6.2.1 GWAS Working Models

Similar to QTL mapping, association mapping also requires the phenotypic and genotypic data along with the genome map. However, due to use of unstructured population, one needs to be careful to consider those variations generated by the unstructured population while running the GWAS. The GWAS model needs to account for population structure in order to avoid getting spurious SNP hits in the analysis. This can be accounted using the Q matrix or the principal components (PCs) in the GWAS model, which can be obtained from the marker information. The next covariate used in the model is the relationship matrix or the kinship matrix

(K), which will account for the variation from the related alleles by identical by descent in the population. K matrix can be calculated either from the pedigree data or from the marker data. With the development of the genotyping platform and the statistical methods, the methodology of GWAS has improved drastically in the recent years.

Association mapping, simply, is a genome-wide scan of the tested molecular markers with the phenotype of interest. The association test idea was brought up on using the simple linear model, where the model is fitted using the SNPs as the predictor variable as fixed effect, and estimates the markers effect for the particular trait of interest using t-statistics. Since, the association mapping involves mostly the use of diverse natural or unstructured panel, the variation due to the diversity of the subpopulation within the population needs to be accounted in the model. The general linear model (GLM) takes into account the population structure in the form of Q matrix or principal components (PCs) and uses it as covariates in the model, which helps to control the spurious association (Price et al. 2006). The very popular and widely used mixed linear model (MLM) is the enhancement of the GLM, in the sense that it also accounts for the genetic relatedness, i.e., the K matrix fitted as random effect in the model. This Q + K matrix strongly helps to control the false positives (Yu et al. 2006). The development of tools and methods for doing association studies continues to grow using EMMA (Kang et al. 2008), CMLM (Zhang et al. 2010), and MLMM (Segura et al. 2012), which were developed especially to reduce the computational time for doing the GWAS. Recently, a new method was developed, FARMCPU, which is reported to completely remove the confounding between the testing markers and both K and Q, by combining MLMM and Fast-LMM-Select, allowing a fixed and random effect model to perform separately, and is also reported to be computationally efficient (Liu et al. 2016). FARMCPU claims to reduce the false positives without losing the true positives.

6.3 Disease Resistance Studies in Maize Using GWAS

6.3.1 Fusarium Ear Rot

Zila et al. (2013) revealed some of the important QTLs in the Fusarium ear rot resistance in maize (Zila et al. 2013), using the maize core diversity panel (Flint-Garcia et al. 2005). Fusarium ear rot, caused by *Fusarium verticillioides* (Sacc) Nirenberg, a common disease of maize affects both the quality of food and feed. The fungus is a hemibiotrophic fungus and endemic to the maize growing regions in the world. The fungus produces mycotoxin fumonisin, a suspected carcinogen associated with the various diseases in livestock and humans. It has been reported that a high genotypic correlation exists between the ear rot resistance and the fumonisin accumulation, indicating effective negative selection on fumonisin in the resistance cultivars. The disease has caused a huge loss of grains and the quality of grains. The best strategy to control the disease is to develop the disease resistance

maize cultivars. Fusarium ear rot resistance is mostly governed by polygenes and strongly influenced by the environment. Hence, there has been no report of the fully immune cultivars being discovered (Clements et al. 2004; Zila et al. 2013). Previous QTL studies have shown that the resistance QTLs for the Fusarium ear rot have small effect size and are not consistent between the populations (Pérez Brito et al. 2001; Ding et al. 2008).

Disease resistance itself is a quantitative trait, governed with multiples genes and affected by the environment. Hence, it is a great challenge to a plant scientist to incorporate the disease resistance genes without any growth or yield penalty. Briefly, the study was conducted using the 267 inbred lines evaluated in two sets of environment and the association mapping was done using 47,445 SNPs (Olukolu et al. 2013), using a mixed model. Three SNPs were found to be significantly associated with disease resistance in at least one subset of environment (Zila et al. 2013). Two of the three identified SNPs were found to be co-localized with the genes related with the programmed cell death. The chromosome 9 SNP explained the largest proportion of the variation in line mean values for ear rot resistance ($R^2 = 11.5$), whereas the SNPs in chromosome 1 and 5 explained 8.8 and 9.6% variation for the Fusarium ear rot resistance; collectively, 26% of the variation is explained by all the 3 SNPs.

Chromosome 9 gene was identified as GRMZM2G178880, which belongs to the cellulose synthase-like family A (*CslA*) protein family. Expression of this gene is found to be highest in the endosperm of the developing seed kernel between 20 and 24 days after flowering (Sekhon et al. 2011). Genes in the *CslA* protein family encode for the noncellulose polysaccharides, such as mannan polymers that form part of the wall matrix in plant cells (Dhugga 2005). Degradation of the mannan-rich cell walls might play an important role in the programmed cell death in the host-pathogen interaction (Rodríguez-Gacio et al. 2012) and may play a role in the disease resistance.

The SNP on chromosome 5 was located downstream of a Heat Shock Protein (HSP60) gene, GRMZM2G111477 (Zila et al. 2013). HSP60s are the chaperonins and are involved in the protein folding when the plants are in stressed condition. In Rice and Arabidopsis, the role of HSP60s is reported to be involved in the programmed cell death (Ishikawa et al. 2003). SNP on chromosome 1 is found within the coding region of the GRMZM2G703598, but has neither gene function predicted nor orthologs with other grass species (Zila et al. 2013).

A major limitation of association mapping in maize is its low linkage disequilibrium (LD) state which requires large number of genetic markers to detect marker-trait associations. Romay et al. (2013) reported that the use of approximately 680,000 GBS markers were sufficient to detect most of the known candidate genes associated with flowering time in maize (Romay et al. 2013). Polymorphism that strongly associated with the lower LD in tropical or subtropical population was more difficult to detect compared to polymorphism that more frequently associated with greater LD in temperate subpopulations. Hence, it indicates that although increased marker coverage and association panel size improves the power of the GWAS, consideration needs to be taken while doing GWAS with low LD subpopulation (tropical/subtropical population), in order to capture the rare allele variants

associated with those subpopulations (Romay et al. 2013). Rapid LD decay along the chromosomes in the maize core diversity panel suggests the use of larger SNP density and large association panels needed to identify the novel loci associated with the ear rot resistance.

6.3.2 Northern Leaf Blight

Poland et al. (2011) studied northern leaf blight in maize (Poland et al. 2011) using GWAS. They evaluated 5000 inbred lines from the nested association mapping lines for the resistance to the northern leaf blight and identified 29 QTLs, and most of them possessed multiple alleles.

Quantitative disease resistance (QDR) is reported to be associated with durable resistance as pathogen that overcomes a single allele of small effect does not gain a large selective advantage, and loss of the allele with small effect does not leave the host completely susceptible (Poland et al. 2009). Northern leaf blight (NLB) is an endemic disease in the maize growing areas of the world causing moderate to severe yield losses (Perkins and Pedersen 1987). NLB is caused by a fungal pathogen *Setosphaeriaturcica* (anamorph *Exserohilumturcicum*), a hemibiotrophic fungus, and is commonly found in the tropical highlands. Previous studies on the NLB have shed light on several QTLs. Among these, three genes confer incomplete race-specific resistance. *Ht1* (Bentolila et al. 1991) located in maize bin 2.08 and *Ht2* (Yin et al. 2003) and *Htn1* (Simcox and Bennetzen 1993) located in the maize bin 8.06. However, as stated earlier, due to the low resolution of the QTL mapping, the positional cloning of these genes was difficult and not widely used in the breeding programs. Hence, this study combined the positive aspect of both the association mapping and the linkage study to unravel the genetic architecture of the NLB.

Briefly, a large NAM population created with 5000 recombinant inbred lines (RILs) was used for the dissection of the complex traits (Yu et al. 2008). Apart from the 25 NAM RILS families, RILs from the intermated B73 X MO17 (IBM) population was included as a 26th family in the study. The NAM RILs were genotyped with 1106 SNP markers and the data are also publicly available in *www.panzea.org*. The study was conducted over three seasons in nurseries artificially inoculated with the single isolate of *S. turcica* race 1. The NAM parent showed the extensive variation on the resistance of the NLB, where the common parent B73 showed 34% of the diseased leaf area being moderately susceptible. The study reported that the GXE interaction of the NLB resistance was minimal; however, the study was carried out only in one location. The author also mentioned that there exists a strong negative correlation between the flowering time (days to anthesis (DTA)) and the NLB resistance in the founder lines (Poland et al. 2011). Joint linkage study was done using stepwise model selection and DTA as a covariate in the model, resulting in the 29 QTLs accounting for 77% of the total variance. Most of the QTLs have a small effect and only few have a large effect. Large effect QTL was identified on chromosome 8 at 152.2 MB segregating in multiple families which were likely to

be *Ht2*, the position was consistent with the physical location identified by fine mapping. The broad sense heritability for the NLB index for the NAM founders was 0.74 (Poland et al. 2011). Most tropical maize lines possess higher level of NLB and other disease resistance than the temperate lines reflecting the favorable conditions for disease development in tropics and thus, useful for a breeder to select for resistance in these environments (Poland et al. 2011). GWAS was done using 1.6 million SNPs, identified 208 significant SNPs association, and 28 of 29 QTLs had one or more SNP associations.

A small subset of the 208 SNP loci found to be associated with the resistance to NLB is shown in Table 6.1, which is adapted from the Poland et al. (2011). The study showed five SNPs associated with the receptor-like kinase (RLK) genes and one additional association with the sixth LRR-related gene. LRR domains have been reported to be associated with the plant disease resistance. Several SNPs detected showed candidate genes with antifreeze domains which has high similarity to the pathogenesis related proteins and were reported to enhance disease resistance. Several serine/threonine protein kinases were identified, and they are also involved in the plant defense responses.

6.3.3 Southern Leaf Blight

Kump et al. (2011) conducted GWAS study on southern leaf blight (SLB) of maize, using nested association mapping population (Kump et al. 2011). SLB is caused by the fungus *Cochliobolus heterostrophus*, a necrotrophic fungus, which tends to occur usually after anthesis. There are limited QTLs and markers identified as the disease resistance QTLs for SLB. They performed joint linkage analysis and identified 32 QTLs, with mostly small additive effects on the SLB resistance. Most of the SNPs detected were previously reported to be near or within the sequence homologues to the genes previously identified in the disease resistance.

Maize NAM represents 135,000 recombination events and hence, good for association mapping as well as linkage study. The study was done across the three environments. GWAS was run using 1.6 M HapMap SNPs that were identified among the founder lines and imputed on the complete NAM panel for the study (Kump et al. 2011). In the study, they used the SLB index values as a phenotypic measurement, which represent the mean of SLB resistance measured across time points and environments. Measurement was done using a standard nine-point rating scale. The B73, common NAM parent, was the most susceptible among all parents. Heritability of the SLB index score was found high, around 87%. The identified SNPs and QTLs and their position in the chromosome were shown in the study (Kump et al. 2011). The 32 QTLs jointly explained 80% of the phenotypic variation of the SLB resistance, as well as 93% of the genotypic variation of the SLB resistance. Additive epistatic interaction between the QTLs was not detected. The QTL with the largest effect estimate was mapped to the bin 3.04, which is known as the previously identified region for the SLB resistance (Balint-Kurti et al. 2007). With the above studies,

Table 6.1 A summary of important SNPs and QTLs of the five major diseases of maize

Disease	Chromosome	Physical position bp	AGP Version	Candidate gene/ (QTLs)	Annotation	Reference
Fusarium Ear Rot	9	151,295,233	AGPv2	GRMZM2G178880	Cellulose synthase-like family A (*CslA*) protein family	Zila et al. (2013)
	5	30,997,717	AGPv2	GRMZM2G111477	Heat Shock Protein	
	1	63,540,590	AGPv2	GRMZM2G703598		
Northern Leaf Blight	1	12,136,678	AGPv1		Serine/threonine protein kinase	Poland et al. (2011)
	2	9,394,756	AGPv1		RLK	
	2	160,834,095	AGPv1		RLK	
	3	3,382,179	AGPv1		Antifreeze	
	5	203,735,206	AGPv1		RLK	
	1	88,927,678	AGPv1		Serine/threonine protein kinase	
	7	125,153,323	AGPv1		Peptidase/serine/threonine protein kinase	
Southern Leaf Blight	1	45,565,372	AGPv1		Mitochondrial carrier protein (programmed cell death); Ran GTPase (plant defense response)	Kump et al. (2011)
	1	80,360,348	AGPv1		Glutathione S-transferase (plant defense)	
	1	210,676,683	AGPv1		LRR receptor kinase (disease resistance25)	
	2	10,687,858	AGPv1		AP2 transcription factor (disease resistance)	
	3	22,604,327			NRR (defense response)	
	4	240,050,394			Pti4, Pti5, and Pti6 ERF transcription factors; ABC transporter (disease resistance)	
	10	1,221,166			NPR1 (disease resistance)	
Head Smut	1	278,884,507	AGPv1	GRMZM2G300990	Serine/threonine protein kinase	Wang et al. (2012)
	2	201,359,446	AGPv1	GRMZM2G140231	Serine/threonine protein kinase	
	2	219,834,173	AGPv1	GRMZM2G166566	Basic leucine zipper transcription factor	

	3	124,139,795	AGPv1	GRMZM2G137289	MADS-box	
	5	188,373,740	AGPv1	GRMZM2G312274	Auxin	
	9	84,924,940	AGPv1	GRMZM2G443953	WD40 repeat	
	10	137,723,733	AGPv1	GRMZM2G117667	Lipase	
Gray Leaf Spot	1	234,309,200	AGPv2	GLS 1.2 (QTLGLSchr1)		Mammadov et al. (2015)
	6	107,475,955	AGPv2	GLS 6.1 (QTLGLSchr6)		
	7	19,500,572	AGPv2	GLS 7.2 (QTLGLSchr7)		
	8	19,550,800	AGPv2	GLS 8.2 (QTLGLSchr8a)		
	8	79,142,282	AGPv2	GLS 8.3 (QTLGLSchr8b)		

it should be noted that plant scientists working in GWAS of diseases that correlated with the flowering time or days to anthesis (DTA) should account for DTA variation as covariate in their GWAS model. In the study, the author found the 30 flowering QTLs, explaining around 85% of the phenotypic variation for the DTA, where 8 pairs of the QTLs for SLB and DTA were found to have overlapping support intervals. The RILs subfamily B73 X CML247 possesses the highest of 15 QTLs with significant allelic effect, whereas the B73 X CML52 possesses 2 QTLs.

A list of the important candidate gene annotation for SLB resistance is shown in Table 6.1, adapted from Kump et al. (2011). Two genes with leucine-rich repeat (LRR) domains were found. Another important gene found was a gene with strong similarity to *NPR1*, which is related to defense response. An SNP was found adjacent to a homolog of the rice gene NRR (negative regulator of the resistance) that encodes a protein which interacts with the NPR1 protein during the defense response (Chern et al. 2005).

6.3.4 Head Smut

A GWAS study on the head smut conducted by Wang et al. (2012) identified 18 novel candidate genes, which were further categorized into resistance genes, disease response genes, and other disease resistance function genes. The author used 45,658 SNPs with an association panel of 144 inbred lines and ran the GWAS in mixed linear model (Q + K) in Tassel V2.1.

Head smut is caused by fungus *Sphacelothecareiliana* (Kühn) Clint, a global maize disease causing moderate to severe loss of both quality and quantity. The study was conducted in different environments with different replications. Artificial inoculation was performed using the previously collected teliospores of *S. reiliana* at a ratio of 1000:1 (teliospores: seed). The percentages of the completely infected plants per plots were scored in either tassels or ears at the mature plant stage. Extensive variation of the susceptibility to head smut was found among the lines, which range from 0% to 83% susceptibility range and the broad sense heritability was high at 88.7% (Wang et al. 2012). The genotype by environment interaction was also significant.

Several QTL mapping studies have been done and have found several QTLs for the head smut (Lu and Brewbaker 1999; Lübberstedt et al. 1999; Li et al. 2008). However, the QTLs were not very consistent among the studies. The QTLs found were reported mainly on chromosome 1, 2, 3, 8, 9, and 10. The major QTL for the head smut resistance, *qHSR1*, has been fine mapped in bin 2.09 using the 68 BC2 recombinants from the cross of Ji1037 and Huangza04 (Chen et al. 2008).

The mixed linear model outputs 19 significant SNPs, which collectively explained 86.5% of the total phenotypic variation ranging from 3.5% to 9.2%. Defense-related gene families such as serine/threonine protein kinases, leucine-rich repeat protein, MADS-box protein (bin 3.05), Auxin (bin 5.05), and WD40 repeat containing protein (bin 9.03) were identified. Two nucleotide-binding sites (NBS) encoding protein were detected on chromosome 8. Bin 2.09 was previously identified as a head

smut resistance region. The SNP on chromosome 2, with physical position 219834173 on AGPv1, was found to be overlapped in the region and was later molecularly validated as resistance gene. This gene GRMZM2G166566 is annotated as a basic leucine zipper transcription factor and has R^2 value of 9.3%.

6.3.5 Gray Leaf Spot

Gray Leaf Spot (GLS) (causal agents *Cercosporazeae-maydis* and *Cercosporazeina*) is one of the most important foliar diseases of maize. In the United States, *C. zeae-maydis* occurs everywhere where corn is being cultivated, whereas *C. zeina* is mainly found on the East coast (Wang et al. 1998). Disease is prevalent in the areas where dewy mornings are followed by a hot humid afternoon and relatively cool nights. Breeding the disease resistance cultivars is the most prominent strategy to control the disease. Mammadov et al. (2015) combined the high QTL detection power of genetic linkage mapping with high resolution power of GWAS to study the resistance of GLS, which is beneficial for the marker-assisted QTLs introgression (Mammadov et al. 2015).

The association study used the 300 maize association panel and was replicated in four different environments. The Association Panel comprised 215 DAS proprietary lines of North and South American origin, 27 ex-PVP lines, 37 CYMMIT lines, and 21 lines from the National Plant Germplasm system. All lines in the association panel were chosen based on their previously known reaction to GLS, which were categorized into four major categories: GLS susceptible, moderately GLS susceptible, moderately GLS resistant, and GLS resistant. For any bi-allelic SNP [A/B], a positive effect suggests that the allele contributing to GLS severity comes from allele A and a negative effect suggests that the allele contributing to GLS severity comes from allele B. GWAS was conducted using ~25,000 SNP markers with minor allele frequencies (MAF) > 0.1.

Besides, they used 72 lines for the biparental QTL mapping developed from the cross between DAS-001 (GLS resistant) and DAS-002 (GLS susceptible). Both DAS are the proprietary maize inbred lines of Dow AgroSciences. The map of 1985 SNP markers was evenly distributed across ten maize chromosomes. Extended composite interval mapping (ECIM) model was used for QTL mapping.

They identified four GLS resistance QTL on the chromosome 1, 6, 7, and 8, which was further validated by GWAS. The genetic linkage – GWAS hybrid mapping system in the study identified one novel GLS resistance QTL (*QTLGLSchr8a)* and confirmed four previously mapped QTL (*QTLGLSchr1*, *QTLGLSchr6*, *QTLGLSchr7*, and *QTLGLSchr8b*) with more refined position. Three minor and one major QTL were detected on chromosomes 1, 6, 7, and 8, respectively. The QTL on chromosome 8 (*QTLGLSchr8*) explained about 26.5% of the variation, while the QTL on chromosomes 1 (*QTLGLSchr1*), chromosome 6 (*QTLGLSchr6*), and chromosome 7 (*QTLGLSchr7*) were responsible for 4.55%, 6.85%, and 5.23% of GLS resistance, respectively. In total, all four identified QTL explained 43.13% of GLS resistance in the DAS-001 inbred line (Mammadov et al. 2015).

6.4 Future Perspective

Genome-wide association studies in crops have tremendously benefitted the farmers, cooperatives, agriculture companies, and the scientific community. It has already been proven that the GWAS studies are beneficial in finding the causal variants of the disease and can be effectively used in developing disease resistance cultivars. However, one needs to be careful in using the GWAS model, as it has a high chance to provide false positive SNPs, given the incorrect model, and we already know that it is affected by several factors such as population structure, kinship, and selection history, hence, it is always useful to have positive control traits/SNPs in running the GWAS model or one can do simulation of the SNPs in the absence of the positive control to avoid those spurious hits. With the decrease in the cost of genotyping, GWAS using high density markers, high population sample size replicated in different environments and years will provide high power to detect the causal variants.

Quantitative traits are governed by polygenes of mostly small effects. Interaction of genes and their associative role in the phenotype is proven to be highly important. However, detecting those epistatic QTLs are still a major challenge for plant scientists. Now it is time to contemplate on designing the mapping population that can dissect the epistatic variation over the additive variation in studying quantitative traits. The other major limitation of GWAS is that it is not capable of detecting the rare allelic variants. The power of detection of marker-trait association depends on allele frequency of the particular QTL. Rare/low frequency alleles having either small or large effect are not detected by GWAS. The functional role of those rare variants has started to shed light in human as well as in plant disease. Hence, the next few decades will be important to understand the functional role of the rare variants/alleles in disease resistance.

The other limitation of GWAS includes the missing heritability concept, where the high heritable traits on the phenotypic variation remain unexplained. One of the reasons is that we tend to ignore the effect of thousands of SNPs under the threshold, which might possess good biological information. Hence, the concept of genomic prediction and selection has evolved in the recent years in the field of plant disease resistance. Genomic prediction utilizes the genomic breeding values of the genotypes obtained from genotypic and phenotypic information from the training set population and used that to predict the phenotype of the breeding set. This is useful, as the genotypic cost is decreasing dramatically, whereas the phenotypic cost is still high. Genomic prediction has already begun in a few diseases in maize (Technow et al. 2013; Gowda et al. 2015) and shown to have good prediction, which helps to reduce the cycle of selection and ease the breeding effort for developing disease-resistant cultivars. Exploring and mitigating the disease resistance challenge using multi-omics integration and system genetics approach is another interesting modern day concept. With all these fascinating developments in tools and concepts, the breeding for the disease-resistant cultivars in the coming decades will be another revolution in mitigating the poverty and malnutrition and for the sustainable agriculture across the globe.

References

Acuna TB, Rebetzke G, He X, Maynol E, Wade L (2014) Mapping quantitative trait loci associated with root penetration ability of wheat in contrasting environments. Mol Breed 34(2):631–642

Balint-Kurti P, Carson M (2006) Analysis of quantitative trait loci for resistance to southern leaf blight in juvenile maize. Phytopathology 96(3):221–225

Balint-Kurti PJ, Johal GS (2009) Maize disease resistance. In: Handbook of maize: its biology. Springer, New York, pp 229–250

Balint-Kurti P, Zwonitzer JC, Wisser RJ, Carson M, Oropeza-Rosas MA, Holland JB, Szalma SJ (2007) Precise mapping of quantitative trait loci for resistance to southern leaf blight, caused by Cochliobolus heterostrophus race O, and flowering time using advanced intercross maize lines. Genetics 176(1):645–657

Bent AF, Kunkel BN, Dahlbeck D, Brown KL, Schmidt R, Giraudat J, Leung J, Staskawicz BJ (1994) RPS2 of Arabidopsis thaliana: a leucine-rich repeat class of plant disease resistance genes. Science 265(5180):1856–1860

Bentolila S, Guitton C, Bouvet N, Sailland A, Nykaza S, Freyssinet G (1991) Identification of an RFLP marker tightly linked to theHt1 gene in maize. Theor Appl Genet 82(4):393–398

Broman KW, Wu H, Sen Ś, Churchill GA (2003) R/qtl: QTL mapping in experimental crosses. Bioinformatics 19(7):889–890

Castro A, Tacaliti M, Giménez D, Tocho E, Dobrovolskaya O, Vasicek A, Collado M, Snape J, Börner A (2008) Mapping quantitative trait loci for growth responses to exogenously applied stress induced hormones in wheat. Euphytica 164(3):719

Causse M, Saliba-Colombani V, Lecomte L, Duffe P, Rousselle P, Buret M (2002) QTL analysis of fruit quality in fresh market tomato: a few chromosome regions control the variation of sensory and instrumental traits. J Exp Bot 53(377):2089–2098

Causse M, Duffe P, Gomez M, Buret M, Damidaux R, Zamir D, Gur A, Chevalier C, Lemaire-Chamley M, Rothan C (2004) A genetic map of candidate genes and QTLs involved in tomato fruit size and composition. J Exp Bot 55(403):1671–1685

Chen Y, Chao Q, Tan G, Zhao J, Zhang M, Ji Q, Xu M (2008) Identification and fine-mapping of a major QTL conferring resistance against head smut in maize. Theor Appl Genet 117(8):1241

Chern M, Canlas PE, Fitzgerald HA, Ronald PC (2005) Rice NRR, a negative regulator of disease resistance, interacts with Arabidopsis NPR1 and rice NH1. Plant J 43(5):623–635

Clements M, Maragos C, Pataky J, White D (2004) Sources of resistance to fumonisin accumulation in grain and Fusarium ear and kernel rot of corn. Phytopathology 94(3):251–260

Dhugga KS (2005) Plant Golgi cell wall synthesis: from genes to enzyme activities. Proc Natl Acad Sci U S A 102(6):1815–1816

Ding J-Q, Wang X-M, Chander S, Yan J-B, Li J-S (2008) QTL mapping of resistance to Fusarium ear rot using a RIL population in maize. Mol Breed 22(3):395–403

Doerge RW (2002) Multifactorial genetics: mapping and analysis of quantitative trait loci in experimental populations. Nat Rev Genet 3(1):43

Flint-Garcia SA, Thuillet AC, Yu J, Pressoir G, Romero SM, Mitchell SE, Doebley J, Kresovich S, Goodman MM, Buckler ES (2005) Maize association population: a high-resolution platform for quantitative trait locus dissection. Plant J 44(6):1054–1064

Flor HH (1971) Current status of the gene-for-gene concept. Annu Rev Phytopathol 9(1):275–296

Foolad M (1999) Comparison of salt tolerance during seed germination and vegetative growth in tomato by QTL mapping. Genome 42(4):727–734

Gowda M, Das B, Makumbi D, Babu R, Semagn K, Mahuku G, Olsen MS, Bright JM, Beyene Y, Prasanna BM (2015) Genome-wide association and genomic prediction of resistance to maize lethal necrosis disease in tropical maize germplasm. Theor Appl Genet 128(10):1957–1968

Hammond-Kosack KE, Jones JD (1997) Plant disease resistance genes. Annu Rev Plant Biol 48(1):575–607

Ishikawa A, Tanaka H, Nakai M, Asahi T (2003) Deletion of a chaperonin 60β gene leads to cell death in the Arabidopsis lesion initiation 1 mutant. Plant Cell Physiol 44(3):255–261

Johal GS, Briggs SP (1992) Reductase activity encoded by the HM1 disease resistance gene in maize. Science 258(5084):985–987

Jones JD, Dangl JL (2006) The plant immune system. Nature 444(7117):323

Kang HM, Zaitlen NA, Wade CM, Kirby A, Heckerman D, Daly MJ, Eskin E (2008) Efficient control of population structure in model organism association mapping. Genetics 178(3):1709–1723

Kump KL, Bradbury PJ, Wisser RJ, Buckler ES, Belcher AR, Oropeza-Rosas MA, Zwonitzer JC, Kresovich S, McMullen MD, Ware D (2011) Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. Nat Genet 43(2):163

Levings CS, Siedow JN (1992) Molecular basis of disease susceptibility in the Texas cytoplasm of maize. In: 10 years plant molecular biology. Springer, Dordrecht, pp 135–147

Li X, Wang Z, Gao S, Shi H, Zhang S, George M, Li M, Xie C (2008) Analysis of QTL for resistance to head smut (Sporisorium reiliana) in maize. Field Crop Res 106(2):148–155

Liu X, Huang M, Fan B, Buckler ES, Zhang Z (2016) Iterative usage of fixed and random effect models for powerful and efficient genome-wide association studies. PLoS Genet 12(2):e1005767

Lu X, Brewbaker J (1999) Molecular mapping of QTLs conferring resistance to Sphacelotheca reiliana (Kühn) Clint. Maize Genetics Cooperation Newsletter (73)

Lübberstedt T, Xia X, Tan G, Liu X, Melchinger A (1999) QTL mapping of resistance to Sporisorium reiliana in maize. Theor Appl Genet 99(3–4):593–598

Mammadov J, Sun X, Gao Y, Ochsenfeld C, Bakker E, Ren R, Flora J, Wang X, Kumpatla S, Meyer D (2015) Combining powers of linkage and association mapping for precise dissection of QTL controlling resistance to gray leaf spot disease in maize (Zea mays L.). BMC Genomics 16(1):916

Martin GB, Brommonschenkel SH, Chunwongse J, Frary A, Ganal MW, Spivey R, Wu T, Earle ED, Tanksley SD (1993) Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science 262(5138):1432–1436

McMullen MD, Kresovich S, Villeda HS, Bradbury P, Li H, Sun Q, Flint-Garcia S, Thornsberry J, Acharya C, Bottoms C (2009) Genetic properties of the maize nested association mapping population. Science 325(5941):737–740

Nimchuk Z, Eulgem T, Holt Iii BF, Dangl JL (2003) Recognition and response in the plant immune system. Annu Rev Genet 37(1):579–609

Nordborg M, Tavaré S (2002) Linkage disequilibrium: what history has to tell us. Trends Genet 18(2):83–90

Olukolu BA, Negeri A, Dhawan R, Venkata BP, Sharma P, Garg A, Gachomo E, Marla S, Chu K, Hasan A (2013) A connected set of genes associated with programmed cell death implicated in controlling the hypersensitive response in maize. Genetics 193(2):609–620

Park KJ, Sa KJ, Kim BW, Koh H-J, Lee JK (2014) Genetic mapping and QTL analysis for yield and agronomic traits with an F2: 3 population derived from a waxy corn× sweet corn cross. Genes Genomics 36(2):179–189

Parlevliet JE (2002) Durability of resistance against fungal, bacterial and viral pathogens; present situation. Euphytica 124(2):147–156

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335(6192):721

Pérez Brito D, Jeffers D, González de León D, Khairallah M, Cortés C, Velázquez C, Azpíroz S, Srinivasan G (2001) QTL mapping of Fusarium moniliforme ear rot resistance in highland maize. Agrociencia, Mexico, 35(2)

Perkins J, Pedersen W (1987) Disease development and yield losses associated with northern leaf blight on corn. Plant Dis 71(10):940–943

Poland JA, Balint-Kurti PJ, Wisser RJ, Pratt RC, Nelson RJ (2009) Shades of gray: the world of quantitative disease resistance. Trends Plant Sci 14(1):21–29

Poland JA, Bradbury PJ, Buckler ES, Nelson RJ (2011) Genome-wide nested association mapping of quantitative resistance to northern leaf blight in maize. Proc Natl Acad Sci 108(17):6893–6898

Pratt RC, Gordon SG (2006) Breeding for resistance to maize foliar pathogens. Plant Breed Rev 27:119

Price AH (2006) Believe it or not, QTLs are accurate! Trends Plant Sci 11(5):213–216

Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D (2006) Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet 38(8):904

Pring DR, Lonsdale DM (1989) Cytoplasmic male sterility and maternal inheritance of disease susceptibility in maize. Annu Rev Phytopathol 27(1):483–502

Quarrie S, Gulli M, Calestani C, Steed A, Marmiroli N (1994) Location of a gene regulating drought-induced abscisic acid production on the long arm of chromosome 5A of wheat. Theor Appl Genet 89(6):794–800

Ray J, Yu L, McCouch S, Champoux M, Wang G, Nguyen H (1996) Mapping quantitative trait loci associated with root penetration ability in rice (Oryza sativa L.). Theor Appl Genet 92(6):627–636

Rodríguez-Gacio MDC, Iglesias-Fernández R, Carbonero P, Matilla ÁJ (2012) Softening-up mannan-rich cell walls. J Exp Bot 63(11):3976–3988

Romay MC, Millard MJ, Glaubitz JC, Peiffer JA, Swarts KL, Casstevens TM, Elshire RJ, Acharya CB, Mitchell SE, Flint-Garcia SA (2013) Comprehensive genotyping of the USA national maize inbred seed bank. Genome Biol 14(6):R55

Sax K (1923) The association of size differences with seed-coat pattern and pigmentation in Phaseolus vulgaris. Genetics 8(6):552–560

Segura V, Vilhjálmsson BJ, Platt A, Korte A, Seren Ü, Long Q, Nordborg M (2012) An efficient multi-locus mixed-model approach for genome-wide association studies in structured populations. Nat Genet 44(7):825

Sekhon RS, Lin H, Childs KL, Hansey CN, Buell CR, de Leon N, Kaeppler SM (2011) Genome-wide atlas of transcription during maize development. Plant J 66(4):553–563

Simcox KD, Bennetzen JL (1993) The use of molecular markers to study Setosphaeria turcica resistance in maize. Phytopathology 83(12):1326–1330

Singh B, Singh AK (2015) Marker-assisted plant breeding: principles and practices. Springer

Song W-Y, Wang G-L, Chen L-L, Kim H-S, Pi L-Y, Holsten T, Gardner J, Wang B, Zhai W-X, Zhu L-H (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, Xa21. Science 270(5243):1804–1806

Tan Y, Sun M, Xing Y, Hua J, Sun X, Zhang Q, Corke H (2001) Mapping quantitative trait loci for milling quality, protein content and color characteristics of rice using a recombinant inbred line population derived from an elite rice hybrid. Theor Appl Genet 103(6–7):1037–1045

Technow F, Bürger A, Melchinger AE (2013) Genomic prediction of northern corn leaf blight resistance in maize with combined or separated training sets for heterotic groups. G3 3(2):197–203

Thornsberry JM, Goodman MM, Doebley J, Kresovich S, Nielsen D, Buckler ES IV (2001) Dwarf8 polymorphisms associate with variation in flowering time. Nat Genet 28(3):286

Tian Y, Zhang H, Xu P, Chen X, Liao Y, Han B, Chen X, Fu X, Wu X (2015) Genetic mapping of a QTL controlling leaf width and grain number in rice. Euphytica 202(1):1–11

Ullstrup A (1972) The impacts of the southern corn leaf blight epidemics of 1970-1971. Annu Rev Phytopathol 10(1):37–50

Wang J, Levy M, Dunkle LD (1998) Sibling species of Cercospora associated with gray leaf spot of maize. Phytopathology 88(12):1269–1275

Wang S, Basten C, Zeng Z (2007) Windows QTL cartographer 2.5. Department of statistics. North Carolina state university, Raleigh

Wang M, Yan J, Zhao J, Song W, Zhang X, Xiao Y, Zheng Y (2012) Genome-wide association study (GWAS) of resistance to head smut in maize. Plant Sci 196:125–131

Wisser RJ, Balint-Kurti PJ, Nelson RJ (2006) The genetic architecture of disease resistance in maize: a synthesis of published studies. Phytopathology 96(2):120–129

Xiao W, Zhao J, Fan S, Li L, Dai J, Xu M (2007) Mapping of genome-wide resistance gene analogs (RGAs) in maize (Zea mays L.). Theor Appl Genet 115(4):501–508

Yin X, Wang Q, Yang J, Jin D, Wang F, Wang B, Zhang J (2003) Fine mapping of the Ht2 (Helminthosporium turcicum resistance 2) gene in maize. Chin Sci Bull 48(2):165–169

Yu J, Pressoir G, Briggs WH, Bi IV, Yamasaki M, Doebley JF, McMullen MD, Gaut BS, Nielsen DM, Holland JB (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nat Genet 38(2):203

Yu J, Holland JB, McMullen MD, Buckler ES (2008) Genetic design and statistical power of nested association mapping in maize. Genetics 178(1):539–551

Zhang Z, Ersoz E, Lai C-Q, Todhunter RJ, Tiwari HK, Gore MA, Bradbury PJ, Yu J, Arnett DK, Ordovas JM (2010) Mixed linear model approach adapted for genome-wide association studies. Nat Genet 42(4):355

Zhu C, Gore M, Buckler ES, Yu J (2008) Status and prospects of association mapping in plants. Plant Genome 1(1):5–20

Zila CT, Samayoa LF, Santiago R, Butrón A, Holland JB (2013) A genome-wide association study reveals genes associated with Fusarium ear rot resistance in a maize core diversity panel. G3 3(11):2095–2104

Chapter 7
Molecular Breeding Approaches for Disease Resistance in Sugarcane

Mehzabin Rahman, Sabira Sultana, Deepshikha Nath, Surya Kalita, Dhiresh Chakravarty, Sahil Mehta, Shabir Hussain Wani, and Md Aminul Islam

7.1 Introduction

At present, sugar is the predominant commodity of the global food market manufactured from sugarcane (*Saccharum officinarum* L.) (Mohan 2016; Augustine 2017). This makes sugarcane an important cash crop grown worldwide (Sengar 2018). About 70% of the world's total sugar is manufactured from sugarcane. In terms of quantity, sugarcane is cultivated on nearly 27 million hectares in more than 120 countries around the globe. For the year 2016–2017, global sugar production amounted to approximately 191.81 million metric tons. Out of all countries, Brazil tops the rank as the largest sugar-producing country in the world followed by India, China, and Thailand. However, Asia is the largest sugar-producing continent

M. Rahman
Bimala Prasad Chaliha College, Nagarbera, Assam, India

Department of Biotechnology, Gauhati University, Jalukbari, Assam, India

S. Sultana
Department of Biotechnology, Gauhati University, Jalukbari, Assam, India

D. Nath
Independent Researcher, Silchar, Assam, India

S. Kalita · D. Chakravarty
Bimala Prasad Chaliha College, Nagarbera, Assam, India

S. Mehta
International Centre for Genetic Engineering and Biotechnology, New Delhi, India

S. H. Wani
Mountain Research Centre for Field Crops, Sher-e-Kashmir University of Agricultural sciences and Technology of Kashmir, Jammu and Kashmir, India

M. A. Islam (✉)
Bimala Prasad Chaliha College, Nagarbera, Assam, India

National Institute of Plant Genome Research, New Delhi, India

S. H. Wani (ed.), *Disease Resistance in Crop Plants*,
https://doi.org/10.1007/978-3-030-20728-1_7

contributing about 40% of the global sugar production (Solomon and Li 2016). Within the Asian region, India, China, Thailand, Sri Lanka, and Bangladesh are the major sugar-producing countries. Within India, Uttar Pradesh and Maharashtra are the largest sugarcane-producing states for the session 2017–2018. The state-wise production and overall yield are depicted in Fig. 7.1.

Sugarcane is one of the most-efficient, perennial monocotyledonous glycophytes which belongs to the family Poaceae which includes all grass species (Mohan 2016; Augustine 2017). Furthermore, it provides raw materials for sugar industries and

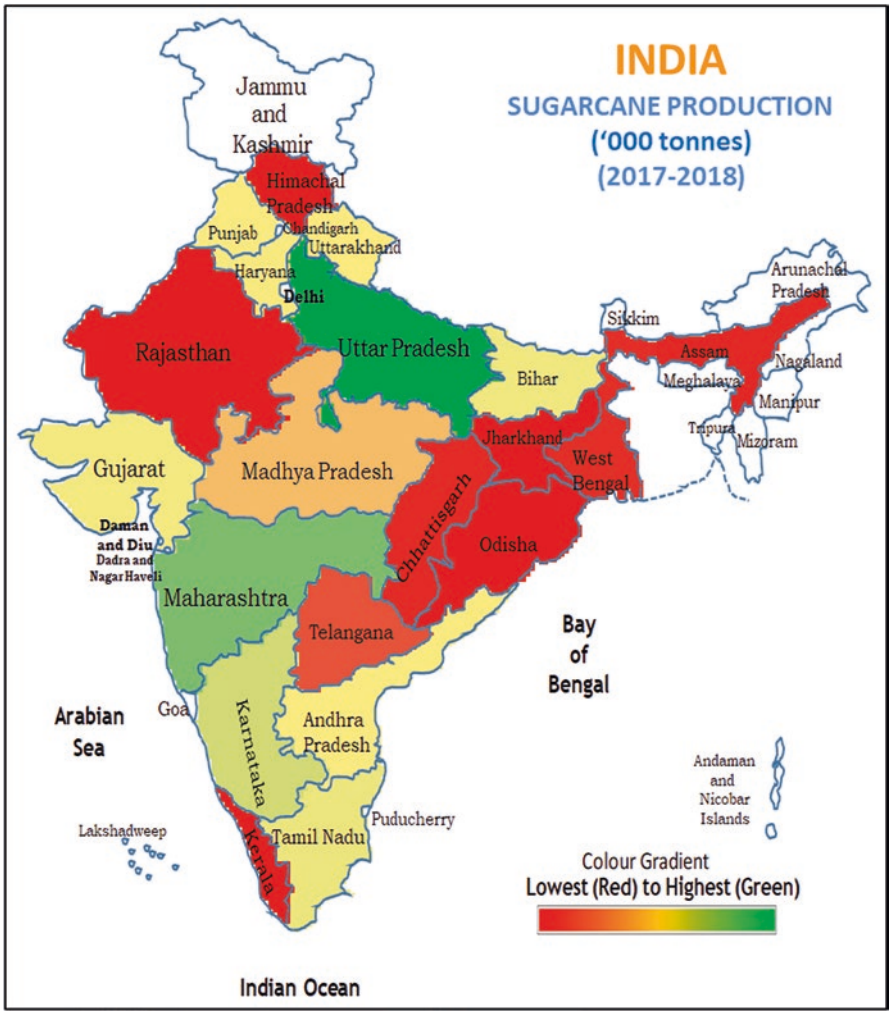

Fig. 7.1 Geographic heat maps of India representing (**a**) sugarcane production and (**b**) sugarcane yield for the 2017–2018. The maps have been generated using IndZara (https://indzara.com). (Accessed on 10th February 2019)

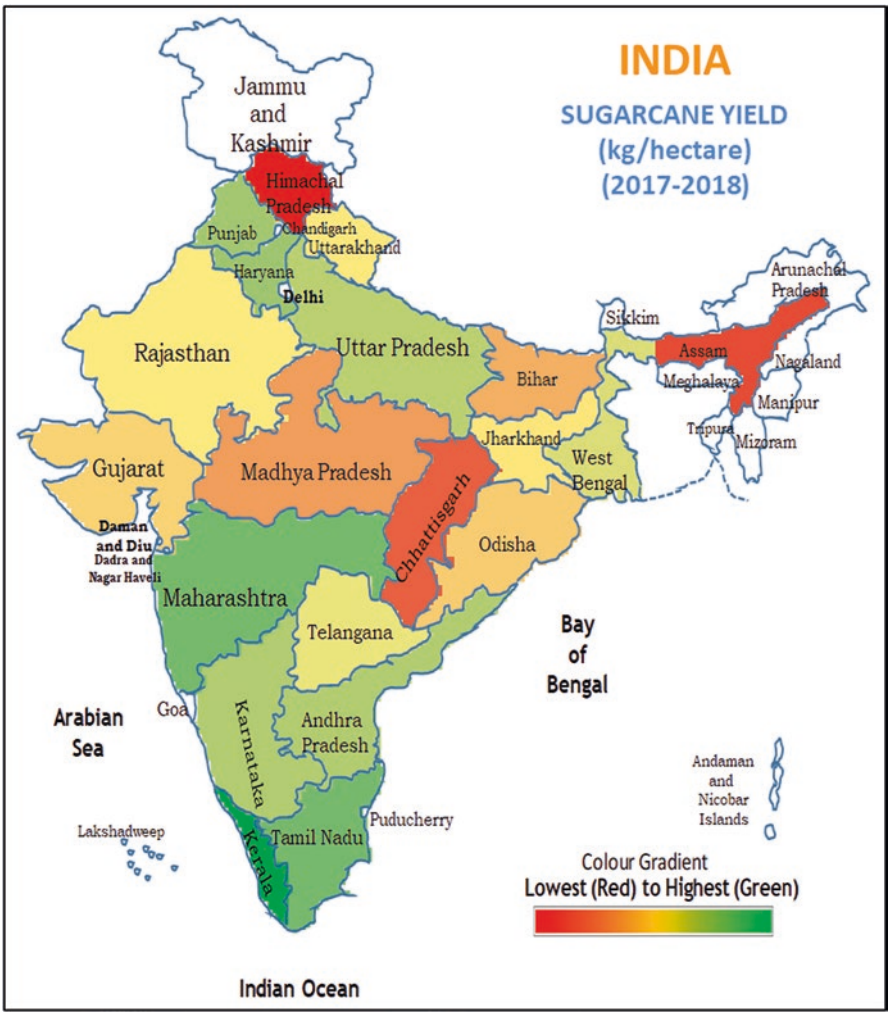

Fig. 7.1 (continued)

allied groups of by-product industries. However, economic importance is much more than its share in the gross cropped area. Recently, it was recognized as an important energy crop due to large-scale molasses-based ethanol production (Fig. 7.2). Furthermore, it is the most efficient biofuel feedstock for the generation of bio-butanol, diesel, and many other valuable by-products (Yadav and Solomon 2006; Solomon 2011; Abdel-Halim 2014). The other important by-products are paper, acetic acid, plywood, and industrial enzymes (Arencibia et al. 1998) (Fig. 7.2). As a result, it is one of the most important gifts from nature's vault for humans. Table 7.1 enlists the data regarding sugarcane production and other related parameters for India.

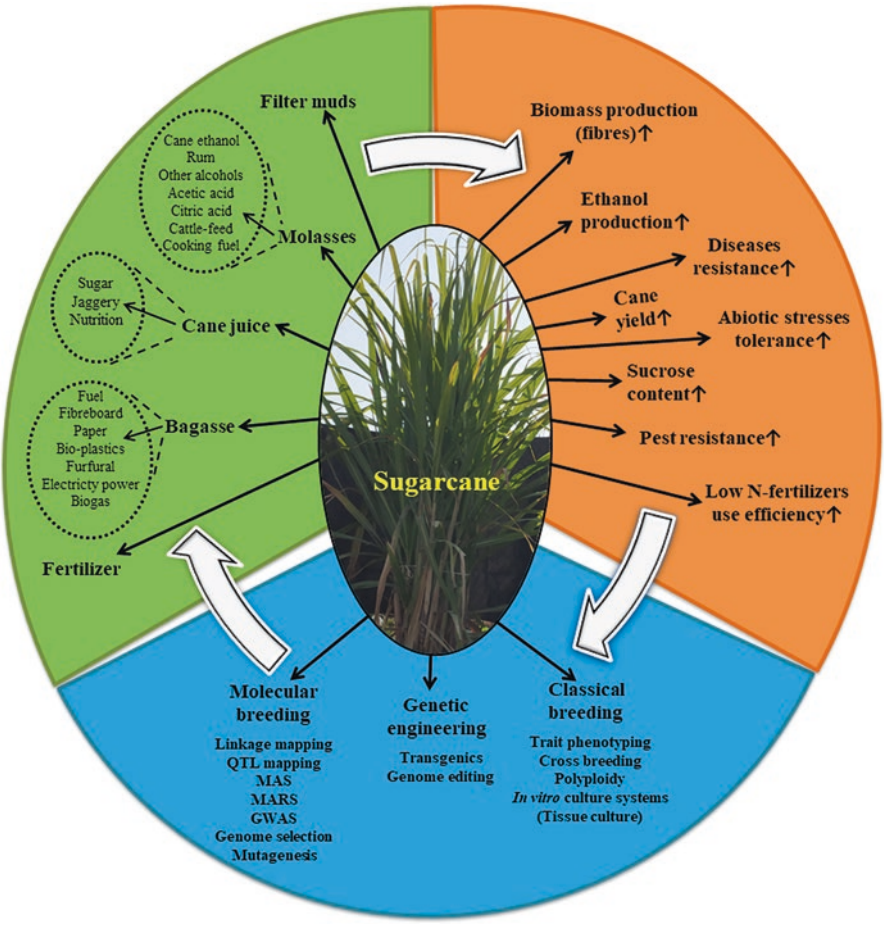

Fig. 7.2 A-B-C of sugarcane breeding programs depicting the uses, breeding aims, and employed approaches

Likewise, other crops, sugarcane production has been affected by global climate change in the past few decades (Ahanger et al. 2013; Pachauri et al. 2014). It has been noted that the global temperature has increased for about 0.8 °C in the past hundred years and it is expected to increase between 0.9 °C and 3.5 °C by 2095. In addition, the global climate changes, as well as anthropogenic activities, have either affected or changed atmospheric CO_2 concentration, temperature, extreme weather phenomena, and precipitation patterns (Régnière 2011). Furthermore, all these changing variables also influence the major elements of disease triangle, i.e., host, host microbiome, pathogen, and environment (Coakley et al. 1999; Ghini et al. 2008; Chakraborty and Newton 2011) which ultimately increases disease incidence as well as severity.

Table 7.1 Comparison of sugarcane productivity and other related parameters for India in the last 4 years. The data have been adapted from Indian Sugar Association Mills (http://www.indiansugar.com). (Accessed on 10th February 2019)

Particulars	2013–2014	2014–2015	2015–2016	2016–2017	2017–2018	2018–2019
Estimates	Final estimates	Final estimates	Final estimates	Final estimates	4th advance estimates	1st advance estimates
Sugarcane production (tons)	352142.9	362333.4	310120.3	306069.0	376904.6	383892.0
Cane acreage (hectares)	4993.2	5067.1	4584.2	4435.7	4732.0	5158.5
Yield (Kg/hectare)	70524.4	71511.6	67669.4	69012.1	79650.3	74419.3
Molasses production (tons)	10,882	12,482	8937	9026	14,036	14,568
No. of factories in production	509	538	526	493	525	515
Fair and remunerative price of sugarcane (Rs/quintal)	210	220	230	230	255	275
Minimum recovery %	9.5.	9.50	9.50	9.50	9.50	10
Premium for every 0.1% increase	2.21	2.32	2.42	2.42	2.68	2.75

Due to the changing climate and surge in reports related to disease incidence, plant breeders have shifted their focus toward modern "molecular breeding" which has experienced significant innovations and advances during the past three decades. This is due to rapid germplasm evaluation, development of molecular markers, genetic mapping, molecular marker-assisted breeding, map-based gene discovery, continuous refinement of molecular assays, and characterization of agronomically important traits in multiple crops (Xu 2010; Jiang 2013). In comparison to conventional breeding methods, molecular breeding has significant advantages; like genotypic assays are faster, cheaper, and more accurate, depending on the traits and conditions. In addition, it has a higher efficiency in terms of time and resources (Jiang 2013).

The molecular breeding has been used in sugarcane varieties for high cane yield, enhanced sucrose content, season-wise maturity, multiple abiotic stress tolerance, insect resistance, and disease resistance (Mohan 2016; Dhansu et al. 2018) (Fig. 7.2). However, disease resistance is a major goal for breeders after cane yield as disease causes considerable losses in sugarcane production. More than 100 pathogens, including bacteria, fungi, viruses, phytoplasmas, and nematodes, have been reported to cause diseases of sugarcane (Rott 2000). Therefore, screening and breeding sugarcane for disease resistance is a very important process for enhancing global sugarcane production.

7.2 Major Diseases of Sugarcane

Being a long duration crop, sugarcane is constantly challenged by different types of biotic and abiotic stresses (Solomon 2014), out of which biotic stress acts as the major limiting factor on sugarcane production. As a result, the sugarcane yield is decreasing worldwide since the reports for disease incidence are increasing at an alarming rate with each year. Moreover, the overuse of chemical pesticides along with climate change is predicted to increase frequency and disease severity (Huang et al. 2018). Therefore, it is prone to many diseases including red rot, wilt, smut, ringspot disease, etc. (Rott 2000) worldwide. Furthermore, the incidence of viral diseases like sugarcane mosaic disease is also increasing; hence, breeding sugarcane for viral diseases is a hot topic for research. Table 7.2 offers brief information about some of the important sugarcane diseases. About 45 sugarcane diseases are reported in India, some of which majorly constraint the sugarcane production and result in yield losses which tune up to 10–15% (Viswanathan and Rao 2011; Solomon 2014). Figure 7.3 enlists the major sugarcane diseases and pests in India.

7.3 Breeding for Resistance to Economically Important Diseases of Sugarcane

Modern cultivated sugarcane (*Saccharum* spp.) is a highly polyploid and complex plant. It is originated from crosses between *S. officinarum* and *S. spontaneum* and in some lineages *S. sinense* Roxb., or *S. barberi* Jesw (Daniels et al. 1975; Le Cunff et al. 2008). Detail information on the members of *Saccharum* species has been discussed in Table 7.3.

Over the many decades, sugarcane breeding has been widely acknowledged as the only method for introducing resistance against common diseases such as smut, common rust, sugarcane mosaic virus, red rot, leaf scald, and many more diseases. However, there are many superior varieties which have succumbed to diseases like red rot, smut, or wild in farmer fields during the course of cultivation, which hitherto at the release time were rated as resistant.

7.3.1 Glimpses of Classical Genetics and Traditional Breeding

Classical genetics and traditional breeding have contributed enormously in the sugarcane breeding approach. The germplasm collection is the first prerequisite for any breeding program and provides information about the target donor genes as well as genetically divergent genotypes to be used in crosses. It takes help of morphological, cytological, and isozyme markers (Eksomtramagel and Pauletl 1992; Pandiyan et al. 2012; You et al. 2013; Ghose et al. 2016) to identify a gene responsible for a

Table 7.2 Brief information on important diseases of sugarcane worldwide

Sl. No.	Disease name	Pathogen	Parts affected	Symptoms	Transmission
Fungal diseases					
1	Red rot	*Colletotrichum falcatum*	Leaves and stalks	Discoloration and drying of young leaves from margin to midrib. Red dots in the leaves; production of red, elongated midrid lesions; leaf sheaths with red patches; and leaf blades with dark red spots slightly acidic, starchy odor and are interrupted by whitish patches along the stalk	Soil-, wind-, or rain-borne spores of the fungus from infected seedcanes
2	Smut	*Sporisorium scitamineum* (formerly known as *Ustilago scitaminea*)	Leaves and stalks	The emergence of a long, elongated whip in the growing tip or lateral bud of the stalk. The whip is surrounded by a layer of black teliospores. Silver membrane ruptures releasing millions of teliospores of the smut. Stunted growth of infected stalks, profuse tillering, erect shoots, narrow leaves and grass-like appearance of the cane	Through infected seedcane or wind-borne teliospore of the fungus
3	Wilt	*Fusarium sacchari*	Leaves, shoots, and stalks	Wilted and stunted growth of the plants, presence of diffused reddish-brown patches on the internal tissues of the plants, appearance of yellowish color in the crown leaves, and the infected leaves lose turgor and gradually wither and dry	Soil, infected seedcanes, wind, rain, and irrigation water spread the spores of the fungus
4	Sugarcane pineapple disease	*Ceratocystis paradoxa*	Seedcane and stalks	Infected tissues firstly become reddish in color and gradually turn brownish black due to the production of fungal spores; infected tissue rots and produces the smell of overripe pineapple. Shoot development and early shoot vigor of the seedcane are also affected. Other symptoms include an appearance of patchy and uneven growth in the young cane crops	Transmitted by fungal spores present in the soil. Also, wind-blown or rain-splashed spores from infected standing cane
5	Sugarcane pokkah boeng disease	*Fusarium moniliforme*	Leaves and stalks	Development of chlorotic areas at the base of the young leaves followed by deformation (wrinkling and twisting), narrowing of leaves and stalk distortion, internal and external portion of the stalk develops lesions, reddish stripes and specks develop within lesions. In acute cases, top rot occurs ultimately resulting in the death of the plant	Through airborne spores or infected seed pieces

(continued)

Table 7.2 (continued)

Sl. No.	Disease name	Pathogen	Parts affected	Symptoms	Transmission
6	Sugarcane ringspot disease	*Leptosphaeria sacchari*	Leaf blade, leaf sheath, and stalk	Appearance of a yellow, oblong spot in the initial stage of infection. Later the lesions enlarge and coalesce to form reddish-brown patches. Ring spots frequently occur on old leaves	Wind- or rain-born spores of the fungus
7	Sugarcane eyespot disease	*Helminthosporium sacchari*	Leaves and uppermost region of the stalks	Appearance of lesions in the form of minute water-soaked spots on the young leaves, straw-colored lesion becomes elongated and gradually turns reddish-brown in color in the center surrounded by straw-colored margins resembling the shape of an eye, dryness of leaf tissues	Wind and rain spread spores (conidia) present on leaf lesions
8	Sugarcane yellow spot disease	*Cercosporakoepkei*	Leaves	Yellow spots of irregular shapes appear over the leaf surface. Spots coalesce on the later stage of infection and appear red. Drying of leaves occurs from tip to base	Wind- or rain-born spores of the fungus
Bacterial diseases					
9	Sugarcane leaf scald	*Xanthomonas albilineans*	Leaves	Appearance of "white pencil line" in leaf veins, necrosis develops from the leaf apex and finally covers the whole leaf. Leaves look burnt and curl inward resulting in the scalded appearance of the leaves, partial or complete chlorosis of the leaves resulting in dark brown coloration. Other symptoms include sprouting of lateral bud outward, sudden wilting of the mature stalks	Infected seedcane and cutting implements, rain- or waterborne suspensions of the bacterial pathogen
10	Sugarcane ratoon stunting disease	*Leifsonia xyli* subsp. *xyli* (*Clavibacter xyli* subsp. *xyli*)	Stalk	No observable external symptoms. Internally there is an appearance of orange-colored pinhead like dots of bacteria in the nodal region of the vascular bundle of the stalk. Other symptoms include thinner stalks, short internodes, stunted growth, and pale yellowish foliage	Infected seedcane, mechanical harvesting machine contaminated by the bacteria from diseases stalks

11	Sugarcane red stripe disease	*Acidovorax avenae* subsp. *avenae*	Leaf base	Appearance of water-soaked chlorotic strips near the midribs of leaves, the strips are reddish in color, and later turns maroon to dark red in color. Leaf lesions sometimes extend to leaf sheath and in others to leaf blades. Whitish flakes appear in the lower leaf surface of lesions	Rain- or waterborne suspensions of the bacterial pathogen from the surface of leaf lesions
Phytoplasma disease					
12	Grassy shoot disease	*Candidatus phytoplasma*	Leaves and stalks	Profuse tillering, the proliferation of side shoots from the base of the stalks, stunted growth of the stalks, reduction in the number, size, length, and soft texture of the leaves. Infected clumps appear bushy like grass and results in the formation of dwarf canes with short internodes and thin chlorotic tillers	From affected seedcane by the aphids
Viral diseases					
13	Sugarcane yellow leaf disease	*Sugarcane yellow leaf virus* (SCYLV)	Leaves	Yellowing of the leaf midrib in the underside of the leaf, yellowing expands from the midrib to the leaf blade and causes necrosis of leaves from the apex toward the base resulting in the drying of the foliage. During severe infection, the virus causes yellowing and extensive drying of the leaves	Infected vegetative cuttings and by aphids
14	Sugarcane bacilliform disease	*Sugarcane bacilliform virus (SCBV)*	Leaves	Appearance of spots or mottles on leaves chlorotic spots or stripes with shrunken leaves. In internodes appearance of cervices, stunted stalks with bunchy tops	Insect vector (*Saccharicoccus sacchari*), infects seedcane
15	Sugarcane streak mosaic virus	*Sugarcane streak mosaic virus (SCSMV)*	Leaf blades, leaf sheaths	Appearance of contrasting shades of green and yellow patches on leaf blades. Leaf redding, necrosis, chlorosis in the leaf base and leaf sheath	Infected stalks, mechanical inoculation
16	Sugarcane mosaic disease	*Sugarcane mosaic virus* (SCMV) and *Sorghum mosaic virus* (SrMV)	Leaves	Appearance of longitudinal short stripes parallel to leaf veins. Stunted stalk growth, less tillering, decrease the amount of sugarcane juice	Infected seedcanes, aphid vectors, also by virus-contaminated cutting knives

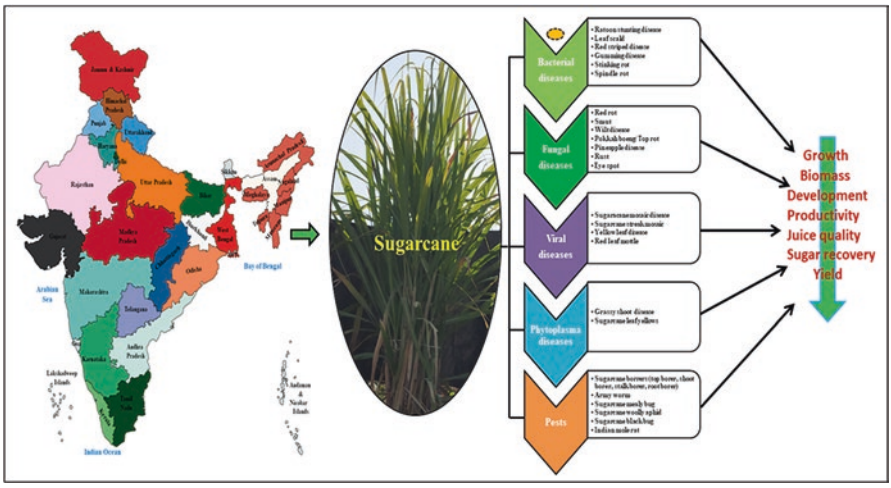

Fig. 7.3 Major sugarcane diseases and pests prevalent in India. The data have been adapted from *V*asantdada *S*ugar *I*nstitute (http://vsisugar.com/india/organisation/index.htm), ICAR-SBI (https://sugarcane.icar.gov.in/index.php/en/), Vikaspedia (http://vikaspedia.in/InDG), EDIS (http://edis.ifas.ufl.edu/), Netafim (http://sugarcanecrops.com/), American Phytopathological Society (http://www.apsnet.org/Pages/default.aspx), AgriFarming (https://www.agrifarming.in/), and International Society for Plant Pathology (http://www.isppweb.org/). (Accessed on 10th February 2019)

particular trait. Furthermore, intergeneric hybridization was among the most useful classical approach. The first successful intergeneric hybrid between *S. officinarum* and *Narenga porphyrocoma* was made by Barber in 1913 (Barber 1996). Few sugarcane linkage maps were shown in Table 7.4. For red rot disease resistant, *S. officinarum* × *Erianthus sara* hybridization was initiated by Rumke in Java (Rumke 1934). At Sugarcane Breeding Institute, Coimbatore, India, the work on intergeneric hybridization involving *S. spontaneum* ($2n$ = 112) and *Erianthus ravennae* ($2n$ = 20) was initiated by Janaki Ammal in 1938 (Janaki-Ammal 1941). However, the process does not imply large changes in the production and still in hope of productivity gains (Sanghera et al. 2017).

During the last three decades, productivity and yield have been increasing at a significant rate. The probable reason is the development of varieties resistant to diseases including smut, wilt, common rust, leaf scald, red rot, etc. through molecular marker-based breeding program and genetic engineering (Xu 2010; Jiang 2013). Molecular markers offer a possibility to study the genetic architecture of quantitative traits in sugarcane, and thus, they are used to speed up artificial selection (Pastina et al. 2012; Sanghera et al. 2017). In addition, the quantitative-trait-based selection is commonly based on information from multi-harvest-location trials (Hoarau et al. 2002; Reffay et al. 2005; Pinto et al. 2010; Pastina et al. 2012).

Table 7.3 Members of genus *Saccharum*

Classification	Species	Chromosome No. (2*n*)	Morphology	Center of origin	Center of diversity	Sugar content	Disease resistance
Wild type	*Saccharum spontaneum*	40–128	The cane is very short and thin, leaves are short and narrow	Colder regions of subtropical India	Widely distributed	Lowest	Yes
	S. robustum	60–200	Thick and long stalk and vigorous growing	New Guinea	New Guinea	Lowest	Yes
Ancient hybrid	*S. barberi*	111–120	Thin stalked	North India	North India	Good	Yes
	S. sinense	80–124	It is thin stalked with long internodes and long and narrow leaves	China	China	Low	No
Noble cane	*S. officinarum*	80	Vigorous and long stalk and low fiber content	New Guinea/ Indo-Burma-China border	New Guinea	Highest	Yes

Table 7.4 List of some publications in the literature regarding linkage maps in sugarcane

Sl. No	No. of population	Cross information	No. of linkage groups	Map length (cM)	Marker distance (cM)	DNA marker types	No of marker	References
1	173 and 168	YC96–40 × HN92–77 × CP84–1198 and YCE01–116 × NJ57–416	38 and 36	1209.7 and 973.9	13.6 and 11	SSR and AFLP	756 and 728	Chen et al. (2015)
2	227	Q165 × IJ76–514	–	9774.4 cM	4.3	DArT, AFLP, SSRs, and SNP	2267	Aitken et al. (2014)
3	188	IAC66-6 × TUC71–7	92	4843.19	8.87	AFLPs, EST-SSRs, and scIvana_1	730	Palhares et al. (2012)
4	300	*S. officinarum* L. × *S. spontaneum*	108	5617	7.16	AFLP, SSR, and TRAP	1111	Andru et al. (2011)
5	100	La Striped × SES 147B	49 and 45	1732 and 1491	12	AFLP, SRAP, and TRAP	344	Alwala et al. (2008)
6	100	SP80-180 × SP80–4966	192	6261.1		RFLP, AFLP, SSR, EST-SSR, and EST-RFLP	2303	Oliveira et al. (2007)
7	100	SP80-180 × SP80–4966	131	2602.4	7.3	RFLP, AFLP, and SSR	1118	Garcia et al. (2006)
8	227	*S. officinarum* × *S. spontaneum*	116	9058.30	–	AFLP, SSR, and RAP	967	Aitken et al. (2005)
9	100	LA Purple × Mol 5829	74 and 65	1881 and 1189	6.65 and 5.74	Arbitrarily primed-PCR, RFLPs, and AFLPs and single-dose DNA markers (SDMs)	341 and 301	Guimarães et al. (1999)
10	90	ADP068 × SES208	44	2107	25	RFLP	216	Silva et al. (1993)

7.3.2 Association Mapping Studies

The identification of markers is associated with traits of interest which depend upon the presence of linkage disequilibrium (LD); attention has been recently focused on determining the extent of LD in large plant populations (Flint-Garcia et al. 2003; Gaut and Long 2003). In addition, LD within the genome depends on mating systems, the structure of the population, admixture, genetic drift, directional selection, and population history (Gaut and Long 2003; Gupta and Rustgi 2004). Since it is propagated vegetatively and combined with the strong founder effect, as a result, it exhibits extensive long-range LD, approximately 10 cM (Jannoo et al. 1999) in spite of its large genome (Henry 2010; Yang et al. 2019). This global disequilibrium is not surprising considering the bottleneck in the breeding history of modern sugarcane cultivars. In the literature, LD in sugarcane was first investigated by Jannoo et al. (1999) using 38 RFLP probes on 59 cultivars in comparison with an RFLP map of a commercial variety. Forty-two cases of locus association among 33 loci were observed. Most of these pairs of loci were separated by less than 10 cM. These studies in sugarcane lay the foundation for association mapping, as LD structure in the genome greatly affects the number and density of markers required, sample size, and many other aspects of study design. However, there are very few reports for targeted gene LD studies in sugarcane (Jannoo et al. 1999; Gupta et al. 2005; Raboin et al. 2008; Gouy et al. 2015; Yang et al. 2019). Due to the limited information on candidate genes, LD studies in sugarcane have focused on genome-wide approaches leading to linkage disequilibrium-based studies (Nordborg and Tavaré 2002; Raboin et al. 2008) assessed LD in 72 sugarcane cultivars using potential AFLP markers technique. A total of 1537 polymorphic markers were surveyed in all the cultivars. Their study highlighted a high level of LD up to 40 cM between AFLP markers among modern sugarcane cultivars.

7.3.3 A Brief Account of Molecular Mapping of Disease Resistance Genes and QTLs

Considering the polyploidy of sugarcane hybrids and the complex properties of their chromosome associations (Jannoo et al. 2004), only single-dose alleles can be readily mapped, with the help of standard methodologies developed for diploid organisms (Sanghera et al. 2017). Various molecular marker systems including RAPD, RFLP, SSR, ESTs, ribosomal RNA, chloroplast, and mitochondrial genes (Glaszmann et al. 1990; Lu et al. 1994; Nair et al. 1999; Cordeiro et al. 2003; Srivastava and Gupta 2008; Virupakshi and Naik 2008; Sanghera et al. 2017) have been reported for analyzing germplasm diversity within the genus *Saccharum*. In addition, ISSR markers have been also used for analyzing the basis of disease resistance in 42 varieties of subtropical India (Srivastava and Gupta 2008). In another study, Virupakshi and Naik (2008) used organellar genome inter-simple sequence repeat markers

(cp ISSR and mt ISSR) to analyze red rot disease-resistant/moderately resistant and susceptible elite sugarcane genotypes.

Furthermore, partial genetic maps have been produced for *S. spontaneum* (Al-Janabi et al. 1993; da Silva et al. 1995; Ming et al. 1998), *S. officinarum* (Mudge et al. 1996; Ming et al. 1998; Guimarães et al. 1999) and modern cultivars (D'Hont et al. 1993; Hoarau et al. 2002; Rossi et al. 2003; Raboin et al. 2006) using different molecular marker technologies (Sanghera et al. 2017).

A series of publications is in the literature regarding linkage mapping of sugarcane (Daugrois et al. 1996; Mudge et al. 1996; Asnaghi et al. 2000; Aitken et al. 2005; Sanghera et al. 2017). A combination approach of direct identification of resistance gene analogs from EST cluster data and de novo PCR from RNA from sugarcane tissues resulted in the determination of map location in sugarcane for 31 RGAs (Sanghera et al. 2017). Even many genes have been identified which play a significant role in disease resistance (Table 7.5.)

In the past century, many varieties were developed with higher yield and high sugar content through breeding approaches; however, combining favorable agronomic traits like high sugar yield and disease resistance is difficult (Sanghera et al. 2017; Thirugnanasambandam et al. 2018). As a result, many QTL studies have been conducted related to other traits in sugarcane (Hoarau et al. 2002; Da Silva and Bressiani 2005; Aljanabi et al. 2007; Aitken et al. 2008; Alwala et al. 2009; Nibouche et al. 2012). However, there were only a few studies which have assessed between markers and traits including resistance to smut, African stalk borer, pachymetra root rot, leaf scald, and Fiji leaf gall (McIntyre et al. 2005; Raboin et al. 2006; Wei et al. 2006; Butterfield 2007).

In a study done by Wei et al. (2006), the linkage between markers and QTL for the disease trait was assessed. They reported very few markers were significant for more than one disease. Furthermore, the number of markers showing association was greatest for smut.

Table 7.5 Few identified disease-resistant genes in sugarcane

Type of disease	Resistant gene	Marker type	Population/cultivar	References
SCMV	Scmv1, Scmv2	RFLP, SSR	Backcross five (BC_5) [FAP1360A (resistant) × F_7 (susceptible)]	Wu et al. (2012)
SCYLV	MB39	SSR	Genotype 6–1 and 6–2 (transformed clones of CP 92–1666)	Gilbert et al. (2009)
SCGS	R2R3-MYB	EST	Co740, Co 62,175	Kawar et al. (2010)
PRR	RGA	RFLP, SSR, AFLP	Q_1 population (Q117 × 74C42)	McIntyre et al. (2005)
Brown rust	Bru1	RFLP, AFLP/BSA	Self-progeny (P_1), population B (R570)	Daugrois et al. (1996), Asnaghi et al. (2004)

7.4 Toward Genetic Engineering

In today's world, there is a boom for gene editing. It refers to any of the processes which enable change/modification of a specific sequence of a chromosome or the targeted DNA in a host genome (Malzahn et al. 2017; Butler et al. 2018). This is accomplished by the utilization of sequence-specific nucleases comprising hybrid DNA/RNA gene repair oligonucleotides, modified meganucleases, ZFNs, TALENs, and the most famous CRISPR/Cas9 system (Mohanta et al. 2017; Yin et al. 2017). In recent years, it has been used for accomplishing reverse genetics, genome engineering, and targeted integration in efficient and precise manner. Due to the genome complexity and low fertility, the conventional breeding methods are very labor-intensive and time-consuming; hence, genetic engineering has become an alternative and useful tool for the production of improved varieties of sugarcane (Bortesi and Fischer 2015; Osakabe et al. 2016; Nerkar et al. 2018). As compared to the other methods, this is considered a user-friendly tool for its ability to generate non-transgenic genome edited crop plants. This method has found its application in a wide range of economically important crops in terms of providing higher yield, high nutritional quality, and weed protection and improving abiotic and biotic stress tolerance (diseases and pests) (Shukla et al. 2009; Li et al. 2012; Sauer et al. 2016; Wang et al. 2016; Aglawe et al. 2018; Jung et al. 2018; Shah et al. 2018). Owing largely to its simplicity, specificity, robustness, cost-effectiveness, and efficiency, the CRISPR/Cas system has surpassed other tools of gene editing like ZFNs and TALENs and has become the most attractive gene editing tool for plant biology (Quétier 2016; Weeks 2017; Zaman et al. 2018).

Like gene editing, there are also other biotechnology tools available including *Agrobacterium* transformation, VIGS, particle bombardment, etc. (Souza et al. 2007; Nayyar et al. 2017; Aslam et al. 2018; Cristofoletti et al. 2018; Gao et al. 2018). However, there are several constraints such as transgene silencing, low transformation efficiency, and time limitations which hinder sugarcane transformation (Mohan 2016). To date, there are few reports in the literature about gene editing for agronomic traits improvement in sugarcane (Jung and Altpeter 2016; Augustine 2017). RNAi is a novel technique for the production of virus-resistant transgenic plants. Guo et al. (2015) reported the production of anti-*Sorghum mosaic virus* transgenic sugarcane plants by using RNAi for suppressing *Sorghum mosaic virus* coat protein gene (SrMV CP). Recently RNAi was applied for the suppression of the *Polyketide* synthase 1 gene (PKS1) in *Colletotrichum falcatum* causal agent of red rot in sugarcane (Scindiya et al. 2018). Also, the expression of the sugarcane mosaic virus coat protein (SCMV CP) gene was downregulated by applying RNAi technology in transgenic sugarcane (Aslam et al. 2018). Furthermore, there are several reports available on the application of microRNAs (miRNAs) as a potential gene regulator in sugarcane. The identification of 19 miRNAs having 46 potential targets involved in the various metabolic process of sugarcane was studied by Zanca et al. (2010). Similarly, Viswanathan et al. (2014) predicted and experimentally validated the targets of sugarcane streak mosaic virus-encoded miRNA in sugarcane.

Ferreira et al. (2012) identified and validated that miR164 and miR399 were associated with drought stress response in sugarcane. The regulatory role of miRNAs in sugarcane in relation to drought tolerance, salinity tolerance, disease resistance, waterlogging, and axillary bud growth has been reviewed by Swapna and Kumar (2017). Thus, the technology of gene silencing holds significant potential for analyzing the functional genes and regulating the gene expression for improving sugarcane productivity.

Furthermore, there are multiple chloroplast genes which play role in disease development; hence, successful chloroplast transformation in sugarcane (Mustafa and Khan 2012) of disease resistance genes is also a good option to enhance disease resistance. Considerable progress that has been achieved in the transformation of sugarcane for inducing resistance genes related to disease resistance is outlined in Table 7.6.

7.5 A Brief Account of the Role of Bioinformatics as a Tool

Sugarcane is the second-last major cultivated crop to have its genome sequenced (Garsmeur et al. 2018); as a result, the sugarcane genomic database was not available until recently. As a result, before 2018, only comparative genomic databases devoted to rice, sorghum, maize, and *Brachypodium distachyon* were used to study the genomic structure of sugarcane (Zhao et al. 2004; Ouyang et al. 2006; Garvin et al. 2008; Liang et al. 2008; Paterson et al. 2009).

Gene expression databases are of two types: (a). Sequence Cluster Databases – Over 250,000 ESTs has been generated from an assortment of sugarcane varieties and tissues (Bower et al. 2005). Private databases organizing EST data were developed by both the SUCEST (Telles et al. 2001) and Australian projects (Casu et al. 2004) to organize project data. The Sugarcane Gene Index is in its second major release (version 2.2), which was re-clustered on 29th July 2008. Input sequences consisted of 255,635 ESTs and 499 mRNAs (all derived from GenBank). The index presents these sequences organized into 40,016 Theoretical Contigs (TCs), 76,529 singleton ESTs, and 43 singleton mRNAs, giving a total of 116,588 unique sequences. Sugarcane sequence clusters have also been produced by the UniGene project at NCBI and by PlantGDB. (b). Transcript expression databases – These have been devised to hold and curate high-throughput experimental data. The Gene Expression Omnibus is a public repository for a variety of macroarray, microarray (both single and dual channel), SAGE, MS-peptide profiling, and quantitative sequence data. Sugarcane high-throughput profiling experiments have so far only been lodged with GEO. At present, 17 experiments have been lodged at GEO, but only one curated dataset is present due to an acknowledged backlog.

Metabolomics studies are in their infancy in sugarcane (Glassop et al. 2007). One example of a possible metabolome database to interact with is the Golm

Table 7.6 Genetic transformation of sugarcane for disease and pest resistance

Genes	Transformation technique	Trait/function tested	Reference
Insect/pest resistance genes			
GNA	*Agrobacterium sp.*	*Ceratovacuna lanigera*	Zhangsun et al. (2007)
Aprotinin	Particle bombardment	Top borer (*Scirpophaga excerptalis*)	Christy et al. (2009)
cry1Ab	Particle bombardment	Shoot borer (*Chilo infuscatellus*) resistance	Arvinth et al. (2010)
Agrobacterium modified cry1Ac	Particle bombardment	Sugarcane stem borer (*Procerasvenosatus*)	Weng et al. (2011)
cry1Ac	Particle bombardment	Sugarcane borer, (*Diatraea saccharalis*)	Gao et al. (2016)
cry1Ab	*Agrobacterium sp.*	Sugarcane borer resistance	Wang et al. (2017)
cry2A	Particle bombardment	Stem borer resistance	Gao et al. (2018)
cry1Ab and cry2Ab	*Agrobacterium sp.*	Sugarcane borer (*Diatraea saccharalis*) resistance	Cristofoletti et al. (2018)
cry1Ac	Particle bombardment	Stem borer (*Diatraea saccharalis*)	Zhou et al. (2018)
Disease resistance genes			
albD	Particle bombardment	Sugarcane leaf scald resistance	Zhang et al. (1999)
FDVS9 ORF 1(*Fiji disease virus* segment 9 ORF 1)	Particle bombardment	*Fiji disease virus* resistance	McQualter et al. (2004)
SrMV CP (coat protein gene of Sorghum mosaic virus (SrMV)	*Agrobacterium sp.*	*Potyvirus sugarcane mosaic virus (SCMV)* and/or *Sorghum mosaic virus (SrMV)* resistance	Guo et al. (2015)
SCMV CP	Particle bombardment	*Sugarcane mosaic virus* (SCMV) resistance	Yao et al. (2017)
β-1,3-glucanase	*Agrobacterium sp.*	Red rot (*Colletotrichum falcatum* Went) resistance	Nayyar et al. (2017)
Barley chitinase II	Particle bombardment	Red rot (*Colletotrichum falcatum* Went) resistance	Tariq et al. (2018)
SCMV CP	Particle bombardment	*Sugarcane mosaic virus* (SCMV) resistance	Aslam et al. (2018)

Metabolome Database (*http://csbdb.mpimp-golm.mpg.de/csbdb/gmd/gmd.html*). This database provides data related to mass spectra libraries, metabolite profiling experiments, and other pertinent data (Kopka et al. 2005). Another possibility is KNApSAcK (*http://kanaya.naist.jp/KNApSAcK/*), a tool for the analysis of metabolites which already contains information pertaining to 17 *Saccharum* genus metabolites. This database emphasizes the biological origins of the compounds contained within it, and the data can be extracted in a variety of ways.

As alluded to above, systems biology requires integrated access to the full spectrum of "omics" data that has been well organized and profiled. Examples are currently restricted to the model plants, especially *A. thaliana*. The best example is TAIR, hosted at *http://www.arabidopsis.org/* (Swarbreck et al. 2008). This resource collects and organizes a wealth of genetic and genomic data and integrates this with information on seed stocks, markers, publication, and information on the *Arabidopsis* research community. This type of data organization extracts maximum benefit from all of the research performed on an organism and will allow for new insights to be more easily gained than if the resources were distributed and not related. Others plant resources integrating various data including genetic and genomics data include Gramene, FLAGdb++, and CSB.DB (Samson et al. 2004; Liang et al. 2008; Steinhauser et al. 2004).

7.6 Brief Account on Social, Political, and Regulatory Issues

The year-wise patenting issue for the sugarcane field indicates that the maximum number of patents is granted to the USA. This may be due to technological advances in the USA research community. However, some countries like India, China, Australia, and the Philippines have less number of patents. In order to increase the yield, it is a crucial time for innovation in the field.

Indian sugar industry provides employment opportunity to nearly 50 million growers, and thus sugarcane farming has a significant role in the agronomy of the country. But the farmers are still facing different problems relating to finance which has made a bad impact on sugarcane farming. The main problem with sugarcane farming is of availability of credit, the problem of apportionment of cost, and resources utilization in an important manner. It is also difficult to measure the financial performance of sugarcane farmers because they do not maintain proper accounting record. There is a need to take proper financial investment decision by the farmers which will increase the economic value of the sugarcane crop and ultimately give benefit to the sugarcane farmers.

Indigenous knowledge systems and informal rural social institutions have contributed a huge role in conservation, management, and sustaining the indigenous biodiversity. Additionally, the indigenous sugarcane varieties have always been found to be more stable in the household economy as well as resource-poor farmers, though less in quantity than the improved varieties. Thus, strengthening the promotion and management policy of location-specific sugarcane varieties will be helpful. In addition, strengthening participatory plant breeding (PPB) programs for sugarcane from breeders to policymakers via consumers will be a good option (Shanthy 2010).

7.7 Future Perspectives

From the traditional times, the main objective for sugarcane breeders had been increasing the sugar yield. However, in accordance with the emergence of new pathovars and races and sustainable society, the focus on disease resistance and biofuels or energy production are gaining much popularity. As a result, sugarcane breeding programs have been reoriented to strengthen the development of new cultivars that fit with this new focus profile. Surely, new germplasm resources will be explored by sugarcane breeders. This even strengthens the efforts to broaden the genetic base of cultivars for enhancing disease resistance in order to increase overall yield, ensuring more durable sugarcane cultivation. With the availability of new genomic resources, genome sequence, advances in molecular biology, and biotechnological and bioinformatic tools, the aim of the research community is toward a better understanding of plant-pathogen interacting genes and mode of interaction and using the knowledge to generate "super sugarcane" that responds to current challenges and future human's needs.

References

Abdel-Halim ES (2014) Chemical modification of cellulose extracted from sugarcane bagasse: preparation of hydroxyethyl cellulose. Arab J Chem 7:362–371

Aglawe SB, Barbadikar KM, Mangrauthia SK, Madhav MS (2018) New breeding technique "genome editing" for crop improvement: applications, potentials and challenges. 3 Biotech 8:336

Ahanger RA, Bhat HA, Bhat TA, Ganie S, Lone AA, Wani IA et al (2013) Impact of climate change on plant diseases. Int J Mod Plant Anim Sci 1:105–115

Aitken KS, Jackson PA, McIntyre CL (2005) A combination of AFLP and SSR markers provides extensive map coverage and identification of homo(eo)logous linkage groups in a sugarcane cultivar. Theor Appl Genet 110:789–801

Aitken K, Hermann S, Karno K, Bonnett G, McIntyre L, Jackson P (2008) Genetic control of yield related stalk traits in sugarcane. Theor Appl Genet 117:1191–1203

Aitken KS, McNeil MD, Hermann S, Bundock PC, Kilian A, Heller-Uszynska K et al (2014) A comprehensive genetic map of sugarcane that provides enhanced map coverage and integrates high-throughput Diversity Array Technology (DArT) markers. BMC Genomics 15:152

Aljanabi SM, Parmessur Y, Kross H, Dhayan S, Saumtally S, Ramdoyal K et al (2007) Identification of a major quantitative trait locus (QTL) for yellow spot (Mycovellosiella koepkei) disease resistance in sugarcane. Mol Breed 19:1–14

Al-Janabi SM, Honeycutt RJ, McClelland M, Sobral B (1993) A genetic linkage map of *Saccharum spontaneum* L.'SES 208'. Genetics 134:1249–1260

Alwala S, Kimbeng CA, Veremis JC, Gravois KA (2008) Linkage mapping and genome analysis in a *Saccharum* interspecific cross using AFLP, SRAP and TRAP markers. Euphytica 164:37–51

Alwala S, Kimbeng CA, Veremis JC, Gravois KA (2009) Identification of molecular markers associated with sugar-related traits in a *Saccharum* interspecific cross. Euphytica 167:127–142

Andru S, Pan Y-B, Thongthawee S, Burner DM, Kimbeng CA (2011) Genetic analysis of the sugarcane (*Saccharum* spp.) cultivar 'LCP 85-384'. I. Linkage mapping using AFLP, SSR, and TRAP markers. Theor Appl Genet 123:77–93

Arencibia AD, Carmona ER, Tellez P, Chan M-T, Yu S-M, Trujillo LE et al (1998) An efficient protocol for sugarcane (*Saccharum* spp. L.) transformation mediated by *Agrobacterium tumefaciens*. Transgenic Res 7:213–222

Arvinth S, Arun S, Selvakesavan RK, Srikanth J, Mukunthan N, Ananda Kumar P et al (2010) Genetic transformation and pyramiding of aprotinin-expressing sugarcane with cry1Ab for shoot borer (*Chilo infuscatellus*) resistance. Plant Cell Rep 29:383–395

Aslam U, Tabassum B, Nasir IA, Khan A, Husnain T (2018) A virus-derived short hairpin RNA confers resistance against sugarcane mosaic virus in transgenic sugarcane. Transgenic Res 27:203–210

Asnaghi C, Paulet F, Kaye C, Grivet L, Deu M, Glaszmann JC et al (2000) Application of synteny across Poaceae to determine the map location of a sugarcane rust resistance gene. Theor Appl Genet 101:962–969

Asnaghi C, Roques D, Ruffel S, Kaye C, Hoarau JY, Télismart H et al (2004) Targeted mapping of a sugarcane rust resistance gene (Bru1) using bulked segregant analysis and AFLP markers. Theor Appl Genet 108:759–764

Augustine SM (2017) CRISPR-Cas9 system as a genome editing tool in sugarcane. In: Mohan C (ed) Sugarcane biotechnology: challenges and prospects. Springer International Publishing, Cham, pp 155–172

Barber CA (1996) Studies in Indian sugarcanes. No. 2. Mem Dept India Bot Ser 8:103–199

Bortesi L, Fischer R (2015) The CRISPR/Cas9 system for plant genome editing and beyond. Biotechnol Adv 33:41–52

Bower NI, Casu RE, Maclean DJ, Reverter A, Chapman SC, Manners JM (2005) Transcriptional response of sugarcane roots to methyl jasmonate. Plant Sci 168:761–772

Butler NM, Jiang J, Stupar MR (2018) Crop improvement using genome editing. In: Goldman I (ed) Plant breeding reviews. WILEY Blackwell, Hoboken, pp 55–102

Butterfield MK (2007) Marker assisted breeding in sugarcane: a complex polyploid. University of Stellenbosch, Stellenbosch

Casu RE, Dimmock CM, Chapman SC, Grof CPL, McIntyre CL, Bonnett GD et al (2004) Identification of differentially expressed transcripts from maturing stem of sugarcane by in silico analysis of stem expressed sequence tags and gene expression profiling. Plant Mol Biol 54:503–517

Chakraborty S, Newton AC (2011) Climate change, plant diseases and food security: an overview. Plant Pathol 60:2–14

Chen J-W, Lao F-Y, Chen X-W, Deng H-H, Liu R, He H-Y et al (2015) DNA marker transmission and linkage analysis in populations derived from a sugarcane (*Saccharum* spp.) x *Erianthus arundinaceus* hybrid. PLoS One 10:e0128865

Christy LA, Arvinth S, Saravanakumar M, Kanchana M, Mukunthan N, Srikanth J et al (2009) Engineering sugarcane cultivars with bovine pancreatic trypsin inhibitor (aprotinin) gene for protection against top borer (*Scirpophaga excerptalis* Walker). Plant Cell Rep 28:175–184

Coakley SM, Scherm H, Chakraborty S (1999) Climate change and plant disease management. Annu Rev Phytopathol 37:399–426

Cordeiro GM, Pan Y-B, Henry RJ (2003) Sugarcane microsatellites for the assessment of genetic diversity in sugarcane germplasm. Plant Sci 165:181–189

Cristofoletti PT, Kemper EL, Capella AN, Carmago SR, Cazoto JL, Ferrari F et al (2018) Development of transgenic sugarcane resistant to sugarcane borer. Trop Plant Biol 11:17–30

D'Hont A, Lu Y, Feldmann P, Glaszmann J-C (1993) Cytoplasmic diversity in sugar cane revealed by heterologous probes. Sugar Cane (United Kingdom)

Da Silva JA, Bressiani JA (2005) Sucrose synthase molecular marker associated with sugar content in elite sugarcane progeny. Genet Mol Biol 28:294–298

da Silva J, Honeycutt RJ, Burnquist W, Al-Janabi SM, Sorrells ME, Tanksley SD et al (1995) Saccharum spontaneum L.'SES 208'genetic linkage map combining RFLP-and PCR-based markers. Mol Breed 1:165–179

Daniels J, Smith P, Paton N, Williams CA (1975) The origin of the genus Saccharum. Sugarcane Breed News 36:24–39

Daugrois JH, Grivet L, Roques D, Hoarau JY, Lombard H, Glaszmann JC et al (1996) A putative major gene for rust resistance linked with a RFLP marker in sugarcane cultivar 'R570'. Theor Appl Genet 92:1059–1064

Dhansu P, Kumar A, Mann A, Kumar R, Meena BL, Sheoran P et al (2018) Insights into biotechnological interventions for sugarcane improvement. In: Sengar K (ed) Biotechnology to enhance sugarcane productivity and stress tolerance. CRC Press, Boca Raton, pp 131–152

Eksomtramagel T, Pauletl F (1992) Development of a cryopreservation process for embryogenic calluses of a commercial hybrid of sugarcane (SACCHARLIM SP.) and application to different varieties. Cryo-Letters 13:239–252

Ferreira TH, Gentile A, Vilela RD, Costa GGL, Dias LI, Endres L et al (2012) microRNAs associated with drought response in the bioenergy crop sugarcane (*Saccharum* spp.). PLoS One 7:e46703

Flint-Garcia SA, Thornsberry JM, Buckler ES IV (2003) Structure of linkage disequilibrium in plants. Annu Rev Plant Biol 54:357–374

Gao S, Yang Y, Wang C, Guo J, Zhou D, Wu Q et al (2016) Transgenic sugarcane with a cry1Ac gene exhibited better phenotypic traits and enhanced resistance against sugarcane borer. PLoS One 11:e0153929

Gao S, Yang Y, Xu L, Guo J, Su Y, Wu Q et al (2018) Particle bombardment of the cry2A gene cassette induces stem borer resistance in sugarcane. Int J Mol Sci 19:1692

Garcia AAF, Kido EA, Meza AN, Souza HMB, Pinto LR, Pastina MM et al (2006) Development of an integrated genetic map of a sugarcane (*Saccharum* spp.) commercial cross, based on a maximum-likelihood approach for estimation of linkage and linkage phases. Theor Appl Genet 112:298–314

Garsmeur O, Droc G, Antonise R, Grimwood J, Potier B, Aitken K et al (2018) A mosaic monoploid reference sequence for the highly complex genome of sugarcane. Nat Commun 9:2638

Garvin DF, Gu Y-Q, Hasterok R, Hazen SP, Jenkins G, Mockler TC et al (2008) Development of genetic and genomic research resources for Brachypodium distachyon, a new model system for grass crop research. Crop Sci 48:69–84

Gaut BS, Long AD (2003) The lowdown on linkage disequilibrium. Plant Cell 15:1502

Ghini R, Hamada E, Bettiol W (2008) Climate change and plant diseases. Sci Agric 65:98–107

Ghose AK, Kuasha M, Razzak MA, Islam MJ, Rahman MA, Hossain MA (2016) Genetic diversity analysis of sugarcane genotypes by SSR markers. Fundam Appl Agric 1:112–117

Gilbert RA, Glynn NC, Comstock JC, Davis MJ (2009) Agronomic performance and genetic characterization of sugarcane transformed for resistance to sugarcane yellow leaf virus. Field Crop Res 111:39–46

Glassop D, Roessner U, Bacic A, Bonnett GD (2007) Changes in the sugarcane metabolome with stem development. Are they related to sucrose accumulation? Plant Cell Physiol 48:573–584

Glaszmann J-C, Lu Y, Lanaud C (1990) Variation of nuclear ribosomal DNA in sugarcane. J Genet Breed 44:191–197

Gouy M, Rousselle Y, Thong Chane A, Anglade A, Royaert S, Nibouche S et al (2015) Genome wide association mapping of agro-morphological and disease resistance traits in sugarcane. Euphytica 202:269–284

Guimarães CT, Honeycutt RJ, Sills GR, Sobral BW (1999) Genetic maps of Saccharum officinarum L. and Saccharum robustum Brandes & Jew. ex grassl. Genet Mol Biol 22:125–132

Guo J, Gao S, Lin Q, Wang H, Que Y, Xu L (2015) Transgenic sugarcane resistant to Sorghum mosaic virus based on coat protein gene silencing by RNA interference. Biomed Res Int 2015:861907

Gupta PK, Rustgi S (2004) Molecular markers from the transcribed/expressed region of the genome in higher plants. Funct Integr Genomics 4:139–162

Gupta PK, Rustgi S, Kulwal PL (2005) Linkage disequilibrium and association studies in higher plants: present status and future prospects. Plant Mol Biol 57:461–485

Henry JR (2010) Basic information on the sugarcane plant. CRC Press, Boca Raton
Hoarau J-Y, Grivet L, Offmann B, Raboin L-M, Diorflar J-P, Payet J et al (2002) Genetic dissection of a modern sugarcane cultivar (Saccharum spp.). II. Detection of QTLs for yield components. Theor Appl Genet 105:1027–1037
Huang Y-K, Li W-F, Zhang R-Y, Wang X-Y (2018) Diagnosis and control of sugarcane important diseases. In: Huang Y-K, Li W-F, Zhang R-Y, Wang X-Y (eds) Color illustration of diagnosis and control for modern sugarcane diseases, pests, and weeds. Springer Singapore, Singapore, pp 1–103
Janaki-Ammal E (1941) Intergeneric hybrids of Saccharum. J Genet 41:217–253
Jannoo N, Grivet L, Dookun A, D'Hont A, Glaszmann JC (1999) Linkage disequilibrium among modern sugarcane cultivars. Theor Appl Genet 99:1053–1060
Jannoo N, Grivet L, David J, D'Hont A, Glaszmann J-C (2004) Differential chromosome pairing affinities at meiosis in polyploid sugarcane revealed by molecular markers. Heredity 93:460
Jiang GL (2013) Molecular markers and marker-assisted breeding in plants. In: Plant breeding from laboratories to fields. IntechOpen. Available at https://www.intechopen.com/books/plant-breeding-from-laboratories-to-fields/molecular-markers-and-marker-assisted-breeding-in-plants
Jung JH, Altpeter F (2016) TALEN mediated targeted mutagenesis of the caffeic acid O-methyltransferase in highly polyploid sugarcane improves cell wall composition for production of bioethanol. Plant Mol Biol 92:131–142
Jung C, Capistrano-Gossmann G, Braatz J, Sashidhar N, Melzer S (2018) Recent developments in genome editing and applications in plant breeding. Plant Breed 137:1–9
Kawar PG, Pagariya MC, Dixit GB, Prasad DT (2010) Identification and isolation of SCGS phytoplasma-specific fragments by riboprofiling and development of specific diagnostic tool. J Plant Biochem Biotechnol 19:185–194
Kopka J, Schauer N, Krueger S, Birkemeyer C, Usadel B, Bergmüller E et al (2005) GMD@CSB. DB: the Golm metabolome database. Bioinformatics 21:1635–1638
Le Cunff L, Garsmeur O, Raboin L-M, Pauquet J, Telismart H, Selvi A et al (2008) Diploid/polyploid syntenic shuttle mapping and haplotype-specific chromosome walking toward a rust resistance gene (Bru1) in highly polyploid sugarcane (2n≈ 12x≈ 115). Genetics 180:649
Li T, Liu B, Spalding MH, Weeks DP, Yang B (2012) High-efficiency TALEN-based gene editing produces disease-resistant rice. Nat Biotechnol 30:390
Liang C, Jaiswal P, Hebbard C, Avraham S, Buckler ES, Casstevens T et al (2008) Gramene: a growing plant comparative genomics resource. Nucleic Acids Res 36:D947–D953
Lu Y, D'Hont A, Walker D, Rao P, Feldmann P, Glaszmann J-C (1994) Relationships among ancestral species of sugarcane revealed with RFLP using single copy maize nuclear probes. Euphytica 78:7–18
Malzahn A, Lowder L, Qi Y (2017) Plant genome editing with TALEN and CRISPR. Cell Biosci 7:21–21
McIntyre CL, Whan VA, Croft B, Magarey R, Smith GR (2005) Identification and validation of molecular markers associated with pachymetra root rot and brown rust resistance in sugarcane using map- and association-based approaches. Mol Breed 16:151–161
McQualter RB, Dale JL, Hardin RH, McMahon JA, Smith GR (2004) Production and evaluation of transgenic sugarcane containing a Fiji disease virus (FDV) genome segment S9- derived synthetic resistance gene. Aust J Agric Res 55:139–145
Ming R, Liu S-C, Lin Y-R, Da Silva J, Wilson W, Braga D et al (1998) Detailed alignment of Saccharum and Sorghum chromosomes: comparative organization of closely related diploid and polyploid genomes. Genetics 150:1663–1682
Mohan C (2016) Genome editing in sugarcane: challenges ahead. Front Plant Sci 7:1542–1542
Mohanta TK, Bashir T, Hashem A, Abd Allah EF, Bae H (2017) Genome editing tools in plants. Genes 8:399
Mudge J, Andersen WR, Kehrer RL, Fairbanks DJ (1996) A RAPD genetic map of Saccharum officinarum. Crop Sci 36:1362–1366

Mustafa G, Khan MS (2012) Prospecting the utility of antibiotics as lethal selection agents for chloroplast transformation in sugarcane. Int J Agric Biol 14:307–310

Nair NV, Nair S, Sreenivasan TV, Mohan M (1999) Analysis of genetic diversity and phylogeny in Saccharum and related genera using RAPD markers. Genet Resour Crop Evol 46:73–79

Nayyar S, Sharma BK, Kaur A, Kalia A, Sanghera GS, Thind KS et al (2017) Red rot resistant transgenic sugarcane developed through expression of β-1,3-glucanase gene. PLoS One 12:e0179723

Nerkar G, Thorat A, Sheelavantmath S, Kassa HB, Devarumath R (2018) Genetic transformation of sugarcane and field performance of transgenic sugarcane. In: Gosal SS, Wani SH (eds) Biotechnologies of crop improvement, volume 2: transgenic approaches. Springer International Publishing, Cham, pp 207–226

Nibouche S, Tibère R, Costet L (2012) The use of Erianthus arundinaceus as a trap crop for the stem borer Chilo sacchariphagus reduces yield losses in sugarcane: preliminary results. Crop Prot 42:10–15

Nordborg M, Tavaré S (2002) Linkage disequilibrium: what history has to tell us. Trends Genet 18:83–90

Oliveira KM, Pinto LR, Marconi TG, Margarido GRA, Pastina MM, Teixeira LHM et al (2007) Functional integrated genetic linkage map based on EST-markers for a sugarcane (*Saccharum* spp.) commercial cross. Mol Breed 20:189–208

Osakabe Y, Watanabe T, Sugano SS, Ueta R, Ishihara R, Shinozaki K et al (2016) Optimization of CRISPR/Cas9 genome editing to modify abiotic stress responses in plants. Sci Rep 6:26685–26685

Ouyang S, Zhu W, Hamilton J, Lin H, Campbell M, Childs K et al (2006) The TIGR rice genome annotation resource: improvements and new features. Nucleic Acids Res 35:D883–D887

Pachauri RK, Allen MR, Barros VR, Broome J, Cramer W, Christ R et al. 2014. Climate change 2014: synthesis report. Contribution of Working Groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change. IPCC

Palhares AC, Rodrigues-Morais TB, Van Sluys M-A, Domingues DS, Maccheroni W Jr, Jordão H Jr et al (2012) A novel linkage map of sugarcane with evidence for clustering of retrotransposon-based markers. BMC Genet 13:51–51

Pandiyan M, Senthil N, Packiaraj D, Jagadeesh S (2012) Greengram germplasm for constituting of core collection. Wudpecker J Agric Res 1:223–232

Pastina M, Malosetti M, Gazaffi R, Mollinari M, Margarido G, Oliveira K et al (2012) A mixed model QTL analysis for sugarcane multiple-harvest-location trial data. Theor Appl Genet 124:835–849

Paterson AH, Bowers JE, Bruggmann R, Dubchak I, Grimwood J, Gundlach H et al (2009) The Sorghum bicolor genome and the diversification of grasses. Nature 457:551

Pinto LR, Garcia AAF, Pastina MM, Teixeira LHM, Bressiani JA, Ulian EC et al (2010) Analysis of genomic and functional RFLP derived markers associated with sucrose content, fiber and yield QTLs in a sugarcane (*Saccharum* spp.) commercial cross. Euphytica 172:313–327

Quétier F (2016) The CRISPR-Cas9 technology: closer to the ultimate toolkit for targeted genome editing. Plant Sci 242:65–76

Raboin LM, Oliveira KM, Lecunff L, Telismart H, Roques D, Butterfield M et al (2006) Genetic mapping in sugarcane, a high polyploid, using bi-parental progeny: identification of a gene controlling stalk colour and a new rust resistance gene. Theor Appl Genet 112:1382–1391

Raboin L-M, Pauquet J, Butterfield M, D'Hont A, Glaszmann J-C (2008) Analysis of genome-wide linkage disequilibrium in the highly polyploid sugarcane. Theor Appl Genet 116:701–714

Reffay N, Jackson PA, Aitken KS, Hoarau J-Y, D'Hont A, Besse P et al (2005) Characterisation of genome regions incorporated from an important wild relative into Australian sugarcane. Mol Breed 15:367–381

Régnière J (2011) Invasive species, climate change and forest health. Forests in development: a vital balance. Springer, Dordrecht, pp 27–37

Rossi M, Araujo PG, Paulet F, Garsmeur O, Dias VM, Chen H et al (2003) Genomic distribution and characterization of EST-derived resistance gene analogs (RGAs) in sugarcane. Mol Gen Genomics 269:406–419

Rott P (ed) (2000) A guide to sugarcane diseases. Editions Quae. Available at https://books.google.co.in/books?hl=en&lr=&id=W4g5NXRvpZ8C&oi=fnd&pg=PA9&dq=rott+et+al.+2000+diseases+of+sugarcane&ots=tzq6gcShRf&sig=Qji0CGBTv0Jd8FCV2pPtUMD2Oa8#v=onepage&q=rott%20et%20al.%202000%20diseases%20of%20sugarcane&f=false

Rumke C (1934) Saccharum-Erianthus bastardan. Arch Suik Ned Indie 42:211–261

Samson F, Brunaud V, Duchêne S, De Oliveira Y, Caboche M, Lecharny A et al (2004) FLAGdb++: a database for the functional analysis of the *Arabidopsis* genome. Nucleic Acids Res 32:D347–D350

Sanghera GS, Singh RP, Tyagi V, Thind KS (2017) Recent genomic approaches for sugarcane improvement: opportunities and challenges. In: Quality and quantum improvement in field crops, pp 109–152

Sauer NJ, Mozoruk J, Miller RB, Warburg ZJ, Walker KA, Beetham PR et al (2016) Oligonucleotide-directed mutagenesis for precision gene editing. Plant Biotechnol J 14:496–502

Scindiya M, Malathi P, Kaverinathan K, Ramesh Sundar A, Viswanathan R (2018) RNA-mediated silencing of PKS1 gene in *Colletotrichum falcatum* causing red rot in sugarcane. Eur J Plant Pathol 153:371. https://doi.org/10.1007/s10658-018-1563-z

Sengar KE (2018) Biotechnology to enhance sugarcane productivity and stress tolerance. CRC Press, Boca Raton. https://doi.org/10.1201/9781315152776

Shah T, Andleeb T, Lateef S, Noor MA (2018) Genome editing in plants: advancing crop transformation and overview of tools. Plant Physiol Biochem 131:12–21

Shanthy RT (2010) Participatory varietal selection in sugarcane. Sugar Tech 12:1–4

Shukla VK, Doyon Y, Miller JC, DeKelver RC, Moehle EA, Worden SE et al (2009) Precise genome modification in the crop species *Zea mays* using zinc-finger nucleases. Nature 459:437

Silva JAGD, Sorrells ME, Burnquist WL, Tanksley SD (1993) RFLP linkage map and genome analysis of *Saccharum spontaneum*. Genome 36:782–791

Solomon S (2011) Sugarcane by-products based Industries in India. Sugar Tech 13:408–416

Solomon S (2014) Sugarcane agriculture and sugar industry in India: at a glance. Sugar Tech 16:113–124

Solomon S, Li Y-R (2016) Editorial-the sugar industry of asian region. Sugar Tech 18:557–558

Souza AJ, Mendes BMJ, Mourão Filho FDAA (2007) Gene silencing: concepts, applications, and perspectives in woody plants. Sci Agric 64:645–656

Srivastava S, Gupta PS (2008) Inter simple sequence repeat profile as a genetic marker system in sugarcane. Sugar Tech 10:48–52

Steinhauser D, Usadel B, Luedemann A, Thimm O, Kopka J (2004) CSB.DB: a comprehensive systems-biology database. Bioinformatics 20:3647–3651

Swapna M, Kumar S (2017) microRNAs and their regulatory role in sugarcane. Front Plant Sci 8:997–997

Swarbreck D, Wilks C, Lamesch P, Berardini TZ, Garcia-Hernandez M, Foerster H et al (2008) The Arabidopsis information resource (TAIR): gene structure and function annotation. Nucleic Acids Res 36:D1009–D1014

Tariq M, Khan A, Tabassum B, Toufiq N, Bhatti MU, Riaz S et al (2018) Antifungal activity of chitinase II against Colletotrichum falcatum Went. causing red rot disease in transgenic sugarcane. Turk J Biol 42:45

Telles GP, Braga MDV, Dias Z, Tzy-Li L, Quitzau JAA, Silva FRD et al (2001) Bioinformatics of the sugarcane EST project. Genet Mol Biol 24:9–15

Thirugnanasambandam PP, Hoang NV, Henry RJ (2018) The challenge of analyzing the sugarcane genome. Front Plant Sci 9:616

Virupakshi S, Naik G (2008) ISSR analysis of chloroplast and mitochondrial genome can indicate the diversity in sugarcane genotypes for red rot resistance. Sugar Tech 10:65–70

Viswanathan R, Rao GP (2011) Disease scenario and management of major sugarcane diseases in India. Sugar Tech 13:336–353

Viswanathan C, Anburaj J, Prabu G (2014) Identification and validation of sugarcane streak mosaic virus-encoded microRNAs and their targets in sugarcane. Plant Cell Rep 33(2):265–276

Wang F, Wang C, Liu P, Lei C, Hao W, Gao Y et al (2016) Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene OsERF922. PLoS One 11:e0154027

Wang WZ, Yang BP, Feng XY, Cao ZY, Feng CL, Wang JG et al (2017) Development and characterization of transgenic sugarcane with insect resistance and herbicide tolerance. Front Plant Sci 8:1535–1535

Weeks DP (2017) Gene editing in polyploid crops: wheat, camelina, canola, potato, cotton, peanut, sugar cane, and citrus. In: Progress in molecular biology and translational science, vol 149. Academic Press, pp 65–80. Available at https://www.sciencedirect.com/science/article/pii/S1877117317300686

Wei X, Jackson PA, McIntyre CL, Aitken KS, Croft B (2006) Associations between DNA markers and resistance to diseases in sugarcane and effects of population substructure. Theor Appl Genet 114:155–164

Weng L-X, Deng H-H, Xu J-L, Li Q, Zhang Y-Q, Jiang Z-D et al (2011) Transgenic sugarcane plants expressing high levels of modified cry1Ac provide effective control against stem borers in field trials. Transgenic Res 20:759–772

Wu L, Zu X, Wang S, Chen Y (2012) Sugarcane mosaic virus – Long history but still a threat to industry. Crop Prot 42:74–78

Xu Y (2010) Molecular plant breeding. Cabi

Yadav RL, Solomon S (2006) Potential of developing sugarcane by-product based industries in India. Sugar Tech 8:104–111

Yang X, Song J, Todd J, Peng Z, Paudel D, Luo Z et al (2019) Target enrichment sequencing of 307 germplasm accessions identified ancestry of ancient and modern hybrids and signatures of adaptation and selection in sugarcane (*Saccharum* spp.), a 'sweet' crop with 'bitter' genomes. Plant Biotechnol J 17:488–498

Yao W, Ruan M, Qin L, Yang C, Chen R, Chen B et al (2017) Field performance of transgenic sugarcane lines resistant to sugarcane mosaic virus. Front Plant Sci 8:104–104

Yin K, Gao C, Qiu J-L (2017) Progress and prospects in plant genome editing. Nat Plants 3:17107

You Q, Xu L, Zheng Y, Que Y (2013) Genetic diversity analysis of sugarcane parents in Chinese breeding programmes using gSSR markers. Sci World J 2013:1–11

Zaman QU, Li C, Cheng H, Hu Q (2018) Genome editing opens a new era of genetic improvement in polyploid crops. Crop J 7(2, April 2019):141–150

Zanca AS, Vicentini R, Ortiz-Morea FA, Del Bem LEV, da Silva MJ, Vincentz M et al (2010) Identification and expression analysis of microRNAs and targets in the biofuel crop sugarcane. BMC Plant Biol 10:260

Zhang L, Xu J, Birch RG (1999) Engineered detoxification confers resistance against a pathogenic bacterium. Nat Biotechnol 17:1021

Zhangsun D, Luo S, Chen R, Tang K (2007) Improved agrobacterium-mediated genetic transformation of GNA transgenic sugarcane. Biologia 62:386

Zhao W, Wang J, He X, Huang X, Jiao Y, Dai M et al (2004) BGI-RIS: an integrated information resource and comparative analysis workbench for rice genomics. Nucleic Acids Res 32:D377–D382

Zhou D, Liu X, Gao S, Guo J, Su Y, Ling H et al (2018) Foreign cry1Ac gene integration and endogenous borer stress-related genes synergistically improve insect resistance in sugarcane. BMC Plant Biol 18:342–342

Chapter 8
Molecular Breeding for Resistance to Economically Important Diseases of Pulses

Parmeshwar K. Sahu, Vinod J. Dhole, and Suvendu Mondal

Abbreviations

AFLP	Amplified Fragment Length Polymorphism
BC	Backcross
BSA	Bulked Segregant Analysis
DAF	DNA Amplification Fingerprinting
DArT	Diversity Arrays Technologies
DHL	Doubled Haploid Lines
ELISA	Enzyme-Linked Immunosorbent Assay
EST	Expressed Sequence Tags
GWAS	Genome-Wide Association Studies
ICRISAT	International Crops Research Institute for the Semi-Arid Topics
InDel	Insertion-Deletion
ISSR	Inter Simple Sequence Repeat
MAB	Marker-Assisted Breeding
MABC	Marker-Assisted Backcrossing
MAGIC Population	Multiparent Advanced Generation Intercross Population
MAS	Marker-Assisted Selection

P. K. Sahu
Department of Genetics and Plant Breeding, Indira Gandhi Krishi Viswavidyalaya, Raipur, Chhattisgarh, India

V. J. Dhole
Nuclear Agriculture & Bio Technology Division, Bhabha Atomic Research Centre, Mumbai, Maharashtra, India

S. Mondal (✉)
Nuclear Agriculture & Bio Technology Division, Bhabha Atomic Research Centre, Mumbai, Maharashtra, India

Homi Bhabha National Institute, Training School Complex, Mumbai, Maharashtra, India

S. H. Wani (ed.), *Disease Resistance in Crop Plants*,
https://doi.org/10.1007/978-3-030-20728-1_8

NAM Population	Nested Association Mapping Population
NIL	Near-Isogenic Lines
PCR	Polymerase Chain Reaction
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RGC	Resistance Gene Candidates
RIL	Recombinant Inbred Lines
SCAR	Sequence-Characterized Amplified Region
SNP	Single Nucleotide Polymorphism
SRAP	Sequence-Related Amplified Polymorphism
SSR	Simple Sequence Repeat
STMS	Sequence-Tagged Microsatellite Sites
STS	Sequence-Tagged Sites
TRAP	Target Region Amplification Polymorphism

8.1 Introduction

Plants are continuously threatened by various pathogens in the environment. In natural condition, some of the wild plants have intrinsic resistance power which helps them to resist such attack for survival (Tanksley and McCouch 1997; Maxted and Kell 2009). Whenever plants got domesticated and further improved for yield attributing traits by humans, they gradually lost the resistance and hence became vulnerable to pathogen attacks (Warschefsky et al. 2014). Even though they contain certain resistance genes, the genetic resistance can also be overcome by the newly evolved strains of pathogen. This continuous co-evolution phenomenon between crop plants and their pathogen demands sustainable plant breeding efforts to generate newer crop varieties or to pyramid resistance genes in well-adapted varieties (Mundt 2014). Another concern is the expected increase in climatic variability (IPCC 2012), which could enhance the occurrence of pathogens in a given locality. Host plant resistance is generally the most favorable control method for environmental, economical, and social reasons (Mundt 2014). Conventional plant breeding method has helped till now to tackle this problem. But demand for newer resistant crop varieties has to be made within a short time frame. Molecular breeding or marker-assisted breeding (MAB) has ample potential to ease such problem and tackle it in a more efficient manner within a shorter time period than conventional breeding (Varshney et al. 2014a, b). Moreover, the selection of resistant plants can easily be achieved without the disease occurrence in the field in MAB. Marker-assisted gene pyramiding is a method of choice for gene stacking within an adapted variety without the need of screening against multiple races of pathogen in different environments.

Pulses are important food crops that balance our diet and are the main principal protein source for the semi-arid topical region of the world. In farming system, pulses are very important crop for restoration of soil fertility and maintenance of

soil health (FAO 2016). Among the major pulses grown in the world, chickpea (*Cicer arietinum* L.), pigeon pea [*Cajanus cajan* (L.) Millsp.], mung bean [*Vigna radiata* (L.) Wilczek], black gram [*Vigna mungo* (L.) Hepper], cowpea [*Vigna unguiculata* (L.) Walp.], lentil (*Lens culinaris* Medik.), pea (*Pisum sativum* L.), and common bean (*Phaseolus vulgaris* L.) are the important sources of protein for humans. Based on their climatic condition for growth, they are divided into tropical pulse crops (pigeon pea, mung bean, urd bean, cowpea, common bean, etc.) and temperate pulse crops (chickpea, lentil, pea, etc.). These pulses are damaged by several plant pathogens that include virus, bacteria, fungus, and pathogenic weed species. Of them, yellow vein mosaic virus is a common problem for tropical legumes like mung bean, urd bean, and cowpea. Both chickpea and lentil are heavily damaged by *Ascochyta* blight. *Fusarium* wilt is a common problem for both chickpea and pigeon pea. Sterility mosaic by a virus is an endemic problem in pigeon pea cultivation in subtropics. To rescue the pulse production from these plant pathogens, the development of resistant cultivars in the above pulse crops is an immediate need. Recent genome sequencing initiative in major pulse crop has generated immense marker data and molecular breeding or genomics platform. The usage of those has helped to generate fewer improved varieties and has great scope in future toward the development of disease-resistant cultivars of pulses. We will discuss here on recent developments and progress on molecular breeding for disease resistance in these pulse crops.

8.2 Development of Molecular Markers in Pulse Crops

DNA-based markers have shown great promises in expediting plant breeding methods. At the present time, exploitation of genetic markers is not a dream to a traditional plant breeder since it is used worldwide in all major cereal crops as a component of plant breeding because of the availability of a large amount of basic genetic and genomic resources (Gupta et al. 2010). In the past few years, major emphasis has also been given to develop similar kind of genomic resources for improving productivity and developing resistance for diseases of pulse crops (Varshney et al. 2009). The use of molecular marker technology can give real output in terms of high-yielding genotypes in pulses because high phenotypic instability for important traits makes them difficult for improvement through conventional breeding methods.

8.2.1 Establishment of Mapping Population

In genetics and breeding, mapping populations are the tools used to identify the genetic loci controlling measurable phenotypic traits. Mapping population is a group of individuals on which genetic analysis is carried out. The decisions on the selection of parents and mating design used for the development of a mapping

population depend mainly on the objectives of the study. The parents of mapping populations must have sufficient variation for the traits of interest at both the DNA sequence and the phenotype level. Mapping population may comprise F_2, backcross (BC), recombinant inbred lines (RIL), doubled haploid lines (DHL), F_2 derived F_3 (F_2:F_3) populations, and near-isogenic lines (NILs). F_2, backcross, and recombinant inbred are the three primary types of mapping populations used for molecular mapping of any traits. In other cases, DHLs are also the products of one meiotic cycle and hence comparable to F_2 in terms of recombination information. DHLs are permanent mapping population and hence can be replicated and evaluated over locations and years and maintained without any genetic change like in RIL. It provides opportunity to induce homozygosity in single generation and instant production of homozygous lines. Since it involves in vitro techniques, relatively more technical skills are required in comparison with the development of other mapping populations. Till now, suitable culturing methods, organogenesis, and haploid production methods are not available for most of the pulse crops, and hence, successful production of DHLs in this crop is not reported much.

A mapping population is essential to develop tightly linked molecular markers for disease resistance gene in any crops. To develop a mapping population, two diverse genotypes should be crossed to each other, and at the same time, they should not be too genetically distant so as to a) cause sterility of the progenies and/or b) show very high levels of segregation distortion during linkage analysis. Thus, wide hybridizations (interspecific or intraspecific crosses) are needed. For example, a chickpea RIL population was made by crossing *Cicer arietinum* x *Cicer reticulatum* (Ratnaparkhe et al. 1998; Winter et al. 2000; Cobos et al. 2005). Similarly *Cicer arietinum* and *Cicer echinospermum* were crossed to produce a mapping population for identification of marker for *Ascochyta* blight resistance (Collard et al. 2003). Eujayl et al. (1998) used an RIL population to identify molecular markers linked to the single dominant gene conditioning *Fusarium* vascular wilt resistance, while Gupta et al. (2008) has developed an RIL population in black gram by crossing a cultivated black gram (*Vigna mungo*) variety with *Vigna mungo* var. *silvestris* for the development of first linkage map in this crop. Sometimes two morphologically distinct genotypes can also be crossed to produce a population like in Desi × Kabuli (Iruela et al. 2006; Tar'an et al. 2007) or Desi × Desi (Radhika et al. 2007) crosses in chickpea. Among the various mapping populations, F_2 population (Bohra et al. 2012), BC population (Skiba et al. 2004; Kongjaimun et al. 2012), and RIL population (Dhole and Reddy 2013; Bhadauria et al. 2017) were much used in pulses. Pulses are generally self-pollinated crops, and thus, artificial hybridization is needed to develop above kind of population for genetic and QTL mapping. The usage of association mapping population is another way to reveal high resolution markers for *Aphanomyces* root rot disease resistance in pea (Dasgroux et al. 2016), *Fusarium* root rot in pigeon pea (Patil et al. 2017), and anthracnose and angular leaf spot resistance in common bean (Perseguini et al. 2016). In a recent example, high resolution mapping for *Ascochyta* blight resistance in pea was achieved by using a heteroge-

neous inbred family's population (Jha et al. 2017). The use of MAGIC and NAM population is another way to capture panoramic view of genetic factors affecting the disease resistance in different pulse cops (Jha et al. 2017).

8.2.2 Development of Genetic Maps

A genetic map, or linkage map, is a map of the frequencies of recombination that occurs between tested markers on homologous chromosomes during meiosis. Recombination frequency between two markers is proportional to the distance separating the markers. The greater is the frequency of recombination, the greater is the distance between two genetic markers. Thus, a genetic map is a depiction of recombination events and frequencies, rather than a physical map. An appropriate mapping population, a suitable marker system, and the software for analyses of data are the key requirements for constructing a genetic linkage map. Genetic map construction requires (i) selection of the most appropriate mapping population(s), (ii) calculation of pairwise recombination frequencies using the population, (iii) establishment of linkage groups and estimation of map distances, and (iv) determination of map order.

Genetic maps are the prerequisite for the identification of linked markers or QTLs for a particular disease resistance in any crops except in association mapping. Projects on development of genetic maps of pulses had started during the 1990s. The first genetic linkage map on pulse crop was made during the 1990s. Havey and Muehlbauer (1989) developed a genetic linkage map of lentil followed by the genetic map of garden pea (Weeden and Wolko 1990). Later, an integrated genetic map was made in chickpea by Simon and Muehlbauer (1997). These maps were later improved by incorporation of new markers in them although the genetic linkage map targeted for tagging of disease resistance gene was started later. Genetic maps targeting for *Ascochyta* blight in chickpea were developed in different years by several scientists (Lichtenzveig et al. 2006; Tar'an et al. 2007; Sabbavarapu et al. 2013). Similarly, transcriptome sequencing studies of lentil have generated an EST database which has delivered large numbers of EST-derived SSR and SNP markers (Kaur et al. 2014). These sequences-derived marker systems have been used to construct dense genetic linkage maps and to identify QTLs for disease resistance in the past few years (Kaur et al. 2014). Further sequence-linked genetic markers facilitated the identification of bridging loci between population specific genetic maps and subsequent integration to produce high-density consensus maps in lentil (Sudheesh et al. 2016). Molecular maps were also developed in cowpea by using various markers like RFLP (Young 1999), AFLP and RAPD (Ouedraogo et al. 2002), SNP (Xu et al. 2011; Muchero et al. 2009; Lucas et al. 2011), and SSR (Anadrgie et al. 2011; Kongjaimum et al. 2012) either in F_2 or RIL populations.

8.2.3 Screening for Disease Resistance

Screening of plants for a particular disease resistance could be achieved by field screening, green house screening, laboratory screening, and bioassay techniques. Since in actual field situation different strains/races of plant pathogens are present, it is wise to screen disease resistance of plant based on multi-environment field screening. It gives an idea about the reaction of plant genotypes to a particular disease in a particular environment, and often, plants with horizontal resistance against a particular disease got isolated through this technique. Thus, plant breeders mostly follow this screening technique in disease resistance breeding scheme. In actual field conditions, a disease was evaluated based on various disease scales (depending on the plant and its type of disease). The disease scale was normally developed by the plant pathologist, and it was based on the percent disease incidence of plant (Reddy et al. 1994). For uniform pathogen distribution around field, infector row (for aerial pathogens) or sick plots (for soil-borne pathogens) must be there in the field-based screening (Rana et al. 2013). Eujayl et al. (1998) demonstrated the use of sick plot technique for screening of mapping population of lentil against *Fusarium* wilt disease toward the study of its genetics and marker development.

But often, field-based disease screening gives error-prone result due to complex interaction of host, pathogen, and environment. The actual susceptible plant may escape the disease symptoms, and hence, interpretation may be wrong. In sick plots or field, there will be always a risk that multiple soil-borne diseases could be present at the same time and interfere with the disease assessment. To meet out this problem, one can go for screening for disease resistance under controlled conditions, i.e., greenhouse or laboratory conditions (Infantino et al. 2006). In such cases, green house screening or laboratory screening or bioassay-based technique is followed. To do so, disease inoculum must be mass multiplied in the laboratory, and certain amount of inoculum should be either injected or sprayed to the plants in congenial weather condition inside the green house or in the laboratory. A mini-dome technique (Chen and Muehlbauer 2003) was used to measure pathogenic variation of different isolates of *Didymella rabiei* for *Ascochyta* blight disease of chickpea by spraying 2×10^5 pycnidiospores ml^{-1} over the plants (Chen et al. 2004), whereas "cloth chamber screening technique" was followed to screen different accession of wild *Cicer* species against *Ascochyta* blight (Gurha et al. 2003). Some of the obligate pathogens may not be culturable, and thus, they cannot be mass multiplied easily. In such cases, a disease should be maintained in susceptible host throughout the year, and infector rows should be maintained in the green house or laboratory condition for spreading of the disease. Another easy protocol called excised/detached leaf technique was also used in mung bean (Reddy et al. 1987) and pea (Warkentin et al. 1995; Fondevilla et al. 2006) for screening powdery mildew resistance in laboratory condition.

In the case of plant virus, different bioassay techniques along with controlled growth facilities are involved in screening for virus resistance in plants. Along with typical symptoms, the resistance screening for virus particle may also involve

ELISA, PCR, and real-time PCR technique to determine the titer value of the virus particle inside the infected plant tissue. Moreover, artificial inoculation based on agro-inoculation technique has been widely used to screen MYMV resistance in mung bean and other pulses (Mandal et al. 1997). In some viral diseases, it is important to inoculate the test plant with the vector for spreading the disease. Such a situation demands "infector hedge row" and "leaf stapling technique" which was followed to screen genotypes resistant to sterility mosaic disease in pigeon pea (Nene and Reddy 1976). The use of hardcore molecular technique toward screening of disease resistance in pulse crop is no longer a dream now. Ghosh et al. (2017) used loop-mediated isothermal amplification (LAMP) assay that targets fungal specific 5.8 S rDNA and partial ITS (internal transcribed spacer) region for visual detection of *Rhizoctonia bataticola* causing dry root rot of chickpea.

8.2.4 Identification of Molecular Markers for Important Disease Resistance in Major Legume Crops

In general, identification of molecular markers for any disease resistance demands development of a segregating mapping population for disease reaction, genotyping of the population with molecular markers, and analysis toward marker identification. Using these approaches, different markers were identified in pulse crops for different disease resistance (Table 8.1). The details of those will be covered "Successful Examples in Tropical/Temperate Pulse Crops" in this chapter.

8.3 Exploitation of Linked Molecular Markers in Marker-Assisted Breeding

8.3.1 Example for MAS in Chickpea

Sequence-tagged microsatellite markers have been used for MAS to isolate near-isogenic lines in chickpea. The SSR markers tightly linked to *foc5* (TA59) and $foc0_1$ (TR59, TS35) were used to assist selection of resistant and susceptible genotypes toward the development of NILs in chickpea (Castro et al. 2010; Jendoubi et al. 2016). MABC lines resistant to *Fusarium* (*foc1* and *foc3*) and *Ascochyta* blight were developed in the genetic background of C 214 cultivar at ICRISAT (Varshney et al. 2014). Similarly, MABC lines resistant to *foc4* were developed in the genetic background of JG 74, Phule G12, and Annigeri 1 at various agricultural universities in India. Another set of MABC lines resistant to *foc2* has been developed in the background of Pusa 256 at Indian Institute of Pulses Research, Kanpur, India (Varshney et al. 2013; Saxena et al. 2016).

Table 8.1 List of identified QTLs and linked markers for various diseases of pulse crops

S. no.	Crop (s)	Trait(s)	QTLs/genes	Type of marker(s)	Reference(s)
1	**Chickpea (*Cicer arietinum* L.)**				
	1.1	*Ascochyta* blight	QTL-1, QTL-2	RAPD and ISSR	Santra et al. (2000)
			QTLs	RAPD	Millan et al. (2003)
			Ar19	RAPD	Rakshit et al. (2003)
			QTLar2b	SSR	Udupa and Baum (2003)
			Ar19	STMS	Cho et al. (2004)
			QTLar1, QTLar2	SSR	Iruela et al. (2006)
			QTL	SSR	Tar'an et al. (2007)
			QTL_{AR3}	SSR	Iruela et al. (2007, 2009)
			QTL2	SSR	Kottapalli et al. (2009)
			QTL	SSR	Anbessa et al. (2009)
			QTL	STMS	Aryamanesh et al. (2010)
			AB-Q-SR-4-1	SSR	Sabbavarapu et al. (2013)
			QTLs	SNP	Daba et al. (2016)
	1.2	*Fusarium* wilt	*Foc3*	SSR	Sharma et al. (2004, 2005)
			TR59	STMS	Cobos et al. (2005)
			QTL_{foc02}, QTL_{foc5}	SSR	Cobos et al. (2009)
			Foc1, foc2, Foc3	SSR	Gowda et al. (2009)
			FW-Q-APR – 6-2	SSR	Sabbavarapu et al. (2013)
			Foc-1	STMS	Barman et al. (2014)
			QTL (GSSR 18-TC14801)	SSR	Jingade and Ravikumar (2015)
			QTL_{CaLG02}	SSR and SNP	Garg et al. (2018)

(continued)

Table 8.1 (continued)

S. no.	Crop (s)	Trait(s)	QTLs/genes	Type of marker(s)	Reference(s)
2	**Pea (*Pisum sativum* L.)**				
	2.1	Powdery mildew	*er*	SCAR	Janila and Sharma (2004)
			er	RFLP	Dirlewanger et al. (1994)
			er (Sc-OPO-18$_{1200}$)	RAPD/SCAR	Tiwari et al. (1998)
			er (OPD-10$_{650}$)	RAPD	Timmerman et al. (1994)
			er (PSMPSAD60, PSMPSAA374e, PSMPA5, PSMPSAA369, PSMPSAD51	SSR	Ek et al. (2005)
			er1-6	SNP	Sun et al. (2016)
			er-1	STMS	Frew et al. (2002)
	2.2	*Ascochyta* blight	QTLs	SSR, RAPD, and CAPS	Miranda (2012)
			QTLs	STS	Timmerman-Vaughan et al. (2004)
			Asc2.1, Asc4.2, Asc4.3 and Asc7.1	Candidate defense-related sequences	Timmerman-Vaughan et al. (2016)
			QTL abIII-1 and abI-IV-2	SNP	Jha et al. (2016)
			abI-IV-2.1 and abI-IV-2.2	SNP	Jha et al. (2017)
			QTLs	SSR	Tar'an et al. (2003a)
			QTLs	SNP	Jha et al. (2015)
			MpII.1, MpIII.5, MpV.2 and MpV.3	SNP	Carrillo et al. (2014)
	2.3	*Fusarium* wilt	*Fw*	RAPD	Dirlewanger et al. (1994)
			Fw	RAPD, AFLP	McClendon et al. (2002)
			Fnp	SSR and RAPD	McPhee et al. (2012)
			Fw_Trap_480, Fw_ Trap_340, and Fw_Trap_220	SCAR	Kwon et al. (2013)
	2.4	Pea common mosaic virus	*mo*	RFLP	Dirlewanger et al. (1994)
	2.5	Pea rust	*Up1*	RAPD	Barilli et al. (2010)
	2.6	Pea seed-borne mosaic virus	*Sbm-1*	STS	Frew et al. (2002)

(continued)

Table 8.1 (continued)

S. no.	Crop (s)	Trait(s)	QTLs/genes	Type of marker(s)	Reference(s)
3	**Lentil (*Lens culinaris*)**				
	3.1	*Ascochyta* blight	QTL	RAPD	Ford et al. (1999)
			Ra/2	RAPD, SCAR	Chowdhery et al. (2001)
			QTL1 QTL 2	RAPD, ISSR, RFLP, AFLP	Tar'an et al. (2003a)
			QTL-1-5 QTL-6-8	RAPD, ISSR, AFLP	Rubeena et al. (2006)
			QTL	RAPD, AFLP, SSR	Tullu et al. (2006)
			QTL 1	EST-SSR/SSR, ISSR, RAPD, ITAP	Gupta et al. (2012)
			AB_IH1 *AB_IH1.2* *AB_NF1*	Genomic DNA-derived SSR, –EST-SSR, SNP	Sudheesh et al. (2016)
	3.2	*Fusarium* wilt	*fw*	RAPD	Eujayl et al. (1998)
			fw	AFLP, SSR	Hamwieh et al. (2005)
	3.3	Anthracnose	*LCt-2*	AFLP, RAPD	Tullu et al. (2003)
			LCt-2, OP-P4$_{400}$	AFLP, RAPD	Tullu et al. (2006)
4	**Common bean (*Phaseolus vulgaris*)**				
	4.1	Common bacterial blight	QTL	RAPD, SCAR, STS, SSR, RFLP	Tar'an et al. (2001)
			QTLs	SSR, SCAR	Zhu et al. (2016)
			QTL	RFLP	Lopez et al. (2003)
	4.2	Bean common mosaic virus	QTL-I	RAPD	Jung et al. (1996)
			QTLs	RAPD	Miklas et al. (1996)
				SCAR	Melotto et al. (1996)
	4.3	Anthracnose	*Are gene*	SCAR	Adam-Blondon et al. (1994)
			QTLs/genes	SSR	Choudhary et al. (2018)
			QTLs/genes	CAPS, SCAR, RAPD	Boersma et al. (2013)
			QTLs	SNP, SSR	Perseguini et al. (2016)
	4.4	White mold	QTLs	RAPD, AFLP	Kolkman and Kelly (2003)

(continued)

Table 8.1 (continued)

S. no.	Crop (s)	Trait(s)	QTLs/genes	Type of marker(s)	Reference(s)
			QTLs	SSR, AFLP, and SRAP	Lara et al. (2014)
			WM1.1, WM2.2, WM3.1, WM5.4, WM6.2, WM7.1, WM7.4, WM7.5, and WM8.3	SNP	Vasconcellos et al. (2017)
			WM2.2, WM8.3, and WM7.3	SRAP and RAPD	Soule et al. (2011)
	4.5	*Fusarium* wilt	*PvPR1, PvPR2*	RAPD	Schneider et al. (2000)
			QTLs	SNP	Hagerty et al. (2015)
			QTLs	RAPD	Fall et al. (2001)
	4.6	Root rot	QTLs	SNP	Hagerty et al. (2015)
			QTLs	SSR	Kamfwa et al. (2013)
			QTLs	RAPD	Schneider et al. (2000)
	4.7	Angular leaf spot	QTL	SSR	Teixeira et al. (2005)
			QTL ALS11^{AS}	SNP, SSR	Bassi et al. (2017)
			ALS10.1^{DG,UC}, ALS5.2	SSR	Oblessuc et al. (2012)
			ALS	SSR	Teixeira et al. (2005)
			ALS4.1$^{GS,\ UC}$	SSR, Tm markers	Keller et al. (2015)
			QTLs	SNP, SSR	Perseguini et al. (2016)
			ALS	RFLP	Lopez et al. (2003)
	4.8	Rust	*Ur-3*	SNP, SSR	Hurtado-Gonzales et al. (2017)
			Ur-13	SCAR	Mienie et al. (2005)
			Ur-7	RAPD	Park et al. (2004)
	4.9	Powdery mildew	*PWM2^{AS} and PWM11^{AS}*	SNP, SSR	Bassi et al. (2017)
5	**Mung bean (*Vigna radiata* L.)**				
	5.1	Powdery mildew	*qPMR-1 and qPMR-2*	SSR	Kasettranan et al. (2010)
			QTLs	RFLP	Humphry et al. (2003)

(continued)

Table 8.1 (continued)

S. no.	Crop (s)	Trait(s)	QTLs/genes	Type of marker(s)	Reference(s)
			QTLs	RAPD, CAP, AFLP	Chen et al. (2007)
	5.2	Mung bean Yellow mosaic India virus	*qYMIV1, qYMIV2, qYMIV3, qYMIV4, and qYMIV5*	SSR	Kitsanachandee et al. (2013)
			OPB07-SCAR_583 (MYMVR-583)	SCAR	Dhole and Reddy (2013)
	5.3	*Cercospora* leaf spot	*qCLS*	SSR	Chankaew et al. (2011)
6	**Black gram (*Vigna mungo* L. Hepper)**				
	6.1	Yellow mosaic virus	Monogenic	STS-RGA	Basak et al. (2004)
	6.2	Mung bean Yellow mosaic India virus	QTL	SSR and RGH markers	Anjum et al. (2010)
	6.3	Powdery mildew	QTL	SSR and RGH markers	Anjum et al. (2010)
7	**Faba bean**				
	7.1	Faba bean rust	*Uvf-1*	RAPD	Avila et al. (2003)
	7.2	*Ascochyta* blight	QTL-1, QTL-2, QTL-3, QTL-4,	SNP, EST-SSR	Kaur et al. (2014)
			Af-1, Af-2, Af-3,	SSR	Atienza et al. (2016)
			Af-1, Af-2	RAPDs, isozymes, ESTs, SCAR, SSRs, STSs, and intron-spanning markers	Díaz-Ruiz et al. (2009)
8	**Cowpea [*Vigna unguiculata* (L.) Walp.]**				
	8.1	Cowpea rust	*Ruv1, Ruv2, Ruv3,*	SNP	Wu et al. (2017)
			QTLs	SSRs	Uma et al. (2016)
	8.2	Cowpea bacterial blight	*CoBB-1, CoBB-2*	SNP	Agbicodo et al. (2010)
	8.3	Cowpea golden mosaic virus	QTLs	AFLP	Rodrigues et al. (2012)
	8.4	Fusarium wilt resistance (*Fot race 3*)	QTLs	SNP	Pottorff et al. (2012)
	8.5	*Fusarium* wilt resistance (*Fot race 4*)	QTLs	SNP	Pottorff et al. (2014)

(continued)

Table 8.1 (continued)

S. no.	Crop (s)	Trait(s)	QTLs/genes	Type of marker(s)	Reference(s)
9	**Pigeon pea (*Cajanus cajan* L. Millsp.)**				
	9.1	Sterility mosaic disease	*qSMD3 qSMD4 qSMD5 qSMD6*	SSR	Gnanesh et al. (2011)
			C.cajan_01839	SNP	Singh et al. (2016a, b)
			CcLG11	SNP	Saxena et al. (2017b)
	9.2	*Fusarium* wilt	*Fw Gene*	RAPD	Kotresh et al. (2006)
			C.cajan_03203	SNP	Singh et al. (2016a, b)
			qFW11.1, qFW11.2 and qFW11.3	SNP	Saxena et al. (2017a)
10	**Lathyrus (*Lathyrus sativus* L.)**				
	10.1	*Ascochyta* blight	QTL	RAPD, STMS	Skiba et al. (2004)

8.3.2 Examples of MAS in Common Bean

Most of the breeding programs for common bean improvement in the world attempted to bring resistance against bean common mosaic virus (BCMV) in most of the released cultivars. Melotto et al. (1996) has developed a SCAR marker SW13 which was found linked to the dominant BCMV resistance I gene in this crop. This SW13 SCAR was much used in various breeding programs to introduce dominant resistance in common bean (Miklas et al. 2006). Similarly SR2 SCAR has been very useful for bringing in bean golden yellow mosaic virus resistance in this plant (Blair et al. 2007; Beebe 2012). A marker SU91 is reported to be linked to a QTL for common bacterial blight (CBB) resistance on linkage group B8. The marker BC420 is linked to another QTL for CBB resistance in B6 linkage group (Miklas et al. 2000; Pedraza et al. 1997; Yu et al. 2000). O'Boyle et al. (2007) demonstrated the usage of those SCAR markers SU91 and BC420 for the successful isolation of CBB resistant lines from 93 $F_{3:4}$ single plant selections. Various resistant common bean germplasm like advanced cranberry, pinto, great northern, and snap bean with resistance to CBB have been developed in the USA using MAS approach (Miklas et al. 2006). In the recent past, three major rust resistance genes, *Ur-5*, *Ur-11*, and *Ur-14*, were pyramided into a high yielding common bean variety "Carioca" through marker-assisted backcrossing method. This improved varieties used to be most consumed in Brazil and representing around 70% of their internal market (Souza et al. 2014).

8.3.3 MAS in Cowpea

Striga, a parasitic weed of cowpea, is important in African countries. Different QTLs conferring *Striga* resistance were identified by using AFLP and SCAR markers (Ouédraogo et al. 2002; Boukar et al. 2004). The large numbers of molecular markers developed for this resistance trait have been used for marker-based backcrossing incorporating foreground and background selection for improved version of local cultivars. At International Institute of Tropical Agriculture, IT93K-452-1 and IT89KD-288 were officially released varieties that are being improved for *Striga* resistance through MAS (Boukar et al. 2016).

8.4 Successful Examples in Tropical Pulse Crops

8.4.1 Mung Bean and Black Gram

Mung bean (*Vigna radiata* (L.) Wilczek) and black gram (*V. mungo* (L.) Hepper) are important legume crops widely cultivated in Indian subcontinent. Low productivity is a major concern in these crops. Of the various agronomic factors, biotic stresses are also responsible for this low productivity. Among biotic stresses, yellow mosaic disease (YMD) caused by mung bean yellow mosaic virus (genus *Begomovirus*, family *Geminiviridae*), powdery mildew (PM) caused by fungus *Erysiphe polygoni* DC., and *Cercospora* leaf spot (CLS) caused by *Cercospora canescens* Illis & Martin are the most important diseases which reduced seed yield considerably depending on the stage at which plant gets infected (Khattak et al. 2000; Pandey et al. 2009). Pathogens of all three diseases are obligate parasites and hence cannot be grown and maintained on the artificial media. In this case, marker-assisted selection will be very useful for development of resistant varieties to diseases like YMD and PM in both mung bean and black gram. Genomic resources are required for tagging the disease resistance genes and their transfer through marker-assisted selection. Until recently genomic resources were very scarce in these neglected pulse crops. The estimated genome size of mung bean and black gram is 579 Mbp (0.60 pg/IC) and 574 Mbp (0.59 pg/IC), respectively (Arumuganathan and Earle 1991). After the availability of mung bean SSR markers, the gene tagging and linkage analysis has started (Kumar et al. 2002a, b; Miyagi et al. 2004; Gwag et al. 2006), which was further strengthened after the availability of 100 Mb genome sequence information of mung bean (Tangphatsornruang et al. 2009). With the availability of draft genome sequence of mung bean, there is an enough scope for acceleration of marker-assisted breeding program in both mung bean and black gram (Kang et al. 2014). Recently, the 993 genic-SSR markers were designed successfully in black gram from immature seed transcriptome (Souframanien and Reddy 2015).

Yellow Mosaic Disease (YMD) In the case of YMD, the virus is not transmitted by sap or seed but transmitted only by insect vector whitefly (*Bemisia tabaci*). Hence, it cannot be created artificially, and screening entirely depends on field screening at hot spot by infector row method. The two different strains, i.e., MYMV and MYMIV, are reported in Indian subcontinents (Hussain et al. 2004; Pant et al. 2001; Ilyas et al. 2010), which leads to further complications in screening for virus resistance. Resistance to YMD in mung bean was reported to be controlled by a single recessive gene (Malik et al. 1986; Reddy and Singh 1995; Saleem et al. 1998; Basak et al. 2004; Reddy 2009), a dominant gene (Sandhu et al. 1985), two recessive genes (Verma and Singh 1988; Pal et al. 1991; Ammavasai et al. 2004), and complementary recessive genes (Shukla and Pandya 1985). In black gram, YMD resistance is reported to be governed by single recessive gene (Souframanien and Gopalakrishna 2006; Kundagrami et al. 2009) and two recessive genes (Verma and Singh 1986). The RAPD markers linked to YMD resistance gene were identified in mung bean (Selvi et al. 2006; Dhole and Reddy 2013) and further converted to SCAR markers (MYMVR-583) for better reproducibility in MAS (Dhole and Reddy 2013). In black gram, ISSR marker linked to YMD resistance was developed into SCAR marker and validated in different resistant black gram genotypes (Souframanien and Gopalakrishna 2006). The resistant gene analog (RGA) markers YR4 and CYR1 were found associated with resistance to YMD in black gram (Maiti et al. 2011). Before the availability of SSR markers in these crops, the markers from cowpea, azuki bean, and common bean were found to be useful in both mung bean and black gram (Gupta and Gopalakrishna 2009; Gupta and Gopalakrishna 2010). The cowpea SSR marker CEDG180 was found to be associated with YMD resistance in black gram (Gupta et al. 2013). For MYMIV resistance, three QTLs, i.e., *qYMIV1*, *qYMIV2*, and *qYMIV3* in India and two QTLs, i.e., *qYMIV4* and *qYMIV5* in Pakistan were identified through composite interval mapping of mung bean (Kitsanachandee et al. 2013). AFLP and SSR markers were used for identification of four major QTLs for MYMIV resistance (Chen et al. 2013). Three markers, ISSR 811_{1357}, YMV1-FR, and CEDG180 were found to discriminate the YMV resistant and susceptible black gram genotypes which can be used for MAS (Gupta et al. 2015).

Powdery Mildew The second most important disease of mung bean and black gram is powdery mildew which can be screened in field as well as in laboratory conditions by using excised leaf technique (Reddy et al. 1987). Three independent dominant genes (Pm_1, Pm_2, *and* Pm_3) governing resistance reaction to powdery mildew disease were identified in mung bean at Bhabha Atomic Research Centre, Mumbai, India (Reddy 2007; Reddy 2009). The RFLP markers were the first markers used in mung bean for identification of linkage between a major powdery mildew resistance locus and the marker (Humphry et al. 2003), while two QTLs, i.e., *qPMR-1* and *qPMR-2*, for powdery mildew resistance were reported in mung bean (Kasettranan et al. 2010). The SSR markers DMBSSR 130 and VM 27 were found to be associated with powdery mildew-resistant plants in F_2 population of black gram (Savithramma and Ramakrishnan 2016).

Cercospora **Leaf Spot (CLS)** It is the third most important disease of mung bean and black gram mainly confined to rainy season (June to September) in India. Field screening at hot spot and that to humid climate is the only method of screening genotypes for CLS. Single dominant gene conferring resistance to *Cercospora* leaf spot disease was identified (Chankaew et al. 2011). Very few studies were carried out on tagging of *Cercospora* resistance gene in mung bean and black gram. Seven SSR markers, i.e., CEDC031, CEDG044, CEDG084, CEDG117, CEDG305, VR108, and VR393, were found to be associated with CLS resistance in F_2 and BC_1F_1 population of mung bean (Chankaew et al. 2011).

8.4.2 Cowpea

Cowpea (*Vigna unguiculata* L. Walp.) is a very important crop cultivated worldwide in each continent. It is used for both vegetable and grain purposes and is a rich source of protein and minerals for humans and livestock. Major yield constraints of cowpea include diseases caused by bacteria, viruses, and fungi. The most important diseases of cowpea are bacterial blight (*Xanthomonas axonopodis* pv. *vignicola* (Xav)) and bacterial pustule (*Xanthomonas* sp.) followed by viral diseases like bean common mosaic virus (BCMV), cowpea aphid-borne mosaic virus (CABMV), cowpea mosaic virus (CPMV), southern bean mosaic virus (SBMV), cowpea mottle virus (CPMoV), cucumber mosaic virus (CMV), and cowpea golden mosaic virus (CGMV). In fungal diseases, anthracnose and brown blotch (*Colletotrichum* sp.), charcoal rot (*Macrophomina phaseolina*), *Cercospora* leaf spot (*Cercospora canescens*), and *Fusarium* wilt are commonly appearing in cowpea. Growing of disease-resistant varieties is the only solution to combat yield losses in cowpea. The development of multiple disease-resistant varieties is a prime breeding objective in cowpea which is the host for so many diseases. Marker-assisted backcrossing and selection can boost the gene pyramiding for resistance to multiple diseases and save time and effort for disease screening. The development of tightly linked molecular markers with disease-resistant gene depends on genomic information available in the target crop. Cowpea is having the chromosome number $2n = 22$ with a genome size of 620 Mb (Varshney et al. 2009). The first attempt to sequence cowpea genome includes sequencing for about 97% of all known cowpea genes by using Illumina paired-end technology on GAII, and then they were assembled together with Sanger BAC-end sequences and "gene-space" sequences (Timko et al. 2008) using SOAPdenovo (Luo et al. 2012). Before the availability of cowpea SSR and SNP markers, RFLP (Fatokun et al. 1993), AFLP (Fang et al. 2007), DAF (Simon et al. 2007), and RAPD (Zannou et al. 2008) markers were used for genetic diversity studies and linkage mapping in cowpea. Molecular maps were developed in cowpea by using various F_2 and RIL populations, and markers like RFLP (Young 1999), AFLP and RAPD (Ouedraogo et al. 2002), SNP (Xu et al. 2011; Muchero et al. 2009; Lucas et al. 2011), and SSR (Anadrgie et al. 2011; Kongjaimum et al. 2012) were used. In cowpea, bacterial blight resistance gene candidate (RGC) loci were

reported to be placed on various locations of LG3, LG5, and LG9 on the integrated cowpea map constructed by using RFLP markers (Kelly et al. 2003). QTLs CoBB-1, CoBB-2, and CoBB-3 represent RGC loci and are present on linkage groups LG3, LG5, and LG9, respectively, on SNP marker-based cowpea genetic map (Agbicodo et al. 2010). A QTL for cowpea yellow mosaic virus (CYMV) resistance was identified and validated using SSR markers (Gioi et al. 2012). Cowpea genetic map showed that blackeye cowpea mosaic potyvirus (B1CMV) and southern bean mosaic virus (SBMV) resistance was mapped to LG8 and LG6, respectively, and resistance to cowpea mosaic virus (CPMV) and cowpea severe mosaic virus (CPSMV) was mapped to opposite ends of LG3, while the CPSMV resistance was mapped near a locus conferring resistance to *Fusarium* wilt (Ouédraogo et al. 2002). Three QTLs were reported for cowpea golden mosaic virus resistance by using AFLP markers in F_2 population (Rodrigues et al. 2012). Nine QTLs for resistance to *Macrophomina* were identified to be located on various linkage groups (Muchero et al. 2010; Muchero et al. 2011). QTLs conferring *Fusarium* wilt resistance against race 3 was found to be located on LG6 and race 4 was on LG8, LG 9, LG3, respectively (Pottorff et al. 2014).

8.4.3 Pigeon Pea

Pigeon pea (*Cajanus cajan* (L.) Millsp.) is an important pulse crop in India that is the largest producer and consumer in the world. The productivity of this crop is severely affected by some major diseases like *Fusarium* wilt (*Fusarium udum* Butler), sterility mosaic disease (SMD) caused by pigeon pea sterility mosaic virus (PPSMV), and *Phytophthora* blight (*Phytophthora drechsleri* f. sp. *cajani*). Other diseases, viz., *Alternaria* blight, sudden death, and root rots, are appearing recently due to climatic changes (Sharma and Ghosh 2016). *Fusarium* wilt (FW) is the most important and destructive disease in Indian Subcontinent (Saxena 2008). Due to five different variants of *Fusarium udum* (Tiwari and Dhar 2011), precise phenotyping at field level becomes difficult for genetic studies. Hence, different reports were found on genetics of FW resistance. A single dominant gene, two duplicate dominant genes, two complementary genes, and multiple genes governing the resistance reaction to FW disease are reported in various studies (Saxena and Sharma 1990; Okiror 2002; Singh et al. 2016a, b). Recently an association-based mapping approach has detected significant association of the SSR marker HASSR18 (accounting for 5–6% phenotypic variation due to wilt resistance across the years) with the genetic resistance against *Fusarium* wilt variant 2 in pigeon pea (Patil et al. 2017).

The PPSMV is transmitted by an eriophyid mite (*Aceria cajani* Channabasavanna) and hence spread rapidly, which leads to epidemics under congenial conditions. Development of varieties resistant to PPSMV becomes very difficult through conventional breeding because pigeon pea is a long-duration and often cross-pollinated crop, and phenotyping is tedious due to the existence of three different strains of

PPSMV in India (Kulkarni et al. 2003) and disease spread depends on mite population. Moreover, it becomes difficult to transfer resistant genes from wild relatives due to linkage drag. In the absence of efficient screening method, phenotyping is based mainly on symptoms which may vary depending on time and stage at which infection occurs. Hence, reports on genetics of resistant gene lead to different results. PSMD resistance was reported to be controlled by single gene (Ganapathy et al. 2009; Murugesan et al. 1997; Srinivas et al. 1997), oligo-genes (Gnanesh et al. 2011; Nagaraj et al. 2004; Sharma et al. 1984), and two genes with inhibitory gene action (Daspute et al. 2014). While four QTLs for Patancheru PSMD isolate and two QTLs for Bangalore PSMD isolate were also identified (Gnanesh et al. 2011). Short-duration pigeon pea varieties are very important for multiple cropping and to avoid terminal drought. Another disease, *Phytophthora* blight, is found to be more severe in short-duration cultivars as compared to long- or medium-duration genotypes (Ratnaparkhe and Gupta 2007). Looking at the losses due to different diseases, the development of disease-resistant varieties is the best means to resolve these problems, but lack of efficient and reliable screening methods limits the use of conventional breeding methods. Recent advances in genomics of pigeon pea pave the way for marker-assisted disease-resistant breeding for pyramiding the resistance genes for different diseases. Earlier, very limited information was available as far as the genomic resources are concerned. With the availability of draft genome sequence in pigeon pea (genome size = 833.07 Mb), this crop became rich for genomic resources (Varshney et al. 2012). Thus, a large number of SSR markers are now available, viz., 3072 SSRs from 88,860 BESs (Bohra et al. 2011), 3583 SSR markers from ESTs (Raju et al. 2010), and 309,052 SSRs from scanning the draft genome sequence of pigeon pea (Varshney et al. 2012). In addition to this, 10,000 SNPs are also available in pigeon pea research community (Varshney et al. 2013). These markers are going to be very useful for saturating the genetic maps with plenty of molecular markers and tagging QTL/genes for important traits like disease resistance. The genetic maps were developed by using an interspecific population and SSR markers (Bohra et al. 2011), DArT-based paternal and maternal-specific genetic maps (Yang et al. 2011), and a dense genetic map with SNP makers (Saxena et al. 2012). Consensus genetic maps have been developed by using SSR markers in six intraspecific populations (Bohra et al. 2012). Several markers linked to resistance genes were reported for these diseases which can be utilized for marker-assisted selection and gene pyramiding for multiple disease resistance. Different types of markers were used and identified to be linked with *Fusarium* wilt (FW) resistance, viz., two RAPD markers ($OPM03_{704}$ and $OPAC11_{500}$) (Kotresh et al. 2006), six SSRs (ASSR-1, ASSR-23, ASSR-148, ASSR-229, ASSR-363, and ASSR-366) (Singh et al. 2016a, b), and five SSR markers (PFW 26, PFW 31, PFW 38, PFW56, and PFW70) (Khalekar et al. 2014), while three important QTLs (qFW11.1, qFW11.2, and qFW11.3) were reported by using SNPs (Saxena et al. 2017a). Two genes, i.e., *C. cajan*_01839 for SMD resistance and *C. cajan*_03203 for FW resistance, were identified through SNP mapping (Singh et al. 2016a, b). For SMD, mapping was attempted by using AFLP markers, and four markers, E-CAA/M-GTG_{150}, E-CAA/M-GTG_{60}, E-CAG/M-GCC_{120}, and E-CAG/M-GCC_{150}, were identified

which were found to be linked with the SMD resistance gene at the distance of 5.7, 4.8, 5.2, and 20.7 cM from the resistance loci (Ganapathy et al. 2009). A single coupling phase short decamer random DNA marker ($IABTPPN7_{414}$) and a repulsion phase marker ($IABTPPN7_{983}$) were reported to be co-segregating with PSMD reaction (Daspute and Fakrudin 2015). Six QTLs (qSMD1, qSMD2, qSMD3, qSMD4, qSMD5, and qSMD6) linked to SMD were identified by using SSR markers in two different populations (Gnanesh et al. 2011). Another 10 QTLs including three major QTLs associated with SMD resistance were identified in three different populations (Saxena et al. 2017b). So far, reported linked markers are not utilized successfully to transfer the resistance genes toward the development of disease-resistant pigeon pea varieties. Validation of these markers across different genetic background is equally important as far as the application of these markers for breeding program is concerned. The tightly linked markers to disease resistance genes should be developed using multi-parent mapping populations (MAGIC) with very precise phenotyping so that it can be applicable across the pigeon pea populations. In the near future, with the availability of throughput approaches and tightly linked markers, the MABC will become very much possible for the resistance breeding to serious diseases like FW and SMD in pigeon pea.

8.4.4 Common Bean

Common bean (*Phaseolus vulgaris* L.) has often been termed as nutritional powerhouse for human diet (Broughton et al. 2003). It is used as food in the form of tender pods, fresh seeds, and dry beans. It originated in Central America and has two centers of domestication (Mesoamerican and Andean) with well-defined types in each gene pool (Singh et al. 1991). The crop used to hamper by different foliar and root diseases like angular leaf spot by *Pseudocercospora griseola* Sacc., common bacterial blight by *Xanthomonas axonopodis* pv. *phaseoli*, bean common mosaic virus, bean common mosaic necrosis virus, anthracnose by *Colletotrichum lindemuthianum*, root rots by *Fusarium solani* and *Rhizoctonia solani*, and rust by *Uromyces fabae*. Genetic resistance against bean common mosaic virus (BCMV) is conditioned by four different recessive loci, bc-1, bc-2, bc-3, and bc-u, along with a dominant gene I in *P. vulgaris*. Although mechanism of resistance of recessive and dominant gene is different, breeders want to pyramid them together for developing durable resistance. Melotto et al. (1996) have developed a SCAR marker (SW13) which was found linked to the dominant BCMV resistance I gene in this crop. Similarly SR2 SCAR has been very useful for bringing in bean golden yellow mosaic virus resistance in the plant (Blair et al. 2007; Beebe 2012). Anthracnose resistance in common bean is also conditioned by multiallelic Co-1 locus. Of these various alleles, Co-4 has been much used in breeding program due to the availability of a tightly linked SCAR marker SB 114 (Miklas et al. 2006).

Common bacterial blight (CBB) disease is caused by *Xanthomonas axonopodis* pv. *phaseoli*. Genetic resistance against this pathogen is quantitatively inherited,

and thus, different QTLs were identified in different linkage group of common bean (Jung et al. 1996; Bai et al. 1997; Miklas et al. 2000). Two dominant SCAR markers, SU91 and BC420, were independently developed from resistant tepary bean germplasm. The marker SU91 is reported to be linked to a QTL for CBB resistance on linkage group B8. The marker BC420 is linked to another QTL in B6 linkage group (Miklas et al. 2000; Pedraza et al. 1997; Yu et al. 2000). Resistance to angular leaf spot (ALS) disease (*Pseudocercospora griseola* Sacc.) is controlled by either dominant or recessive genes in common bean. These genes are independent as different types of molecular markers were identified for each resistance and they were placed in different chromosomes. The resistance gene *Phg-1 in* Andean cultivar AND 277 was mapped to chromosome 1 (Goncalves-Vidigal et al. 2011). Another major resistance locus on linkage group Pv04 was identified in other Andean accession G5686 (Mahuku et al. 2009). This locus was later confirmed and named ALS4.1$^{GS,\ UC}$ (Oblessuc et al. 2012). In addition, Mahuku et al. (2009) reported two complementary resistance genes in G5686 on Pv09 (ALS9.1^{GS}) and Pv04 (ALS4.2^{GS}). Further QTL studies also supported a more quantitative nature of ALS resistance (Lopez et al. 2003; Teixeira et al. 2005; Mahuku et al. 2011; Oblessuc et al. 2012). A major QTL explaining 75.3% of ALS resistance in the G5686 × Sprite population was validated, mapped to 418 kbp on chromosome Pv04, and tagged with two closely linked SNP markers (Marker50 and 4M437). These findings have enough potential to be used in MAS. ALS4.1$^{GS,\ UC}$ defines a region of 36 genes including 11 STPKs, which are likely candidates for the resistance gene. Additionally, three minor QTLs were identified (Keller et al. 2015). Bean rust is distributed around the world, but it effectively causes major production problems in humid tropical and subtropical areas (Souza et al. 2014). Several RAPD markers associated with genes conferring resistance to rust in common bean have been identified, and some of them were converted into SCAR markers to increase the reproducibility of the markers (Souza et al. 2007; Souza et al. 2008).

8.5 Successful Examples in Temperate Pulse Crops

8.5.1 Chickpea

Chickpea (*Cicer arietinum* L.) is the second most important pulse crop in the world after common bean. It is mainly used as a dietary protein source in Mediterranean region, India, Pakistan, and North Africa. The main biotic constraints for increasing yield in these countries are the susceptibility of the crop to foliar diseases like *Ascochyta* blight and *Fusarium* wilt. In addition, dry root rot (DRR) along with *Fusarium* wilt has emerged as a highly devastating root disease in central and southern India in context with climate change. A recent report described the identification of two flanking SSR markers for a dominant DRR resistance gene in chickpea (Talekar et al. 2017). There are eight different *Fusarium oxysporum* f. sp. *ciceri*

races that are present globally. Of these, the presence of resistance gene against *foc 1A* or *foc 1B/C* can protect the chickpea plant from early wilting, while resistance genes against *foc 0*, *foc 2*, *foc 3*, *foc 4*, and *foc 5* impart complete resistance over all the growing stages of the plant (Sharma et al. 2005; Sharma and Muehlbauer 2007). Marker information on all these different *Fusarium* wilt resistance genes of chickpea is summarized in Table 8.2. Most of the markers identified earlier were of RAPD, ISSR, or AFLP markers, but their usage in MAS is limited due to problem of reproducibility. Thus, most of the recent works were focused on the usage of SSR and SNP markers for this cause. A molecular map based on intraspecific cross (Kabuli-Desi cross) was developed and used to tag genes for resistance to *Fusarium* wilt. Two SCAR markers and two RAPD markers (Mayer et al. 1997) were found associated with resistance to race 1 and one ISSR marker with resistance to race 4 (Ratnaparkhe et al. 1998). The genes for resistance to races 4 and 5 were found to be linked and located close to one STMS and one SCAR marker (Winter et al. 2000). Recently eight QTLs were found associated with *Ascochyta* blight resistance in chickpea. Of them, a cluster of QTLs were found in chromosome 8 at a map interval of 8.5 cM (Daba et al. 2016). Li et al. (2017) identified 100 kb region in chromosome 4 that is significantly associated with *Ascochyta* blight in chickpea through genome-wide association mapping in Australian breeding population. Chetukuri et al. (2011) identified three QTLs for *Botrytis* gray mold disease of chickpea. Of these, QTL 3 (flanked by TA 159 and TA 118) in linkage group 3 explained 48% of the phenotypic variation due to botrytis grey mold disease reaction. Two sequence-tagged microsatellite sites (STMS) markers, TA18 and TA180 (3.9 cM apart), were identified as the flanking markers for rust resistance gene in chickpea (Madrid et al. 2007). These findings could be the starting point for a marker-assisted selection (MAS) program for rust resistance in chickpea.

Using traditional bi-parental populations, several QTLs for AB resistance have been identified on linkage groups LG2 (Udupa and Baum 2003; Cho et al. 2004), LG3 (Tar'an et al. 2007), LG4 (Lichtenzveig et al. 2006; Tar'an et al. 2007; Sabbavarapu et al. 2013; Stephens et al. 2014), LG5 (Sabbavarapu et al. 2013), LG6 (Tar'an et al. 2007; Sabbavarapu et al. 2013), and LG8 (Lichtenzveig et al. 2006). One major QTL has been repeatedly reported in a similar region of LG4 across several studies and therefore makes this locus a good candidate region for improving AB resistance in chickpea (Lichtenzveig et al. 2006; Tar'an et al. 2007; Sabbavarapu et al. 2013; Stephens et al. 2014).

8.5.2 Lentil

Lentil (*Lens culinaris* Medik) is a self-pollinating diploid ($2n = 2x = 14$) grain legume. It is cultivated globally and is valued for its quality protein and mineral content (particularly Fe content). Its production is limited by many biotic stresses including infection by the pathogen causing *Ascochyta* blight (*Ascochyta lentis* Vassilievsky), *Fusarium* wilt (*Fusarium oxysporum* f. sp. *lentis*), anthracnose

Table 8.2 Detailed information of linked markers, mapping population, and types of resistance genes for *Fusarium* wilt disease of chickpea

Foc genes	Nature of markers	Name of markers	Mapping population	Linkage group	References
foc 0	RAPD	$OPJ20_{600}$	RIL (CA2139 × JG 62)	LG 2	Rubio et al. (2003)
	RAPD and SSR	$OPJ20_{600}$ TR 59	RIL (CA2139 × JG 62)	LG 3	Cobos et al. (2005)
foc 0_2	SSR	–	RIL (CA2139 × JG 62) (CA 2156 × JG 62)	LG 2	Halila et al. (2009)
Foc 1A	RAPD	UBC 170	RIL (WR315 × C104)	LG 2	Tullu (1996)
	SCAR	CS 27 $CS27_{700}$			Mayer et al. (1997)
foc 1	SSR	TA 110	RIL JG 62 × Vijay	LG 2	Sant (2001)
	SSR	H3A12 TA 110	RIL (Vijay × JG 62)	LG 2	Gowda et al. (2009)
	SSR	QTLs: FW-Q-APR-6-2 FW-Q-APR-6-1	$F_{2:3}$ (C214 × WR 315)	LG 6	Sabbawarapu et al. (2013)
	SSR	TA 37 TA 200 TA 2	RIL (WR315 × C 104)	LG 2	Barman et al. (2014)
	SSR	QTLs: Wilt-1 (30 DAS) Wilt-2 (60 DAS)	RIL (JG 62 × WR 315)	LG 2	Patil et al. (2014)
	SSR and SNP	Five QTLs	RIL (JG 62 × ICCV05530	LG 2 LG 4 LG 6	Garg et al. (2018)
Foc 1 (H_2 locus)	SSR	QTL in between GSSR 18 and TC 14801	RIL (K 850 × WR 315)	LG 1	Jingade and Ravikumar (2015)
foc 2	SSR	TA 96 TA 27 TA 19	RIL (WR 315 × C 104)	LG 2	Sharma and Muehlbauer (2005)
	SSR	TA 96 H3A12	RIL (Vijay × JG 62)	LG 2	Gowda et al. (2009)
foc 3	SSR and STS	TA 96 TA 27 CS 27A (STS)	RIL (WR 315 × C 104)	LG 2	Sharma et al. (2004)
	SSR	H1B06y TA 194	RIL (Vijay × JG 62)	LG 2	Gowda et al. (2009)
	SSR and SNP	Two QTLs	RIL (JG 62 × ICCV05530	LG 2 LG 4	Garg et al. (2018)

(continued)

Table 8.2 (continued)

Foc genes	Nature of markers	Name of markers	Mapping population	Linkage group	References
foc 4	ISSR	UBC 855_{500}	RIL (ICC 4958 × *C. reticulatum* (PI489777))	LG 2	Ratnaparkhe et al. (1998)
	RAPD	UBC 170_{500} CS 27_{700}	RIL (C104 × WR 315)	LG2	Tullu et al. (1998)
	RAPD	CS 27_{700}	F_2 and F_3 (JG 62 × Surutato 77)	LG2	Tullu et al. (1999)
	SCAR or ASAP	$CS27_{700}$	RIL (ICC 4958 × *C. reticulatum* (PI489777))	LG2	Tekeoglu et al. (2000)
	SSR and AFLP	CS 27 TA 96 EAAMCTA12	RIL (ICC 4958 × *C. reticulatum* (PI489777))	LG 2	Winter et al. (2000)
	DAF	R 2609-1	RIL (*C. arietinum* × *C. reticulatum*)	LG 2	Benko-Iseppon et al. (2003)
foc 5	SSR and AFLP	ECAMCTA07	RIL (ICC 4958 × *C. reticulatum* (PI489777))	LG 2	Winter et al. (2000)
	SCAR or ASAP	$CS27_{700}$	RIL (ICC 4958 × *C. reticulatum* (PI489777))	LG2	Tekeoglu et al. (2000)
	SSR	QTL_AR3 TA110 TA 89	RIL	LG 2	Iruela et al. (2007)

(*Colletotrichum truncatum*), stemphylium blight (*Stemphylium botryosum*), rust (*Uromyces viciae-fabae*), botrytis gray mold (*Botrytis cinerea* and *B. fabae*), and white mold (*Sclerotinia sclerotiorum*). A SRAP marker F7XEM4a was identified for rust resistance gene in lentil by Saha et al. (2010a). This marker was placed 7.9 cM from the rust resistance gene. Later, an SSR marker Gllc 527 was identified to be linked to rust-resistant locus at a genetic distance of 5.9 cM in lentil (Dikshit et al. 2016). Toward the marker development on stemphylium blight resistance, two SRAP markers, ME5XR10 and ME4XR16c, were identified to be significantly associated with the QTLs for disease resistance in lentil (Saha et al. 2010b). Taran et al. (2003) developed RAPD ($OPE06_{1250}$ and UBC 704_{700}) and AFLP markers ($EMCTTACA_{350}$, $EMCTTAGG_{375}$, and $EMCTAAAG_{175}$) which were linked to LCt-2 locus for disease resistance against *Colletotrichum truncatum* (causal organism of anthracnose disease). In another study, a QTL (explained 41% of the variation in the

reaction to *Ascochyta* blight) was identified on the linkage group 6. This QTL was localized between an AFLP marker (ctcaccB) and LCt2 (Tullu et al. 2006). Eujayl et al. (1998) used an RIL population to identify molecular markers linked to the single dominant gene conditioning *Fusarium* vascular wilt resistance. On the other hand, resistance to *Fusarium* vascular wilt was mapped on linkage group 6, and this resistance gene was found flanked by a microsatellite marker SSR59-2B and an AFLP marker p17m30710 at distances of 8.0 cM and 3.5 cM, respectively (Hamwieh et al. 2005).

Among various diseases in lentil, *Ascochyta* blight is the most economically concerned in the majority of lentil-producing regions of the world. From 1999 onward, various molecular markers were identified for this disease resistance in lentil. Ford et al. (1999) identified two RAPD markers (RB18 and RV01) for a dominant *Ascochyta* blight disease resistance gene *AbR1*. Andrahennadi (1994) reported that a recessive gene *ral2* conditioned the resistance against *A. lentis* in cv. Indianhead. Later, this finding was confirmed by Choudhury et al. (2001) who have developed two RAPD markers ($UBC227_{1290}$ and $OPD10_{870}$) that are linked to *ral2* in lentil. Very recently genomic DNA-derived SSRs and SNP markers were developed based on the seedling (at 14 days) disease reaction and QTL analysis. Of the four QTLs identified by the authors, an SNP marker (SNP_20005010) was consistently found in two different mapping populations (Sudheesh et al. 2016). These particular SNP markers along with other flanking markers identified in the above QTL study showed promise for marker-assisted selection in the future. An international sequencing effort for lentil cultivar "CDC Redberry" is presently undergoing in full swing. The availability of an improved and well-annotated genome sequence assembly will allow development of more markers for *Ascochyta* blight resistance in the future. Till now, the utilization of these markers in MAS is very limited in lentil. In the past, Taran et al. (2003) used markers linked to *ral2* (UBC 227_{1290}), to *Ab*R1 ($RB18_{680}$), and to the major gene for resistance to anthracnose ($OPO6_{1250}$) to isolate RILs which were resistant to the disease.

8.5.3 Pea

Pea (*Pisum sativum* L.) is an important legume mainly grown as spring crop in temperate regions. It is the cheap source of high-quality vegetable proteins both for human food and animal feed and is able to fix atmospheric nitrogen symbiotically, improves soil fertility, and reduces the need for nitrogen fertilizers (Sun et al. 2015; Ghafoor and McPhee 2012). However, pea frequently suffers from various diseases throughout its lifecycle which severely affects its yield and seed quality. *Ascochyta* blight, *Fusarium* wilt, downy and powdery mildew, bacterial blight, root rot and damping off, etc., are the major diseases that occur in pea.

Powdery Mildew Powdery mildew disease is caused by *Erysiphe pisi* which reduces the pea yield up to 25–50%. Several pea germplasm lines had been identified

and characterized for resistance to *E. pisi* and their resistance genes. Two recessive genes (*er1* and *er2*) and one dominant gene (*Er*3) have been identified for resistance to powdery mildew in pea germplasm (Fondevilla et al. 2007). Genetic analyses of resistance to *E. pisi* indicated that gene *er1* is the most commonly present in all resistant pea genotypes whereas *er2* is found in only few resistant individuals. The newly identified dominant gene *Er3* is now characterized and transferred into cultivated pea for powdery mildew resistance (Sun et al. 2015; Tiwari et al. 1997). To aid MAS in pea breeding programs, several studies have been carried out to identify the genomic regions associated with *er1* locus by RFLP, RAPD, SCAR, and SSR markers (Shrivastava et al. 2012). Sarala (1993) and Timmerman et al. (1994) stated that the *er1* gene was present on pea linkage group (LG) VI based on their linkage study by using both morphological and molecular markers. Dirlewanger et al. (1994) found the position of *er1* gene at 9.8 cM distance from RFLP marker p236, whereas Timmerman et al. (1994) found that the RAPD marker, $OPD10_{650}$, was positioned at 2.1 cM from *er1* gene. Janila and Sharma (2004) converted the RAPD marker ($OPD10_{650}$) into a SCAR marker, which was mapped at a distance of 3.4 cM from *er1* gene. Three SSR markers, viz., PSMPSAD60, PSMPSAA374, and PSMPA5, were developed by Ek et al. (2005) which are linked with *er1* gene at a distance of 10.4, 11.6, and 14.9 cM, respectively. According to Tonguc and Weeden (2010), the *er1* locus is positioned between two markers, BC210 and BA9. They found that *er1* was 8.2 cM away from the marker BC210, and further they confirmed the presence of *er1* locus on LG VI of the genetic map of pea. The efficacy of MAS for powdery mildew was investigated by Nisar and Ghafoor (2011) in the F_2 population of the hybrid Fallon (*er1*)/11760-3(*ER1*) with RAPD marker $OPB18_{430}$ which is linked to *er1* gene at 11.2 cM distance. Recently, Sun et al. (2016) discovered a novel *er1* allele designated as *er1–6*, conferring powdery mildew resistance in Chinese pea. They found that resistance effect of *er1–6* was consistent with those of *er1–2* allele through transcript analysis.

Marker-assisted breeding for powdery mildew resistance in pea was performed by Rakshit et al. (2001) using an RAPD marker OPD 10_{650} which was linked to powdery mildew resistance locus at 3.6 cM. However, Tiwari et al. (1998) did not find $OPD10_{650}$ to be useful for MAS in progeny derived from a cross of the resistant cultivar Highlight (*er1*) and the susceptible cultivar Radley. Since *er1* is a recessive gene, therefore, introgression of *er1* requires a generation of selfing after every backcross generation to obtain homozygous resistant BC_nF_2 parents for the next backcross cycle. Marker-assisted selection provides an ideal strategy for transferring *er1* gene into superior cultivars having powdery mildew susceptibility (Ghafoor and McPhee 2012). Thus, several marker-trait associations for powdery mildew resistance have been identified with varying degrees of linkage which needs to reconfirm the marker-trait association for use in MAS-based breeding pea for powdery mildew resistance in the future.

Ascochyta Blight Ascochyta blight or black spot is the most destructive disease of field peas, and it is distributed throughout the world (Bretag et al. 2006). The disease *Ascochyta* blight in pea is caused by a complex of three fungal pathogens, commonly

referred to the *Ascochyta* complex, including *Ascochyta pinodes* L.K. Jones (teleomorph: *Mycosphaerella pinodes* (Berk. & Blox.) Vestergr.), *Phoma medicaginis* var. *pinodella* (L.K. Jones) Morgan-Jones & K.B. Burch, *Ascochyta pisi* Lib. (teleomorph: *Didymella pisi* sp. nov.), and *Phoma koolunga* Davidson et al. sp. nov. (Davidson et al. 2009; Liu et al. 2013). It reduces the grain yield up to 10–40% and causes damage on the leaves, stems, and roots limiting proper plant metabolism and also reduces grain quality (Liu et al. 2016). Among various management strategies, genetic resistance is the reasonably and ecologically sound approach to control *Ascochyta* blight in field pea (Fondevilla et al. 2011). Several linkage maps have been developed in pea using AFLP, RAPD, SSR, STS, and EST-SSR markers for the identification of genomic regions associated with *Ascochyta* blight resistance (Prioul et al. 2004; Fondevilla et al. 2008). Scientists are continuously working on *Ascochyta* blight resistance in pea and found more than 30 QTLs associated with *Ascochyta* blight resistance on all the seven linkage groups (LGs) (Prioul et al. 2004; Tar'an et al. 2003a, b; Timmerman-Vaughan et al. 2002, 2004). Timmerman-Vaughan et al. (2002, 2004) reported 19 QTLs for AB resistance on LGs I, II, III, IV, V, and VII and Group A in two pea mapping populations, whereas Tar'an et al. (2003a) identified three QTLs on LGs II, IV, and VI. Prioul et al. (2004) reported six QTLs on LGs III, V, VI, and VII and 10 QTLs on LGs II, III, V, and VII under controlled and field conditions, respectively. In *P. sativum* ssp. *syriacum*, six QTLs were reported on LGs II, III, IV, and V by Fondevilla et al. (2008), whereas three additional QTLs were identified by Fondevilla et al. (2011) on LGs III and VI. Carrillo et al. (2014) identified four new QTLs on LGs II, III, and V controlling cellular mechanisms involved in *Ascochyta* blight resistance in *P. sativum* ssp. *syriacum*. Fondevilla et al. (2011) indicated that QTLs MpIII.1, MpIII.3, and MpIII.2 detected in *P. sativum* ssp. *syriacum* corresponded to the QTLs mpIII-1, mpIII-3, and mpIII-5 identified in *P. sativum* by Prioul et al. (2004). Co-localization of QTLs for disease resistance with candidate genes including RGAs (resistance gene analogs), PsDof1 (a putative transcription factor), and DRR230-b (a pea defensin) involved in defense responses to *P. pinodes* was reported in pea (Timmerman-Vaughan et al. 2002, 2016; Prioul-Gervais et al. 2007). Further, Jha et al. (2015) reported significant association of SNPs detected within candidate genes *PsDof1* (PsDof1p308) and *RGA-G3A* (RGA-G3Ap103) with *Ascochyta* blight scores. Most recently, nine QTLs were identified for *Ascochyta* blight resistance in an interspecific pea population (PR-19) developed from a cross between Alfetta (*P. sativum*) and wild pea accession P651 (*P. fulvum*) (Jha et al. 2016). QTLs abI-IV-2 and abIII-1 were further fine mapped in RIL-based HIF populations through SNP-based GBS by Jha et al. (2017). They found two new QTLs, abI-IV-2.1 and abI-IV-2.2 within abI-IV-2 QTL for *Ascochyta* blight resistance, and these QTLs were individually explained 5.5 to 14% of the total phenotypic variation.

Fusarium **Wilt** Fusarium wilt (*Fusarium oxysporum* f. sp. *pisi* (*Fop*)) of pea is one of the most widespread diseases worldwide and causes a vascular wilt resulting in significant crop losses. Based on the differential pathogenicity on pea genotypes, mainly four races, viz., *Fop*1, *Fop* 2, *Fop* 5, and *Fop* 6, of *Fusarium oxysporum* f. sp. *pisi*

were identified (Kraft and Pfleger 2001). According to McClendon et al. (2002), resistance to most *Fop* races are governed by single gene. Resistance to *Fusarium* wilt race 1 was reported as a single gene, *Fw*, located on linkage group III. Resistance to *Fop* race 2 was postulated to be qualitative and was assigned a single gene (*Fnw*) called *Fusarium* near wilt. The major locus *Fnw* has now been mapped to LG IV of pea and named *Fnw4.1* (McPhee et al. 2012). Two other significant minor QTLs, viz., *Fnw 3.1* and *Fnw 3.2*, on LG III for *Fop* race 2 have been also identified by McPhee et al. (2012). Gene *Fwf* conferring resistance to *Fop* race 5 has been placed on LG II (McClendon et al. 2002; Okubara et al. 2005). The genetics of resistance to *Fop* race 6 is not clear, but few scientists believed that it is governed by single dominant gene (Haglund and Kraft 2001).

McClendon et al. (2002) identified one AFLP marker, ACG: CAT_222 at 1.4 cM away from Fw locus. A RAPD maker, Y15_1050 (4.6 cM from *Fw*) was developed into a dominant 999 base-pair (bp) SCAR marker which identified a Y15 allele linked in coupling phase to susceptibility (McClendon et al. 2002; Okubara et al. 2005). Later on, Loridon et al. (2005) mapped the *Fw* locus on the pea SSR consensus map between AA5-235 (3.3 cM) and AD134-213 (2.5 cM). Kwon et al. (2013) successfully developed SCAR markers tightly linked to *Fw* in pea using the TRAP marker technology in conjunction with BSA. They described the production of three useful SCAR markers linked to *Fop* race 1 resistance in pea. Using a combination of two SCARs, Fw_Trap_480 and Fw_Trap_220, in a multiplex PCR, the accuracy for marker-assisted selection was improved later (Kwon et al. 2013).

8.6 Major Bottlenecks

Since the inception of molecular markers in crop plants, several genetic linkage maps were developed in pulses. Many markers for disease resistance are available in common bean, lentil, chickpea, and some tropical legumes. But, most of them are RAPD, SCAR, or AFLP markers. Report on SSR markers in these pulses has started appearing since last 10 years. Availability of high-resolution genetic linkage map in pulse crops is lacking. Information on genome sequences, expression databases, and genomics platform are available for most of these major pulse crops in this decade. With this advent, the development of high-resolution maps of major pulse crops like pigeon pea, chickpea, lentil, etc. is needed. Availability of reference genome sequences in pulses triggers adoption of re-sequencing and GWAS approach in some pulses. Such re-sequencing approaches have ample scope for the development of breeder-friendly markers (like InDel, STMS, and SNP markers). The usage of these new markers for the development of high-resolution maps is of immediate need. Moreover, such markers could be better utilized in tagging disease resistance genes through bi-parental mapping. The generation of high-resolution bi-parental mapping population in some of the pulse crops (like lentil and chickpea) is cumbersome due to their inherent low pod setting per artificial cross. To avoid this problem,

future thrust should be given on GWAS approaches utilizing available global germplasm, mini-core collection, diversity panels, MAGIC population, etc. Another important bottleneck in disease resistance breeding is the frequent evolution of pathogen races and breakdown of genetic resistance. To overcome such unavoidable situations in the field, breeding efforts must be directed toward incorporation of horizontal resistance or bringing in recessive resistances which have broad-spectrum activity in the field (Ning et al. 2017; Ning and Wang 2018). Moreover, improved varieties in pulses should be pyramided with various disease resistances with the help of MAS in the future.

8.7 Conclusion and Perspective

The reproduction rate of pathogen is higher than its host. In nature, pathogen can generate variability through mutation, sexual recombination, heterokaryosis, and parasexual cycle. To keep the pace with this continuous load of pathogenic strains in the field, resistance breeding should be well focused for economical crops like pulses. Research should be focused on development of quick/fast disease screening protocol, rapid identification of resistant genotypes and molecular markers, and pyramiding of various disease resistance genes through marker-assisted selection procedure. At present, genomic pipelines in most of the major pulse crops have been generated (Varshney 2016). It is utmost need to develop complementary genomic pipelines in pathogen too. Generation of genomic pipelines and expression data in pathogen will help in genome-wide identification of effector repertoires. Such effectors can be used for effector-mediated screening of germplasm for disease resistance through agro-infection or virus-mediated infection in plants. This "effectoromics" approach will be a potent contributor in modern disease resistance breeding for pulse crops (Vleeshouwers and Oliver 2014). Although enough markers were developed in pulses for various disease resistance traits, their exploitation in field remains elusive due to the problem in reproducibility, unreliability, and larger map distance between the marker and the targeted resistance genes. In the era of genomics technologies, reliable marker-trait association should be established through GWAS in diversity panel or in MAGIC or NAM populations. NGS technologies along with the above approaches will help to develop various SNP markers within a close proximity to candidate gene or within gene itself. Such developments will trigger high-throughput germplasm screening, MAS, and pyramiding of different resistances through the usage of various SNP platforms in the future.

Acknowledgments The authors sincerely acknowledge the encouragement from the Associate Director (A), Bioscience Group, and Head of Nuclear Agriculture and Biotechnology Division of Bhabha Atomic Research Centre.

Conflict of Interest The authors of this chapter declare that there are no conflict of interest and no financial gain from it.

References

Adam-Blondon AF, Sevignac M, Bannerot H, Dron M (1994) SCAR, RAPD and RFLP markers linked to a dominant gene (*Are*) conferring resistance to anthracnose in common bean. Theor Appl Genet 88:865–870

Agbicodo EM, Fatokun CA, Bandyopadhyay R, Wydra K, Diop NN, Muchero W, Ehlers JD, Roberts PA, Close TJ, Visser RGF, van der Linden CG (2010) Identification of markers associated with bacterial blight resistance loci in cowpea [*Vigna unguiculata* (L.) Walp.]. Euphytica 175:215–226

Ammavasai S, Phogat DS, Solanki IS (2004) Inheritance of resistance to mungbean yellow mosaic virus (MYMV) in green gram (*Vigna radiata* L. Wilczek). Ind J Genet 64:146

Anbessa Y, Tara'n B, Warkentin TD, Tullu A, Vandenberg A (2009) Genetic analyses and conservation of QTL for Ascochyta blight resistance in chickpea (*Cicer arietinum* L.). Theor Appl Genet 4:757–765

Andargie M, Pasquet RS, Gowda BS, Muluvi GM, Timko MP (2011) Construction of a SSR-based genetic map and identification of QTLs for yield and domestication traits using recombinant inbred lines from a cross between wild X cultivated cowpea (*V. unguiculata* (L.) Walp.). Mol Breed 28:413–420

Andrahennadi CP (1994) Genetic linkage of isozyme markers and resistance to seed borne *Ascochyta* infection in lentil. M.Sc Thesis, Department of Crop Science and Plant Ecology, University of Saskatchewan, SK., Canada

Anjum T, Gupta SK, Datta S (2010) Mapping of Mungbean Yellow Mosaic India Virus (MYMIV) and powdery mildew resistant gene in black gram [*Vigna mungo* (L.) Hepper]. Elect J Plant Breed 1(4):1148–1152

Arumuganathan K, Earle DE (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–218

Aryamanesh N, Nelson MN, Yan G, Clarke HJ, Siddique KHM (2010) Mapping a major gene for growth habit and QTLs for Ascochyta blight resistance and flowering time in a population between chickpea and *Cicer reticulatum*. Euphytica 173:307–319

Atienza SG, Palomino C, Gutiérrez N, Alfaro CM, Rubiales D, Torres AM, Ávila CM (2016) QTLs for ascochyta blight resistance in faba bean (*Vicia faba* L.): validation in field and controlled conditions. Crop Pasture Sci 67(2):216–224

Avila CM, Sillero JC, Rubiales D, Moreno MT, Torres AM (2003) Identification of RAPD markers linked to the *Uvf*-1 gene conferring hypersensitive resistance against rust (*Uromuces viciae-fabae*) in *Vicia Faba* L. Theor Appl Genet 107:353–358

Bai Y, Michaels TE, Pauls KP (1997) Identification of RAPD markers linked to common bacterial blight resistance genes in *Phaseolus vulgaris* L. Genome 40:544–551

Barilli E, Satovic Z, Rubiales D, Torres AM (2010) Mapping of quantitative trait loci controlling partial resistance against rust incited by *Uromyces pisi* (Pers.) Wint. in a *Pisum fulvum* L. intraspecific cross. Euphytica 175:151–159

Barman P, Handique AK, Tanti B (2014) Tagging STMS markers to Fusarium wilt race-1 resistance in chickpea (*Cicer arietinum* L.). Indian J Biotechnol 13:370–375

Basak J, Kundagrami S, Ghose TK, Pal A (2004) Development of yellow mosaic virus (YMV) resistance linked DNA marker in *Vigna mungo* from populations segregating for YMV reaction. Mol Breed 14:375–383

Bassi D, Briñez B, Rosa JS, Oblessuc PR, Almeida CP, Nucci SM, Silva LCD, Chiorato AF, Vianello PR, Camargo LEA, Blair MW, Lasry L, Reis B (2017) Linkage and mapping of quantitative trait loci associated with angular leaf spot and powdery mildew resistance in common beans. Genet Mol Biol 40(1):109–122

Beebe S (2012) Common bean breeding in the tropics. In: Janick J (ed) Plant breeding reviews 36. Wiley, Hoboken, pp 357–426

Benko-Iseppon AM, Winter P, Huettel B, Staginnus C, Muehlbauer FJ, Kahl G (2003) Molecular markers closely linked to Fusarium resistance genes in chickpea show significant alignments

to pathogenesis-related genes located on *Arabidopsis* chromosomes 1 and 5. Theor Appl Genet 107:379–386

Bhadauria V, Ramsay L, Bett KE, Banniza S (2017) QTL mapping reveals genetic determinants of fungal disease resistance in the wild lentil species *Lens ervoides*. Sci Rep 7:3231

Blair MW, Rodriguez LM, Pedraza F, Morales F, Beebe S (2007) Genetic mapping of the bean golden mosaic geminivirus resistant gene Bgm-1 and linkage with potyvirus resistance in common bean (Phaseolus vulgaris L.). Theor Appl Genet 107:1362–1374

Boersma JG, Conner RL, Balasubramanian PM, Yu K, Hou A (2013) Marker-assisted dissection of anthracnose resistance in the dry bean cultivar Morden003. Can J Plant Sci 93:1115–1123

Bohra A, Dubey A, Saxena RK, Penmetsa RV, Poornima KN, Kumar N et al (2011) Analysis of BAC-end sequences (BESs) and development of BES-SSR markers for genetic mapping and hybrid purity assessment in pigeonpea (*Cajanus spp.*). BMC Plant Biol 11:56

Bohra A, Saxena RK, Gnanesh BN, Saxena KB, Byregowda M, Rathore A et al (2012) An intra-specific consensus genetic map of pigeonpea [*Cajanus cajan* (L.) Millspaugh] derived from six mapping populations. Theor Appl Genet 125:1325–1338

Boukar O, Kong L, Singh BB, Murdock L, Ohm HW (2004) AFLP and AFLP-derived SCAR markers associated with *Striga* gesnerioides resistance in cowpea. Crop Sci 44:1259–1264

Boukar O, Fatokun CA, Huynh BL, Roberts PA, Close TJ (2016) Genomic tools in cowpea breeding programs: status and perspectives. Front Plant Sci 7:757. https://doi.org/10.3389/fpls.2016.00757

Bretag TW, Keane PJ, Price TV (2006) The epidemiology and control of ascochyta blight in field peas: a review. Aust J Agr Res 57:883–902

Broughton WJ, Hernandez G, Blair M, Beebe S, Gepts P, Vanderleyden J (2003) Beans (*Phaseolus* spp.) – model food legumes. Plant Soil 252:55–128

Carrillo E, Boucherot K, Satovic Z, Rubiales D, PAubert G, Fondevilla S (2014) Identification of quantitative trait loci and candidate genes for specific cellular resistance responses against *Didymella pinodes* in pea. Plant Cell Rep 33:1133–1145

Castro P, Pistón F, Madrid E, Millán T, Gil J, Rubio J (2010) Development of chickpea near-isogenic lines for fusarium wilt. Theor Appl Genet 121:1519–1526

Chankaew S, Somta P, Sorajjapinun W, Srinives P (2011) Quantitative trait loci mapping of *Cercospora* leaf spot resistance in mungbean, *Vigna radiata* (L.) Wilczek. Mol Breed 28:255–264

Chen W, Muehlbauer FJ (2003) An improved technique for virulence assay of *Ascochyta rabiei* on chickpea. Intl Chickpea Pigeonpea Newslett 10:31–33

Chen W, Coyne CJ, Peever TL, Muehlbauer FJ (2004) Characterization of chickpea differentials for pathogenicity assay of ascochyta blight and identification of chickpea accessions resistant to *Didymella rabiei*. Plant Pathol 53:759–769

Chen HM, Liu CA, Kuo CG, Chein CM, Sun HC, Huang CC, Lin YC, Ku HM (2007) Development of a molecular marker for a bruchid (*Callosobruchus chinensis* L.) resistance gene in mungbean. Euphytica 157:113–122

Chen HM, Ku HM, Schafleitner R., Bains TJ, Kuo CG, Liu CA, Nair R (2013) The major quantitative trait locus for mungbean yellow mosaic Indian virus resistance is tightly linked in repulsion phase to the major bruchid resistance locus in a cross between mungbean (*Vigna radiata* (L.) Wilczek) and its wild relative Vigna radiata ssp. sublobata. Euphytica 192:215–216

Chetukuri A, Gaur PM, Pande S, Gali KK, Ganesh M, Kumar J, Varshney RK (2011) Mapping QTL for resistance to botrytis grey mould in chickpea. Euphytica 182:1–9

Cho SH, Chen WD, Muehlbauer FJ (2004) Pathotype-specific genetic factors in chickpea (*Cicer arietinum* L.) for quantitative resistance to ascochyta blight. Theor Appl Genet 109:733–739

Choudhary N, Bawa V, Paliwal R, Singh B, Bhat A, Mir JI, Gupta M, Sofi PA, Thudi M, Varshney RK, Mir RR (2018) Gene/QTL discovery for Anthracnose in common bean (*Phaseolus vulgaris* L.) from North-western Himalayas. PLoS One 13(2):e0191700

Choudhury MA, Andrahennadi CP, Slinkard AE, Vandenberg A (2001) RAPD and SCAR markers for resistance to ascochyta blight in lentil. Euphytica 118:331–337

Cobos MJ, Fernandez M, Rubio J, Kharat M, Moreno MT, Gil J, Millan T (2005) A linkage map of chickpea (*Cicer arietinum* L.) based on populations from Kabuli x Desi crosses: location of genes for resistance to *Fusarium* wilt race 0. Theor Appl Genet 110:1347–1353

Cobos MJ, Winter P, Kharrat M, Cubero JI, Gil J, Millan T, Rubio J (2009) Genetic analysis of agronomic traits in a wide cross of chickpea. Field Crop Res 111:130–136

Collard BCY, Pang ECK, Ades PK, Taylor PWJ (2003) Preliminary investigation of QTLs associated with seedling resistance to ascochyta blight from Cicer echinospermum, a wild relative of chickpea. Theor Appl Genet 107:719–729

Daba K, Deokar A, Banniza S, Warkentin TD, Taran B (2016) QTL mapping of early flowering and resistance to ascochyta blight in chickpea. Genome 59:413–425

Dasgroux A, L'Anthoëne V, Roux-Duparque M, Rivière J-P, Aubert G et al (2016) Genome-wide association mapping of partial resistance to *Aphanomyces euteiches* in pea. BMC Genomics 17:124

Daspute A, Fakrudin B, Bhairappanavar SB, Kavil SP, Narayana YD, Muniswamy Kaumar A, Krishnaraj PU, Yerimani A, Khadi BM (2014) Inheritance of pigeonpea sterility mosaic disease resistance in pigeonpea. Plant Pathol J 30:188–194

Daspute A, Fakrudin B (2015) Identification of coupling and repulsion phase DNA marker associated with an allele of a gene conferring host plant resistance to pigeonpea sterility mosaic virus (PPSMV) in pigeonpea (*Cajanus cajan* L. Millsp.). Plant Pathol J 31:33–40

Davidson JA, Hartley D, Priest M, Herdina MKK, McKay A, Scott ES (2009) A new species of *Phoma* causes ascochyta blight symptoms on field peas (*Pisum sativum*) in South Australia. Mycologia 101:120–128

Dhole VJ, Reddy KS (2013) Development of a SCAR marker linked with a MYMV resistance gene in mungbean (*Vigna radiata* L. Wilczek). Plant Breed 132:127–132

Díaz-Ruiz R, Satovic Z, Ávila CM, Alfaro CM, Gutierrez MV, Torres AM, Román B (2009) Confirmation of QTLs controlling Ascochyta fabae resistance in different generations of faba bean (*Vicia faba* L.). Crop Pasture Sci 60:353–361

Dikshit HK, Singh A, Singh D, Aski M, Jain N, Hegde VS, Basandrai AK, Basandrai D, Sharma TR (2016) Tagging and mapping of SSR markers for rust resistance gene in lentil (*Lens culinaris* Medik *sub sp. culinaris*) Ind. J Exp Bot 54:394–399

Dirlewanger E, Isaac PG, Ranade S, Beldeaux M, Cousin R, deVienne D (1994) Restriction fragment length polymorphism analysis of loci with disease resistance genes and developmental traits in *Pisum sativum* L. Theor Appl Genet 88:17–27

Ek M, Eklund M, Von Post R, Dayteg C, Henriksson T, Weibull P, Ceplitis A, Isaac P, Tuvesson S (2005) Microsatellite markers for powdery mildew resistance in pea (*Pisum sativum* L.). Hereditas 142:86–91

Eujayl I, Erskine W, Bayaa B, Baum M, Pehu E (1998) Fusarium vascular wilt in lentil: inheritance and identification of DNA markers for resistance. Plant Breed 117:497–499

Fall AL, Byrne PF, Jung G, Coyne DP, Brick MA, Schwartz HF (2001) Detection and mapping of a major locus for Fusarium wilt resistance in common bean. Crop Sci 41:1494–1498

Fang JG, Chao CT, Roberts PA, Ehlers JD (2007) Genetic diversity of cowpea (*Vigna unguiculata*) in four West African and USA breeding programme as determined by AFLP analysis. Genet Resour Crop Evol 54:1197–1209

FAO (2016) In: Lucrezia C, Ronald V, Liesi W (eds) Soil and Pulses Symbiosis for Life. Food and Agriculture Organization of the United Nations, Rome. ISBN 978-92-5-109501-0.

Fatokun CA, Danesh D, Menancio-Hautea DI, Young ND (1993) A linkage map for cowpea [*Vigna unguiculata* (L) Walp] based on DNA markers (2n = 22). In: O'Brien JS (ed) Genetic maps 1992. A compilation of linkage and restriction maps of genetically studied organisms. Cold Spring Harbor Laboratory Press, Cold Spring Harbour, pp 6256–6258

Fondevilla S, Carver TLWQ, Moreno MT, Rubiales D (2006) Macroscopic and histological characterization of gene er1 and er2 for powdery mildew resistance in pea. Eur J Plant Pathol 115:309–321

Fondevilla S, Torres AM, Moreno MT, Rubiales D (2007) Identification of a new gene for resistance to powdery mildew in *Pisum fulvum*, a wild relative of pea. Breed Sci 57:181–184

Fondevilla S, Satovic Z, Rubiales D, Moreno MT, Torres AM (2008) Mapping of quantitative trait loci for resistance to *Ascochyta pinodes* in *Pisum sativum* subsp. *syriacum*. Mol Breed 21:439–454

Fondevilla S, Küster H, Krajinski F, Cubero JI, Rubiales D (2011) Identification of genes differentially expressed in a resistant reaction to *Ascochyta pinodes* in pea using microarray technology. BMC Genomics 12:28

Ford R, Pang ECK, Taylor PWJ (1999) Genetics of resistance to ascochyta blight of lentil and the identification of closely linked markers. Theor Appl Genet 98:93–98

Frew TJ, Russell AC, Timmerman-Vaughan GM (2002) Sequence tagged site markers linked to the *smb1* gene for resistance to pea seed borne mosaic virus in pea. Plant Breed 121:512–516

Ganapathy KN, Byregowda M, Venkatesh SC, Ramachandra R, Gnanesh BN, Girish G (2009) Identification of AFLP markers linked to sterility mosaic disease in pigeonpea *Cajanus cajan* (L.) Millsp. Int J Integr Biol 7:145–149

Garg T, Mallikarjuna BP, Samineni S, Singh S, Sandhu JS, Kaur L, Singh I, Sirari A, Basandrai AK, Basandrai D, Varshney RK, Gaur PM (2018) Identification of QTLs for resistance to Fusarium wilt and Ascochyta blight in a recombinant inbred population of chickpea (*Cicer arietinum* L.). Euphytica 214:45. https://doi.org/10.1007/s10681-018-2125-3

Ghafoor A, McPhee K (2012) Marker assisted selection (MAS) for developing powdery mildew resistant pea cultivars. Euphytica 186:593–607

Ghosh R, Tarafdar A, Sharma M (2017) Rapid and sensitive diagnoses of dry root rot pathogen of chickpea (*Rhizoctonia bataticola* (Taub.) Butler) using loop-mediated isothermal amplification assay. Sci Rep 7:42737

Gioi TD, Boora KS, Chaudhary K (2012) Identification and characterization of SSR markers linked to yellow mosaic virus resistance genes in cowpea (*Vigna unguiculata*). Int J Plant Res 2:1–8

Gnanesh BN, Bohra A, Sharma M, Byregowda M, Pandey S, Wesley V, Saxena RK, Saxena KB, KaviKishor PB, Varshney RK (2011) Genetic mapping and quantitative trait locus analysis of resistance to sterility mosaic disease in pigeonpea [*Cajanus cajan* (L.) Millsp.]. Field Crop Res 123:53–61

Goncalves-Vidigal MC, Cruz AS, Garcia A, Kami J, Vidigal Filho PS, Sousa LL, McClean P, Gepts P, Pastor-Corrales MA (2011) Linkage mapping of the Phg-1 and Co-1 (4) genes for resistance to angular leaf spot and anthracnose in the common bean cultivar AND 277. Theor Appl Genet 122:893–903

Gowda SJM, Radhika P, Kadoo NY, Mhase LB, Gupta VS (2009) Molecular mapping of wilt resistance genes in chickpea. Mol Breed 24:177–183

Gupta SK, Gopalakrishna T (2009) Genetic diversity analysis in blackgram (*Vigna mungo* (L.) Hepper) using AFLP and transferable microsatellite markers from azuki bean (*Vigna angularis* (Willd.) Ohwi & Ohashi). Genome 52:120–128

Gupta SK, Gopalakrishna T (2010) Development of unigene-derived SSR markers in cowpea (*Vigna unguiculata*) and their transferability to other *Vigna species*. Genome 53:508–523

Gupta SK, Souframanien J, Gopalakrishna T (2008) Construction of a genetic linkage map of black gram, *Vigna mungo* (L.) Hepper, based on molecular markers and comparative studies. Genome 51:628–637

Gupta PK, Langridge P, Mir RR (2010) Marker-assisted wheat breeding: present status and future possibilities. Mol Breed 26:145–161

Gupta D, Taylor PWJ, Inder P, Phan HTT, Ellwood SR, Mathur PN et al (2012) Integration of EST-SSR markers of *Medicago truncatula* into intraspecific linkage map of lentil and identification of QTL conferring resistance to ascochyta blight at seedling and pod stages. Mol Breed 30:429–439

Gupta S, Gupta DS, Anjum KT, Pratap A, Kumar J (2013) Transferability of simple sequence repeat markers in blackgram (*Vigna mungo* L. Hepper). Aust J Crop Sci 7:345–353

Gupta SK, Souframanien J, Reddy KS (2015) Validation of molecular markers linked to yellow mosaic virus disease resistance in diverse genetic background of black gram [*Vigna mungo* (L.) Hepper]. Electron J Plant Breed 6:755–763

Gurha SN, Singh G, Sharma YR (2003) Diseases of chickpea and their management. In: Ali M, Kumar S, Singh NB (eds) Chickpea research in India. Indian Institute of Pukse research, Kanpur, pp 195–227

Gwag JG, Chung JW, Chung HK, Lee JH, Ma KH, Dixit A, Park YJ, Cho EG, Kim TS, Lee SH (2006) Characterization of new microsatellite markers in mungbean, *Vigna radiata* (L.). Mol Ecol Resour 6:1132–1134

Hagerty CH, Cuesta-Marcos A, Cregan PB, Song Q, McClean P, Noffsinger S, Myers JR (2015) Mapping and root rot resistance and root architecture quantitative trait loci in common bean. Crop Sci 55:1969–1977

Haglund WA, Kraft JM (2001) Fusarium wilt. In: Kraft JM, Pfleger FL (eds) Compendium of pea diseases, 2nd edn. APS Press, St. Paul, pp 14–16

Halila I, Cobos MJ, Rubio J, Millan T, Kharrat M, Gil J (2009) Tagging and mapping a second resistance gene for Fusarium wilt race 0 in chickpea. Eur J Plant Pathol 124:87–92

Hamwieh A, Udupa S, Choumane W, Sarker A, Dreyer F, Jung C, Baum M (2005) A genetic linkage map of *Lens* sp. based on microsatellite and AFLP markers and the localization of fusarium vascular wilt resistance. Theor Appl Genet 110:669–677

Havey MJ, Muehlbauer FJ (1989) Linkages between restriction fragment length, Isozyme, and morphological markers in lentil. Theor Appl Genet 77:395–401

Humphry ME, Magner T, McIntyre CL, Aitken EAB, Liu CJ (2003) Identification of a major locus conferring resistance to powdery mildew (*Erysiphe polygoni* DC) in mungbean (*Vigna radiata* L. Wilczek) by QTL analysis. Genome 46:738–744

Hurtado-Gonzales OP, Valentini G, Song O, Pastor-Corrales MA (2017) Fine mapping of Ur-3, a historically important rust resistance locus in common bean. G3 7:557–569

Hussain M, Qazi J, Mansoor S, Iram S, Bashir M, Zafar Y (2004) First report of mungbean yellow mosaic India virus on mungbean in Pakistan. Plant Pathol 53:518

Ilyas M, Qazi J, Mansoor S, Briddon RW (2010) Genetic diversity and phylogeography of begomoviruses infecting legumes in Pakistan. J Gen Virol 91:2091–2101

Infantino A, Kharrat M, Riccioni L, Coyne CJ, McPhee KE, Grunwald NJ (2006) Screening techniques and sources of resistance to root diseases in cool season food legumes. Euphytica 147:201–222

IPCC (Intergovernmental Panel on Climate Change) (2012) Managing the risks of extreme events and disasters to advance climate change adaptation. In: Field CB, Barros V, Stocker TF, Qin D, Dokken DJ, Ebi KL, Mastrandrea MD, Mach KJ, Plattner GK, Allen SK, Tignor M, Midgley PM (eds) A Special Report of Working Groups I and II of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge/New York, pp 3–21. Summary for policy makers

Iruela M, Rubio J, Barro F, Cubero JI, Millan T, Gil J (2006) Detection of two quantitative trait loci for resistance to ascochyta blight in an intra-specific cross of chickpea (*Cicer arietinum* L.): development of SCAR markers associated with resistance. Theor Appl Genet 112:278–287

Iruela M, Castro P, Rubio J, Cubero JI, Jacinto C, Millan T, Gil J (2007) Validation of a QTL for resistance to Ascochyta blight linked to resistance to Fusarium wilt race 5 in chickpea (*Cicer arietinum* L.). Eur J Plant Pathol 119:29–37

Iruela M, Piston F, Cubero JI, Millan T, Barro F, Gil J (2009) The marker SCK13 associated with resistance to Ascochyta blight in chickpea is located in a region of a putative retrotransposon. Plant Cell Rep 28:53–60

Janila P, Sharma B (2004) RAPD and SCAR markers for powdery mildew resistance gene *er* in pea. Plant Breed 123:271–274

Jendoubi W, Bouhadida M, Millan T, Kharrat M, Gil J, Rubio J, Madrid E (2016) Identification of target region including the $Foc0_1$ /$foc0_1$ gene and development of near isogenic lines for resistance to *Fusarium* wilt race 0 in chickpea. Euphytica 210:119–133

Jha AB, Tar'an B, Diapari M, Sindhu A, Shunmugam A, Bett K, Warkentin TD (2015) Allele diversity analysis to identify SNPs associated with ascochyta blight resistance in pea. Euphytica 202:189–197

Jha AB, Tar'an B, Stonehouse R, Warkentin TD (2016) Identification of QTLs associated with improved resistance to ascochyta blight in an interspecific pea recombinant inbred line population. Crop Sci 56:2926–2939

Jha AB, Gali KK, Tar'an B, Warkentin TD (2017) Fine mapping of QTLs for ascochyta blight resistance in Pea using heterogeneous inbred families. Front Plant Sci 8:765

Jingade P, Ravikumar RI (2015) Development of molecular map and identification of QTLs linked to Fusarium wilt resistance in chickpea. J Genet 94:723–729

Jung G, Coyne DP, Scroch PW, Nienhuis J, Arnaud-Santana E, Bokosi J, Ariyarathne HM, Steadman, Beaver JS, Kaeppler SM (1996) Molecular markers associated with plant architecture and resistance to common blight, web blight, and rust in common bean. J Am Soc Hortic Sci 121:794–803

Kamfwa K, Mwala M, Okori P, Gibson P, Mukankusi C (2013) Identification of QTL for Fusarium Root Rot Resistance in Common Bean. J Crop Improv 27:406–418

Kang YJ et al (2014) Genome sequence of mungbean and insights into evolution within *Vigna* species. Nat Commun 5:5443. https://doi.org/10.1038/ncomms6443

Kasettranan W, Somta P, Srinives P (2010) Mapping of quantitative trait loci controlling powdery mildew resistance in Mungbean (*Vigna radiata* (L.) Wilczek). J Crop Sci Biotech 3:155–161

Kaur S, Kimber RBE, Cogan NOI, Materne M, Forster JW, Paull JG (2014) SNP discovery and high-density genetic mapping in faba bean (*Vicia faba* L.) permits identification of QTLs for ascochyta blight resistance. Plant Sci 217:47–55

Keller B, Manzanares C, Jara C, Lobaton JD, Studer B, Raatz B (2015) Fine-mapping of a major QTL controlling angular leaf spot resistance in common bean (*Phaseolus vulgaris* L.). Theor Appl Genet 128:813–826

Kelly JD, Gepts P, Miklas PN, Coyne DP (2003) Tagging and mapping of genes and QTL and molecular markers assisted selection for traits of economic importance in bean and cowpea. Field Crop Res 82:135–154

Khalekar GD, Akhare AA, Gahukar SJ, Singh NK, Kumar M (2014) Identification of simple sequence repeat markers associated with wilt resistance in pigeonpea. J Environ Biol 35:955–960

Khattak GSS, Haq MA, Rana SA, Abass G, Irfag M (2000) Effect of mungbean yellow mosaic virus (MYMV) on yields and yield components of mungbean (*Vigna radiata* L. Wilczek). Kasetsart J (Nat Sci) 34:12–16

Kitsanachandee R, Somta P, Chatchawankanphanich O, Akhtar KP, Shah TM, Nair RM, Bains TS, Sirari A, Kaurand L, Srinives P (2013) Detection of quantitative trait loci for mungbean yellow mosaic India virus (MYMIV) resistance in mungbean (*Vigna radiata* (L.) Wilczek) in India and Pakistan. Breed Sci 63:367–373

Kolkman JM, Kelly JD (2003) QTL conferring resistance and avoidance to white mold in common bean. Crop Sci 43:539–548

Kongjaimun A, Kaga A, Tomooka N, Somta P, Shimizu T, Shu Y, Isemura T, Vaughan DA, Srinives P (2012) An SSR-based linkage map of yardlong bean (*Vigna unguiculata* (L.) Walp. subsp. unguiculata sesquipedalis group) and QTL analysis of pod length. Genome 55:81–92

Kotresh H, Fakrudin B, Punnuri SM, Rajkumar BK, Thudi M, Paramesh H et al (2006) Identification of two RAPD markers genetically linked to a recessive allele of a *Fusarium* wilt resistance gene in pigeonpea (*Cajanus cajan* L. Millsp.). Euphytica 149:113–120

Kottapalli P, Gaur PM, Katiyar SK, Crouch JH, Buhariwalla HK, Pande S, Gali KK (2009) Mapping and validation of QTLs for resistance to an Indian isolate of Ascochyta blight pathogen in chickpea. Euphytica 165:79–88

Kraft JM, Pfleger FL (2001) Compendium of Pea diseases, 2nd edn. American Phytopathological Society Press, St. Paul

Kulkarni NK, Reddy AS, Kumar PL, Vijaynarasimha J, Rangaswamy KT, Muniyappa V, Reddy LJ, Saxena KB, Jones AT, Reddy DVR (2003) Broad-based resistance to pigeonpea sterility mosaic disease in accessions of *Cajanus scarabaeoides* (L.) Benth. Indian J Plant Prot 31:6–11

Kumar SV, Tan SG, Quah SC, Yusoff K (2002a) Isolation and characterization of seven tetranucleotide microsatellite loci in mungbean, *Vigna radiata*. Mol Ecol Notes 2:293–295

Kumar SV, Tan SG, Quah SC, Yusoff K (2002b) Isolation of microsatellite markers in mungbean, *Vigna radiata*. Mol Ecol Notes 2:96–98

Kundagrami S, Basak J, Maiti S, Kundu A, Das B, Ghose TK, Pal A (2009) Agronomic, genetic and molecular characterization of MYMV tolerant mutant lines of *Vigna Mungo*. Int J Plant Breed Genet 3:1–10

Kwon SJ, Smykal P, Hu J, Wang M, Kim SJ, McGee RJ, McPhee K, Coyne CJ (2013) User-friendly markers linked to *Fusarium* wilt race 1 resistance Fw gene for marker-assisted selection in pea. Plant Breed 132:642–648

Lara LAC, Santos JB, Veloso JS, Balestre M, Alves FC, Leite ME (2014) Identification of QTLs for Resistance to Sclerotinia sclerotiorum in Carioca Common Bean by the Moving Away Method. Hindawi Publishing Corporation. ISRN Molecular Biology, p 7.

Li Y, Ruperao P, Batley J, Edwards D, Davidson J, Hobson K, Sutton T (2017) Genome analysis identified novel candidate genes for ascochyta blight resistance in chickpea using whole genome re-sequencing data. Front Plant Sci 8:359

Lichtenzveig J, Bonfil DJ, Zhang HB, Shtienberg D, Abbo S (2006) Mapping quantitative trait loci in chickpea associated with time to flowering and resistance to Didymella rabiei the causal agent of Ascochyta blight. Theor Appl Genet 113:1357–1369

Liu JF, Cao TS, Feng J, Chang KF, Hwang SF, Strelkov SE (2013) Characterization of the fungi associated with ascochyta blight of field pea in Alberta, Canada. Crop Prot 54:55–64

Liu N, Xu S, Yao X, Zhang G, Mao W, Hu Q, Feng Z, Gong Y (2016) Studies on the Control of *Ascochyta* blight in field peas (*Pisum sativum* L.) caused by *Ascochyta pinodes* in Zhejiang Province, China. Front Microbiol 7:481

Lopez CE, Acosta IF, Jara C, Pedraza F, Gaitan-Solis E, Gallego G, Beebe S, Tohme J (2003) Identifying resistance gene analogs associated with resistances to different pathogens in common bean. Phytopathology 93:88–95

Loridon K, McPhee KE, Morin J, Dubreuil P, Pilet-Nayel ML, Aubert G, Rameau C, Baranger A, Coyne CJ, Lejeune-Henault I, Burstin J (2005) Microsatellite marker polymorphism and mapping in pea (*Pisum sativum* L.). Theor Appl Genet 111:1022–1031

Lucas MR, Diop NN, Wanamaker S, Ehlers JD, Roberts PA, Close TJ (2011) Cowpea–soybean synteny clarified through an improved genetic map. Plant Genome 4:218–225

Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J et al (2012) SOAPdenovo2: an empirically improved memory-efficient short-read denovo assembler. Giga Sci 1:18. https://doi.org/10.1186/2047-217X-1-18

Madrid E, Rubiales D, Moral A, Moreno MT, Millán T, Gil J, Rubio J (2007) Mechanism and molecular markers associated with rust resistance in a chickpea interspecific cross (Cicer arietinum × Cicer reticulatum). European J Plant Pathol 121(1):43–53

Mahuku GS, Maria Iglesias A, Jara C (2009) Genetics of angular leaf spot resistance in the Andean common bean accession G5686 and identification of markers linked to the resistance genes. Euphytica 167:381–396

Mahuku GS, Antonia Henriquez M, Montoya C, Jara C, Teran H, Beebe S (2011) Inheritance and development of molecular markers linked to angular leaf spot resistance genes in the common bean accession G10909. Mol Breed 28:57–71

Maiti S, Basak J, Kundagrami S, Kundu A, Pal A (2011) Molecular marker assisted genotyping of mungbean yellow mosaic India virus resistant germplasm of mungbean and urdbean. Mol Biotechnol 47:95–104

Malik IA, Sarwar G, Ali Y (1986) Genetic studies in mung bean (*Vigna radiata* (L) Wilczek). 1. Inheritance of tolerance to mungbean yellow mosaic virus and some morphological characters. Pak J Bot 18:189–198

Mandal B, Verma A, Malathi VG (1997) Systemic infection of *Vigna mungo* using the cloned DNAs of the blackgram isolate of mungbean yellow mosaic geminivirus through agroinoculation and transmission of the progeny virus by whitefly. J Phytopathol 145:505–510

Maxted N, Kell SP (2009) Establishment of a global network for the in situ conservation of crop wild relatives: status and needs. FAO Commission on Genetic Resources for Food and Agriculture, Rome

Mayer MS, Tullu A, Simon CJ, Kumar J, Kaiser WJ, Kraft JM, Muehlbauer FJ (1997) Development of a DNA marker for fusarium wilt resistance in chickpea. Crop Sci 37:1625–1629
McClendon MT, Inglis DA, McPhee KE, Coyne CJ (2002) DNA markers linked to *Fusarium* wilt race 1 resistance in pea. J Am Soc Hortic Sci 127:602–607
McPhee KE, Inglis DA, Gundersen B, Coyne CJ (2012) Mapping QTL for *Fusarium* wilt race 2 partial resistance in pea (*Pisum sativum*). Plant Breed 131:300–306
Melotto M, Afanador L, Kelly JD (1996) Development of a SCAR marker linked to the I gene in common bean. Genome 39:1216–1219
Mienie CMS, Liebenberg MM, Pretorius ZA, Miklas PN (2005) SCAR markers linked to the common bean rust resistance gene *Ur-13*. Theor Appl Genet 111:972–979
Miklas PN, Johnson E, Stone V, Beaver JS, Montoya C, Zapata M (1996) Selective mapping of QTL conditioning disease resistance in common bean. Crop Sci 36:1344–1351
Miklas PN, Smith JR, Riley R, Grafton KF, Singh SP, Jung G, Coyne DP (2000) Marker-assisted breeding for pyramided resistance to common bacterial blight in common bean. Annu Rep Bean Improv Coop 43:39–40
Miklas PN, Kelly JD, Beebe SE, Blair MW (2006) Common bean breeding for resistance against biotic and abiotic stresses: from classical to MAS breeding. Euphytica 147:105–131
Millan T, Rubio J, Iruela M, Daly K, Cubero JI, Gil J (2003) Markers associated with Ascochyta blight resistance in chickpea and their potential in marker assisted selection. Field Crop Res 84:373–384
Miranda ALR (2012) Genome mapping and molecular markers for Ascochyta blight resistance in pea (*Pisum stivum* L.). MSc Thesis submitted to North Dakota State University. p 116
Miyagi M, Humphry M, Ma ZY, Lambrides CJ, Bateson M, Liu CJ (2004) Construction of bacterial artificial chromosome libraries and their application in developing PCR-based markers closely linked to a major locus conditioning bruchid resistance in mungbean (*Vigna radiata* L. Wilczek). Theor Appl Genet 110:151–156
Muchero W, Diop NN, Bhat PR, Fenton RD, Wanamaker S, Pottorff M, Hearne S, Cisse N, Fatokun CA, Ehlers JD, Roberts PA, Close TJ (2009) A consensus genetic map of cowpea [*Vigna unguiculata* (L) Walp.] and synteny based on EST-derived SNPs. Proc Natl Acad Sci U S A 106:18159–18164
Muchero W, Ehlers JD, Roberts PA (2010) QTL analysis for resistance to foliar damage caused by *Thrips tabaci* and *Frankliniella schultzei* (Thysanoptera: *Thripidae*) feeding in cowpea [*Vigna unguiculata* (L.) Walp.]. Mol Breed 25:47–56
Muchero W, Ehlers JD, Close TJ, Roberts PA (2011) Genic SNP markers and legume synteny reveal candidate genes underlying QTL for *Macrophomina phaseolina* resistance and maturity in cowpea [*Vigna unguiculata* (L) Walp.]. BMC Genomics 12:8
Mundt CC (2014) Durable resistance: a key to sustainable management of pathogen and pests. Infect Genet Evol:446–455. https://doi.org/10.1016/j.meegid.2014.01.011
Murugesan S, Murugan E, Nadarajan N (1997) Inheritance of duration, leaf colour, sterility mosaic disease resistance and growth habit in pigeonpea. Madras Agric J 84:10–12
Nagaraj KM, Chikkadevaiah, Kulkarni RS (2004) Inheritance of resistance to sterility mosaic virus in pigeonpea (*Cajanus cajan* (L.) Millsp.). Ind J Genet 64:118–120
Nene YL, Reddy MV (1976) Screening for resistance to sterility mosaic of pigeon pea. Plant Dis Rep 60:1034–1036
Ning Y, Liu W, Wang GL (2017) Balancing immunity and yield in crop plants. Trends Plant Sci 22:1069–1079
Ning Y and Wang GL (2018) Breeding plant broad-spectrum resistance without yield penalties. Proc Natl Acad Sci 115(12):2859–2861
Nisar M, Ghafoor A (2011) Linkage of a RAPD marker with powdery mildew resistance *er-1* gene in *Pisum sativum* L. Russ J Genet 47:300–304
O'Boyle PD, Kelly JD, Kirk WW (2007) Use of marker-assisted selection to breed for resistance to common bacterial blight in common bean. J Am Soc Hort Soc 132:381–386
Oblessuc PR, Baroni RM, Garcia AAF, Chioratto AF, Carbonell SAM, Camargo LEA, Benchimol LL (2012) Mapping of angular leaf spot resistance QTL in common bean (*Phaseolus vulgaris* L.) under different environments. BMC Genet 13:50

Okiror MA (2002) Genetics of resistance to *Fusarium udum* in pigeonpea [*Cajanus cajan* (L.) Millsp.]. Ind J Genet 62:218–220

Okubara PA, Keller KE, McClendon MT, Inglis DA, McPhee KE, Coyne CJ (2005) Y15_999Fw, a dominant SCAR marker linked to the *Fusarium* wilt race 1 (*Fw*) resistance gene in pea. Pisum Genet 37:30–33

Ouédraogo JT, Gowda BS, Jean M, Close TJ, Ehlers JD, Hall AE, Gillaspie AG, Roberts PA, Ismail AM, Bruening G, Gepts P, Timko MP, Belzile FJ (2002) An improved genetic linkage map for cowpea (*Vigna unguiculata* L.) combining AFLP, RFLP, RAPD, biochemical markers and biological resistance traits. Genome 45:175–188

Pal SS, Dhaliwal HS, Bains SS (1991) Inheritance of resistance to yellow mosaic virus in some *Vigna* species. Plant Breed 106:168–171

Pandey S, Sharma M, Kumari S, Gaur PM, Chen W, Kaur L, Macleod W, Basandrai AK, Basandrai D, Bakr A, Sandhu JS, Tripathi HS, Gowda CLL (2009) Integrated foliar diseases management of legumes. In: Ali M et al (eds) Grain legumes: genetic improvement, management and trade. Indian Society of Pulses Research and Development, Indian Institute of Pulses Research, Kanpur, pp 143–161

Pant V, Gupta D, Choudhury NR, Malathi VG, Varma A, Mukherjee SK (2001) Molecular characterization of the Rep protein of the blackgram isolate of Indian mungbean yellow mosaic virus. J Gen Virol 82:2559–2567

Park SO, Coyne DP, Steadman JR, Crosby KM, Brick MA (2004) RAPD and SCAR markers linked to the Ur-6 Andean gene controlling specific rust resistance in common bean. Crop Sci 44:1799–1807

Patil BS, Ravikumar RL, Bhat JS, Soregaon CD (2014) Molecular mapping of QTLs for resistance to early and late Fusarium wilt in chickpea. Czech J Genet Plant Breed 50:171–176

Patil PG, Dubey J, Bohra A, Mishra RK, Saabale PR, Das A, Rathore M, Singh NP (2017) Association mapping to discover significant marker-trait association for resistance against fusarium wilt variant 2 in pigeon pea [*Cajanus cajan* (L.) Millsp.] using SSR markers. J Appl Genet 58:307–319

Pedraza F, Gallego G, Beebe S, Tohme J (1997) Marcadores SCAR y RAPD para la Resistencia a la bacteriosis cmun (CBB). In: Singh SP, Voysest O (eds) Taller de Mejoramiento de Frijol papa el Siglo XXI: bases para una estrategia para America Latina. International Centre for Tropical Agriculture, Cali, pp 130–134

Perseguini JMKC, Oblessuc PR, Rosa JRBF, Gomes KA, Chiorato AF, Carbonell SAM et al (2016) Genome-wide association studies of anthracnose and angular leaf spot resistance in common bean (*Phaseolus vulgaris* L.). PLoS One 11(3):e0150506

Pottorff M, Wanamaker S, Ma YQ, Ehlers JD, Roberts PA, Close TJ (2012) Genetic and physical mapping of candidate genes for resistance to *Fusarium oxysporum* f.sp. *tracheiphilum* race 3 in cowpea [*Vigna unguiculata* (L.) Walp]. PLoS One 7(7):e41600

Pottorff MO, Li G, Ehlers JD, Close TJ, Roberts PA (2014) Genetic mapping, synteny, and physical location of two loci for *Fusarium oxysporum* f.sp. *tracheiphilum* race 4 resistance in cowpea [*Vigna unguiculata* (L.) Walp]. Mol Breed 33:779–791

Prioul S, Frankewitz A, Deniot G, Morin G, Baranger A (2004) Mapping of quantitative trait loci for partial resistance to *Ascochyta pinodes* in pea (*Pisum sativum* L.) at the seedling and adult plant stages. Theor Appl Genet 108:1322–1334

Prioul-Gervais S, Deniot G, Receveur EM, Frankewitz A, Fourmann M, Rameau C, Baranger A (2007) Candidate genes for quantitative resistance to *Mycosphaerella pinodes* in pea (*Pisum sativum* L.). Theor Appl Genet 114:971–984

Radhika P, Gowda SJM, Kadoo NY, Mhase LB, Jamadagni BM, Sainani MN, Chandra S, Gupta VS (2007) Development of an integrated intraspecific map of chickpea (*Cicer arietinum* L.) using two recombinant inbred line populations. Theor Appl Genet 115:209–216

Raju NL, Gnanesh BN, Lekha P, Jayashree B, Pande S, Hiremath PJ et al (2010) The first set of EST resource for gene discovery and marker development in pigeonpea (*Cajanus cajan* L Millsp). BMC Plant Biol 10:45

Rakshit S, Mohapatra T, Mishra SK, Dasgupta SK, Sharma RP, Sharma B (2001) Marker assisted breeding for powdery mildew resistance in pea (*Pisum sativum* L.). J Genet Breed 55:343–348

Rakshit S, Winter P, Tekeoglu M, Munoz JJ, Pfaff T, BenkoIseppon M, Muehlbauer FJ, Kahl G (2003) DAF markers tightly linked to a major locus for Ascochyta blight resistance in chickpea (*Cicer arietinum* L.). Euphytica 132:23–30

Rana JC, Banyal DK, Sharma KD, Sharma MK, Gupta SK, Yadav SK (2013) Screening of pea germplasm for resistance to powdery mildew. Euphytica 189:271–282

Ratnaparkhe MB, Gupta VS (2007) Pigeonpea. In: Kole C (ed) Genome mapping and molecular breeding in plants: pulses, sugar and tuber crops. Springer, Berlin, pp 133–142

Ratnaparkhe MB, Tekeoglu M, Muehlbauer FJ (1998) Inter-simple-sequence-repeat (ISSR) polymorphisms are useful for Wnding markers associated with disease resistance gene clusters. Theor Appl Genet 97:515–519

Reddy KS (2007) Identification by genetic analysis of two races of *Erysiphe polygoni* DC. causing powdery mildew disease in mungbean. Plant Breed 126:603–606

Reddy KS (2009) A new mutant for yellow mosaic virus resistance in mungbean (*Vigna radiata* L. Wilczek) variety SML-668 by recurrent Gamma-ray irradiation. In: Shu QY (ed) Induced plant mutation in the genomics era. Food and Agriculture Organization of the United Nations, Rome, pp 361–362

Reddy KR, Singh DP (1995) Inheritance of resistance to mungbean yellow mosaic virus. Madras Agric J 88:199–201

Reddy KS, Pawar SE, Bhatia CR (1987) Screening for powdery mildew (*Erysiphe polygoni* DC) resistance in mungbean (*Vigna radiata* (L.) Wilczek) using excised leaves. Proc Indian Acad Sci (Plant Sci) 99:365–369

Reddy KS, Pawar SE, Bhatia CR (1994) Inheritance of powdery mildew (*Erysiphe polygoni* DC) resistance in mungbean (*Vigna radiata* (L.) Wilczek). Theor Appl Genet 88:945–948

Rodrigues MA, Santos CAF, Santana JRF (2012) Mapping of AFLP loci linked to tolerance to cowpea golden mosaic virus. Genet Mol Res 11:3789–3797

Rubeena A, Taylor PWJ, Ades PK, Ford R (2006) QTL mapping of resistance in lentil (*Lens culinaris* ssp. culinaris) to ascochyta blight (*Ascochyta lentis*). Plant Breed 125:506–512

Rubio J, Haji-Moussa E, Kharrat M, Moreno MT, Millan T, Gil J (2003) Two genes and linked RAPD markers involved in resistance to Fusarium oxysporum f. sp. Ciceri race 0 in chickpea. Plant Breed 122:188–191

Sabbavarapu MM, Sharma M, Chamarthi SK, Swapna N, Rathore A, Thudi M, Gaur PM, Pande S, Singh S, Kaur L, Varshney RK (2013) Molecular mapping of QTLs for resistance to *Fusarium* wilt (race 1) and *Ascochyta* blight in chickpea (*Cicer arietinum* L.). Euphytca 193:121–133

Saha GC, Sarker A, Chen W, Vandemark GJ, Muehlbauer FJ (2010a) Identification of markers associated with genes for rust resistance in *Lens culinaris* Medik. Euphytica 175:261–265

Saha GC, Sarker A, Chen W, Vandemark GJ, Muehlbauer FJ (2010b) Inheritance and linkage map positions of genes conferring resistance to stemphylium blight in lentil. Crop Sci 50:1831–1839

Saleem M, Haris WA, Malik IA (1998) Inheritance of yellow mosaic virus resistance in mungbean. Pak J Phytopathol 10:30–32

Sandhu TS, Brar JS, Sandhu SS, Verma MM (1985) Inheritance of resistance to mungbean yellow mosaic virus in greengram. J Res Punjab Agric Univ 22:607–611

Sant VJ (2001) Genetic diversity and linkage analysis in chickpea using DNA markers, PhD thesis, University of Pune, Pune, India

Santra DK, Tekeoglu M, Ratnaparkhe M, Kaiser WJ, Muehlbauer FJ (2000) Identification and mapping of QTLs conferring resistance to Ascochyta blight in chickpea. Crop Sci 40:1606–1612

Sarala K (1993) Linkage studies in pea (*Pisum sativum* L.) with reference to er gene for powdery mildew resistance and other genes. Ph.D. Thesis, Indian Agricultural Research Institute, New Delhi, India

Savithramma DL, Divya Ramakrishnan CK (2016) Single marker analysis in mungbean (*Vigna radiata* (L) Wilczek) for powdery mildew disease resistance and yield attributing traits. Proteomics Bioinform 9(12):45

Saxena KB, Sharma D (1990) Pigeonpea genetics. In: Nene YL, Hall SD, Sheila VK (eds) The pigeonpea. Wallingford, Oxon

Saxena KB (2008) Genetic Improvement of Pigeon Pea- A Review. Tropical Plant Biol 1:159–178

Saxena RK, Penmetsa RV, Upadhyaya HD, Kumar A, Carrasquilla-Garcia N, Schlueter JA et al (2012) Large-scale development of cost-effective single nucleotide polymorphism marker assays for genetic mapping in pigeonpea and comparative mapping in legumes. DNA Res 19:449–461

Saxena R, Thudi M, Varshney RK (2016) Genomics, trait mapping and molecular breeding in pigeonpea and chickpea. Ind J Genet 76:504–511

Saxena RK, Singh V, Kale SM, Parupali S, Joshi S, Tathineni R, Parupali S, Kumar V, Garg V, Das RR, Sharma M, Yamini KN, Muniswamy S, Ghanta A, Rathore A, Sameerkumar CV, Saxena KB, Kavikishore PB, Varshney RK (2017a) Construction of genotyping-by-sequencing based high-density genetic maps and QTL mapping for fusarium wilt resistance in pigeonpea. Sci Rep 7:1911

Saxena RK, Kale SM, Kumar V, Parupali S, Joshi S, Singh V, Garg V, Das RR, Sharma M, Yamini KN, Ghanta A, Rathore A, Sameerkumar CV, Saxena KB, Varshney RK (2017b) Genotyping-by-sequencing of three mapping populations for identification of candidate genomic regions for resistance to sterility mosaic disease in pigeonpea. Sci Rep 7:1813

Schneider KA, Grafton KF, Kelly JD (2000) QTL analysis of resistance to Fusarium root rot in bean. Crop Sci 41(2):535–542

Selvi R, Muthiah AR, Manivannan N, Raveendran TS, Manickam A, Samiyappan R (2006) Tagging of RAPD marker for MYMV resistance in mungbean (*Vigna radiata* L. Wilczek). Asian J Plant Sci 5:277–280

Sharma M, Ghosh R (2016) An update on the host plant resistance to pigeonpea diseases. Legume Perspect 11:21–23

Sharma KD, Muehlbauer FJ (2005) Genetic mapping of *Fusarium oxysporum* f. sp. *ciceris* race-specific resistance genes in chickpea (*Cicer arietinum* L.). In: Abstract of the International Food Legume Research Conference-IV. Indian Agricultural Research Institute, New Delhi, pp 18–22

Sharma KD, Muehlbauer FJ (2007) Fusarium wilt of chickpea: physiological specialization, genetics of resistance and resistance gene tagging. Euphytica 157:1–14

Sharma D, Gupta SC, Rai GS, Reddy MV (1984) Inheritance of resistance to sterility mosaic disease in pigeonpea. Indian J Genet 44:84–90

Sharma KD, Winter P, Kahl G, Muehlbauer FJ (2004) Molecular mapping of *Fusarium oxysporum* f. sp. ciceris race 3 resistance gene in chickpea. Theor Appl Genet 108:1243–1248

Sharma KD, Chen W, Muehlbauer FJ (2005) Genetics of chickpea resistance to five races of *Fusarium* wilt and a concise set of race differentials for *Fusarium oxysporum* f. sp. *ciceris*. Plant Dis 89:385–390

Shukla GP, Pandya BP (1985) Resistance to yellow mosaic in greengram. SABRAO J 17:165–171

Simon CJ, Muehlbauer FJ (1997) Construction of a chickpea linkage map and its comparison with map of pea and lentil. J Hered 88:115–119

Simon MV, Benko Iseppon AM, Resende LV, Winter P, Kahl G (2007) Genetic diversity and phylogenetic relationships in *Vigna* Savi germplasm revealed by DNA amplification finger printing. Genome 50:538–547

Singh SP, Gepts P, Debouck DG (1991) Races of common bean (*Phaseolus vulgaris*, Fabaceae). Econ Bot 45:379–396

Singh D, Sinha B, Rai VP, Singh MN, Singh DK, Kumar R, Singh AK (2016a) Genetics of *Fusarium* wilt resistance in pigeonpea (*Cajanus cajan*) and efficacy of associated SSR markers. Plant Pathol J 32:95–101

Singh VK, Khan AW, Saxena RK, Kumar V, Kale SM, Chitikineni A, Pazhamala LT, Garg V, Sharma M, Sinha P, Kumar CVS, Parupalli S, Vechalapu S, Patil S, Muniswamy S, Ghanta A, Yamini M, Dharmaraj PS, Varshney RK (2016b) Next-generation sequencing for identification of candidate genes for Fusarium wilt and sterility mosaic disease in pigeonpea (*Cajanus cajan*). Plant Biotech J 14:1183–1194

Skiba B, Ford R, Pang ECK (2004) Construction of a linkage map based on a *Lathyrus sativus* backcross population and preliminary investigation of QTLs associated with resistance to Ascochyta blight. Theor Appl Genet 109:1726–1735

Souframanien J, Gopalakrishna T (2006) ISSR and SCAR marker linked to the mungbean yellow mosaic virus (MYMV) resistance gene in blackgram (*Vigna mungo* L. Hepper). Plant Breed 125:619–622

Souframanien J, Reddy KS (2015) De novo assembly, characterization of immature seed transcriptome and development of genic-SSR markers in black gram [*Vigna mungo* (L.) Hepper]. PLoS One 10(6):e0128748

Soule M, Porter L, Medina J, Santana GP, Blair MW, Miklas PN (2011) Comparative QTL map for white mold resistance in common bean, and characterization of partial resistance in dry bean lines VA19 and I9365-31. Crop Sci 51:123–139

Souza TLPO, Alzate-Marin AL, Dessaune SN, Nunes ES, Queiroz VT, Moreira MA, Barros EG (2007) Inheritance study and validation of SCAR molecular marker for rust resistance in common bean. Crop Breed Appl Biotechnol 7:11–15

Souza TLPO, Alzate-Marin AL, Faleiro FG, Barros EG (2008) Pathosystem common bean—*Uromyces appendiculatus*: host resistance, pathogen specialization, and breeding for rust resistance. Pest Technol 2:56–69

Souza TLPO, Ragagnin VA, Dessaune SN et al (2014) DNA marker-assisted selection to pyramid rust resistance genes in "carioca" seeded common bean lines. Euphytica 199:303–316

Srinivas T, Reddy MV, Jain KC, Reddy MSS (1997) Studies on inheritance of resistance and allelic relationships for strain 2 of pigeonpea sterility mosaic pathogen. Ann Appl Biol 130:105–110

Srivastava RK, Mishra SK, Singh K, Mohapatra T (2012) Development of a coupling-phase SCAR marker linked to the powdery mildew resistance gene *er1* in pea (*Pisum sativum* L.). Euphytica 186:855–866

Stephens A, Lombardi M, Cogan NOI, Forster JW, Hobson K, Materne M et al (2014) Genetic marker discovery, intraspecific linkage map construction and quantitative trait locus analysis of ascochyta blight resistance in chickpea (*Cicer arietinum* L.). Mol Breed 33:297–313

Sudheesh S, Rodda MS, Davidson J, Javid M, Stephans A, Slater AT et al (2016) SNP-based linkage mapping for validation of QTLs for resistance to ascochyta blight in lentil. Front Plant Sci 7:1604

Sun S, Wang J, Fu H, Duan C, Wang X, Zhu Z (2015) Resistance to powdery mildew in the pea cultivar Xucai-1 is conferred by the gene *er1*. Crop J 3:489–499

Sun S, Fu H, Wang Z, Duan C, Zong X, Zhu Z (2016) Discovery of a novel er1 allele conferring powdery mildew resistance in Chinese Pea (*Pisum sativum* L.) landraces. PLoS One 11(1):e0147624

Talekar SC, Viswanatha KP, Lohithaswa HC (2017) Assessment of genetic variability, character association and path analysis in F2 segregating population for quantitative traits in chickpea. Int J Curr Microbiol App Sci 6(12):2184–2192

Tangphatsornruang S, Somta P, Uthaipaisanwong P, Chanprasert J, Sangsrakru D, Seehalak W, Sommanas W, Tragoonrung S, Srinives P (2009) Characterization of microsatellites and gene contents from genome shotgun sequences of mungbean (*Vigna radiata* (L.) Wilczek). BMC Plant Biol 9:137

Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277:1063–1066

Tar'an B, Michaels TE, Pauls KP (2001) Mapping genetic factors affecting the reaction to *Xanthomous axonopodis* pv. *Phaseoli* in *Phaseolus vulgaris* L. under field conditions. Genome 44:1045–1056

Tar'an B, Warkentin T, Somers DJ, Miranda D, Vandenberg A, Blade S, Penner G (2003a) Quantitative trait loci for lodging resistance, plant height and partial resistance to *Mycosphaerella blight* in field pea (*Pisum sativum* L.). Theor Appl Genet 107:1482–1491

Tar'an B, Buchwaldt L, Tullu A, Banniza S, Warkentin TD, Vandenberg A (2003b) Using molecular markers to pyramid genes for resistance to ascochyta blight and anthracnose in lentil (*Lens culinaris* Medik.). Euphytica 134:223–230

Tar'an B, Warkentin TD, Tullu A, Vandenberg A (2007) Genetic mapping of Ascochyta blight resistance in chickpea (Cicer arietinum L.) using a simple sequence repeat linkage map. Genome 50:26–34

Taran B, Buchwald L, Tullu A, Banniza S, Warkantin TD, Vandenberg A (2003) Using molecular markers to pyramid genes for resistance to Ascochyta blight and anthracnose in Lentil (*Lens culinaris* medic). Euphyica 134:223–230

Teixeira FF, Bosco dos Santos J, Patto Ramalho MA, Barbosa Abreu ÂF, Teixeira Guimarães C, Carlos de Oliveira A (2005) QTL mapping for angular leaf spot in common bean using microsatellite markers. Crop Breed Appl Biotechnol 5:272–278

Tekeoglu M, Tullu A, Kaiser WA, Muehlbauer FJ (2000) Inheritance and linkage of two genes that confers resistance to *Fusarium* wilt in chickpea. Crop Sci 40:1247–1251

Timko MP, Rushton PJ, Laudeman TW, Bokowiec MT, Chipumuro E, Cheung F, Town CD, Chen X (2008) Sequencing and analysis of the gene-rich space of cowpea. BMC Genomics 9:103

Timmerman GM, Frew TJ, Weeden NF, Miller AL, Goulden DS (1994) Linkage analysis of *er-1*, a recessive *Pisum sativum* gene for resistance to powdery mildew fungus (*Erysiphe pisi* D.C.). Theor Appl Genet 88:1050–1055

Timmerman-Vaughan GM, Frew TJ, Russell AC, Khan T, Butler R, Gilpin M, Falloon K (2002) QTL mapping of partial resistance to field epidemics of ascochyta blight of pea. Crop Sci 42:2100–2111

Timmerman-Vaughan GM, Frew TJ, Butler R, Murray S, Gilpin M, Falloon K, Khan T (2004) Validation of quantitative trait loci for *Ascochyta* blight resistance in pea (*Pisum sativum* L.), using populations from two crosses. Theor Appl Genet 109:1620–1631

Timmerman-Vaughan GM, Moya L, Frew TJ, Murray SR, Crowhurst R (2016) Ascochyta blight disease of pea (*Pisum sativum* L.): defence-related candidate genes associated with QTL regions and identification of epistatic QTL. Theor Appl Genet 129:879–896

Tiwari S, Dhar V (2011) Prevalence of new variants of *Fusarium udum* in India. Indian Phytopathol 64:243–246

Tiwari KR, Penner GA, Warkentin TD (1997) Inheritance of powdery mildew resistance in pea. Can J Plant Sci 77:307–310

Tiwari KR, Penner GA, Warkentin TD (1998) Identification of coupling and repulsion phase markers for powdery mildew resistance genes *er1* in pea. Genome 41:440–444

Tonguc M, Weeden NF (2010) Identification and mapping of molecular markers linked to *er1* gene in pea. J Plant Mol Biol Biotechnol 1(1):1–5

Tullu A (1996) Genetics of fusarium wilt resistance in chickpea. PhD dissertation. Crop and Soil Science Department. Washington State University, Pullman

Tullu A, Muehlbauer FJ, Simon CJ, Mayer MS, Kumar J, Kaiser WJ, Kraft JM (1998) Inheritance and linkage of a gene for resistance to race 4 of fusarium wilt and RAPD markers in chickpea. Euphytica 102:227–232

Tullu A, Kaiser WJ, Kraft JM, Muehlbauer FJ (1999) A second gene for resistance to race 4 of *Fusarium* wilt in chickpea and linkage with a RAPD marker. Euphytica 109:43–50

Tullu A, Buchwaldt L, Warkentin T, Taran B, Vandenberg A (2003) Genetics of resistance to anthracnose and identification of AFLP and RAPD markers linked to the resistance gene in PI 320937 germplasm of lentil (*Lens culinaris* Medikus). Theor Appl Genet 106:428–434

Tullu A, Taran B, Breitkreutz C, Buchwaidt L, Banniza S, Warkentin TD et al (2006) A quantitative-trait locus for resistance to ascochyta blight *Ascochyta lentis* maps close to a gene for resistance to anthracnose *Colletotrichum truncatum* in lentil. Can J Plant Pathol 28:588–595

Udupa SM, Baum M (2003) Genetic dissection of pathotype-specific resistance to Ascochyta blight disease in chickpea (*Cicer arietinum* L.) using microsatellite markers. Theor Appl Genet 106:1196–1202

Uma MS, Hegde N, Hittalmani S (2016) Identification of SSR marker associated with rust resistance in cowpea (*Vigna unguiculata* L.) using bulk segregant analysis. Legum Res 39(1):39–42

Varshney RK (2016) Exciting journey of 10 years from genome to fields and markets: some success stories of genomics-assisted breeding in chickpea, pigeonpea and groundnut. Plant Sci 242:98–107

Varshney RK, Close TJ, Singh NK, Hoisington DA, Cook DR (2009) Orphan legume crops enter the genomics era. Curr Opin Plant Biol 12:202–210

Varshney RK, Chen W, Li Y, Bharti AK, Saxena RK, Schlueter JA et al (2012) Draft genome sequence of pigeonpea (*Cajanus cajan*), an orphan legume crop of resource-poor farmers. Nat Biotechnol 30:83–89

Varshney RK, Mohan M, Gaur PM, Gangarao NVPR, Pandey MK, Bohra A et al (2013) Achievements and prospects of genomics-assisted breeding in three legume crops of the semi-arid tropics. Biotechnol Adv 31:1120–1134

Varshney RK, Mohan SM, Gaur PM, Chamarthi SK, Singh VK, Srinivasan S, Swapna N, Sharma M, Singh S, Kaur L, Pande S (2014) Marker-assisted backcrossing to introgress resistance to *Fusarium* wilt (FW) race 1 and *Ascochyta* blight (AB) in C 214, an elite cultivar of chickpea. Plant Genome 7:1

Varshney RK, Terauchi R, McCouch SR (2014a) Harvesting the promising fruits of genomics: applying genome sequencing technologies to crop breeding. PLoS Biol 2:e1001883

Varshney RK, Thudi M, Nayak SN, Gaur PM, Kashiwagi J, Krishnamurthy L et al (2014b) Genetic dissection of drought tolerance in chickpea (*Cicer arietinum* L.). Theor Appl Genet 127:445–462

Vasconcellos RCC, Oraguzie OB, Soler A, Arkwazee H, Myers JR, Ferreira JJ, Song Q, McClean P, Miklas PN (2017) Meta-QTL for resistance to white mold in common bean. PLoS One 12(2):e0171685

Verma RPS, Singh DP (1986) The allelic relationship of genes giving resistance to mungbean yellow mosaic virus in blackgram. Theor Appl Genet 72:737–738

Verma RPS, Singh DP (1988) Inheritance of resistance to mungbean yellow mosaic virus in greengram. Ann Agric Res 9:98–100

Vleeshouwers VGAA, Oliver RP (2014) Effectors as tools in disease resistance breeding against biotrophic, hemibiotrophic, and necrotrophic plant pathogens. MPMI 27:196–206

Warkentin TD, Rashid KY, Zimmer RC (1995) Effectiveness of a detached leaf assay for determination of the reaction of pea plant to powdery mildew. Can J Plant Pathol 17:87–89

Warschefsky E, Verma Penmetsa R, Cook DR, van Wettberg EJB (2014) Back to the wild: Tapping evolutionary adaptations for resilient crops through systematic hybridization with crop wild relatives. Am J Bot 101(10):1791–1800

Weeden NF, Wolko B (1990) Linkage map for the garden pea (*Pisum sativum*). In: O'Brien SJ (ed) Genetic maps. Locus maps of complex genomes. Cold Spring Harbor Laboratory Press, New York, pp 6.106–6.112

Winter P, Benko-Iseppon AM, Huttel B, Ratnaparkhe M, Tullu A, Sonnante G, PfaV T, Tekeoglu M, Santra D, Sant VJ, Rajesh PN, Kahl G, Muehlbauer FJ (2000) A linkage map of the chickpea (*Cicer arietinum* L.) genome based on recombinant inbred lines from a *C. arietinum* x *C. reticulatum* cross: localization of resistance genes for Fusarium wilt races 4 and 5. Theor Appl Genet 101:1155–1163

Wu X, Wang B, Wu X, Lu Z, Li G, Xu P (2017) SNP marker-based genetic mapping of rust resistance gene in the vegetable cowpea landrace ZN016. Legum Res 387:1–4

Xu P, Wu X, Wang B, Liu Y, Ehlers JD, Close TJ, Roberts PA, Diop NN, Qin D, Hu T, Lu Z, Li G (2011) A SNP and SSR based genetic map of Asparagus bean (*Vigna unguiculata* ssp. *sesquipedalis*) and comparison with the broader species. PLoS One 6(1):e15952

Yang S, Saxena RK, Kulwal PL, Ash GJ, Dubey A, Harper JD et al (2011) First genetic map of pigeonpea based on diversity array technology (DArT) markers. J Genet 90:103–109

Young ND (1999) A continuously optimistic vision for marker assisted breeding. Mol Breed 5:505–510

Yu K, Park SJ, Poysa V (2000) Marker-assisted selection of common beans for resistance to common bacterial blight: efficacy and economics. Plant Breed 119:411–415

Zannou A, Kossou DK, Ahanchede ZJ, Agbicodo E, Struik PC (2008) Genetic variability of cultivated cowpea in Benin assessed by random amplified polymorphic DNA. Afr J Biotechnol 7:4407–4414

Zhu J, Wu J, Wang L, Blair MW, Zhu Z, Wang S (2016) QTL and candidate genes associated with common bacterial blight resistance in the common bean cultivar Longyundou 5 from China. Crop J 4:344–352

Chapter 9
RETRACTED CHAPTER: Molecular Breeding for Resistance to Economically Important Diseases of Fodder Oat

Pawan Saini, Mudasir Gani, Pooja Saini, Javaid Akhter Bhat, Rose Mary Francies, Narender Negi, and S. S. Chauhan

9.1 Introduction

Oat (*Avena* sp.) is a cereal grain best known for its hardiness. It thrives and withstands poor and adverse conditions that may otherwise prove a challenge for other cereal crops, mostly cultivated as a fodder crop across the globe (Loskutov and Rines 2011). Oats serves as a balanced feed for cattle, sheep and other domestic animals. Green fodder contain about 10–12 per cent protein and 30–35 per cent dry matter (Hand Book of Agriculture 2007). Besides its use as a fodder and forage crop, the straw is used for bedding, hay, haylage and silage chaff, while for human consumption, the grains are most commonly rolled or crushed into oatmeal or ground into fine oat flour (Ahmad et al. 2014). Presently, oat cereals are important constituent of breakfast in most developed countries since they are the excellent source of β-gluten proteins owing to their low content of prolamines (Gorash et al. 2017). It

The original version of this chapter was retracted: The retraction note to this chapter is available at https://doi.org/10.1007/978-3-030-20728-1_12

P. Saini · M. Gani · S. S. Chauhan
CSB-Central Sericultural Research & Training Institute (CSR&TI), Pampore, Jammu & Kashmir, India

P. Saini
Department of Biotechnology, Eternal University, Baru Sahib, Himachal Pradesh, India

J. A. Bhat (✉)
School of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu, Chatha, Jammu (J&K), India

R. M. Francies
Kerala Agricultural University, Vellanikara, Thrissur, Kerala, India

N. Negi
ICAR-National Bureau of Plant Genetic Resources (NBPGR), Regional Station – Phagli, Shimla, Himachal Pradesh, India

S. H. Wani (ed.), *Disease Resistance in Crop Plants*,
https://doi.org/10.1007/978-3-030-20728-1_9

proves helpful in the treatment of coronary heart disease (Mellen et al. 2008; Thies et al. 2014; Nwachukwu et al. 2015; Schuster et al. 2015) through the reduction of serum cholesterol and control of obesity (Chen et al. 2006a, b; Zdunczyk et al. 2006; Chang et al. 2013 and Shebini El et al. 2014). Consumption of oats may aid in the treatment of type II diabetes through stabilization of blood sugar levels (Tapola et al. 2005; Priebe et al. 2008; Zhang et al. 2014; Ho 2015; Hou et al. 2015) and certain cancer (Egeberg et al. 2010; Boffetta et al. 2014) as oats are an excellent source of antioxidants (Peterson 2001; Rasane et al. 2015; Vanden Broeck et al. 2016).

9.1.1 The Production Scenario

Although oat is a crop produced on a global scale, the crop ranks behind the staple food crops such as wheat, rice, maize and millets (Stewart and McDougall 2014). Oat stands sixth (6th) in production statistics following wheat, maize, rice, barley and sorghum (Ivanov 2006) and seventh (7th) in the cultivated area among world cereals (FAO 2013). It contributes 0.86 per cent to the global cereal production. During the period 2009–2013, the average area under oat crop globally was about 9.6 mha in comparison with major cereal crops wheat (220 mha) and barley (50 mha) (FAO 2015). In 2012, the global oat production reached 19.6 megatonnes (faostat.fao.org). As per the US Department of Agriculture (USDA) statistics, total oat production was about 23.6 megatonnes in the year 2013–2014, amounting to a 10.6 per cent increase over the 2012–2013 harvest. However, this production statistics also revealed a significant reduction in global oat production from 46.9 megatonnes in 1961 which is clearly indicative of progressive decline in production at global level (Stewart and McDougall 2014). During the period of 1960–2005, the yield increase in oats was the least (39%) among all of the main cereals. Over the same period the yield increase for wheat was 147 per cent and 143 per cent for corn (Menon et al. 2016). A parallelism can be drawn between the downhill slide in oat production scenario and the substantial decline in the oat production in major oat producing countries such as the USA, Canada, Russia, China, Argentina and Brazil.

9.1.2 Origin and Distribution

Cultivation of oats is in vogue across the temperate to tropical regions of the world. The wide edaphoclimatic environments and variation in agricultural practices have all contributed to the diversity of forms. The highest genetic diversity of the *Avena* spp. is observed in Mediterranean, Black and Caspian seas with diverse ecological conditions (Loskutov and Rines 2011).

Oat (*Avena* sp.) is a self-pollinated crop belonging to grass family Poaceae (Gramineae). The genus *Avena* consists of 31 species which were described by a number of authors from time to time since Carl Linnaeus (1753) proposed binary system of nomenclature. These include both the wild and cultivated species and have been categorized based on the genome, ploidy level and distribution

(Loskutov and Rines 2011) (Table 9.1). Oat is considered as a secondary crop, because it is derived from the weed of primary cereal domesticates. It is an allohexaploid that arose through a cycle of interspecific hybridization and polyploidization. It comprises of seven chromosome pairs (x = 7) from each of the three diploid genomes designated AA, CC and DD (Rajhathy and Thomas 1974). Oats form a polyploid series of diploids [*Avena strigosa* L., black oat ($2n = 2x = 14$, AsAs), and *Avena nuda* L., naked oat ($2n = 2x = 14$, AsAs)], tetraploids [*Avena abyssinica*, Ethiopian oat ($2n = 4x = 28$, AABB)] and hexaploids [*Avena sativa* L., white oat ($2n = 6x = 42$, AACCDD), and *Avena byzantina*, red oat ($2n = 6x = 42$, AACCDD)] (Table 9.1).

Table 9.1 Chromosome number, ploidy, genome constitution and distribution of *Avena* species

Sl. no.	Species	Chromosome number	Genome constitution	Distribution
Diploid				
1.	*Avena clauda*	2*x* = 14	CpCp	Bulgaria, Greece, Turkey, Iran, Iraq, Uzbekistan, Azerbaijan, Jordan, Israel, Lebanon, Syria, Algeria, Morocco
2.	*A. pilosa* syn. *A. eriantha*	2*x* = 14	CpCp	Spain, Greece, Bulgaria, Ukraine, Russia, Iran, Turkey, Iraq, Uzbekistan, Syria, Jordan, Israel
3.	*A. ventricosa*	2*x* = 14	CvCv	Cyprus, Algeria, Iraq
4.	*A. bruhnsiana*	2*x* = 14	CvCv	Azerbaijan
5.	*A. longiglumis*	2*x* = 14	AlAl	Spain, Portugal, Greece, Italy, Syria, Libya, Morocco, Algeria, Israel, Jordan
6.	*A. damascena*	2*x* = 14	AdAd	Syria, Morocco
7.	*A. prostrata*	2*x* = 14	ApAp	Spain, Morocco
8.	*A. canariensis*	2*x* = 14	AcAc	Canary Islands
9.	*A. wiestii*	2*x* = 14	AsAs	Spain, Azerbaijan, Turkey, Iraq, Iran, Syria, Jordan, Israel, Algeria, Egypt, Northern Sahara, Arabic Peninsula
10.	*A. hirtula*	2*x* = 14	AsAs	Spain, Portugal, France, Italy, Greece, Algeria, Morocco, Tunisia, Israel, Turkey, Syria, Jordan
11.	*A. atlantica*	2*x* = 14	AsAs	Morocco
12.	*A. brevis*	2*x* = 14	AA	
13.	*A. nuda*	2*x* = 14	AA	
14.	*A. strigosa*	2*x* = 14	AsAs	Europe
15.	*A. hispanica*	2*x* = 14	AA	
16.	*A. barbata*	4*x* = 28	AABB	Mediterranean Basin, European Atlantic coast, Asia Minor, Himalayas, Ethiopia, Brazil, Japan, Australia
Tetraploid				
17.	*A. vaviloviana*	4*x* = 28	AABB	Ethiopia, Saudi Arabia, Algeria
18.	*A. magna* syn. *A. moroccana*	4*x* = 28	AACC	Morocco

(continued)

Table 9.1 (continued)

Sl. no.	Species	Chromosome number	Genome constitution	Distribution
19.	*A. murphyi*	4*x* = 28	AACC	Spain, Morocco
20.	*A. abyssinica*	4*x* = 28	AABB	Ethiopia, Eritrea, Yemen
21.	*A. insularis*	4*x* = 28	–	Sicily, Tunisia
22.	*A. macrostachya*	4*x* = 28	–	Algeria Atlas Mountains
Hexaploid				
23.	*A. sativa*	6*x* = 42	AACCDD	All over the world
24.	*A. sterilis*	6*x* = 42	AACCDD	Spain, Portugal, Italy, Switzerland, France, Iraq, Turkey, Ukraine, North Africa, Ethiopia, Japan, South Korea
25.	*A. fatua*	6*x* = 42	AACCDD	All over the world
26.	*A. occidentalis*	6*x* = 42	AACCDD	Canary Islands, Portugal, Egypt, Ethiopia, Azores, Madeira, Algeria
27.	*A. atherantha*	6*x* = 42	AACCDD	
28.	*A. hybrida*	6*x* = 42	AACCDD	
29.	*A. trichophylla*	6*x* = 42	AACCDD	
30.	*A. byzantina*	6*x* = 42	AACCDD	Spain, Portugal, North Africa, Brazil, Australia
31.	*A. ludoviciana*	6*x* = 42	AACCDD	Europe, Ukraine, Russia, Azerbaijan, Central and South-Western Asia, Iran, Asia Minor, Afghanistan, Northern Africa, Mediterranean Basin, Australia, New Zealand

Although considered a hardy crop, similar to other cereal crops, oat is also susceptible to several plant pathogens that invariably reduce the crop yield and also hamper its quality for human as well as livestock consumption. Genetic uniformity among varieties is yet another factor that contributes to increased vulnerability of the oat crop to disease epidemics and insect infestation. The oat gene pool encompasses the limitless array of diversity of forms that exhibit varied quality and quantity of grains as well as responses to biotic and abiotic stresses. Hence, the diverse forms serve as a rich source of genes for crop improvement activities that focus on its hitherto though untapped potential for climate resilience.

9.1.3 Oat Production in the Changing Climate Perspective

The predictions by the Intergovernmental Panel on Climate Change (IPCC 2007) gave birth to several speculations of the climatic changes that one could expect in the coming decades. The change in climate is leading to manifestation of various types of stresses in plants imposed by either environmental factors or biological factors. Stress, viz. abiotic (drought, heat, cold and salinity) and biotic stresses (diseases, pests and weeds), ultimately affects the growth and development of crop

plants which leads to reduction in crop yield through reduced water uptake, photosynthesis, etc. Among biotic stresses, occurrence of disease epidemics is the most severe factor for reduction in crop productivity. Pathogens and plants have co-evolved, and as a result of this type of co-evolution, interaction between plant (host) and pathogen leads towards either development of resistance or susceptibility to diseases. The diseases are the main cause of farmers' yield loss in a large and diverse form.

Diversity is fundamental for the improvement of current and future cultivars. Hence, it is prerequisite to meet the diverse goals of plant breeding such as producing cultivars with increasing yield, genetic adoption, desirable quantity and pest and disease resistance (Nevo et al. 1982). The landraces in oats have built in genetic variability over several generations of growing and selection by farmers. Divergence among genotypes or populations serves as a sound basis of breeding cultivars that possess durable resistance either through conventional or molecular breeding approaches. In lieu of this, the chapter aims to provide a detailed comprehension on economically important oat diseases and omics-based molecular strategies to meet out the future type of high-yielding as well as disease-resistant varieties for sustainable growth and production in the present climate change era.

9.2 Oat Diseases: An Overview

9.2.1 Losses due to Oat Diseases

Oat grain has always been an important form of livestock feed and serves as a good source of excellent protein, fibre and minerals. However, the world oat production has declined from 26.30 million metric tonnes in 2003 to projected production of 23.16 metric tonnes in 2018/2019 (USDA, Foreign Agricultural Service, Commodity production, supply and disposition database, https://apps.fas.usda.gov/psdonline/circulars/production.pdf) owing to several factors discussed earlier.

Roughly, direct yield losses caused by pathogens, animals and weeds are altogether responsible for losses ranging between 20 and 40 per cent of global agricultural productivity (Teng and Krupa 1980; Teng 1987; Oerke et al. 1994; Oerke 2006). The phrase 'losses between 20 and 40 per cent' therefore inadequately reflects the true costs of crop losses to consumers, public health, societies, environments, economic fabrics and farmers. On an average it is estimated that 20–30 per cent losses occur due to diseases, while yield loss will be complete in case of severe disease epidemics. Plant protection in general and the protection of crops against plant diseases in particular have an obvious role to play in meeting the growing demand for food quality and quantity (Strange and Scott 2005).

In 350 B.C. Theophrastus, the father of botany, first recorded occurrence of plant diseases and differences among oat plants with respect to the disease reactions. Among the diseases in oats that significantly reduce production the world over, the most important ones are crown rust, stem rust, powdery mildew, *Fusarium* head

blight, leaf blotch, smut and barley yellow dwarf virus (BYDV). Diseases reduce total biomass production by either causing death of plants, killing of branches, general stunting, damage to leaf tissues or damage to reproductive organs including fruits and seeds.

The crown rust disease is considered the most serious and destructive disease. The annual yield losses averaged 5.1 per cent on account of this disease during the period 2001–2005 in Canada (Chong et al. 2011), with highest losses of 11.2 per cent and 8.8 per cent in 2001 and 2005, respectively (McCallum et al. 2007). In the USA, average yield losses for the 10-year period of 1999–2005 were 2.7–20 per cent in individual years and states (Carson 2009). Similarly, stem rust owing a prime place among oat disease caused severe disease epidemics in major oat-producing countries, viz. the USA, Canada and Australia. During 2002, stem rust caused 5–10 per cent yield loss in Canada (Fetch 2005). In China approximately 10–15 per cent yield losses were reported in 2012–2013, while the annual crop losses due to powdery mildew reported were 5–10 per cent in the United Kingdom (Clifford 1995). The *Fusarium* head blight (scab) is yet another destructive disease of oat. In the year 2007–2008, 87 to 93 per cent oat grains were found to be infected with *Fusarium* sp. in the North-Western region of Russia (Gagkaeva et al. 2011). There are several examples of major famines or food losses of crop plants associated with pest and disease epidemics in the past. The prevention of epidemics and ultimately the reduction of losses in yield have been of great concern.

9.2.2 Disease Susceptibility in Oats and Severity

The optimum conditions for a disease to occur and develop are a combination of three factors – susceptible host, virulent pathogen and favourable environmental conditions. A change in any of the factors causes corresponding changes in the expression of disease. In traditional agriculture owing to the presence of genetic heterogeneity and natural biological control, the natural population and wild species of crop plants rarely shows epidemics. On the contrary, modern agriculture technology has introduced important changes: (1) it has narrowed down the genetic base of cultivars, which alters the dynamic imbalance between host and parasites, which in turn results in epidemics; (2) it has generated more or less continuously distributed populations and has changed the whole ecosystem, creating habitats profoundly altered for host and parasites.

Many diseases cause serious direct damage, mainly by reduction of the fodder yield. Among them diseases such as crown rust, stem rusts and leaf blotch caused by *Pyrenophora* spp., *Septoria* spp. and BYDV, respectively, cause severe direct damage through reduction of the fodder yield, while other diseases like SCAB and ERGOT cause indirect damage by compromising the quality of the product. They produce toxins in grains and make them unsuitable for consumption by either animals or humans. Based on the causal organism, the diseases occurring in oats can be categorized into three classes based on the type of pathogen (Table 9.2).

Table 9.2 Diseases of common occurrence in oats

Sl. no.	Type of disease	Diseases	Causal organism
1.	Fungal	Crown rust	*Puccinia coronata* f. sp. *avenae*
		Stem rust	*Puccinia graminis* f. sp. *avenae*
		Helminthosporium leaf blotch	*Drechslera avenae*
		Septoria leaf blotch	*Septoria avenae*
		Powdery mildew	*Erysiphe graminis avenae*
		Loose smut	*Ustilago avenae*
		Fusarium head blight (Scab)	*Fusarium graminearum*
		Anthracnose	*Colletotrichum graminicola*
2.	Bacterial	Halo blight	*Pseudomonas coronafaciens*
		Seed and seedling diseases	*Bipolaris sorokiniana, Fusarium* and *Pythium* spp.
		Bacterial stripe blight	*Pseudomonas syringae* p. *striajaciens*
3.	Viral	Soil-borne oat mosaic	*Oat mosaic virus*
		Barley yellow dwarf	*Barley yellow dwarf virus*

9.2.3 Oat Diseases: Characteristics and Symptoms

9.2.3.1 Crown Rust

Crown rust caused by *Puccinia coronata* f. sp. *avenae* is the most serious disease of oats throughout the world (Simons 1985). This disease attacks several plant species other than oats. Infection by the pathogen induces several structural, biochemical and physiological changes in its host. Disease symptoms appear as yellow pustules containing masses of uredospores, which are exposed after the rupture of the epidermis. These lesions are circular or oblong and occur on both surfaces of the foliage and can reach other green parts of the plant, when the epidemic becomes more severe. After a few weeks, the borders of the uredo pustules can turn black, with teliospore formation. When the infected plants reach maturity, production of uredospores ceases and they are then replaced by teliospores (Simons 1985; Harder and Haber 1992).

9.2.3.2 Stem Rust

Stem rust is caused by *Puccinia graminis* Pers. f. sp. *avenae* Eriks. and Henn. It attacks all species of oats, including wild oats. It is a widespread disease of oats, occurring almost everywhere they are grown (Zillinsky 1983). Disease symptoms most commonly appear on the stems and leaf sheaths, but leaf blades and spikes may also become infected. Uredospores develop in pustules (uredia) that rupture the epidermis and expose masses of reddish brown spores. The pustules are larger than those of crown rust, oval or elongated, with loose or torn epidermal

tissue along their margins. They may appear on both surfaces of the leaf. They continue to be produced until the plants approach maturity. After that, teliospores develop, either in the same uredia or in other fruiting structures called telia. Epidemics are more likely when weather is warm (15–30 °C) and conditions moist (Wallwork 1992).

9.2.3.3 Pyrenophora Leaf Blotch

The causal agent of leaf blotch and darkening on oat grains is the fungus *Pyrenophora chaetomioides* Speg. Briosi and Cavara. Pyrenophora leaf blotch has been frequently reported from most areas of the world where oats are grown. The most commonly observed symptoms of leaf blotch of oats appear on the leaves and under favourable conditions for the disease; they can reach the sheaths and appear soon after their emergence (Ivanoff 1963). Symptoms initially start with appearance of small spots (1–3 × 1–2 mm) with a white centre surrounded by a reddish brown halo on leaves that later coalesce and expand, forming small longitudinal stripes (Ellis 1971). Another symptom, called black stem or stem break, is characterized by darkening of the nodes and by the ease with which stems break. These symptoms start appearing as lesions on the leaf sheaths that are in direct contact with the nodes, become dark and make a more severe infection process. When infection is more severe, a mycelial mass of fungus can be seen in the stem cavity and the stem breaks easily between the third and fourth internodes. Besides the symptoms described above, other symptoms associated with *P. chaetomioides* are the 'spikelet drop' described by Ivanoff (1963) and spots on stems, which can be elongated and narrow or expand themselves irregularly (Harder and Haber 1992).

9.2.3.4 Scab

Scab or *Fusarium* head blight (FHB) is caused mainly by *Fusarium graminearum* (teleomorph = *Gibberella zeae* Schwabe Petch.) (Schroeder and Christensen 1963). Other species such as *Fusarium culmorum*, *F. avenaceum*, *F. moniliforme*, *F. oxysporum*, *F. poae* and *Microdochium nivale*, can also constitute a complex with the disease, although they are usually less important than *F. graminearum* (Warren and Kommedahl 1973; Wiese 1987). Isolates of *F. graminearum* differ in virulence and there is no evidence of the existence of stable races of the pathogen (Bai et al. 1991; Mesterhazy 1987). The characteristic symptoms of scab in oats are discoloured spikelets, pale or whitish in colour, which contrast with normal green healthy panicles. Under favourable climatic conditions for disease development, salmon-pink signs of the pathogen are easily observed on infected spikelets, as well as at the base and edges of the glumes. In infected panicles, the grains are light, wrinkled and wilted with a white rosy or pale brown colour.

9.2.3.5 Smut

Smut disease caused by *Ustilago* spp. is one among the most destructive diseases of oats throughout the world. Despite the use of resistant cultivars and chemical control to reduce disease levels, loose smut occurs most years in many areas. On oats, there are two forms of smut: loose, caused by *Ustilago avenae* (Pers.) Rostr., and covered, caused by *Ustilago kolleri* Wille (Wallwork 1992). Infected plants may be somewhat shorter than healthy ones, but smut symptoms are mainly visible on the panicle. Infected panicles emerge at the same time as healthy ones and usually have a narrower and erect habit. Loose smut destroys seeds, hulls and glumes and replaces them with a powdery mass of dark brown spots. As the crops ripen, most of the spores are blown away or washed off by rain, leaving only a few spores and small, light grey fragments of host tissue on the panicle. In covered smut, the somewhat compacted spores are enclosed in the remains of hulls and glumes, which turn a light grey towards maturity (Martens et al. 1985).

9.2.3.6 Barley Yellow Dwarf Virus (BYDV)

Barley yellow dwarf virus (BYDV) is a member of the luteovirus group. Luteoviruses are characterized by inducing 'yellowing' symptoms and are restricted to phloem and thus not mechanically transmissible; they are persistently and specifically transmitted by aphids (Mathews 1982). BYDV is diagnosed in the field by the presence of yellowish to reddish stunted plants grouped singly or in small patches among normal plants. Early infection of any of the cereals may result in severe stunting, excessive or reduced tillering, bright yellowing or reddening of older leaves, delayed heading or ripening, increased sterility and fewer and lighter kernels. In some oat cultivars, leaves become bronzed. The leaves of plants infected with BYDV are shorter than normal and the flag leaf may be severely shortened. Leaves are often stiffer and more erect. Root systems are reduced and diseased plants are more easily pulled up than healthy ones (Wallwork 1992; Watkins and Lane 2004). Symptoms vary according to the variety, the virus strain, the growth stage of the plant at the time of infection, the general health of the plant, the temperature and other environmental factors.

9.2.3.7 Halo Blight

Halo blight of oats is caused by *Pseudomonas coronafaciens* (Elliot) Young, Dye and Wilkie. Lesions occur mainly on leaf blades, but they are also found on stems, coleoptiles and leaf sheaths. Halo blight produces light green, oval spots, the centres of which become water-soaked and darker than the margins. Spots seem to be surrounded by pale green halos. Later, the whole spot, including the halo, turns brown. Spots may coalesce to form an irregular blotch. There are usually little bacterial

exudates from the lesions. Exceptionally, if conditions remain particularly favourable, the entire plant may be defoliated, or the bacteria may reach the crown, killing the plants (Martens et al. 1985; Harder and Haber 1992; Wallwork 1992). Bacteria causing halo blight are seed-borne and can survive on infected crop residues. The first seedling infections develop from bacteria on the surface of the seeds. From these infections, the bacteria can spread readily from leaf to leaf and from plant to plant during moist spring weather. In late spring, the disease in some fields may look severe, but often a spell of warm, dry weather will check the development of blight and new growth will be relatively free from infection. During the growing season, infection takes place through pores at the tips of the leaves, through stomata distributed over the surface of the leaves and through wounds. Rain, wind and insects, particularly aphids, are the agents responsible for disease spread (Martens et al. 1985; Wallwork 1992).

9.2.3.8 Septoria Blotch

Septoria disease of oats is caused by the fungus *Septoria avenae* f. sp. *avenae* (perfect state *Phaeosphaeria* [Leptosphaeria] *avenaria* f. sp. *Avenaria*). Other common names for the disease are septoria leaf blotch, speckled leaf blotch and septoria black stem. Generally, the disease is sporadic in its occurrence from season to season and from area to area. Septoria fungus is capable of attacking all aboveground portions of the oat plant at most stages in its development. Under appropriate environmental conditions, characteristic leaf, leaf sheath, culm, glume and kernel infections are produced. Leaf infections and culm breakage reduce yields and cause lodging. Kernel infections reduce milling quality. Infected straw may have reduced feeding value. The symptoms of the disease are small, dark brown to purple, oval or elongated spots on leaves. These spots grow into larger light or dark brown blotches up to 20 mm in diameter with surrounding yellow areas that can cover and kill the entire leaf. The infection may spread to leaf sheaths and through them to stems, where greyish-brown or shiny black lesions form. Severe infection may cause lodging. Dark brown blotches can also occur on the head and grain.

9.3 Status of Oat Genetic Resources to Combat Disease

Crop genetic resource refers to the biological diversity existed among the crop plants found in a distinct ecosystem of habitats. Genetic resources are the rich source of genetic diversity and serve as an essential raw material for improving crops and developing new value-added products. A wide spectrum of genetic diversity exists in oats with respect to morphological differentiation at both genus and species level.

Based upon agro-morphological parameters, several researchers have described oats' genetic resources for the benefit of human kind. The collection of genotypes

as well as the conservation of gene pools of cultivated and wild species is essential for genetics and plant breeding research. According to the Food and Agriculture Organization (FAO), the world's oat collections have been estimated to be about 131,000 accessions stored by 125 institutions in 63 countries which are considered as eighth most numerous germplasm collections after wheat, rice, barley, maize, bean, sorghum and soybean. The largest world collection of cultivated oats is maintained by Canada (~40,000), followed by the USA (~22,000) and Vavilov Institute of Plant Industry (VIR, Russia) (~12,000) (Boczkowska et al. 2016) which has a collection of about 10,000 accessions of 4 cultivated and 2000 accessions of 21 wild species (Loskutov and Rines 2011). About 2 per cent of total oat accessions (2100) of world's collection (WC) are held in India. In India, National Bureau of Plant Genetic Resources (NBPGR), New Delhi, and Indian Grassland and Fodder Research Institute (IGFRI), Jhansi, are maintaining 940 (13 species) and 150 oat accessions, respectively.

Conservation of wild gene pool of any crop plant is of utmost importance as they carry valuable genes for desirable traits such as yield, quality and biotic and abiotic stresses for crop improvement programmes. Wild species are helpful in providing basic information on species relationship and evolution pattern of crop plants. More than 24 per cent of accessions in world's oat collections are classified as wild species. Mostly the wild species of oats comprised of numerous hexaploid species which are included into primary gene pool (Leggett and Thomas 1995). Nearly 31,000 accessions of oat wild species are maintained in 29 oat collections, of which 13 hold more than 20 accessions (Brazil, Canada, China, Germany, Israel, Morocco, Norway, Poland, Russia, Spain, Sweden, UK and USA) (Table 9.3) (FAO/WIEWS).

The cultivated species of oats are *A. sativa*, *A. byzantina*, *A. strigosa* and *A. abyssinica*. Around 75,000 accessions of cultivated species are conserved in the world collections (Boczkowska et al. 2016).

9.3.1 Oat Gene Pools

Gene pool consists of all the genes and their alleles present in all such individuals which can hybridize with each other. Gene pool helps in the wider utilization of crop genetic resources. Basing upon the concept of gene pool of Harlan and de Wet (1971), the oat has been classified into three gene pools, i.e. primary, secondary and tertiary gene pool, by Leggett and Thomas (1995) (Table 9.4 and Fig. 9.1). The wild species of oats contain many qualitative and quantitative traits which would be advantageous if incorporated into the cultivated crop. These characteristics include resistance to crown rust, stem rust, powdery mildew, nematodes, yellow dwarf virus and agronomic traits including flowering, abiotic stresses and yield and grain quality traits. The ease of utilization of such variation is dependent upon the relationship between the wild and cultivated species. Examples of the incorporation of useful traits into the *Avena* gene pool are given in Table 9.5.

Table 9.3 Wild *Avena* species maintained in ex situ collections in the world

Sl. no.	Species	Number of accessions
1	*Avena atlantica*	18
2	*A. brevis*	87
3	*A. canariensis*	70
4	*A. damascene*	17
5	*A. hirtula*	75
6	*A. hispanica*	16
7	*A. longiglumis*	85
8	*A. nuda*	35
9	*A. prostrate*	02
10	*A. strigosa*	697
11	*A. wiestii*	76
12	*A. bruhnsiana*	01
13	*A. clauda*	111
14	*A. pilosa* (Syn. *A. eriantha*)	156
15	*A. ventricosa*	08
16	*A. macrostachya*	1
17	*A. abyssinica*	415
18	*A. barbata*	2,126
19	*A. lusitanica*	30
20	*A. vaviloviana*	248
21	*A. agadiriana*	18
22	*A. insularis*	14
23	*A. magna* (syn. *A. murphyi ssp.*)	97
24	*A. murphyi*	12
25	*A. diffusa*	08
26	*A. fatua*	2341
27	*A. hybrida*	24
28	*A. ludoviciana*	444
29	*A. macrocarpa*	02
30	*A. occidentalis*	71
31	*A. sterilis*	22,951
Total		**30,868 ~31,000**

Source: Germeier (2008)

9.3.1.1 Primary Gene Pool (GP1)

The primary gene pool consists of the taxa comprising the cultivated, weedy and wild forms of a crop. The crossing between the members of GP1 is easy and the hybrids produced will be fertile with normal meiotic chromosome pairing and recombination. The exchange of desirable genes between two accessions is straightforward with no crossing/sterility barriers. All the hexaploid species of oats belong

Table 9.4 Primary, secondary and tertiary gene pool of oat

Primary gene pool	Secondary gene pool	Tertiary gene pool
A. sativa	*A. magna* syn. *A. moroccana*	*A. clauda*
A. sterilis	*A. murphyi*	*A. pilosa* syn. *A. eriantha*
A. fatua	*A. insularis*	*A. ventricosa*
A. occidentalis		*A. bruhnsiana*
A. atherantha		*A. longiglumis*
A. hybrida		*A. damascena*
A. trichophylla		*A. prostrata*
A. byzantina		*A. canariensis*
A. ludoviciana		*A. wiestii*
		A. hirtula
		A. atlantica
		A. brevis
		A. nuda
		A. strigosa
		A. hispanica
		A. abyssinica
		A. macrostachya
		A. barbata
		A. vaviloviana

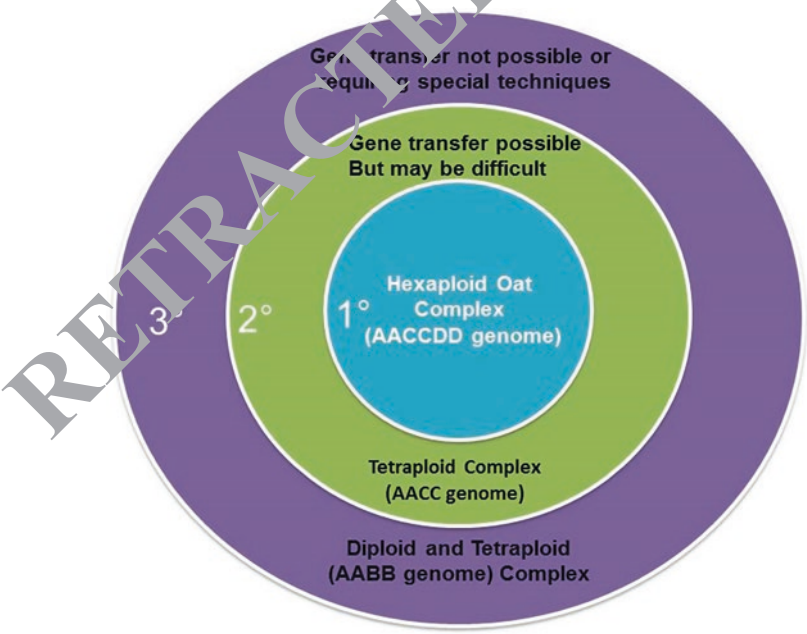

Fig. 9.1 Schematic diagram of three gene pools in oat

Table 9.5 Utilization of primary, secondary and tertiary gene pools

Traits incorporated	Resulting germplasm	Reference
Primary gene pool		
A. sterilis, $2n = 2x = 42$ (AACCDD)		
Crown rust resistance	Multiline cultivars E68, E69 and E70	Frey et al. (1971a)
	Multiline cultivars M68, M69 and M70	Frey et al. (1971b)
	Multiline cultivars E72, E73 and E74	Frey and Browning (1976a)
	Multiline cultivars M72 and M73	Frey and Browning (1976b)
	Multiline cultivars E76 and E77	Frey et al. (1985)
	Multiline cultivar Webster	Frey et al. (1988)
	Dumont cultivar	McKenzie et al. (1984)
	Tam 0-301 cultivar	McDaniel (1974a)
	Tam 0-312 cultivar	McDaniel (1974b)
	Coker's Pedigreed Seed Co. cultivars	Frey (1991)
	IN09201 cultivar	Ohm et al. (1995)
	Milton cultivar	Stuthman et al. (1995)
	Ensiler cultivar	Forsberg (Personal Communication)
	Fidler cultivar	McKenzie et al. (1981)
Crown rust and smut resistance	Riel cultivar	McKenzie et al. (1986)
	Steele, Valley, Newdak cultivars	McMullen and Patterson (1992)
	BC_1F_2 lines	Martens et al. (1980)
Powdery mildew resistance	BC5-derived line	Lawes and Hayes (1965)
Nematode resistance	Nelson and Panama cultivars	Marshall and Shaner (1992)
	Aline of Sol II cultivar	Mattsson (1988)
Partial resistance to crown rust	BC_1F_2 and F_5 lines (25, 50% *sterilis* parentage)	Harder and McKenzie (1984)
	3 germplasm lines (12–50% *sterilis* parentage)	Simons et al. (1987)
Partial resistance to stem rust	Aojss germplasm line	Rothman (1984)
	Alpha germplasm line	Rothman (1976) and Rothman (1984)
	Omega germplasm line	Martens et al. (1981)
Partial resistance to crown rust	Starter cultivar	Stuthman et al. (1990)
Barley yellow dwarf virus resistance	F_2 lines (50% parentage)	Landry et al. (1984)
A. fatua, $2n = 2x = 42$ (AACCDD genome)		
Crown rust resistance	F_3 lines (50% *fatua* parentage)	Sebesta and Kuhn (1990)
Secondary gene pool		
A. moroccana (=*A. magna*) $2n = 2x = 28$ (AACC genome)		

(continued)

Table 9.5 (continued)

Traits incorporated	Resulting germplasm	Reference
Crown rust resistance	Amagalon germplasm lines	Rothman (1984) and Rothman (1986)
Tertiary gene pool		
A. barbata, $2n = 2x = 28$ (AABB genome)		
Stem rust resistance	BC_3F_3 lines with translocation containing Pg-16 gene	Brown et al. (1986)
Powdery mildew resistance	BC_2-derived lines	Thomas et al. (1980)
A. strigosa, $2n = 2x = 14$ (As genome)		
Crown rust resistance	Lines incorporating *strigosa-sativa* chromosome translocation	Sharma and Forsberg (1977)
	Dane, Bay, Belle cultivars	Forsberg (Personal Communication)
	Horicon cultivar	Forsberg et al. (1991a)
	Centennial cultivar	Forsberg et al. (1991b)
	OAC Woodstock cultivar	Rothbergs (1983)
Crown and stem rust resistance	BC_5F_9 disomic addition line X117	Frey et al. (1973)
	Obee germplasm line	Rothman (1984)
Stem rust resistance	Delredsa germplasm line	Rothman (1984)
Smut resistance	Tibor cultivar	Burrows (1986)
A. longiglumis, $2n = 2x = 14$ (Al genome)		
Stem rust resistance	Amagalon derivatives	Rothman (1986)
A. pilosa, $2n = 2x = 14$ (A???? genome)		
Powdery mildew resistance	8x *A. pilosa*/*A. sativa* amphiploids, 6x backcrosses with unstable expression of resistance	Sebesta et al. (1986)

Source: Holland (1997)

to primary gene pool including the wild (*A. sterilis*)/weedy (*A. fatua*) species with cultivated species *A. sativa* in a single biological species (Ladizinsky and Zohary 1971). The useful desirable traits can be easily transferred from hexaploid wild species through conventional crossing and backcrossing methods as the hybrids developed will have complete fertile seeds without meiotic abnormalities. Interspecific hybrids between *A. sativa/A. sterilis* and *A. sativa/A. fatua* result in univalent or micronuclei at higher rate as such abnormalities are not observed in intraspecific hybrids. *Avena sterilis* is a progenitor of cultivated oats and serves as a source of crown rust resistance as well as good protein source and is commonly used in several oat breeding programmes at many breeding centres across the world (Frey 1985).

Majority of oat breeding programmes depends upon utilization of crown rust resistance (*Pc*) gene for development of resistance cultivars. More than 30 crown rust resistance (*Pc*) genes are identified from *A. sterilis* (Chong et al. 2000; Carson 2008). At present the Pc genes provide race-specific resistance and many resistance

varieties are developed by utilizing Pc genes. *A. sterilis* germplasm have been utilized by several workers for disease resistance and other useful traits related to yield and quality.

9.3.1.2 Secondary Gene Pool

The secondary gene pool consists of all taxa that will cross with GP1. The hybrids produced are usually sterile but with some fertility. The difficulty in hybridization is due to ploidy differences, chromosome alterations or genetic barriers. Gene transfer is possible but with considerable difficulties. According to Leggett and Thomas (1995) classification, the secondary gene pool of oats involves the tetraploid (AACC) species *A. magna* (*moroccana*) and A. *murphyi* which do not hybridize as readily with members of primary gene pool (GP1) *A. sativa* and produce highly self-sterile F_1s. This enables the F_1 to backcross to recurrent hexaploid parent to produce some fertile seeds and it will allow some sort of recombination between hexaploid and tetraploid species. More recently a new tetraploid (AACC) species *A. insularis* has been discovered and included in the secondary gene pool (Ladizinsky 1998). The members of the secondary gene pool provide a valuable source of variation for disease resistance and elevated protein content in grains.

9.3.1.3 Tertiary Gene Pool

The tertiary gene pool members are more distantly related to the primary gene pool. Gene transfer from tertiary to primary gene pool is very difficult and requires special techniques like embryo rescue, chromosome doubling and use of bridge species. As defined by Leggett and Thomas (1995), the tertiary gene pool consists of diploid and tetraploid oat species *A. barbata*, *A. vaviloviana*, *A. abyssinica* and *A. macrostachya*. These do not hybridize as readily with *A. sativa* and produced highly sterile F_1s. To overcome this problem Rajhathy and Thomas (1974) suggested the use of lower ploidy level species as a female parent to enhance the frequency and quality of seed set. To make hybrid production successful between diploid and hexaploid cross, frequent utilization of embryo rescue is necessary. Usually the F_1s resulting from wide crosses are sterile and in order to restore fertility, the F_1 plants should be treated with colchicine to induce chromosome doubling to make F_1 fertile. After doubling of chromosome, the doubled hybrid can be successively backcrossed using *A. sativa* as a recurrent parent to develop chromosome addition/substitution lines with trait introgressed.

The widening of the oat gene pool has become critical from the point of imparting disease resistance in oats. This is of considerable importance in the current scenario of crop improvement programmes implemented in oats aiming to develop climate smart and high yielding varieties using molecular breeding tools.

9.4 Developing Disease-Resistant Varieties in Oats: A Brief History

Oats have been cultivated for at least 2000 years (Coffman 1961; Murphy and Hoffman 1992). Oat improvement has been practised since the time the species was first cultivated. According to the report of Hunter (1924), the first oat plant selection was made in 1788 in a potato field in Northern England and resulted in the long famous variety 'Potato'. Further, Lawson and Son (1852) selected another single plant and named it as Sandy variety. This was the first selection in *Avena byzantina* L. It was Patrick Shirreff (1873) who first crossed oat. However, the performance of first cross was not exemplary. The four reports of Sheppherd (1896), de Vries (1900), Correns (1900), Tschermak (1900) and Johannsen (1903) gave a new momentum for oat breeding. Meanwhile, Rose (1903) had observed smut resistance among oat genotypes. Mendel's classical work and Johannsen's pure line theory led people to think that hybridization and mutation could lead towards development of variability which is the major prerequisite for crop improvement. This marked the initiation of search for the discovery of genes for disease resistance. Norton (1907) stated that 'Oat breeding in the United States in general is question of breeding for resistance'. But unfortunately the US oat breeders did not recognized the Norton's report.

Initially, hybridization became popular in Europe with the introduction of several rust-resistant cultivars. Breeding for disease-resistant varieties through hybridization in oats dates back to the year 1927 with the development of Victoria variety (*Avena byzantina*) of oat that could tolerate crown rust, the most destructive disease of oat. Prior to introduction of Victoria variety in the USA, White cross and Lee were selected from the progeny of crosses. During 1927, Victoria oat was introduced from Uruguay. Subsequently, the efforts to impart greater disease resistance to varieties were initiated at the Department of Agriculture, New South Wales. Many varieties were developed from Victoria (Table 9.6). A new variety named Bond was derived from the cross between wild red oat (*Avena sterilis*) and Golden Rain (*A. sativa*). Both Bond and Victoria varieties were found to be resistant to certain smuts. Victoria and Bond were found to be excellent breeding material for their further utilization in breeding programmes for development of superior oat varieties with disease resistant in the USA.

Bond served as an excellent source of disease resistance in breeding programme as one of the parents. In 1932, crosses between Iowa D69 and Bond were made at Ames, Iowa, and several productive high-quality strains having resistance for rust and smuts were selected and their performance was tested at Indiana and Illinois stations during 1939 and 1940. The selection 1335-3 was found to be outstanding for disease resistance, high yield, quality and lodging resistance. Selection 1335-3 was named as Clinton. Clinton and Benton are the first two varieties developed from Bond crosses. Clinton variety was found superior to Victoria-derived varieties Tama, Boone, Cedar and Vicland with respect to better resistance to crown rust, stem rust, leaf spot, lodging resistance and yield wise in Iowa during 1945 and 1946. Benton,

Table 9.6 Disease-resistant oat varieties released between 1925 and 1946

Sl. no.	Varieties	Parentage	Year	Resistance
1	Boone, Control, Tama, Vicland, Cedar and Vikota	Victoria × Richland	1930	Crown rust, stem rust and oat smuts
2	Clinton and Benton	Iowa D69 × Bond	1932	
3	Eaton	Iogold × Bond		
4	Bonda	Bond × Anthony		
5	Mindo	Bond × [(Minota-White Russian) × Black Mesdag]		
6	Osage, Neosho and Ventura	Fulton × Victoria-Richland and Markton-Fulghum × Victoria-Richland	1935	Rust and smuts
7	Letoria, Lelina, Lega, Lelate, Levic, Leroy, Florille and DeSoto	Lee × Victoria		
8	Traveler	Victoria × Custis	193	
9	Quincy Red (Quincy No. 1)	Fulghum × Victoria	930	Crown rust
11.	Fultex			Rust and moderately to smut
11	Fulgrain	Norton 20-93 (Big Boy × Navarro	1925	Oat smut
12	Fulgrain strain 4,5,6,7 and 8) and Victorgrain	Fulgrain × Victoria		Crown rust and smut
13	Ranger, Rustler, Rangler and Carolina red	Norton × Victoria	1930	
14	Quincy Grey (Quincy No. 2/Quincy White)	Victoria-Norton × Red Rustproof	1940	
15	Verde	Back cross of Red Rustproof × Victoria-Richland	1934	Rusts and smuts
16	Camellia	Bond × Alber	1933	Crown rust and smut

a sister selection of Clinton, was tested at Purdue University Agricultural Experiment Station in Indiana as selection 1239-1 in 1939. The Bond-derived varieties have advantage over Victoria-derived varieties in terms of higher productivity, test weight and better resistance to crown rust and stem rust. Eaton, Bonda and Mindo were other three varieties derived from Bond crosses and exhibited resistance against crown rust, stem rust and oat smuts. Traveler is a most promising variety developed from Victoria-Custis cross at Arkansas Agricultural Experiment Station resistant to crown rust and smuts in 1937. Adult plant resistance was first reported by Peturson (1944) and he advised not to discard hybrid lines on the basis of their seedling rust reaction.

In 1946 the Victoria-derived varieties suffered from the attack of a new *Helminthosporium* blight and devastated oat plantation in the USA. This widespread epidemics forced oat breeders to look for a new source of crown rust

resistance which was also resistant to blight. Plants of Columbia/Victoria/Richland cross were found to be resistant to blight (Poehlman and Kingsolver 1950). Varieties developed from Bond crosses were resistant to this disease and continued to be outstanding for yield, quality, disease resistance and stiffness of straw.

During the last decade (2008–2018), remarkable progress has been achieved towards the development of disease-resistant varieties and improved varieties with resistant genes for different pathogen strains of crown rust, stem rust, smuts, powdery mildew, *Fusarium* blight, etc., by the oat breeders across the globe. The National Oat Breeding Program of Western Australia has developed many improved oat varieties coupled with higher gluten content as well as resistant to diseases. Among the developed varieties by National Oat Breeding Program are Forrester, Tammar, Mulgara, Tungoo, Yallara, Wombat, Dunnart, Bannister, Kowari, Mitika, 05096-32 and recently developed Williams (Oat Breeding Newsletter 2018). Another variety named Jupiter-INIA developed by Chile Institute of Agricultural Research, Chile, also found to exhibit disease resistance against crown rust and other diseases like stem rust, smuts and BYDV (Mathias-Ramirell et al. 2016). Aberystwyth University, UK, is working towards the generation of oat varieties with enhanced crown rust and mildew resistance through marker-assisted breeding programme. In India, many state agricultural universities such as Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana; Punjab Agricultural University, Ludhiana, Punjab; GB Pant University of Agricultural and Technology, Pantnagar; Indian Agricultural Research Institute, New Delhi; and national level research institutes Indian Grassland and Fodder Research Institute (IGFRI), Jhansi, and Central Arid Zone Research Institute (CAZRI), Jodhpur, are working in the direction of development of fodder oat varieties. During the last decade, few fodder oat varieties viz., JHO 2000-4, JHO-19 and JHO 99-1 were developed with disease resistant to crown rust, leaf blight, root rot and powdery mildew.

9.5 Role of Plant Breeding in Developing Resistant Oat Cultivars

9.5.1 Harnessing Genetic Variation for Disease Resistance

Genetic variation is the prerequisite in any breeding programme. The breeding of disease-resistant varieties of crop has perhaps received more attention than any other phase of plant breeding. Use of fungicide and other methods of disease control has given effective control of diseases. However, host plant resistance is the most preferable means of crop protection of all kinds as it combines the advantages of cost-effectiveness and ecological soundness. In case of breeding resistant varieties to diseases and pests, it is imperative to search for source of resistance, i.e. the donors from which the resistant gene(s) may be transferred.

The supply of genes for resistance, for disease(s), insect pest(s) and nematode(s), is the first concern in an ongoing resistance breeding programme. The primary and secondary centre of origin (gene centres) of cultivated plants is the best places to find genuine resistance to common diseases and pests. Resistance to diseases may be obtained from germplasm collection, wild/weedy relative species, mutations, somaclonal variations and unrelated organisms. Often the genes from wild species have resistance against a wide range of races. Such genes have been called 'super genes'. Therefore, resistance available from wild relative is attractive even when other sources of resistance are available. I. A. Watson (1970) noted that the new races/biotypes of parasites overcome the resistant gene(s) being used in the cultivars. Thus, it is emphasized that the wild relative or species become increasingly important sources of germplasm in the breeding of many crops.

Several resistance genes against the major diseases, i.e. crown rust, stem rust, powdery mildew, BYDY, etc., from oat gene pool (Table 9.7) have been discovered

Table 9.7 Source of disease resistance among wild oat species

Species	Genome	Sources of resistance to					
		Powdery mildew	Crown Rust	Stem rust	BYDV	Smut	Septoria leaf blight
A. bruhnsiana	Cv	+	+				
A. ventricosa	Cv	+	+	+			
A. clauda	Cp	+	+	+	+		
A. pilosa	Cp	+	+	+			
A. prostrata	Ap	+	+				
A. damascena	Ad	+		+	+		+
A. longiglumis	Al	+	+	+	+		+
A. canariensis	Ac		+	+	+		+
A. wiestii	As	+	+			+	+
A. hirtula	As		+	+	+		+
A. atlantica	As	+					+
A. strigosa	As	+	+	+	+	+	+
A. barbata	AB	+	+	+	+	+	+
A. vaviloviana	AB	+	+		+	+	+
A. abyssinica	AB	+	+	+		+	
A. agadiriana	AB?	+					
A. maroccana	AC		+	+	+		
A. murphyi	AC	+	+		+		+
A. insularis	AC?		+	+			
A. macrostachya	CC?	+	+	+	+		+
A. fatua	ACD	+	+	+	+	+	+
A. occidentalis	ACD	+	+	+	+		
A. ludoviciana	ACD	+	+	+	+		+
A. sterilis	ACD	+	+	+	+	+	+

Source: Loskutov and Rines (2011)

in over 31 wild oat species. *A. sterilis* exhibits multiple resistance to several oat diseases, i.e. crown rust, stem rust, powdery mildew and cereal cyst nematode. Powdery mildew resistance has been transferred into variety HiFi through synthetic amagalon of *A. magna* × *A. longiglumis* cross. During 1925–1946, several disease-resistant cultivars were developed utilizing the Victoria and Bond which are derived from wild red oat and cultivated hexaploid species *A. sativa*. This served as a source for development of many disease-resistant oat varieties for different regions.

9.5.2 Durability of Disease Resistance

Resistance is directly useful in plant breeding if it is available in the same species of varieties. Resistance is a means of disease control by natural, rather than by physical or chemical means or that resistance connotes a hereditary struggle against some specific causal agent. When new diseases or races of established diseases appear, search through the diversity of germplasm represented in the world collections of varieties of crop plants has almost always been successful in locating adequate sources of resistance. In disease resistance breeding collection and conservation of genetic variability including the wild/weedy relative(s) insect(s) is the preliminary step. The next step is to screen the available gene pool against important parasites. The resistant genotype(s)/accession(s) of immediate use may be included in the core germplasm which is used in hybridization.

The cultivation of resistant varieties has been recognized as the most effective, ideal and economical method of reducing crop losses (Stakman and Harrar 1957). The breeding for resistance is generally no way different than breeding for other traits. However, in resistance breeding the two biological entities, host plant and parasites, are involved, whereas in breeding for other traits the breeder deals with the variability in test material only. When genes for resistance occur in existing commercial varieties, selection within these varieties will almost always provide the easiest and most satisfactory method of developing resistant strains. But when resistance gene is not found in commercial varieties, either the backcross or pedigree methods of breeding are selected. If the resistant parent is a wholly unadapted type, the backcross method is the logical choice as a breeding procedure. If, on the other hand, the breeder is satisfied that the resistant parent can also contribute to improved adaptation, quality or yield than either pedigree or bulk, selection procedure will be suitable.

The pedigree method has been widely used in breeding for disease-resistant varieties and practised in oat breeding as given in the US agriculture yearbooks. Resistance is natural and resistance varieties may become susceptible when new pathogenic race of pathogen arises as a result of hybridization, heterocaryosis and mutation. The race between the pathogen and plant is never-ending because some pathogens have a wide range of host plant and some have a narrow range of host plant for disease development. The pathogenic organisms have enormous potential in developing new virulent forms. Ug99 strain of *Puccinia graminis* f. sp. *tritici* of

wheat that first appeared in Uganda in 1999 is an example of new virulent forms of black stem rust. Floor (1956) was the first to show there was a 'gene for gene' relationship between avirulent (*Avr*) gene of pathogen and resistance (*R*) gene of host. Floor's hypothesis of gene for gene provided an explanation for Priestley's concept of boom and bust. From this it is clear that plant breeders must be prepared to face breakdown of resistance due to the increase in pathogenic races. For example, in oats the average usefulness of resistant varieties to stem and crown rust was 5 years in the Corn Belt of the USA. Some varieties survived but a single year. From this Stevens and Scott (1950) concluded that a new oat variety would be needed every 4 or 5 years to meet the threat of new races of stem and crown rust. Thus, durability of resistance is of great concern for plant breeders and pathologists.

Adult plant resistance (APR) is also an effective means of reducing the rust epidemics in oats as APR can be durable or non-durable. Diploid and tetraploid oat accessions and cultivars with durable APR resist have been identified (Cabral et al. 2011). Vander Plank (1963) classified resistance into two categories, viz. horizontal and vertical resistance. Horizontal resistance (polygenic/incomplete/quantitative/non-gene specific/durable) is generally conditioned by multiple genes of partial effect; on the other hand, vertical resistance (complete/qualitative/oligogenic/gene specific/non-durable) is conditioned by a single major gene. The quantitative resistance varied in a continuous range from weak to quite strong and it results in partial resistance. Partial resistance has been reported in almost all the major field crops, i.e. bacterial leaf blight in rice and rusts and powdery mildew in wheat and crown rust in oat. Hence, quantitative resistance (QR) is present almost everywhere. The durability of resistance is a great concern for everyone as durability varies from crop to crop. Sometimes resistance is neutralized in the early stage of the breeding programme and may be effective for more than hundreds of years. Quantitative resistance (QR) appeared to be durable on the basis of several resistance genes. But till date not so much progress has been achieved towards the development of varieties with durable resistance because still we have not fully understood the actual cause of resistance. According to Rubiales and Niks (2000), a combination of genes with different resistance mechanisms can greatly enhance the durability of resistance.

In 1952 Jensen suggested the use of multilines or composite varieties as such varieties would consist of a blend of compatible lines, each selected for similarity of height, maturity and other agronomical traits, but differ for resistance genes. Several near-multiline varieties M68, M69, M70, E68, E69 and E70 have been released from Iowa Agricultural Experiment Station, USA, for crown rust resistance. Backcross method involves hybridization which is most commonly used for breeding resistance cultivars as it involves transfer of disease-resistant gene from the donor parent to the susceptible but superior in agronomy recipient parent through successive backcrossing of F_1 hybrid with the recipient parent for 5–6 generations. Introgression/transfer of resistant genes from unadapted germplasm (wild species) to adapted varieties backcross is performed. At Purdue Agricultural Experiment Station, backcrossing was used to transfer crown rust resistance genes and several resistance varieties (Clintland, Clintland 60 and Clintland 64) were developed.

9.6 Exploitation of Oat Germplasm for Disease Resistance Through Traditional and Molecular Breeding

9.6.1 Traditional Breeding

In the past, much effort was devoted to enhancing grain yield and improving agronomic characteristics and resistance to diseases in oat breeding. The main goal of the complex oat breeding programme is to develop new winter and spring high-yielding varieties with good grain quality and resistance against oat disease complex. As oat is a self-pollinated crop, the basic breeding procedure of selection, introduction and hybridization followed by selection are practised worldwide. According to the reports, the successful oat hybridization took place in the year 1870 and not the year 1930. During 1930s, the oat cultivars cultivated in the USA were all introductions or selection from those introduced cultivars from other parts of the world. After that hybridization was widely utilized by oat breeders to develop high-yielding grain quality with resistance to a wide range of diseases. During 1912–1940, the basic selection and breeding procedures for self-pollinated crops were described by several workers; i.e. Newman (1912) described bulk selection, Harlan and Pope (1922) described backcross method and then Love (1927) described pedigree selection in detail. These methods are being widely used by the oat breeders under different oat improvement programmes till date.

Wide crosses between different plo[illegible]y species contributed greatly towards oat improvement through gene and genome mapping, understanding chromosome behaviour and evolution. Oat diseases are still the major constraints for reduction in yield and grain quality. The main focus of oat breeding to this date is restoration of diversity for disease resistance in cultivated oats through introgression of resistance genes which remained unselected from wild progenitors during domestication due to genetic bottlenecks. Therefore, it is necessary to give more preference to phytopathological studies among wild oat complex to identify the new sources of resistance for broadening of genetic breath of cultivated oats.

9.6.2 Molecular Breeding

Traditional breeding relied upon the generation of new genetic combinations by controlled hybridization and subsequently phenotypic selection in the segregating populations. The protocols of traditional breeding are usually based upon the phenotypic selection for the desired trait of interest with desired gene combinations. The practices of traditional breeding have increased the yielding capacity of major food crops experiencing the difficulties arising through genotype × environment interactions which reduce the effectiveness of phenotypic selection and hindered the identification of superior genotypes. Moreover, pathotyping for disease resistance also becomes a difficult task for plant breeders owing to the complexity of genes

involved in imparting resistance. Introgression of a resistance gene into a susceptible variety from resistant one through traditional hybridization is a difficult and time-consuming procedure. Over the years, due to developmental activities and industrialization, the genetic diversity among the crop species has dwindled while owing to the change in climate pathogens and insect pests are evolving continuously. This has led to breakdown of host resistance which makes breeding varieties combining high yield and resistance the need of the hour.

The availability of genomics tools and resources has opened new vistas in plant breeding as they facilitate more precise study of the genotype and its relationship with the phenotype which is all the more important while dissecting complex traits. Molecular markers are valuable tools to plant breeders to understand complex polygenic traits, dissecting genes responsible for desired traits, characterization, development of genetic linkage map which aids in gene tagging and gene mapping and further development of new cultivars with different kinds of marker-assisted selection (MAS) schemes, viz. marker-assisted backcross breeding (MABB), marker-assisted gene pyramiding and marker-assisted recurrent selection. The development of a wide array of DNA (molecular) markers technology and genetic mapping in major crops has facilitated the identification of a source of variation and served as a reliable tool for identification and selection of disease resistance individuals.

9.6.2.1 Role of Molecular Markers in Genetic Mapping of Disease Resistance Genes

Very few genes have been identified in oats based upon visual selection and mapped because of difficulties associated with mapping in hexaploid genome (Marshall and Sorrells 1992) and problems arising due to several chromosomal rearrangements (O'Donoughue et al. 1995). Oat scientists are also involved in the development of genetic linkage maps composed of a wide array of DNA markers to facilitate the identification of agronomic and other desired genes like disease resistance genes. The position of genes mapped on diploid maps can be used to infer the map location of homologous loci on hexaploid maps. The first genetic linkage map was constructed in diploid species *A. atlantica* × *A. hirtula* by O'Donoughue et al. (1992). Rayapati et al. (1994) developed a second genetic oat map from F_2 cross of *A. strigosa* × *A. wiestii* and it was used successfully for the mapping of *Pca* locus which confers resistance against nine isolates of *Puccinia coronata*. Genetic mapping is a powerful approach which provides a foundation for the identification of disease resistance genes (O'Donoughue et al. 1996), the localization of QTLs (Siripoonwiwat et al. 1996; Ronald et al. 1997; Jin et al. 1998; Kianian et al. 1999, 2000; Groh et al. 2001) and the development of molecular markers for utilization in breeding programmes. The *Pca* locus identified from second linkage map of oats was defined as a cluster of five resistance loci (*R54*, *R263*, *R290*, *R62* and *R 202)* by Wise et al. (1996), and later on Yu and Wise (2000) renamed these loci as *Pc81*, *Pc82*, *Pc83*, *Pc84* and *Pc85*.

Restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), sequence-characterized amplified repeat (SCAR) and diversity array technology (DArT) markers have been developed by several researchers for genetic mapping of economically important diseases of oats on linkage groups. Several workers have identified and developed many markers using near isogenic lines (NILs) and bulked segregant analysis (BSA) which confers resistance for crown rust and stem rust (Table 9.8) and also mapped the resistance genes. In a study conducted on near isogenic lines (NILs) of hexaploid oat, three crown rust resistance genes were identified using RFLP markers. Three markers were found to be linked with crown rust race 203 resistance loci in D494 and X466-1 lines. One marker in D526 and Y345 conferred resistance to crown rust race 345 and D486 and

Table 9.8 Gene mapping and gene tagging of crown rust and stem rust resistance genes

Gene mapped	Population used	Strategy	Reference
Crown rust			
Pc38	Pendek-48 × Pendek-38; OT328 × Dumont	Bulked segregant analysis	Wight et al. (2004)
Pc39	Pendek-39 × Pendek-48; OT328 × Dumont		
Pc48	Pendek-39 × Pendek-48		
Pc68	F_3 of a line with Pc68 × Rodney 0	BSA and cosegregation	Penner et al. (1993c)
Pc71	NILs D526 and Y345; BC_1F_2 of NIL D526 with recurrent parent Lang	BSA	Bush et al. (1994), Bush and Wise (1998)
Pc264B	NILs D486 and X434-II; BC_1F_2 to C237-89 or Lang	NILs and cosegregation	Bush et al. (1994)
R203	NILs D494 and X466-1; BC_1F_2 to C237-89 or Lang		
Pc91	F_2 and BC_1F_6 of donor Amagalon and recurrent parent Ogle or Starter	NILs and cosegregation and aneuploids	Rooney et al. (1994)
Pc92	F_2 and BC_1F_6 of donor Obee/Mid-South and recurrent parent Ogle or Starter		
Pc94	F_2 of Calibre × S42 for BSA; Ibid and MakuraSun-Pc68 × S42 for cosegregation	BSA and cosegregation	Chong et al. (2004)
Stem rust			
Pg3	NILs and F_2 of Rodney 0 × Rodney 0-Pg3	NILs and cosegregation	Penner et al. (1993b)
Pg9	NILs and F3 of Rodney 0 × Rodney 0-Pg9; F_3 of OT328 × Dumont	NILs and BSA and mapping and comparative mapping	O'Donoughue et al. (1996)
Pg13	NILs and F3 of Rodney 0 × Rodney 0-Pg13; F_3 of OT328 × Dumont		
Pg9	–	–	Cheng et al. (2002)

Source: Rines et al. (2006)

X434-II to 264B (Bush et al. 1994). Further, Bush and Wise (1998) developed a high-resolution mapping population of 440BC1F2 from the cross of D526 and Lang and developed a high-resolution RFLP map for *Pc71* which confers resistance to crown rust. Zhu and Kaeppler (2003b) identified two QTLs *Pcq1* and *Pcq2* utilizing Ogle/MAM17-5 linkage map in MAM17-5 oat line which shows total phenotypic variation for crown rust resistance suggesting marker-assisted selection would be more useful for targeting Pcq1 for efficient selection of crown rust resistance. Two sequence-characterized amplified region (SCAR) markers from an AFLP fragment, linked with *Pc94* gene in Caliber/S42 population, were located. These SCAR markers could be further utilized for marker-assisted selection and gene pyramiding leading to development of new crown rust resistance cultivar (Chong et al. 2004). Wight et al. (2004) identified 18 markers linked to resistance against crown rust out of 23 RFLP markers studied. Among the identified markers six were identified for *Pc48* and *Pc39* in Pendek3948 and nine for *Pc38* in OT328Du population. The two RFLP markers identified for *Pc38* coded for a putative leucine-rich repeat transmembrane protein kinase and a *cre3* resistance gene analogue.

Fetch and Fetch (2011) determined inheritance of stem rust in Ronald and AC Gwen varieties and found that both Ronald and AC Gwen possess dominant gene *Pg2* and recessive gene *Pg13*. For crown rust also scientists determined the genetics of crown rust resistance. Portyanko et al. (2005) from hexaploid genetic map of partial resistance line MN841801-1 and Noble-1 detected four major QTLs (*Prq1a*, *Prq1b*, *Prq2* and *Prq7*) and three minor QTLs (*Prq3*, *Prq5* and *Prq6*) which confer partial resistance to crown rust. Six crown rust isolates were tested in $F_{6:7}$-derived RIL population from Ogle × TAM O-301 and genetic segregation indicated three genes conditioning resistance to crown rust in TAM O-301 (Hoffman et al. 2006). Jackson et al. (2007) identified the major gene for resistance against crown rust in Ogle using qPCR, digital image analysis and visual rating and mapped on OT6 linkage group. Further from qualitative as well as quantitative mapping, Jackson et al. (2008) identified an allele for resistance to crown rust conferred by TAM O-301 on OT11 and two major QTLs on OT-11 and OT-32 linkage group. Satheeskumar et al. (2011) detected the seed storage protein loci closely linked with *Pc68* and *Orga 1* and three RGA loci loosely linked with Pc68.

Acevedo et al. (2010) identified eight QTLs associated with MN841801-1 alleles and out of these eight QTLs, seven QTLs were previously identified and a new QTL named *Prq8* was detected on linkage group MN13 conferring resistance to crown rust disease. The *Pc91* is a seedling crown rust resistance gene and was mapped using F7 RIL population of CDC Sol-Fi/HiFi with diversity array technology (DArT), and the DArT markers were successfully converted to sequence-characterized amplified region (SCAR) markers. Gnanesh et al. (2013) developed allele-specific KASP-SNP markers for marker-assisted selection for crown rust resistance gene *Pc91* which resides on translocated oat chromosome 7C-17A. A major QTL located on chromosome 14D designated as QPc.crc-14D confers adult plant resistance to crown rust flanked by two SNP markers GMI_GBS_90753 and GMI_ES14_c1439_83 (Lin et al. 2014). Babiker et al. (2015) identified four QTLs (QCr.cdl9-12D, Qcr.cdl9-191A, QCr.cdl9lsu9-19A and QCr.cdl11013A)

conditioning resistance to crown rust from CDC Boyer and 94197A1-9-2-2-2-5 using newly developed SNP markers. Gnanesh et al. (2015) evaluated OT3019 × Morton population, reported that the resistance provided by Morton is governed by a single gene designated as PcKM and also mapped PcKM gene using TaqMan assay and KASP markers. Recently, Rines et al. (2017) identified effective resistance to crown rust in diploid *A. strigosa* and also developed KASP-SNP markers associated with resistance gene.

The very first report on mapping of powdery mildew resistance in hexaploid oat was made by Yu and Herrmann (2006) utilizing comparative mapping. They reported that the resistance for powdery mildew is governed by a single dominant gene *Pm5*. Also, *A. macrostachya* was identified as a new source of resistance. Hamm et al. (2014) using monosomics analysis identified five powdery mildew resistance genes (*Pm1*, *Pm3*, *Pm6*, *Pm7* and *Pm8*) in four cultivated oats *A. sativa*. Out of these five genes, *Pm6* exhibits recessive mode of inheritance and rest genes showed dominant mode of inheritance and genetic map was prepared for *Pm1*, *Pm3* and *Pm7* using RFLP markers. Using host-pathogen tests Okon et al. (2016) identified ten *Avena sterilis* genotypes out of 350 *A. sterilis* genotypes resistant towards oat powdery mildew disease. Powdery mildew was also observed on *A. sativa* caused by *Blumeria graminis* in China (Xue et al. 2017). Okon et al. (2018) identified *Pm4* powdery mildew-resistant gene using DArTseq technology. To date, eight powdery mildew-resistant genes have been described in oat, but only four of them provide the resistance to the current *B. graminis* f. sp. *avenae* pathotypes (Okon 2015; Okon and Ociepa 2017). Recently, Okon and Ociepa (2018) have identified *Avena sterilis* as a new source of powdery mildew resistance.

9.6.2.2 Mapping of Resistance Gene Analogues (RGAs)

Similar to other crop genus *Avena* suffers from a narrow genetic base for disease resistance and wild *Avena* species *A. strigosa*, *A. sterilis* and *A. barbata* served as a valuable source of resistance for crown rust, stem rust and powdery mildew diseases. Eight soybean-based resistance gene analogues (RGAs) (Yu and Wise 2000) and five RGAs from maize, sorghum and wheat (Kremer et al. 2001) have been mapped in the *A. strigosa* × *A. wiestii* diploid oat mapping population. Using comparative mapping Cheng et al. (2002) mapped kinase gene on five loci of KO linkage group 4_12, 5, 6, 13 and one unlinked locus and using RFLP mapping in Dupont xOT328, one locus is found to be tightly linked with *Pg9* stem resistance gene. From this it became evident that both crown and stem rust resistance genes are clustered together. Based on degenerate primers a set of 15 RGAs were isolated and mapped from the diploid species *A. strigosa* (Irigoyen et al. 2004). Further, 33 sequences analogous to RGAs of NBS-LRR were cloned from 11 different *Avena* species (Irigoyen et al. 2006). In continuation to this Loarce et al. (2009) mapped RGAs into diploid (*A. strigosa*, *A. wiestii*) and hexaploid (MN841801 × Noble-2) RIL populations that segregate for crown rust resistance with STS markers and QTLs associated with resistance were also identified.

9.6.2.3 Other Marker Systems

Simple sequence repeat (SSR) markers can detect several alleles of a single locus simultaneously and therefore prove advantageous for genetic mapping. Because of a finite number and low polymorphism compared to RFLP, Zhu and Kaeppler (2003a) reported SSR markers to be of limited use in oat breeding. Currently, a single nucleotide polymorphism (SNP) has become the most prominent marker of choice among all the marker systems. Developed by KASP, TaqMan assay and genome sequencing platform, this system helps scientists in precisely distinguishing between resistance and susceptible oat accessions and identifying the allelic series for disease resistance. The first generation of DNA markers (RFLP, RAPD and AFLP) has been converted into PCR-based SCAR/CAPS markers (Table 9.9) to reveal the polymorphism for resistance gene among the oat accessions of cultivated as well as wild species by many scientists (Rines et al. 2006; Kapoor and Batra 2016).

Though marker-assisted breeding has not been well established in oats, there are a few examples of DNA markers linked with BYDV resistance (Jin et al. 1998, 1999; Pal et al. 2002) and crown rust resistance (Chen et al. 2004) which acts as a curtain raiser to marker-assisted breeding in oat.

9.6.2.4 Marker-Assisted Breeding

With the discovery of DNA markers, plant breeding has experienced a new technological revolution by the development of a large array of DNA markers which makes breeders task easy for the selection of complex traits especially those which are difficult to assess phenotypically. Marker-assisted breeding (MAB) serves as boon to breeders to carry out effective and speedy selection based upon the DNA markers. With the development of a wide variety of DNA markers and genetic maps, MAB can be used for traits conditioned by qualitative as well as quantitative genes. By practising MAB in breeding programmes, the rate of genetic gain is twice the genetic gain obtained from traditional phenotypic selection. It includes several breeding strategies such as marker-assisted selection (MAS), marker-assisted backcrossing (MABC), marker-assisted gene pyramiding and marker-assisted recurrent selection (MARS). The success of marker-assisted breeding depends on the availability of a tightly linked trait-molecular marker (disease resistance gene).

Since oat genomic resources are not as developed as in other cereal crops (rice, wheat, maize, etc.), the DNA marker system is less developed too. The first molecular marker RFLP developed by Botstein et al. (1980) has been utilized by many oat researchers and strengthens the linkage and comparative mapping for the discovery of crown rust and stem rust resistance genes. Marker-assisted breeding for disease resistance and other agronomic traits has been well discussed by Rines et al. (2006) and Kapoor and Batra (2016).

Table 9.9 Molecular markers linked with crown rust and stem rust resistance

Gene	Marker	Linked marker/QTL	Reference
Crown rust			
Pc38	RFLP	Cdo673, wg420	Wight et al. (2004)
Pc39	RFLP	Cdo666	
Pc48	RFLP	cdo337	
Pc54	RFLP	cdo1435B	Bush and Wise (1996)
Pc58a,b,c	RFLP	PSR637, RZ516D	Hoffman et al. (2006)
Pc59	RFLP	Cdo549B	Bush and Wise (1996)
Pc68	RAPD	ubc269	Penner et al. (1993b)
	SNP	Pc68-SNP1, PC68-SNP2	Chen et al. (2006a, b)
	AFLP	U8PM22, U8PM25	Kulcheski et al. (2010)
	SDS-PAGE	AveX, AveY, AveZ	Satheeskumar et al. (2011)
	RGA/RFLP	Orga1	
	SCAR	ubc269a SCAR	Personal Communication
Pc71	RFLP	cdo783, cdo1502	Bush and Wise (1998)
Pc81,82	AFLP	isu2192, OP C18	Yu and Wise (2000)
Pc83,84,85	STS	Agx4, Agx9, Agx7	
Pc91	RFLP	UMN145	Rooney et al. (1994)
	DArT	oPT-0350	McCartney et al. (2011)
	SCAR	oPT-0350-cdc	
	KASP	oPT-0350-KOM4-2	Gnanesh et al. (2013)
Pc92	RFLP	OG176	Rooney et al. (1994)
Pc94	AFLP	AF94a	Chong et al. (2004)
	SCAR	SCAR94-1, SCAR94-2	
	SNP	Pc94-SNP1a	Chen et al. (2007)
Pca	RGA/RFLP	isu2192	Kremer et al. (2001)
		17M22	Irigoyen et al. (2004)
		B9-1	Sanz et al. (2012)
Pcx	RFLP, RAPD	Xcdo1385F, XpOP6(A), Xacor458A	O'Donoughue et al. (1996)
Stem rust			
Pg3	RAPD	ACOpR-1, ACOpR-2	Penner et al. (1993a)
	SCAR/CAPS	Pg3 SCAR/CAPS	Personal Communication
Pg4	SCAR/CAPS	Ubc254s SCAR	Personal Communication
Pg9	Acid-PAGE	avenin band	Chong et al. (1994)
	RFLP, RAPD	Xcdo1385F, Xacor458A	O'Donoughue et al. (1996)
	SCAR/CAPS	Pg9 SCAR/CAPS	Personal Communication
Pg13	SDS-PAGE	56.6-kDa polypeptide locus	Howes et al. (1992)
	RFLP, RAPD	Xmog12B, Xacor254C	O'Donoughue et al. (1996)
	SCAR	Pg13SCAR	Personal Communication
Sr_57130	AFLP	PacgMcga370	Zegeye (2008)

Source: Gnanesh et al. (2014)

9.6.2.5 Genomics Perspectives and Future Scope for Disease Resistance Breeding

Staple crops such as wheat, rice, barley, maize, pearl millet and sorghum are enriched with a large number of genomic resources in comparison to oats. The progress made in molecular genetic research in oats in comparison to other staples is less owing to the genomic complexity and non-availability of complete genome sequence. DNA marker-based genetic linkage maps developed in various oat genetic populations reveal marker-trait association useful for the identification of genes/QTLs for their further utilization in marker-assisted breeding. But most of the identified genes/QTLs/markers in oats are linked to agronomic and nutritional value traits as the crop is considered as an important food grain crop owing to its high protein content.

Although several linkage maps have been developed in oats by many scientists using numerous mapping populations till date, only one consensus map (Chaffin et al. 2016) is available in oats which depicts the genetic location of several resistance genes. A major reason for this lacuna may be the lack of oat genome sequence which could provide insights into the plant architecture and genomic relationship between different oat genomes. Deducing the complex oat sequence is a challenge for scientists mainly because of polyploidy nature of oats. Highly precise and reliable next-generation sequencing DNA markers like SNP (single nucleotide polymorphism) which are widely used in the present era of genomics can however prove useful in delineating the genome sequence of oats. With the advancement of new sequencing technologies and a rapid development in bioinformatics, complete oat genome sequencing is no longer out of reach. High-throughput genotyping is a prerequisite for marker-assisted breeding (MAB), genomic selection (GS), genome-wide association studies (GWAS), TILLING which is the next-generation mutagenesis technique and the CRISPR/Cas9, most recently developed genome editing platform. In this regard sequencing of oat genome would be highly beneficial. It will enable fine mapping and cloning the disease resistance genes which is a challenge to DNA marker technology aimed for disease resistance.

Marker assisted breeding (MAB) is the most suitable methodology in plant breeding for disease resistance breeding. It is highly useful in the selection of desirable individuals with major disease resistance genes/QTLs. However, minor genes/QTLs also played a major role in disease resistance and tend to produce more durable varieties. As the genetic architecture of resistance shifts from single major R genes to a diffused architecture of many minor genes, the best approach for molecular breeding will shift from marker-assisted selection to genomic selection.

Genomic selection (GS) or genome-wide selection (GWS) is also a form of marker-assisted selection which is based on the statistical prediction models and selection methodology. These statistical models will be able to predict accurately for disease resistance and will outperform the multiple linear regressions applied in marker-assisted breeding. GS has become feasible in plants due to the discovery and development of a large number of SNP markers by genome sequencing (Dhillon and Chhuneja 2014). Thus, use of GS in oats for disease resistance becomes a pow-

erful approach in oat breeding programmes. Many QTLs have been identified using the bi-parental population utilizing DNA markers in crop plants, but such QTLs have limited application in MAS as parental genotypes because these genotypes are often not representatives of germplasm pool which is actively used in breeding programmes, and markers linked to QTL are not always transferable to other genetic backgrounds (Snowdon and Friedt 2004). Genome-wide association studies (GWAS) emerged as an alternative approach which has overcome the limitations of bi-parental linkage mapping. GWAS is most commonly used for detecting the variants for complex human diseases. Recently, it has been utilized in maize, rice, wheat and sorghum for the identification of marker-trait association for agronomic traits (Huang et al. 2010; Jia et al. 2013; Li et al. 2013; Morris et al. 2013), but there are some reports available where GWAS have been utilized for identifying disease resistance genes in maize, rice and wheat (Kump et al. 2011; Gurung et al. 2014; Wang et al. 2014). In oats, few GWAS are available where marker-trait associations have been determined for grain quality traits (Newell et al. 2012; Asoro et al. 2013). But there are some studies where GWAS have been utilized for the identification of QTLs/loci linked with disease resistance. Montilla-Bascón et al. (2015) used GWAS for crown rust and powdery mildew resistance in some oat varieties. Winkler et al. (2016) identified two novel loci associated with crown rust resistance utilizing population structure and genotype-phenotype association in oat landraces and historic cultivars. Klos et al. (2017) identified 29 SNPs on 12 linkage groups related to crown rust reaction and *Pc48*, *Pc58a*, *Pc68*, *Pc71*, *Pc91* and *PcKM* QTLs shown to be linked with seedling resistance genes using genome-wide association mapping (GWAM). Presently, GWAS has become a potential approach which will open new frontiers in disease resistance research in oats.

TILLING (targeting induced local lesions in genomes) is a reverse genetic approach which utilizes traditional mutagenesis to discover spontaneous mutation. It is helpful in generating an allelic series of genes for a particular trait of interest. TILLING has been exploited for many agronomical traits in many crop plants. Despite its exploitation for agronomic traits, some scientists (Menda et al. 2004; Talamè et al. 2008; Pigola et al. 2009; Fitzgerald et al. 2010) have used TILLING for disease resistance in tomato, barley and wheat. There is a plenty of scope of utilization of TILLING for the identification of genes involved in regulatory pathways of defence-related genes in oats. Till date, there are no reports available on utilization of TILLING in oats for disease resistance. Recently, CRISPR/Cas9, a new genome editing technology, is used worldwide among the plants as well as animals for different traits for their improvization. Once oat genomes become a reality, there would be ample opportunity for precise site-directed mutagenesis using CRISPR/Cas9. CRISPR/Cas9 system can also be utilized for defect elimination at specific position in oat genome which regulates pathogenesis genes and would be useful for correcting disease resistance in susceptible oat cultivars. Considering the above, delineating the complete genome sequence in oats would open up new vistas in disease resistance breeding and help accelerate ‘precision oat breeding’.

9.7 Conclusion

Oat genome sequencing would pave new pathways for breeders to develop a large number of sequence-based markers such as SNPs which will help in identifying the disease resistance genes through exploiting linkage disequilibrium mapping and genomic selection. Exploring new genome editing techniques would not only allow precise breeding but also provide a remarkable new opportunity for oat breeders. Integrating traditional breeding methodologies with modern genomics-assisted breeding to develop consensus linkage maps would open new vistas for the identification and precise mapping of major as well as minor genes/QTLs governing resistance against the economically important diseases. Meticulous planning and effective utilization of oat genetic resources would therefore provide ample scope for breeders to develop disease resistance cultivars in oats.

References

Acevedo M, Jackson EW, Chong J, Rines HW, Harrison S, Bonman JM (2010) Identification and validation of quantitative trait loci for partial resistance to crown rust in oat. Phytopthal 100:511–521

Ahmad M, Gul-Zaffer Dar ZA, Habib M (2014) A review on Oat (*Avena sativa* L.) as a dual-purpose crop. Sci Res Essays 9(4):52–59

Asoro FG, Newell MA, Scott MP, Beavis WD, Jannink JL (2013) Genome-wide association study for beta-glucan concentration inelite North American oat. Crop Sci 53:542–553. https://doi.org/10.2135/cropsci2012.01.0039

Babiker EM, Gordon TC, Jackson EW, Chao S, Harrison SA, Carson ML, Obert DE, Bonman JM (2015) Quantitative trait loci from two genotypes of oat (*Avena sativa*) conditioning resistance to *Puccinia coronata*. Phytopathology 105:239–245

Bai GH, Shaner G, Ohm H (19[illegible]) Effects of moist period on response of wheat cultivars to infection by *Fusarium graminearum*. (Abstract). Phytopathology 81:1145–1146

Boczkowska M, Podyma W, Lapinski B (2016) Oat. In: Singh M, Upadhyaya HD (eds) Genetic and genomic resources for cereal improvement, pp 159–225. https://doi.org/10.1016/B978-0-12-802000-5.00004-6

Boffetta P, Thun F, Kris-Etherton P (2014) Epidemiological studies of oats consumption and risk of cancer and overall mortality. Br J Nutr 112:S14–S18

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32(3):314–331

Browder RD, Forsberg RA, McKenzie RIH, Martens JW (1986) The use of disomic alien addition lines in the transfer of stem rust resistance to hexaploids oat. In: Lawes DA, Thomas H (eds) World crops production, utilization and description. Processings of second international oat conference. Martinus, Nijhoff Publishers, Dordrecht, pp 16–20

Burrows VD (1986) Tibor oat. *Can J Plant Sci* 66:403–405

Bush AL, Wise RP (1996) Crown rust resistance loci on linkage groups 4 and 13 in cultivated oat. J Hered 87:427–432

Bush AL, Wise RP (1998) High-resolution mapping adjacent to the *Pc71* crown-rust resistance locus in hexaploid oat. Mol Breed 4:13–21

Bush AL, Wise RP, Rayapati PJ, Lee M (1994) Restriction fragment length polymorphisms linked to genes for resistance to crown rust (*Puccinia coronata*) in near isogenic lines of hexaploid oat (*Avena sativa*). Genome 37:823–831

Cabral AL, Singh D, Park RF (2011) Identification and genetic characterisation of adult plant resistance to crown rust in diploid and tetraploid accessions of *Avena*. Ann Appl Biol 159:220–228

Carson M (2008) Virulence frequencies in oat crown rust in the United States from 2001 through 2005. Plant Dis 92:379–384

Carson ML (2009) Crown rust development and selection for virulence in *Puccinia coronata* f. sp. *avenae* in an oat multiline cultivar. Plant Dis 93:347–353

Chaffin AS, Huang YF, Smith S, Bekele WA, Babikar E, Gnanesh BN et al (2016) A consensus map in cultivated hexaploid oat reveals conserved grass synteny with substantial sub-genome rearrangement. Plant Genome 9. https://doi.org/10.3835/plantgenome2015.10.0102

Chang HC, Huang CN, Yeh DM, Wang SJ, Peng CH, Wang CJ (2013) Oat prevents obesity and abdominal fat distribution, and improves liver function in humans. Plant Foods Hum Nutr 68:18–23

Chen G, Chong J, Gray M, Prashar S, Procunier JD (2004) Single nucleotide polymorphisms as next generation markers for high throughput screening for crown rust resistance in oat. In: Peltonen-Saino P, Topi-Hulmi M (eds) Proc 7th international oat conference, Helsinki, p 86. www.mtt.fi/met/pdf/met51.pdf

Chen G, Chong J, Gray M, Prashar S, Procunier JD (2006a) Identification of single-nucleotide polymorphisms linked to resistance gene *Pc68* to crown rust in cultivated oat. Can J Plant Pathol 28:214–222

Chen J, He J, Wildman RP, Reynolds K, Streiffer RH, Whelton PK (2006b) A randomized controlled trial of dietary fiber intake on serum lipids. Eur J Clin Nutr 60:62–68

Chen G, Chong J, Prashar S, Procunier JD (2007) Discovery and genotyping of high-throughput SNP markers for crown rust resistance gene *Pc94* in cultivated oat. Plant Breed 126:379–384

Cheng DW, Armstrong KC, Tinker N, Wight CP, Hoard S, Lybaert A, Fedak G, Molnar SJ (2002) Genetic and physical mapping of *Lrk10*-like receptor kinase sequences in hexaploid oat (*Avena sativa* L.). Genome 45:100–109

Chong J, Howes NK, Brown PD, Harder DE (1994) Identification of the stem rust resistance gene *Pg9* and its association with crown rust resistance and endosperm proteins in 'Dumont' oat. Genome 37(3):440–447

Chong J, Leonard KJ, Salmeron JJ (2000) A North American system of nomenclature for *Puccinia coronata* f. sp. *avenae*. Plant Dis 84:580–585

Chong J, Reimer E, Somers DJ, Aung T, Penner GA (2004) Development of sequence-characterized amplified region (SCAR) markers for resistance gene *Pc94* to crown rust in oat. Can J Plant Pathol 26:89–96

Chong J, Gruenke J, Dueck R, Mayert W, Fetch JM, McCartney C (2011) Virulence of *Puccinia coronata* f. sp. *avenae* in the Eastern Prairie Region of Canada during 2007–2009. Can J Plant Pathol 33:77–87

Clifford BC (1995) Diseases, pests and disorders of oats. In: Welch RW (ed) The oat crop: production and utilization. Chapman & Hall, London, pp 252–278

Coffman FA (ed) (1961) Oats and oat improvement. American Society of Agronomy, Madison

Correns C (1900) G. Mendels Regel tiber das Verhalten der Nachkommenschaft der Rassenbastarde. Berdeutsch botan Gesellsch 18:158–168

de Vries H (1900) Das Spaltungsgesetz der Bastarde (Vorlaufige Mitteilung). Ber deutsch bot Ges 18:83–90

Dhillon BS, Chhuneja P (2014) New initiatives for precision plant breeding. In: National symposium on crop improvement for inclusive sustainable development, 7th–9th November, 2014. PAU, Ludhiana, pp 9–16

Egeberg R, Olsen A, Loft S, Christensen J, Johnsen NF, Overvad K, Tjønneland A (2010) Intake of whole grain products and risk of colorectal cancers in the diet, cancer and health cohort study. Br J Cancer 103:730–734

Ellis MB (1971) Dematiaceous hyphomycetes. Kew, CAB, 608 pp
FAO (2013) FAOSTAT database. Agricultural crops: wheat: area harvested/yield. URL http://faostat.fao.org/
FAO (2015) FAOSTAT database. Agricultural crops: wheat and oat: prices (USD/ton). URL http://faostat3.fao.org/
Fetch J, Fetch T (2011) Inheritance of resistance to oat stem rust in the cultivars Ronald and AC Gwen. Can J Plant Sci 91(2):419–423
Fetch TG Jr (2005) Races of *Puccinia graminis* on wheat, barley, and oat in Canada in 2002 and 2003. Can J Plant Pathol 27:572–580
Fitzgerald TL, Kazan K, Li Z, Morell MK, Manners JM (2010) A high-throughput method for the detection of homologous gene deletions in hexaploid wheat. BMC Plant Biol 10:264
Floor HH (1956) The complementary genetic systems in flax and flax rust. Adv Genet 8:29–54
Forsberg RA, Brinkman MA, Bunch RA, Duerst RD (1991a) Registration of Horicon oat. Crop Sci 31:1087–1088
Forsberg RA, Brinkman MA, Karow RS, Duerst RD (1991b) Registration of Centennial oat. Crop Sci 31:1087–1087
Frey KJ (1985) Genetic resources and their use in oats breeding. In: Proceedings of the 2nd international oats conference, 15–18 July 1985, Aberystwyth, pp 7–15
Frey KJ (1991) Genetic resources of oats. In: Shands HL, Wiesner L (eds) Use of plant introductions in cultivar development Part-I, CSSA Special Publication No. 17. Crop Science Society of America, Madison, pp 15–24
Frey KJ, Browning JA (1976a) Registration of multiline M72 and multiline M73 oat cultivars. Crop Sci 16:311
Frey KJ, Browning JA (1976b) Registration of multiline M72, multiline M73 and multiline M74 oat cultivars. Crop Sci 16:311–312
Frey KJ, Browning JA, Grindeland RL (1971a) Registration of multiline E68, multiline E69 and multiline E70 oat cultivars. Crop Sci 11:939–940
Frey KJ, Browning JA, Grindeland RL (1971b) Registration of multiline M68, multiline M69 and multiline M70 oat cultivars. Crop Sci 11:940–941
Frey KJ, Browning JA, Grindeland RL (1973) Registration of X117 oat germplasm. Crop Sci 13:290
Frey KJ, Browning JA, Simons MD (1985) Registration of multiline E76 and multiline E77 oats. Crop Sci 25:1125
Frey KJ, Simons MD, Michel L, Murphy JP, Browning JA (1988) Registration of Webster oat isolines as parental lines. Crop Sci 28:386–387
Gagkaeva T, Gavrilova O, Yli-Mattila T, Loskutov I (2011) Evaluation of oat germplasm for resistance to fusarium head blight. Plant Breed Seed Sci 64:15–22
Germeier CU (2008) Global strategy for the *ex situ* conservation for oats (*Avena* spp.). http://www.croptrust.org/documents/web/Oat-Strategy-DRAFT-07April08.pdf
Gnanesh BN, Fetch JM, Menzies JG, Beattie AD, Eckstein PE, McCartney CA (2013) Chromosome location and allele-specific PCR markers for marker-assisted selection of the oat crown rust resistance gene *Pc91*. Mol Breed:1–8. https://doi.org/10.1007/s11032-013-9900-6
Gnanesh BN, Fetch JM, Zegeye T, McCartney CA, Fetch T (2014) Oat. In: Pratap A, Kumar J (eds) Alien gene transfer in crop plants, vol 2. Springer, New York, pp 51–73
Gnanesh BN, McCartney CA, Eckstein PE, Fetch JWM, Menzies JG, Beattie AD (2015) Genetic analysis and molecular mapping of a seedling crown rust resistance gene in oat. Theor Appl Genet 128:247–258
Gorash A, Armoniene R, Fetch JM, Liatukas Ž, Danyte V (2017) Aspects in oat breeding: nutrition quality, nakedness and disease resistance, challenges and perspectives. Ann Appl Biol:1–22
Groh S, Zacharias A, Kianian SF, Penner GA, Chong J, Rines HW, Phillips RL (2001) Comparative AFLP mapping in two hexaploid oat populations. Theor Appl Genet 102:876–884
Gurung S, Mamidi S, Bonman JM, Xiong M, Brown-Guedira G, Adhikari TB (2014) Genome-wide association study reveals novel quantitative trait loci associated with resistance to

multiple leaf spot diseases of spring wheat. PLoS One 9:e108179. https://doi.org/10.1371/journal.pone.0108179
Handbook of Agriculture (2007) Forage crops and grasses. pp 1354–1357
Harder DE, Haber S (1992) Oat diseases and pathologic techniques. In: Marshall HG, Sorrells ME (eds) Oat science and technology. Crop Science Society of America, Madison, pp 307–402. [Agronomy Monograph, No. 33]
Harder DE, McKenzie RIH (1984) Complex additive-type of resistance to *Puccinia coronata* in *Avena sterilis*. Can J Plant Pathol 6:135–138
Harlan JR, de Wet JMJ (1971) Toward a rational classification of cultivated plants. Taxon 20:509–517
Harlan HY, Pope MN (1922) The use and value of backcrosses in small grain breeding. J Hered 13:319–322
Hasm SLK, Mohler V, Zeller FJ (2014) The genetics of resistance to powdery mildew in cultivated oats (*Avena sativa* L.): current status of major genes. J Appl Genet. https://doi.org/10.1007/s13353-014-0196-y
Ho HVT (2015) The effect of oat and barley *β*-glucan on LDL-C and emerging clinical lipid targets for cardiovascular disease. PhD Thesis, Toronto, Canada: University of Toronto
Hoffman DL, Chong J, Jackson EW, Obert DE (2006) Characterization and mapping of a crown rust resistance gene complex (*Pc58*) in TAM O-301. Crop Sci 46:2630–2635
Holland JB (1997) Oat improvement. In: Crop improvement for the 21st century. Journal paper no. J-16942 of the Iowa Agriculture and Home Economics Experiment Station, Ames. Project no.3368, pp 57–98
Hou Q, Li Y, Li L, Cheng G, Sun X, Li S, Tian H (2015) The metabolic effects of oats intake in patients with type 2 diabetes: a systematic review and meta-analysis. Nutrients 7:10369–10387
Howes NK, Chong J, Brown PD (1992) Oat endosperm proteins associated with resistance to stem rust of oats. Genome 35:120–125
Huang X, Wei X, Sang T, Zhao Q, Feng Q, Zhao Y, Li C, Zhu C, Lu T, Zhang Z, Li M, Fan D, Guo Y, Wang A, Wang L, Deng L, Li W, Lu Y, Weng Q, Liu K, Huang T, Zhou T, Jing Y, Li W, Lin Z, Buckler ES, Qian Q, Zhang Q, Li J, Han B (2010) Genome-wide association studies of 14 agronomic traits in rice landraces. Nat Genet 42:961–967. https://doi.org/10.1038/ng.695
Hunter H (1924) Oats: their varieties and characteristics. Ernest Benn, London
IPCC (2007) Climate change — the physical sciences basis: summary for policymakers. Intergovernmental Panel on Climate Change, Genebra 18
Irigoyen ML, Loarce Y, Fominaya A, Ferrer E (2004) Isolation and mapping of resistance gene analogs from the *Avena sterilis* genome. Theor Appl Genet 109:713–724
Irigoyen ML, Loarce Y, Fierro E, Fominaya A, Ferrer E (2006) Identification of resistance gene analogs as markers of disease resistance loci in oats, using near-isogenic lines. Plant Breed 125(4):347–351. https://doi.org/10.1111/j.1439-0523.2006.01258.x
Ivanoff SS (1933) The cause of spikelet drop of oats. Plant Dis Rep 3:206–207
Ivanova R (2006) Trends for the production of oats in the world, the European Union and Bulgeria. Maize the Agronomist. 25–26
Jackson EW, Obert DE, Menz M, Hu G, Avant JB, Chong J, Bonman JM (2007) Characterization and mapping of oat crown rust resistance genes using three assessment methods. Phytopathology 97(9):1063–1070
Jackson EW, Obert DE, Menz M, Hu G, Bonman JM (2008) Qualitative and quantitative trait loci conditioning resistance to *Puccinia coronata* pathotypes NQMG and LGCG in the oat (*Avena sativa* L.) cultivars Ogle and TAM O-301. Theor Appl Genet 116:517–527
Janseen NF (1952) Intra-varietal diversification in oat breeding. Agron J 44:30–34
Jia G, Huang X, Zhi H, Zhao Y, Zhao Q, Li W, Chai Y, Yang L, Liu K, Lu H, Zhu C, Lu Y, Zhou C, Fan D, Weng Q, Guo Y, Huang T, Zhang L, Lu T, Feng Q, Hao H, Liu H, Lu P, Zhang N, Li Y, Guo E, Wang S, Wang S, Liu J, Zhang W, Chen G, Zhang B, Li W, Wang Y, Li H, Zhao B, Li J, Diao X, Han B (2013) A haplotype map of genomic variations and genome-wide association studies of agronomic traits in foxtail millet (*Setaria italica*). Nat Genet 45:957–961. https://doi.org/10.1038/ng.2673

Jin H, Domier LL, Kolb FL, Brown CM (1998) Identification of quantitative loci for tolerance to barley yellow dwarf virus in oat. Phytopathology 88:410–415

Jin H, Domier LL, Kolb FL, Brown CM (1999) Conversion of AFLP markers associated with BYDV tolerance in oats to non-radioactive PCR markers. In: Plant and animal genome VII Conference, San Diego, p 396. www.intl-pag.org

Johannsen WL (1903) Ueber Erblichkeit in Population en und in reinen Leinen. Gustav Fischer, Jena

Kapoor R, Batra C (2016) Oats. In: Singh M, Kumar S (eds) Broadening the genetic base of grain cereals, pp 127–162. https://doi.org/10.1007/978-81-322-3613-9_6

Kianian SF, Egli MA, Phillips RL, Rines HW, Somers DA, Gengenbach BG, Webster FH, Livingston SM, Groh S, O'-Donoughue LS, Sorrells ME, Wesenberg DM, Stuthman DD, Fulcher RG (1999) Association of a major groat oil content QTL and an acetyl-CoA carboxylase gene in oat. Theor Appl Genet 98:884–894

Kianian SF, Phillips RL, Rines HW, Fulcher RG, Webster FH, Stuthman DD (2000) Quantitative trait loci influencing β-glucan content in oat (*Avena sativa*, 2n = 6x = 42). Theor Appl Genet 101:1049–1055

Klos KE, Yimer BA, Babikar EM, Beattie AD, Bonman JM, Carson ML, Chong J, Harrison SA, Ibrahim AMH, Kolb FL, McCartney CA, McMullen M, Fetch JM, Mohammadi M, Murphy JP, Tinker NA (2017) Genome-wide association mapping of crown rust resistance in oat elite germplasm. Plant Genome 10(2). https://doi.org/10.3835/plantgenome2016.10.0107

Kremer CA, Lee M, Holland JB (2001) A restriction fragment length polymorphism based linkage map of a diploid *Avena* recombinant inbred line population. Genome 44:192–204

Kulcheski FR, Graichen FAS, Martinelli JA, Locatelli AB, Federizzi LC, Delatorre CA (2010) Molecular mapping of *Pc68*, a crown rust resistance gene in *Avena sativa*. Euphytica 175:423–432

Kump KL, Bradbury PJ, Wisser RJ, Buckler ES, Belcher AR, Oropeza-Rosas MA, Zwonitzer JC, Kresovich S, McMullen MD, Ware D, Balint-Kurti PJ, Holland JB (2011) Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. Nat Genet 43:163–168. https://doi.org/10.1038/ng.747

Ladizinsky G (1998) A new species of *Avena* from Sicily, possible the tetraploid progenitor of hexaploid oats. Genet Resour Crop Evol 45:263–269

Ladizinsky G, Zohary D (1971) Notes on species delimitation, species relationships and polyploidy in *Avena* L. Euphytica 20:380–395

Landry B, Comeau A, Minvielle F, St-Pierre CA (1984) Genetic analysis of resistance to barley yellow dwarf virus in hybrids between *Avena sativa* Lamer and virus resistant lines of *Avena sterilis*. Crop Sci 24:337–340

Lawes DA, Hayes JD (1965) The effect of mildew (*Erysiphe graminis* f.sp. *avenae*) on spring oats. Plant Pathol 14:125–128

Lawson P, Son (1852) Synopsis of the vegetable products of Scotland in the museum of the Royal Botanic Gardens of Kew. Cereal Grains - *Avena* or oat. Private Press of Peter Lawson and Son, pp 92–95

Leggett JM, Thomas H (1995) Oat evolution and cytogenetics. In: Welch W (ed) The oat crop: production and utilization. Chapman & Hall, London, pp 121–149

Li Q, Peng Z, Yang X, Wang W, Fu J, Wang J et al (2013) Genome-wide association study dissects the genetic architecture of oil biosynthesis in maize kernels. Nat Genet 45:43–50. https://doi.org/10.1038/ng.2484

Lin Y, Gnanesh BN, Chong J, Chen G, Beattie AD, Fetch JWM, Kutcher HR, Eckstein PE, Menzies JG, Jackson EW, McCartney CA (2014) A major quantitative trait locus conferring adult plant partial resistance to crown rust in oat. BMC Plant Biol 14:250

Linnaeus C (1753) A facsimile of the first edition. In: Species plantarum, vol 1, London, pp 1957–1959

Loarce Y, Sanz MJ, Irigoyen ML, Fominaya A, Ferrer E (2009) Mapping of STS markers obtained from oat resistance gene analog sequences. Genome 52:608–619

Loskutov I, Rines H (2011) Avena. In: Kole C (ed) Wild crop relatives: genomic and breeding resources. Springer, Berlin/Heidelberg, pp 109–183
Love HH (1927) A program for selecting and testing small grains in successive generations following hybridization. J Am Soc Agron 19:705–712
Marshall HG, Shaner GE (1992) Genetics and inheritance in oat. In: Marshall HG, Sorrells ME (eds) Oat science and technology, agronomy monograph number 33. American Society of Agronomy and Crop Science Society of America, Madison, pp 509–571
Marshall HG, Sorrells ME (1992) Oat science and technology, Agron Monogr 33. ASA and CSSA, Madison, WI
Martens JW, McKenzie RIH, Harder DE (1980) Resistance to *Puccinia graminis avenae* and *P. coronata avenae* in the wild and cultivated *Avena* populations of Iran, Iraq and Turkey. Can J Genet Cytol 22:641–649
Martens JW, Rothman PG, McKenzie RIH, Brown PD (1981) Evidence for complementary gene action conferring resistance to *Puccinia graminis avenae* in *Avena sativa*. Can J Genet Cytol 23:591–595
Martens JW, Seaman WL, Atkinson TG (1985) Diseases of field crops. In: Canada: an illustrated compendium. Canadian Phytopathological Society, Harrow, 160 pp
Mathews REF (1982) Classification and nomenclature of plant viruses. Intervirology 17(1–3):76–91
Mathias-Ramwell M, Salvo-Garrido H, Reyes-Rebolledo M, Montenegro I, Barriga A (2016) Júpiter-INIA: a new oat variety with improved β-glucan and protein contents. Chilean J Agric Sci 76(4):401–408
Mattsson B (1988) The development of oat germplasm at Svalöv. In: Mattsson B, Lyhagen L (eds) Proceedings of 3rd international oat conference, 4–8 July 1988, Lund, pp 35–38
McCallum BD, Fetch T, Chong J (2007) Cereal rust control in Canada. Aus J Agric Res 58:639–647
Mccartney CA, Stonehouse RG, Roseenagel BG, Eckstein PE, Scoles GJ, Zatorski T, Beattie AD, Chong J (2011) Mapping of the oat crown rust resistance gene *Pc91*. Theor Appl Genet 122:317–325
McDaniel ME (1974a) Registration of TAM 0-301 oats. Crop Sci 14:127–128
McDaniel ME (1974b) Registration of TAM 0-312 oats. Crop Sci 14:128
McKenzie RIH, Martens JW, Brown PD, Harder DE, Nielsen J, Boughton GR (1981) Registration of Fidler oats. Crop Sci 21:632–63
McKenzie RIH, Brown PD, Harder DE, Chong J, Nielsen J, Gill CC, Boughton GR (1984) Registration of Dumont oats. Crop Sci 24:207
McKenzie RIH, Brown PD, Harder DE, Chong J, Nielsen J, Haber S, Martens JW, Noll JS, Boughton GR (1986) Registration of Riel oat. Crop Sci 26:1256
McMullen MS, Patterson FL (1992) Oat cultivar development in the U.S.A. and Canada. In: Sorrells ME, Marshall HG (eds) Oat science and technology. Agronomy monograph No. 33. ASA, CSSA, SSSA, Madison, pp 573–612
Mellen PB, Walsh TF, Herrington DM (2008) Whole grain intake and cardiovascular disease: a meta-analysis. Nutr Metab Cardiovasc Dis 18:283–290
Mendel N, Semel Y, Peled D, Eshed Y, Zamir D (2004) *In silico* screening of a saturated mutation library of tomato. Plant J 38:861–872
Mer[illegible] P, Gonzalez T, Ferruzzi M, Jackson E, Winder D, Watson J (2016) Oats – from farm to fork. Adv Food Nutr Res 77:1–55
Mesterhazy A (1987) Selection of head blight resistant wheat though improved seedling resistance. Plant Breed 98:23–36
Montilla-Bascón G, Rispail N, Sánchez-Martín J, Rubiales D, Mur LAJ, Langdon T, Howarth CJ, Prats E (2015) Genome-wide association study for crown rust (*Puccinia coronata* f. sp. *avenae*) and powdery mildew (*Blumeria graminis* f. sp. *avenae*) resistance in an oat (*Avena sativa*) collection of commercial varieties and landraces. Front Plant Sci 6:103
Morris GP, Ramub P, Deshpandeb SP, Hashc CT, Shah T, Upadhyaya HD, Riera-Lizarazu O, Brown JP, Acharya CB, Mitchell SE, Harriman J, Glaubitz JC, Buckler ES, Kresovich S (2013) Population genomic and genome-wide association studies of agroclimatic traits in sorghum. Proc Natl Acad Sci U S A 110:453–458. https://doi.org/10.1073/pnas.1215985110

Murphy JP, Hoffman LA (1992) The origin, history, and production of oat. In: Marshall HG, Sorrells ME (eds) Oat science and technology. American Society of Agronomy, Madison, pp 1–28

Nevo E, Golenberg E, Beilies A, Brown AHD, Zohary D (1982) Genetic diversity and environmental association of wild wheat, *Triticum diococcoides* in Israel. Theor Appl Genet 62:241–254

Newell MA, Asoro FG, Scott MP, White PJ, Beavis WD, Jannink JL (2012) Genome-wide association study for oat (*Avena sativa* L.) beta-glucan concentration using germplasm of worldwide origin. Theor Appl Genet 125:1687–1696. https://doi.org/10.1007/s00122-012-1945-0

Newman LH (1912) Plant breeding in Scandinavia. Canadian Seed Growers, Ottawa

Norton JB (1907) Notes on breeding oats. Proceedings American Breeders' Association 3:280–285

Nwachukwu ID, Devassy JG, Aluko RE, Jones PJH (2015) Cholesterol-lowering properties of oat *β*-glucan and the promotion of cardiovascular health: did Health Canada make the right call? Appl Physiol Nutr Metabol 40:535–542

O'Donoughue LS, Wang Z, Röder M, Kneen B, Leggett M, Sorrells ME, Tanksley SD (1992) An RFLP-based linkage map of oats based on a cross between two diploid taxa (*Avena atlantica* × *A. hirtula*). Genome 35:765–771

O'Donoughue LS, Kianian SF, Rayapati PJ, Penner GA, Sorrells ME, Tanksley SD, Phillips RL, Rines HW, Lee M, Fedak G, Molnar SJ, Hoffman D, Salas CA, Wu B, Dupique E, VanDeynze A (1995) A molecular linkage map of cultivated oat. Genome 38:368–38

O'Donoughue LS, Chong J, Wight CP, Fedak G, Molnar SJ (1996) Location of stem rust resistance genes and associated molecular markers in cultivated oat. Phytopathology 86:719–727

Oat Breeding Newsletter (October, 2018) pp 1–53. [Online; available: http://pir.sa.gov.au/__data/assets/pdf_file/0018/334332/SARDI_Oat_Newsletter_2018.pdf]

Oerke EC (2006) Crop losses to pests. J Agric Sci 144:31–43

Oerke EC, Dehne HW, Schönbeck F, Weber A (1994) Crop production and crop protection. Estimated losses in major foodand cash crops. Elsevier, Amsterdam

Ohm HW, Shaner G, Buechley G, Aldridge W, Bockwick DE, Ratcliffe RH (1995) Registration of IN09201 spring oat. Crop Sci 35:940

Okon SM (2015) Effectiveness of resistance genes to powdery mildew in oat. Crop Prot 74:48–50. https://doi.org/10.1016/j.cropro.2015.04.004

Okon SM, Ociepa T (2017) Virulence structure of the *Blumeria graminis* DC. f. sp. *avenae* populations occurring in Poland across 2010–2013. Eur J Plant Pathol 149:1–8. https://doi.org/10.1007/s10658-017-1220-y

Okon SM, Ociepa T (2018) Effectiveness of new sources of resistance against oat powdery mildew identified in *A. sterilis*. Plant Dis Prot 125:505–510

Okon SM, da Paczos-Grzeda E, Ociepa T et al (2016) *Avena sterilis* L. Genotypes as a potential source of resistance to oat powdery mildew. Plant Dis 100:2145–2151. https://doi.org/10.1094/PDIS-11-15-1300-RE

Okon S, Ociepa T, Nucia A (2018) Molecular identification of *Pm4* powdery mildew resistant gene in oat. Not Bot Horti Agrobo 46(2):350–355

Pal N, Rathjen JS, Domier LL, Kolb FL (2002) Development and characterization of microsatellite and RAPD-derived PCR markers in oat. Crop Sci 42:912–918

Penner GA, Bush A, Wise R, Kim W, Dormier L, Kasha K, Laroche A, Scoles G, Molnar SJ, Fedak G (1993a) Reproducibility of random amplified polymorphic DNA (RAPD) analysis among laboratories. PCR Methods Appl 2:341–345

Penner GA, Chong J, Levesque-Lemay M, Molnar SJ, Fedak G (1993b) Identification of a RAPD marker linked to the oat stem rust gene *Pg3*. Theor Appl Genet 85:702–705

Penner GA, Chong J, Wight CP, Molnar SJ, Fedak G (1993c) Identification of an RAPD marker for the crown rust resistance gene *Pc68* in oats. Genome 36:818–820

Peterson DM (2001) Oat antioxidants. J Cereal Sci 33:115–129

Peturson B (1944) Adult plant resistance of some oat varieties to physiologic races of crown rust. Can J Res Sci 22:287–289

Poehlman JM, Kingsolver CH (1950) Disease reaction and agronomic qualities of oats selections from a Columbia × Victoria-Richland cross. Agron J 42:498–502

Portyanko VA, Chen G, Rines HW, Phillips RL, Leonard KJ, Ochocki GE, Stuthman DD (2005) Quantitative trait loci for partial resistance to crown rust, *Puccinia coronata*, in cultivated oat, *Avena sativa* L. Theor Appl Genet 111:313–324

Priebe MG, van Binsbergen JJ, de Vos R, Vonk RJ (2008) Whole grain foods for the prevention of type 2 *diabetes mellitus*. Cochrane Database Syst Rev 1. https://doi.org/10.1002/14651858.CD006061.pub2

Rajhathy T, Thomas H (1974) Cytogenetics of oats, vol 2. (Miscellaneous Publication) Genetics Society of Canada, Ottawa, pp 1–90

Rasane P, Jha A, Sabikhi L, Kumar A, Unnikrishnan VS (2015) Nutritional advantages of oats and opportunities for its processing as value added foods – a review. J Food Sci Technol 52:662–675

Rayapati PJ, Gregory JW, Lee M, Wise RP (1994) A linkage map of diploid *Avena* based on RFLP loci and a locus conferring resistance to nine isolates of *Puccinia coronata* var. *avenae*. Theor Appl Genet 89:831–837

Reinbergs E (1983) OAC Woodstock oats. Can J Plant Sci 63:543–544

Rigola D, van Oeveren J, Janssen A, Bonne A, Schneiders H, van der Poel HJ, van Orsouw NJ, Hogers RC, de Both MT, van Eijk MJ (2009) High throughput detection of induced mutations and natural variation using KeyPoint technology. PLoS One 4:e4761

Rines HW, Molnar SJ, Tinker NA, Phillips RL (2006) Oat. In: Kole C (ed) Genome mapping and molecular breeding in plants, Volume 1 Cereals and Millets. Springer, New York, pp 211–242

Rines HW, Miller ME, Carson M, Chao S, Tiede T, Wiersma J, Kianian SF (2017) Identification, introgression, and molecular marker genetic analysis and selection of a highly effective novel oat crown rust resistance from diploid oat, *Avena strigosa*. Theor Appl Genet. https://doi.org/10.1007/s00122-017-3031-0

Ronald PS, Penner GA, Brown PD, Brule-Babel A (1997) Identification of RAPD markers for percent hull in oat. Genome 40:873–878

Rooney WL, Rines HW, Phillips RL (1994) Identification of RFLP markers linked to crown rust resistance genes *Pc 91* and *Pc 92* in oat. Crop Sci 34:940–944

Rose O (1903) Der F1ugbrand der Sommergetreidearten und Massnahmen zur Bekampfung dieses Pilzes in der landwirtschaftIichen Praxis. Inaugural Diss, Rostock

Rothman PG (1976) Registration of oat germplasm. Crop Sci 16:315

Rothman PG (1984) Registration of four stem rust and crown rust resistant oat germplasm lines. Crop Sci 24:1217–1218

Rothman PG (1986) Adequate rust resistance in oats. In: Lawes DA, Thomas H (eds) World crops: production, utilization and description. In: Proceedings of second international oat conference. Martinus, Nijhoff Publishers, Dordrecht, pp 72–76

Rubiales D, Niks RE (2000) Combination of mechanisms of resistance to rust fungi as a strategy to increase durability. Options Méditérr 40:333–339

Sanz MJ, Lource Y, Fominaya A, Vossen JH, Ferrer E (2012) Identification of RFLP and NBS/PK profiling markers for disease resistance loci in genetic maps of oats. Theor Appl Genet 126:203–218

Sathe Ashok S, Sharp PJ, Lagudah ES, McIntosh RA, Molnar SJ (2011) Genetic association of crown rust resistance gene *Pc68*, storage protein loci, and resistance gene analogues in oats. Genome 54:484–497

Schroeder HW, Christensen JJ (1963) Factors affecting resistance of wheat to scab caused by Gibberella zeae. Phytopathology 53:831–838

Schuster J, Beninca G, Vitorazzi R, Bosco SMD (2015) Effects of oats on lipid profile, insulin resistance and weight loss. Nutr Hosp 32:2111–2116

Sebesta J, Kuhn F (1990) *Avena fatua* L. subsp. *fatua* v. *glabrata* Peterm. subv. *Pseudo basifixa* Thele. as a source of crown rust resistance genes. Euphytica 50:51–55

Sebesta J, Harder DE, Jones IT, Kummer M, Clifford BC, Zwatz B (1986) Pathogenicity of crown rust, stem rust and powdery mildew on oats in Europe and sources of resistance. In: Lawes DA, Thomas H (eds) World crops: production, utilization and description. Processings of second international oat conference. Martinus, Nijhoff Publishers, Dordrecht, pp 67–71

Sharma DC, Forsberg RA (1977) Spontaneous and induced interspecific gene transfer for crown rust resistance in *Avena*. Crop Sci 17:855–860
Shebini El SM, Moaty MI, Tapozada ST, Ahmed NH, Mohamed MS, Hanna LM (2014) Effect of whole wheat (*Triticum aestivum*) and oat (*Avena sativa*) supplements on body weight, insulin resistance and circulating omentin in obese women exhibiting metabolic syndrome criteria. World J Med Sci 11:373–338
Sheppherd JH (1896) Grain and forage crops. North Dakota Agric Exp Station Bull 23:31–54
Shirreff P (1873) Improvement of the cereals. Cereals: 1–26. William Blackwood and Sons, Edinburgh
Simons MD (1985) Crown rust. In: Rolfs AP, Bushnell WR (eds) The Cereal rusts: diseases, distribution, epidemiology, and control. Academic Press, New York, pp 131–172
Simons MD, Michel LJ, Frey KJ (1987) Registration of three oat germplasm lines resistant to the crown rust fungus. Crop Sci 27:369
Siripoonwiwat W, O'Donoughue LS, Wesenberg D, Hoffman DL, Barbosa-Neto JF, Sorrells ME (1996) Chromosomal regions associated with quantitative traits in oat. J Agric Genome. http://www.cabi-publishing.org/gateways/jag/index.html
Snowdon RJ, Friedt W (2004) Molecular markers in Brassica oilseed breeding: current status and future possibilities. Plant Breed 123:1–8. https://doi.org/10.1111/j.1439-0523.2003.00968.x
Stakman EC, Harrar JG (1957) Principles of plant pathology. Ronald Press, New York
Stevens NE, Scott WO (1950) How long will present spring oat varieties last in the central corn belt? Agron J 42:307–309
Stewart D, McDougall G (2014) Oat agriculture, cultivation and breeding targets: implications for human nutrition and health. Br J Nutr 112:S50–S57
Strange RN, Scott PR (2005) Plant disease: a threat to global food security. Annu Rev Phytopathol 43:83–116
Stuthman DD, Wilcoxson RD, Rines HW (1990) Registration of Starter oat. Crop Sci 30:1365–1366
Stuthman DD, Stage J, Rines H, McVey D (1995) Mention. Oat Newsletter 43:74
Talamè V, Bovina R, Sanguineti MC, Tuberosa R, Lundqvist U, Salvi S (2008) TILLMore, a resource for the discovery of chemically induced mutants in barley. Plant Biotechnol J 6:477–485
Tapola N, Karvonen H, Niskanen L, Mikola M, Sarkkinen E (2005) Glycemic responses of oat bran products in type 2 diabetic patients. Nutr Metab Cardiovasc Dis 15:255–261
Teng PS (ed) (1987) Crop loss assessment and pest management. APS Press, St Paul
Teng PS, Krupa SV (eds) (1980) Assessment of losses which constrain production and crop improvement in agriculture and forestry. In: Proceedings of the E. C. Stackman Commemorative Symposium. St. Paul: University of Minnesota
Thies F, Masson LF, Boffetta P, Kris-Etherton P (2014) Oats and CVD risk markers: a systematic literature review. Br J Nutr 112:S19–S30
Thomas H, Powell W, Aung T (1980) Interfering with regular meiotic behavior in *Avena sativa* as a method for incorporating the gene for mildew resistance from *Avena barbata*. Euphytica 29:633–640
Tschermak E (1900) Ober kiinstliche Kreuzung bei *Pisum sativum. Ber.deutsch.botan*. Gesellsch 18:232–239
Van den Broeck HC, Londono DM, Timmer R, Smulders MJM, Gilissen LJWJ, Vander Meer IM (2016) Profiling of nutritional and health-related compounds in oat varieties. FoodReview 5:1–11
Vander Plank JE (1963) Plant diseases: epidemics and control. Academic Press, New York
Wallwork H (1992) Cereal leaf and stem diseases. Grains Research and Development Corporation, Barton, 102p
Wang C, Yang Y, Yuan X, Xu Q, Feng Y, Yu H et al (2014) Genome-wide association study of blast resistance in *indica* rice. BMC Plant Biol 14:311. https://doi.org/10.1186/s12870-014-0311-6
Warren HL, Kommedahl T (1973) Fertilization and wheat refuse affects on Fusarium species associated with wheat roots in Minnesota. Phytopathology 63:103–108

Watkins JE, Lane LC (2004) Barley yellow dwarf disease of barley, oats, and wheat. In: NebGuide. University of Nebraska. http://ianrpubs.unl.edu/plantdisease/g906.htm

Watson IA (1970) The utilization of wild species in the breeding of cultivated crops resistant to plant pathogens. In: Frankel OH, Bennett E (eds) Genetic resources in plants: their exploitation and conservation. IBP Handbook No. 11. Oxford, Blackwell, pp 441–457

Wiese MV (1987) Scab (head blight). In: Compendium of wheat diseases, 2nd edn. American Phytopathological Society, St. Paul, pp 16–18

Wight CP, O'Donoughue LS, Chong J, Tinker NA, Molnar SJ (2004) Discovery, localization, and sequence characterization of molecular markers for the crown rust resistance gene *Pc38*, *Pc39*, and *Pc48* in cultivated oat (*Avena sativa* L.). Mol Breed 14:349–361

Winkler LR, Bonman JM, Chao S, Yimer BA, Bockelman H, Esvelt Klos K (2016) Population structure and genotype–phenotype associations in a collection of oat landraces and historic cultivars. Front Plant Sci. https://doi.org/10.3389/fpls.2016.01077

Wise RP, Lee M, Rayapati PJ (1996) Recombination within a 5-centimorgan region in diploid *Avena* reveals multiple specificities conferring resistance to *Puccinia coronata*. Phytopathology 86:340–346

Xue LH, Li CJ, Zhao GQ (2017) First report of powdery mildew caused by *Blumeria graminis* on *Avena sativa* in China. Plant Dis 101:1954. https://doi.org/10.1094/PDIS-05-17-0678-PDN

Yu J, Herrmann M (2006) Inheritance and mapping of a powdery mildew resistance gene introgressed from *Avena macrostachya* in cultivated oat. Theor Appl Genet 113:429–437

Yu GX, Wise RP (2000) An anchored AFLP- and retrotransposon-based map of diploid *Avena*. Genome 43:736–749

Zdunczyk Z, Flis M, Zielinski H, Wroblewska M, Antoszkiewicz Z, Juskiewicz J (2006) *In vitro* antioxidant activities of barley, husked oat, naked oat, triticale and buckwheat wastes and their influence on the growth and biomarkers of antioxidant status in rats. J Agric Food Chem 54:4168–4175

Zegeye T (2008) Stem rust resistance in *Avena strigosa* Schreb.: inheritance, gene transfer, and identification of an amplified fragment length polymorphism (AFLP) marker. Ph. D. Thesis, University of Manitob

Zhang X, McGeoch SC, Megson IL, Mackay SM, Johnstone AM, Abraham P, Pearson DWM, deRoos B, Holtrop G, O'Kennedy N, Lobley GE (2014) Oat-enriched diet reduces inflammatory status assessed by circulating cell-derived microparticle concentrations in type 2 diabetes. Mol Nutr Food Res 58:1322–1332

Zhu S, Kaeppler HF (2003a) A genetic linkage map for hexaploid, cultivated oat (*Avena sativa* L.) based on an intraspecific cross 'Ogle/MAM17-5'. Theor Appl Genet 107:26–35

Zhu S, Kaeppler HF (2003b) Identification of quantitative trait loci for resistance to crown rust in oat line MAM17-5. Crop Sci 43:358–366

Zillinsky FJ (1983) Common diseases of small grain cereals: a guide to identification. CIMMYT, Mexico, D.F. 141p

Chapter 10
Charcoal Rot Resistance in Soybean: Current Understanding and Future Perspectives

Vennampally Nataraj, Sanjeev Kumar, Giriraj Kumawat, M. Shivakumar, Laxman Singh Rajput, Milind B. Ratnaparkhe, Rajkumar Ramteke, Sanjay Gupta, Gyanesh K. Satpute, Vangala Rajesh, Viraj Kamble, and Subhash Chandra

10.1 Introduction

Soybean (*Glycine max* L.) is the foremost source of protein (40%) and oil (20%) (Talukdar et al. 2009). It is a multipurpose crop having been used for human consumption, protein feed ingredient and industrial applications. Soybean production is challenged by various forms of biotic and abiotic stresses. Charcoal rot is the second most economical disease of soybean after brown spot (*Septoria glycines*) (Wrather et al. 2001).

Soybean charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid. is an economically significant disease throughout the world. In addition to soybean, this pathogen has a wide range of hostage including some economical crops like sorghum (*Sorghum bicolor* (L.) Moench) and maize (*Zea mays,* L.) (Mengistu et al. 2007). Charcoal rot disease in soybeans was first observed in the United States in 1949 (Young 1949). Severity of the disease increases with the increase in soil and air temperature (28–35 °C) (Mengistu et al. 2014). Under limited soil moisture conditions, synergistic yield losses occur due to both environmental stress and charcoal rot disease (Mengistu et al. 2011a). Confounding effects of drought make it difficult to estimate the yield loss per se by charcoal rot disease incidence. Under irrigated conditions, about 6–33% yield loss in susceptible cultivars is attributed to charcoal rot disease, indicating the importance of the disease even under irrigated conditions (Mengistu et al. 2011b, 2018).

Under field condition, infection is carried by microsclerotia present in the soil or through conidia present on infected plant tissue or debris to the host through rain splashes (Dhingra and Sinclair 1978). A reddish-brown discolouration at the emerging portion of the hypocotyls may be seen in the infected seedlings (Smith and

V. Nataraj (✉) · S. Kumar · G. Kumawat · M. Shivakumar · L. S. Rajput · M. B. Ratnaparkhe
R. Ramteke · S. Gupta · G. K. Satpute · V. Rajesh · V. Kamble · S. Chandra
ICAR- Indian Institute of Soybean Research, Indore, MP, India

S. H. Wani (ed.), *Disease Resistance in Crop Plants*,
https://doi.org/10.1007/978-3-030-20728-1_10

Wyllie 1999). Root infection is evident through discolouration at the soil line and above (Smith and Wyllie 1999). Under hot and dry conditions, discoloured area turns dark brown to black and the infected seedlings may die. Under wet and cool conditions, infected seedlings survive and carry latent infection through the reproductive stages (Smith and Wyllie 1999). Infection often starts with the biotrophic phase with no visible symptoms and changes in environmental conditions, and plant stress and maturation can promote necrotic phase of infection (Twizeyimana et al. 2012). This phase is evident from visible symptoms such as yellowing, wilting and flagging of leaves due to blockage of water and nutrient transportation to the leaves and shoots by mechanical plugging of root vascular tissue by the fungus and necrosis caused by phytotoxins and enzymatic action (Smith and Wyllie 1999; Luna et al. 2017). A light grey or silvery discolouration develops on epidermal and subepidermal tissues of the taproot and lower part of the stem after flowering. Development of microsclerotia becomes so numerous that it gives a greyish-black colour to the stem pith tissues resembling a sprinkling of finely powdered charcoal (Smith and Wyllie 1999). Reddish-brown discolouration of the taproot vascular tissues is evident which later progresses to the vascular and pith tissues of the stem. Black streaks in the woody portion of the crown are observed in the split open taproot (Smith and Wyllie 1999).

10.2 Screening of Soybean Germplasm for Charcoal Rot Resistance

Host plant resistance is the only feasible method to prevent soybean yield losses by charcoal rot disease (Smith and Carvil 1997; Smith and Wyllie 1999; Silva et al. 2019). Lack of reliable, repeatable and efficient screening systems against charcoal rot is hindering the progress towards development of resistant soybean varieties (Mengistu et al. 2007).

There are six screening methods mainly used for soybean charcoal rot disease assessment: colony-forming unit index (CFUI); root stem severity (RSS); percent height of stem discolouration (PHSD); foliar symptoms (FS) taken at R7 stage; foliar symptoms taken at R1, R3, R5 and R7 and calculating the AUDPC; and cut-stem inoculation method (Mengistu et al. 2007; Twizeyimana et al. 2012).

Briefly, in the CFUI method, the lower stem and root portion of genotypes under study are excised just below the cotyledonary node at R7 stage. Ten such samples were taken from each plot. Thoroughly washed samples were ground and each 0.005 g ground sample was treated with 100 mL of 0.525% NaOCl for 3 min. The triturate was washed thoroughly with sterile distilled water and transferred to a 100 ml autoclaved selective media containing PDA, rifampicin (100 mg L^{-1}) and Tergitol (0.1 mL). Three days after incubation, CFU were counted and CFU per gram was estimated. A CFUI was developed for each genotype by dividing the CFU of the genotype with the CFU of the genotype producing the highest CFU (Mengistu et al. 2007). The genotypes were then classified in percentage based on this CFUI as

resistant (0 to <10), moderately resistant (10 to ≤30), moderately susceptible (>30 to 60) and susceptible (>60) (Schmitt and Shannon 1992; Mengistu et al. 2007).

Disease severity estimation by root stem severity (RSS) is done at R7 stage (Fehr et al. 1971) using the scale (1–5) established by Paris et al. (2006): 1 = no discolouration and no microsclerotia visible; 2 = no discolouration of vascular tissue, with very few microsclerotia visible in the pith, vascular tissue, or under the epidermis; 3 = partially discoloured vascular tissue, with microsclerotia partially covering the tissue; 4 = discoloured vascular tissue, with numerous microsclerotia visible in the tissue under the outer epidermis, in stem and in root sections; and 5 = vascular tissue with numerous microsclerotia producing a dark colour inside and outside of the stem and root tissue.

Percent height of stem discolouration (PHSD) is based on microsclerotial stem discolouration at R7 stage. Length of internal vascular necrosis above the ground level divided by plant height × 100 is percent height of vascular discolouration due to charcoal rot (Mengistu et al. 2007). Foliar symptoms (FS) at R7 stage for disease estimation is done by using Horsfall-Barratt scale (James 1974). The symptom is generally necrosis of soybean leaves at R7 stage (Mengistu et al. 2007). On the scale of 0 to 11 used for FS, 0 = no symptoms; 1 = 0 to 3%, 2 = 3 to 6%, 3 = 6 to 12%, 4 = 12 to 25%, 5 = 25 to 50%, 6 = 50 to 75%, 7 = 75 to 87%, 8 = 87 to 94%, 9 = 94 to 97%, 10 = 97 to 100% and 11 = 100%. Using this scale, the genotypes were classified into four categories: resistant (zero), moderately resistant (>0 and <5), moderately susceptible (≥5 and <8) and susceptible (≥8). Foliar symptoms taken at R1, R3, R5 and R7 were used to calculate the AUDP. In this method, foliar symptoms were recorded on weekly basis from the beginning of the foliar symptoms up to R7 stage (Mengistu et al. 2007). The percentage of affected plants in each plot and the infection intensity was rated and the foliar symptoms over time were used to calculate AUDPC (Tooley and Grau 1984; Mengistu et al. 2007).

In brief, in the cut-stem inoculation technique (Twizeyimana et al. 2012), the soybean plants were grown to V2 stage (Fehr et al. 1971) and a sharp laser blade was used to cut the stem 25 mm (or 40 mm, Coser et al. 2017) above the unifoliate node. The open end of a 10–200 μL pipette tip was pushed into actively growing margins of fungal culture growing on PDA medium, and a circular disk of mycelia plug along with agar was obtained. The fungal mycelium was immediately placed on open end cut stem and pressed to ensure the mycelia is embedded into the stem. Disease ratings are based on the length of stem necrosis. Measurements are recorded 3 days after inoculation and followed for every 3 days until 13–15 days after inoculation. The linear stem necrosis measured over time was used to calculate AUDPC.

Colony-forming unit index (CFUI) has been reported to be a reliable method of rating host compatibility between soybean genotypes and *M. phaseolina* (Paris et al. 2006; Mengistu et al. 2007; Smith and Carvil 1997). Except for CFUI, there was significant genotype by year interaction for other disease assessment methods such as root and stem severity (RSS), percent height of internal stem discolouration (PHSD) and foliar symptoms (FS) (Mengistu et al. 2007). Though it is time consuming, the CFUI method of disease assessment is considered to be the good measure of disease resistance across environments and is recommended when more

accurate and precise classification across the genotypes is needed for genetics of host plant resistance (Schmitt and Shannon 1992; Mengistu et al. 2007). Owing to its rapidity, compromising the accuracy, the root and stem severity (RSS) method of disease assessment is considered to be suitable for breeding programs where there is a need to screen a large number of breeding lines against *M. phaseolina* (Mengistu et al. 2007).

Disease incidence measured by CFUI and RSS is based on extent of colonisation by the fungus. However, since severity of charcoal rot incidence is much influenced on environmental factors like temperature and rainfall and plant maturity, they must be taken into consideration while interpreting field studies to screen soybean genotypes of different maturity groups for their resistance to charcoal rot (Pawlowski et al. 2015). Genotypes can be better screened for resistance at specific growth stages than at specific times after sowing (Pearson et al. 1984). Disease progress is slow throughout the vegetative and reproductive growth stages and is more at R7 stage since the population density of *M. phaseolina* is increased rapidly from R6 to R7 stage (Mengistu et al. 2011b; Mengistu et al. 2018). Dry matter accumulation is ceased at R7 stage (Ritchie et al. 1989), and at R8 stage saprophytic action of the fungus negates the differences among the genotypic reactions to it (Mengistu et al. 2018). Therefore, R7 stage is critical for identification of resistant sources for charcoal rot (Mengistu et al. 2018).

Till date, most of the studies on evaluating resistance to *M. phaseolina* are based on field screening by either inoculating the field plots or relying on the disease incidence history of the field. Variability among soil characteristics, soil microflora and their interaction with *M. phaseolina* and other genotypes by environment interactions may result in inconsistent results between field screening experiments. Field screening relying on field inoculum may not measure the true disease reaction of a genotype because of non-uniform concentration and non-random distribution of inoculums in the field plot. Furthermore, differences in plant maturity duration lead to confounding results of field evaluation usually done at R7 stage (Mengistu et al. 2011b). In such cases, genotypes under study may not reach R7 stage at a time failing to screen all the genotypes under identical environmental conditions. Screening under controlled or semi-controlled conditions will overcome most of the limitations of field evaluation. Cut-stem inoculation technique developed by Twizeyimana et al. 2012 is a screening technique under controlled environmental conditions for more precise comparison of partial resistance to charcoal rot across maturity groups (Pawlowski et al. 2015). Unlike in field evaluation, a uniform amount of inoculum can be applied to the genotypes for infection which minimises the diseases from escaping and experimental error and improves screening precision. Length of necrosis in this technique is a direct measure of disease level considering which will improve the precision of disease evaluation over indirect disease measures such as CFUI (Twizeyimana et al. 2012). The results of cut-stem inoculation technique and CFU index ratings showed similar ranks for the genotypes screened (Twizeyimana et al. 2012; Pawlowski et al. 2015) indicating the efficiency and effectiveness of

cut-stem inoculation technique in identifying the potential sources of charcoal rot resistance (Pawlowski et al. 2015).

M. phaseolina is a pycnidia-producing fungus (Smith and Wyllie 1999). Pycnidia production is common on garden beans and jute beans and occasional on soybean (Smith and Wyllie 1999). Ma et al. (2010) developed a screening technique using conidial suspension. Pycnidia production is not common in most of the culture media (Ma et al. 2010). Out of seven semi-defined media tested for pycnidia production, they found greater pycnidia and conidia production in peanut butter extract-saturated filter paper placed over soynut butter extract agar (PESEA). Conidia from the most aggressive isolate (pine tree, AR) were used to test their infectivity on the soybean radicles**.** Conidial suspension significantly differentiated susceptible genotype LS98-0358 from the moderately resistant genotype DT97-4290. Lesion length produced on LS98-0358 is significantly higher than that of DT97-4290 (Ma et al. 2010). Based on these results, Ma et al. (2010) concluded that PESEA can be used to produce conidia for inoculum for high-throughput evaluation of soybean genotypes for resistance.

Reznikov et al. (2019) developed an in vitro method of soybean root infection by *M. phaseolina* to evaluate charcoal rot disease reaction in soybean germplasm. Soybean seeds were surface sterilised for 1 min with 5% (v/v) NaClO followed by 70% (v/v) ethanol for 30 seconds and rinsed with sterile distilled water thrice, each for 1 min. Disinfected seeds were placed on a petri dish with a layer of filter paper soaked with 15 mL of distilled water. Seeds in the petri dish were incubated for 48 h in darkness at 28 °C to induce germination. Five germinated healthy seedlings were placed in autoclaved flasks having 3 cm layer of cotton and filter paper soaked with 50 mL of sterile distilled water. Three toothpick pieces each of 2 cm long, colonised with an isolate derived from a single microsclerotium, were added to each flask. The flasks having inoculated seedlings were kept in a growth chamber under a 16-h light (600 μE m-2s-1)/8-h dark regime and a temperature of 30 °C. Root disease severity was evaluated after every 48 h for 12 days using images captured with a digital camera. The length of necrosis in the root system of each infected seedling was measured with an image processing program and the disease severity was measured in terms of percentage of necrosis in the root system. The area under disease progress curve (AUDPC) was calculated using disease severity data (Madden et al. 2007). In addition, the in vitro assay as measured by AUDPC correlated with CFU/g (square root transformed) with a Spearman's rank correlation coefficient $r = 0.62$ ($P = 0.0004$), and the AUDPC data is correlated with the field disease severity data (ln-transformed) with a rank correlation coefficient $r = 0.59$ ($P = 0.0009$) thus validating the in vitro phenotyping method to screen genotypes for disease reaction.

So far, no soybean genotype having a high level of resistance to *M. phaseolina* has been identified (Mengistu et al. 2018), and investigation of disease reactions in available soybean germplasm is not extensive (Coser et al. 2017). Those reported genotypes showing moderate resistance to charcoal rot are presented in Table 10.1. Pedigree information of some of the reported moderately resistant sources is presented in Table 10.2.

Table 10.1 Soybean genotypes identified as moderately resistant to charcoal rot using different screening techniques

S. no	Genotypes	Screening	Reference
1	Asgrow 4715, DeltaPineLand 3478, Hamilton and Jackson II	Field screening based on CFU	Smith and Carvil (1997)
2	DT97-4290	Field screening based on RSS and CFU	Paris et al. (2006)
3	DT98-7553, DT99-17483 and DT99-17554	Field screening based on CFU	Mengistu et al. (2007) and Mengistu et al. (2011a)
4	DT99-16864	Field screening based on RSS and CFU	Mengistu et al. (2007), Mengistu et al. (2011a) and Gillen et al. (2016)
5	DG3905, Manokin	Field screening based on CFU	Mengistu et al. (2011a)
6	PI 594302, PI 567562A, PI 506764 and PI 567334	Field screening based on CFU	Mengistu et al. (2013)
7	PI 548302, PI 548414 and PI 548178	Percentage of seedling survival 8 days after inoculation	Pawlowski et al. (2015)
8	PI 548302 and PI 548414	Cut-stem inoculation technique	Pawlowski et al. (2015)
8	PI379559D	Field screening through RSS	Coser et al. (2017)
9	PI567241	Cut-stem inoculation technique	Coser et al. (2017)
10	Y 227-1	Field screening based on CFU	Smith et al. (2018)
11	Munasqa RR	In vitro phenotyping through root infection	Reznikov et al. (2019)

Table 10.2 Pedigree information of some of the moderately resistant sources

S. no	Genotype	Pedigree	Reference
1	DT97-4290	Asgrow 'A5979' 3 Delta Pine 'DP3478'	Paris et al. (2006)
2	DT99-16864	S59-60′ × 'Bolivar'	Gillen et al. (2016)
3	JTN-4307	S97-1688 (PI 633736) × V94-0198	Arelli et al. (2017)
4	Y227-1	SS93-6181 × DT97-4290	Smith et al. (2018)

10.3 Factors Effecting Charcoal Rot Incidence

Charcoal rot disease incidence is influenced by climatic and other factors. Studies on effect of drought, maturity and soybean cyst nematode on charcoal rot disease severity are reviewed in the following.

10.3.1 Drought

Charcoal rot disease is likely to become more predominant owing to climate change scenarios of increased heat and drought stress (Saleh et al. 2010). Increased air and soil temperature and limited soil moisture aggravate the disease (Smith et al. 2018; Mengistu et al. 2011b, 2018; Gary et al. 1991; Pearson et al. 1984; Smith and Wyllie 1999). Low soil moisture will enhance the growth and survival of the pathogen (Short et al. 1980). Drought is a common stress for rain-fed soybean. Drought stress in soybean is more often due to limited rainfall but may also happen due to poor root growth. Research is underway to develop cultivars having resistance to both charcoal rot and drought (Mengistu et al. 2011a). Wrather et al. (2008) studied the disease reaction in seven drought-tolerant soybean genotypes and suggested that not all drought-tolerant genotypes necessarily are resistant to charcoal rot but some drought-tolerant genotypes may resist root colonisation by the fungus. Mengistu et al. (2018) reported that the relationship between drought tolerance as measured by stress tolerance index (STI) and charcoal rot resistance as measured by colony-forming units (CFU) is very weak and found that not all drought-tolerant genotypes under his study exhibited charcoal rot resistance. Therefore, no stronger relationship between drought tolerance and charcoal rot resistance has been reported yet. However, different, diverse drought-tolerant genotypes in a more stressful environment might produce a stronger relationship between the two traits (Mengistu et al. 2018).

Resistance to *M. phaseolina* is associated with drought tolerance in common bean (*Phaseolus vulgaris*) (Pastor-Corrales and Abawi 1988). Drought aggravates the disease in other crops like sorghum (*Sorghum bicolor* (L.) Moench) and sunflower (*Helianthus annuus* L) (Manici et al. 1995; Gary et al. 1991). In case of cowpea (*Vigna unguiculata* (L) Walp.), Muchero et al. (2011) identified three resistance governing quantitative trait loci (QTL), *Mac-4, Mac-5* and *Mac-9*, co-located with seedling drought-tolerant QTLs *Dro-5*, *Dro-10* and *Dro-7* (Muchero et al. 2009), respectively. In each case, the *M. phaseolina*-resistant haplotype corresponded with the seedling drought-tolerant haplotype. On the contrary, in crops such as sorghum reports of no interdependence of non-senescence drought tolerance and charcoal rot resistance are available (Tenkouano et al. 1993).

10.3.2 Maturity

Mengistu et al. (2018) studied the effect of maturity on charcoal rot disease severity. They evaluated six genotypes of MG IV and seven genotypes of MG V for disease severity as measured by AUDP and CFU and found that there is no significant difference in either measure under irrigated or non-irrigated environments.

Confounding effects of genetic backgrounds can be eliminated by using near-isogenic lines (NILs). The true effect of maturity on charcoal rot disease severity as

measured by CFU was first studied by Mengistu et al. (2014). Two sets of NILs, one set with 9 isogenic lines in the background of 'Clark' (Johnson 1958) and the other set with 7 isogenic lines in the background of 'Horosoy' (Weiss and Stevenson 1955), were used to assess disease severity in terms of CFU. Isogenic lines in each set have maturity differences due to different maturity gene combinations but are otherwise genetically homogenous. Field experiment was conducted on two soil types, sandy loam and clay for 2 years, and CFU was estimated for each isogenic line at its physiological maturity. Regression analysis investigating the relationship between maturity and diseases severity indicated that there was no significant relationship between maturity and disease severity.

In other related crops like cowpea (*Vigna unguiculata* (L) Walp.), Muchero et al. (2011) identified resistance governing QTLs *Mac-6* and *Mac-7* co-located with maturity-related senescence QTLs *Mat-2* and *Mat-1*, respectively, suggesting the association between earliness and susceptibility to *M. phaseolina*. Such studies can be done in soybean in evaluating the relationship between charcoal rot resistance and the reported genes governing early maturity.

*10.3.3 Soybean Cyst Nematode (*Heterodera glycines*)*

Interaction between soybean cyst nematode (*Heterodera glycines*) and charcoal rot has been documented long ago (Todd et al. 1987; Meyer et al. 1974), and it was reported that nematode infection increases the colonisation of soybean roots by *M. phaseolina.* Disruption of vascular tissues owing to the nematode infection results in host susceptibility to moisture stress which in turn aggravates the fungal colonisation (Radwan et al. 2014). While SCN is a biotroph, *M. phaseolina* is a necrotroph. Both diseases have different resistant pathways. Hypersensitive reaction induced by the host in response to SCN infection will help *M. phaseolina* to infect the host roots. Understanding the mechanisms underlying the molecular interactions between these two pathogens is essential to design a breeding program to control both the diseases (Radwan et al. 2014). On contrary, some studies have shown no interaction between the two organisms under field conditions (Francl et al. 1988).

10.4 Host Specialisation

M. phaseolina is a generalist clonal plant pathogen (Saleh et al. 2010; Radwan et al. 2014). Host specialisation is very less in this pathogen (Su et al. 2001; Saleh et al. 2010; Zveibil et al. 2012). Few studies have reported host specialisation with host species like corn (Su et al. 2001). Cloud and Rupe (1991) reported host specialisation of *M. phaseolina* with soybean but not with sorghum. They used one isolate each of soybean and sorghum in a cross-inoculation experiment and found that

soybean root infection was significantly greater when inoculated with soybean isolate than with the sorghum isolate, whereas no significant differences in colonisation of sorghum roots were observed when inoculated with either isolate. Su et al. (2001) conducted a cross-inoculation experiment to confirm differential colonisation of soybean roots by isolates from different host species. They used 7 isolates of soybean, 9 isolates of sorghum, 9 isolates of cotton and 6 isolates of corn in their study and came out with the conclusion that soybean root colonisation was significantly more by corn isolates than the isolates from any other hosts. Therefore, no extensive study has been done to confirm the specialisation of *M. phaseolina* with soybean.

10.5 Host-Pathogen Interaction (HPI)

The existence of host-pathogen-specific interactions among soybean genotypes and *M. phaseolina* isolates was for the first time demonstrated by Reznikov et al. (2019). When seven *M. phaseolina* isolates (Mp15, Mp17, Mp18, Mp32, Mp37, Mp42 and Mp48) were used to infect four soybean genotypes (DM 6.2i RR, CRIA 4, DT 97-4290 and Munasqa RR) under both field conditions and in vitro conditions, significant genotype × isolate interactions for both in vitro assay (as measured by AUDPC) ($P = 0.0277$) and field conditions (as measured by CFU) ($P = 0.0025$) were observed. Under field study, the lowest value of CFU/g was observed for the combination Munasqa RR × MP 15 (33.3) and the highest value of CFU/g was observed for the combination DM6.2iRR × MP15 (2366.7). Under in vitro conditions, the lowest value of AUDPC was noticed for the combination Munasqa RR × MP 17 (4.21) and the highest value of AUDPC was noticed for the combination DM6.2iRR × MP37 (233.3). Till date, no study on molecular interactions between soybean and *M. phaseolina* has been done (Radwan et al. 2014). Gene expression profiling of *M. phaseolina*-infected roots of *Medicago truncatula* identified genes involved in jasmonic acid and ethylene pathways that are important for plant defence against necrotrophic fungi. Also, genes involved in auxin homeostasis, polar auxin transport and auxin signalling were found to be regulated by the infection process (Mah et al. 2012). Differential expression of auxin-related genes suggested that the host susceptibility may be partially due to suppression of auxin response in the host by the pathogen (Mah et al. 2012). Such transcriptomic studies must be done in soybean to identify the molecular basis of host-pathogen interaction.

10.6 Genome of *Macrophomina phaseolina*

Genome of *M. phaseolina* was sequenced in 2012 (Islam et al. 2012). Genomes of *M. phaseolina* and *Fusarium oxysporum* Schltdl. have shared many syntenic regions suggesting the similarities in both the pathogens with the pathways to infect hosts

(Islam et al. 2012). A large number of shared genes and syntenic regions observed with the comparative genomics between *M. phaseolina* and *Fusarium oxysporum* may reflect the common infection strategies in the two phytopathogens having a broad host range. To penetrate into the host tissue, the pathogen degrades the host cell wall polysaccharides and lignocelluloses by producing abundant secreted oxidases, peroxidases and hydrolytic enzymes. To overcome the plant defence mechanism, it encodes a significantly higher number of P450s, MFS-type membrane transporters, glycosidases, transposases and secondary metabolites than any other fungi. Being a wide host range pathogen, its genome has several host-pathogen interaction genes including those encoding for adhesion, signal transduction, cell wall breakdown and purin and patulin biosynthesis (Islam et al. 2012). Loss of function mutations in the avirulence gene may result in gain of virulence further, in development of new races (Kang et al. 2001). Wide virulence potential of a fungal genome is often associated with transposon-mediated deletion or inactivation of PAMP (pathogen-associated molecular patterns) encoding genes whose products trigger the host plant's adaptive immune system (Kang et al. 2001; Islam et al. 2012). In this respect, *M. phaseolina* genome comprises 3.98% transposable elements; most of them are DNA transposases (Islam et al. 2012) having potential in evolving virulence and resulting in development of new races.

10.7 Breeding for Charcoal Rot Resistance

Breeding for resistance is the most effective way to combat soybean yield losses due to charcoal rot disease. Insufficient information regarding genetic mechanisms to charcoal rot resistance (CR) is hindering the progress in resistance breeding (Coser et al. 2017). Identification of transgressive lines in breeding and mapping populations indicates the potential for selecting novel resistance forms in the population (Muchero et al. 2011). Till date, no breeding program is expended in combining drought tolerance and charcoal rot resistance in soybean (Mengistu et al. 2018). For environments where both charcoal rot and drought are the problems, selection criterion for breeding programs should include both charcoal rot resistance and drought tolerance. In such case, for screening of segregating population, charcoal rot infestation of the soil and drought stress must be properly measured and strategically employed (Mengistu et al. 2018). Simultaneous screening for drought tolerance and charcoal rot resistance can be done in an infested field under non-irrigated conditions, but screening alone for one trait may not necessarily select for the other (Mengistu et al. 2018). Developing varieties having high yielding potential, moderate resistance to CR and some level of drought tolerance would be the optimum selection criterion to maximise the farmer's produce with limited soil moisture (Mengistu et al. 2018). In order to target yield and resistance, screening under irrigated conditions is recommended (Mengistu et al. 2011b).

Yield is the major criterion in any breeding program. No extensive study has been done in correlating soybean charcoal rot resistance with its yield. Mengistu

et al. (2018) attempted to study the effect of CFU at R7 on seed yield of 13 soybean genotypes over 3 years (2011 and 2012) under both irrigated and non-irrigated conditions. Regression of seed yield on CFU at R7 stage indicated that a significant ($P \leq 0.05$) negative relationship (i.e., as CFU increases, seed yield decreased) between CFU at R7 and seed yield was found only in 2012 non-irrigated environment and a significant ($P \leq 0.10$) negative linear relationship between the two traits was found in other two environments (2011 irrigated, 2012 irrigated) environment. A pooled (global) slope calculated for the six independent regressions (3 years × 2 irrigation environments) indicated a yield loss of 11.5 kg/h for every 1000 CUs at R7. These results indicate that there is a potential relationship between CFU at R7 and seed yield in at least some environments. On the other hand, Smith et al. (2018) studied the effect of charcoal rot on yield losses of six soybean genotypes in an infested plot under irrigated environment over 3 years (2011–13). Regression analysis indicated that only one genotype (LG03-4561-14) showed negative linear relationship between CFU and yield loss in two [2011 ($r^2 = 0.43$; $P = 0.0403$) and 2013 ($r^2 = 0.71$; $P = 0.0023$)] of the three environments. This is the first study to demonstrate the negative linear relationship between CFU and yield loss for a particular genotype. None of the other five genotypes showed significant ($P \leq 0.05$) linear relationship, thus concluding that not all soybean genotypes that were colonised by *M. phaseolina* show yield penalty. Therefore, it seems that no consistent and significant negative linear relationship between CFU and seed yield has been reported and the trends in relationship cannot be generalised across all the genotypes and environments. Tolerance is defined as the condition where the yield of a susceptible genotype is not affected significantly by the colonisation of *M. phaseolina* (Smith et al. 2018). Smith et al. (2018) in his study considered LG03-4561-14 to be intolerant to colonisation by *M. phaseolina*, whereas the other five genotypes are considered to be tolerant to colonisation with different threshold CFU levels. Therefore, it is understood that those genotypes which are susceptible may be tolerant and need not show yield losses (Smith et al. 2018).

In crops like sorghum, there are reports indicating no significant relationship (Williams et al. 2009) or with a perfect positive correlation (i.e., as lesion length increases, yield decreased) (Das and Prabhakar Indira 2008; Bandara et al. 2015) of lesion length (a measure for resistance) with yield and yield-attributing traits. In such cases, screening of genotypes exclusively for resistance is not appropriate to assess the yield under disease pressure (Bandara et al. 2015). Keeping the yield in view, an improved method of screening using *resistance-tolerance index* ($Index_{RT}$) (Bandara et al. 2015) was developed by considering yield-related plant tolerance indicators under disease pressure along with plant resistance against the pathogen. In order to take the tolerance into consideration to breed for high yielding under disease pressure, such indices should be developed and employed in soybean for selection of high-yielding lines under disease pressure.

Recombinant inbred lines (RILs) are a kind of mapping population developed for high-resolution mapping of QTL. It has an added advantage of recovering transgressive lines having higher resistance levels than the resistant parent (Muchero et al. 2011). In a common bean (*Phaseolus vulgaris* L.) breeding program, using a RIL

population derived from two parental lines, BAT 477 (resistant to charcoal rot and drought)/Pinto UI-114 (susceptible to charcoal rot and drought), Garcia-Olivares et al. (2012) identified RILs having stable, high yield and resistance to charcoal rot and drought in charcoal-rot-infected field under rain-fed or terminal heat stress conditions. Such populations should be developed in soybean to recover transgressive lines having improved resistance and yield.

Possible association between high levels of phenolic compounds, sugars and boron in seeds with charcoal rot resistance is demonstrated by Bellaloui et al. (2012). Selection of seeds having high levels of these substances in breeding populations may complement charcoal rot resistance breeding.

10.8 Genetics of Charcoal Rot Resistance and QTL Mapping

Understanding the mode of inheritance and heritability of trait are important in any breeding program. No extensive studies have been done to know whether charcoal rot resistance in soybean is a monogenic or oligogenic trait with high heritability or a polygenic trait with low heritability (Silva et al. 2019). Broad-sense heritability for charcoal rot resistance in soybean is reported as 0.06 by Coser et al. (2017) and 0.45 by Silva et al. (2019) indicating the significant influence of environment on disease reaction and necessitating the need for multilocation evaluation of advanced-generation inbred lines such as RILs in a replicated trial for selection of stable sources of resistance. Talukdar et al. (2009) demonstrated the polygenic gene action for charcoal rot resistance through a continuous distribution of disease reaction, ranging from highly susceptible through moderately resistant to highly resistant. Normal distribution for disease reaction as expressed in terms of length of necrosis is reported by Silva et al. (2019). These studies indicate that charcoal rot resistance in soybean is controlled by multiple loci. Correspondingly, in case of sorghum, inheritance of CR was studied in F2 and backcross populations of the cross 1202A × CSV-5 and concluded that the gene action was polygenic in nature having a major role played by epistatic interaction in inheritance (Rao and Shinde 1985). On the contrary, epistatic gene action is reported in crops like common beans (*Phaseolus vulgaris* L.). Inheritance of charcoal rot resistance in common beans was studied by Olaya et al. (1996) in F2 population of the cross BAT-477 (R)/A--70(S) and observed the disease reaction segregated in 9:7 ratio. Results suggested that the resistance in BAT-477 was governed by two dominant complementary genes. Bulked segregant analysis (BSA) identified two unlinked RAPD (random amplification of polymorphic DNA) markers linked to the resistance. From the F2 population of the cross BATT-477/Pinto UI-114, Hernández-Delgado et al. (2009) concluded that the charcoal rot resistance in BATT 477 was governed by two dominant genes with double recessive epistasis. One possible QTL was found on LG1 in BATT 477.

QTL mapping of charcoal rot resistance is done in a soybean accession PI 567562A (Silva et al. 2019). An F2:3 family (N = 140) derived from the cross PI

567562A (R)/ PI 567437 (S) was used to identify genomic regions conditioning charcoal rot resistance. Three QTLs governing resistance against *M. phaseolina* were identified, one QTL on chromosome 15 and two QTL on chromosome 16. QTL on chromosome 15 was mapped within a confidence interval of 1209 kb between SNPs (single-nucleotide polymorphism) *Gm15_01842053* and *Gm15_03051337* (LOD = 5.25; R^2 = 29.4%). On chromosome 16, the first QTL was mapped in a 1533-kb interval between SNPs, *Gm16_28961127* and *Gm16_30493887* (LOD = 4.32; R^2 = 25.4%). The second QTL on chromosome 16 was mapped into 1105-kb interval between SNPs, Gm16_35973543 and *Gm16_37078478* (LOD = 3.6; R^2 = 8.84%). Resistant alleles for all the three QTLs were contributed by the resistant parent PI 567562A. This is the first report of QTL mapping in a bi-parental mapping population.

10.9 Genomics of Charcoal Rot Resistance

Breeding for polygenic resistance is challenging. Genome-wide association studies (GWAS) provide an insight into the genetic architecture of any trait and provides parental choice in QTL mapping (Korte and Farlow 2013). GWAS is very effective in identifying genetic variants underpinning complex traits such as disease resistance (Iquira et al. 2015). In soybean, GWAS is being used for identifying genes governing several forms of biotic stresses including Sclerotina stem rot (Bastien et al. 2014; Iquira et al. 2015; Zhao et al. 2015; Moellers et al. 2017; Wei et al. 2017), Phytophthora root rot (Sun et al. 2014; Schneider et al. 2016; Qin et al. 2017), sudden death syndrome (Wen et al. 2014; Bao et al. 2015; Zhang et al. 2015), soybean cyst nematode (Bao et al. 2014; Vuong et al. 2015; Zhang et al. 2016), tobacco ring spot virus (Chang et al. 2016), soybean aphid (Hanson et al. 2018) and charcoal rot (Coser et al. 2017; Vinholes et al. 2019). Coser et al. (2017) attempted to decipher the genetic architecture of charcoal rot resistance and to identify the genes responsible for resistance. Both field screening and glasshouse screening was done in a diverse collection of 459 plant introductions of the USDA soybean germplasm core collection. Five significant SNPs and putative candidate genes governing biotic and abiotic stress response were identified in field screening, while in glasshouse screening, eight loci associated with eight candidate gene families controlling the functions of plant defence response were identified. Intriguingly, no commonality of genes or markers has been identified between field and glasshouse screenings indicating the complexity of the mechanism underlying the resistance to CR across different environments (Coser et al. 2017).

Vinholes et al. (2019) attempted to identify genomic regions conferring CR in a soybean association mapping panel through GWAS, using SNP markers and haplotype information. An association mapping panel (Contreras-Soto et al. 2017) containing 169 core Brazilian varieties used by farmers from 1991 to 2010 was used for field evaluation of CR. Phenotyping was based on percent mortality. The evaluation of plant mortality is started 4 weeks after sowing when infection symptoms began

to appear. The number of dead plants within each plot was counted every 7 days for 12 weeks. The data was expressed in terms of percentage of mortality. Through genome-wide association analysis, six SNPs were identified for association with CR in soybean (Vinholes et al. 2019). Two haplotypes, of three SNP markers *Gm08_44422211_T_C*, *Gm08_18909193_A_G* and *Gm19_34320762_A_C,*were identified where genotypes having haplotype TAC had lesser mortality percentage than genotypes possessing haplotype CGA (Vinholes et al. 2019).

Developments in genomics since the last decade allowed the use of new breeding strategies for crop improvement. Understanding complex biological systems in legumes is facilitated by comparative genomics using model plants such as *Medicago truncatula* and *Lotus japonicas* (Li et al. 2015). Candidate genes for CR were identified in cowpea based on annotated genic SNPs and comparative genomic studies with soybean and *Medicago truncatula*. Out of nine QTLs identified for resistance based on plant mortality in field and glasshouse experiments, QTL peak of the major QTL *Mac-2* is co-located with a SNP marker derived from a gene inhibiting pectin esterase (Muchero et al. 2011). Comparative genome analysis of the QTL *Mac-2* revealed that the corresponding soybean genomic region on chromosome 8 had a pectin esterase inhibitor gene and two copies of a gene encoding pectin esterase. Another major QTL *Mac-1* coincided with a MATE efflux family protein encoding gene which was highly syntenic to homeologous regions on chromosomes 10 and 20 of soybean (*Glycine max* L*)*. The syntenic region on chromosome 10 harboured nine copies of the MATE efflux family protein gene, whereas the syntenic region on chromosome 20 carried three copies of the same gene. Three resistance QTLs, *Mac-4, Mac-5 and Mac-9*, were syntenic to the soybean genomic regions harbouring osmotic-stress-responsive genes such as heat shock, calcium sensing and sodium hypersensitive genes (Muchero et al. 2011).

10.10 Conclusion and Future Perspectives

Macrophomina phaseolina (Tassi) Goid is a generalist plant pathogen having a wide host range. Economic importance of this pathogen is likely to increase with increase in heat and drought stress under climate change scenarios. Quality and quantity of inoculums and standardisation and repeatability of screening technique are crucial for determining the disease reaction of individual plants in breeding and mapping populations (Ma et al. 2010). Reliable, repeatable and high-throughput screening methods have to be developed for speeding of development of resistant cultivars. Though greenhouse screening is robust, correlation between resistance observed in field and glasshouse conditions is not consistent in some cases (Coser et al. 2017). Several factors such as environmental conditions, growth stages, resistance mechanisms, amount and distribution of inoculums and plant part inoculated must be considered while standardising and correlating different screening techniques. In spite of several limitations, field screening which represents the ideal crop environments must be still considered for disease evaluation. A glasshouse screening technique

that positively and consistently correlates with field screening experiment must be developed. Such technique can prescreen the genotypes prior to a detailed investigation in the field (Coser et al. 2017). An extensive investigation into genome-based host-pathogen interaction (Islam et al. 2012) will be effective in designing breeding strategies for disease control. Charcoal rot resistance is a quantitative trait (Talukdar et al. 2009; Silva et al. 2019). Due to its quantitative nature, information on marker trait association can be well applied in genomic selection rather than marker-assisted selection (MAS). MAS are ineffective in improving polygenic traits. Since many small-effect loci are controlling the CR, genomic selection would be suggested (Coser et al. 2017). Since large-scale precise phenotyping for disease reaction is challenging and heritability of the trait is considerably low, genomic selection can be effective. Precise phenotyping data and marker effects are used to develop a prediction model in a training population. Such prediction models can be applied in 'testing population' to indirect prediction of the phenotypes using only marker genotype. Breeding must aim at high yielding potential, moderate resistance and at least some drought tolerance to maximise the produce even under low soil moisture regimes (Mengistu et al. 2018). Broad-sense heritability of charcoal rot resistance is low (Coser et al. 2017; Silva et al. 2019) and influenced much by environmental factors. In such cases, selection of stable sources of resistance and identification of stable QTL and epistatic QTL interactions can only be done under multilocation environments in replicated trials. Soybean has a narrow germplasm. In such cases, populations like MAGIC (multiparent advanced-generation intercross)-derived RILs resulting from three generations of recombination events among eight diverse parents can have a broad genetic base (Shivakumar et al. 2018). Nested association mapping populations, where a charcoal-rot-tolerant genotype is used as common parent, may be utilised for identifying genetic background effect on QTLs conditioning charcoal rot resistance. Such populations are needed to be developed for soybean improvement against charcoal rot disease for fine mapping of QTL or candidate genes governing resistance and for selection of lines having resistance to charcoal rot. Since charcoal rot disease aggravates under drought conditions, parents selected for MAGIC RIL development must include both charcoal-rot-resistant genotypes and drought-tolerant genotypes. The resultant advanced inbred lines can have both charcoal rot resistance and drought tolerance.

References

Arelli PR, Shannon JG, Mengistu A et al (2017) Registration of conventional soybean germplasm JTN-4307 with resistance to nematodes and fungal diseases. Journal of Plant Registrations 11: 192–199

Bandara YMAY, Perumal R, Little CR (2015) Integrating resistance and tolerance for improved evaluation of sorghum lines against Fusarium stalk rot and charcoal rot. Phytoparasitica. https://doi.org/10.1007/s12600-014-0451-0

Bao Y, Vuong T, Meinhardt C, Tiffin P et al (2014) Potential of association mapping and genomic selection to explore PI 88788 derived soybean cyst nematode resistance. Plant Genome 7:1–13. https://doi.org/10.3835/plantgenome2013.11.0039

Bao Y, Kurle JE, Anderson G, Young ND (2015) Association mapping and genomic prediction for resistance to sudden death syndrome in early maturing soybean germplasm. Mol Breed 35:128. https://doi.org/10.1007/s11032-015-0324-3

Bastien M, Sonah H, Belzile F (2014) Genome wide association mapping of Sclerotinia sclerotiorum resistance in soybean with a genotyping-by-sequencing approach. Plant Genome 7:1–13. https://doi.org/10.3835/plantgenome2013.10.0030

Bellaloui N, Mengistu A, Zobiole L et al (2012) Resistance to toxin-mediated fungal infection: role of lignins, isoflavones, other seed phenolics, sugars, and boron in the mechanism of resistance to charcoal rot disease in soybean. Toxin Rev 31(1–2):16–26

Chang HX, Brown PJ, Lipka AE et al (2016) Genome-wide association and genomic prediction identifies associated loci and predicts the sensitivity of tobacco ring spot virus in soybean plant introductions. BMC Genomics 17:153. https://doi.org/10.1186/s12864-016-2487-7

Cloud GL, Rupe JC (1991) Morphological instability on a chlorate medium of isolates of *Macrophomina phaseolina* from soybean and sorghum. Phytopathology 78:1563

Contreras-Soto RI, de Oliveira MB, Costenaro-da-Silva D et al (2017) Population structure, genetic relatedness and linkage disequilibrium blocks in cultivars of tropical soybean (*Glycine max*). Euphytica. https://doi.org/10.1007/s10681-017-1966-5

Coser SM, Reddy RVC, Zhang J et al (2017) Genetic architecture of charcoal rot (*Macrophomina phaseolina*) resistance in soybean revealed using a diverse panel. Front Plant Sci 8:1626. https://doi.org/10.3389/fpls.2017.01626

Das IK, Prabhakar Indira S (2008) Role of stalk-anatomy and yield parameters in development of charcoal rot caused by Macrophomina phaseolina in winter sorghum. Phytoparasitica 36:199–208

Dhingra OD, Sinclair JB (1978) Biology and pathology of *Macrophomina phaseolina*. Universidade Federal de Viçosa, Viçosa

Fehr WR, Caviness CE, Burmood DT, Pennington JS (1971) Stage of development descriptions for soybeans, *Glycine max* (L.) Merr. Crop Sci 11:929–931

Francl LJ, Wyllie TD, Rosenbrock SM et al (1988) Influence of crop rotation on population density of *Macrophomina phaseolina* in soil infested with *Heterodera glycines*. Plant Dis 72:760–764

Garcia-Olivares JG, Lopez-Salina EL, Cumpian-Gutierrez J et al (2012) Grain yield and charcoal rot resistance in common beans under terminal drought conditions. J Phytopathol 160:98–105

Gary FA, Mihail JD, Lavigne RJ et al (1991) Incidence of charcoal rot of sorghum and soil populations of *Macrophomina phaseolina* associated with sorghum and native vegetation in Somalia. Mycopathologia 114:145–151

Gillen AM, Mengistu A, Smith JR et al (2016) Registration of DT99 16864 soybean germplasm line with moderate resistance to Charcoal Rot [*Macrophomina phaseolina* (Tassi) Goid.]. J Plant Reg 10:309–315. https://doi.org/10.3198/jpr2016.01.0002crg

Hanson AA, Lorenz AJ, Hesler LS et al (2018) Genome-wide association mapping of host-plant resistance to soybean aphid. Plant Genome 11:180011. https://doi.org/10.3835/plantgenome2018.02.0011

Hernández-Delgado S, Reyes-Valdés MH, Rosales-Serna R et al (2009) Molecular markers associated with resistance to *Macrophomina phaseolina* (tassi) goid. in common bean. J Plant Pathol 91(1):163–170

Iquira E, Humira S, Francois B (2015) Association mapping of QTLs for sclerotinia stem rot resistance in a collection of soybean plant introductions using a genotyping by sequencing (GBS) approach. BMC Plant Biol 15:5. https://doi.org/10.1186/s12870-014-0408-y

Islam MS, Haque MS, Islam MM et al (2012) Tools to kill: genome of one of the most destructive plant pathogenic fungi *Macrophomina phaseolina*. BMC Genomics 13:493

James WC (1974) Assessment of plant disease losses. Annu Rev Phytopathol 12:27–48

Johnson HW (1958) Registration of soybean varieties: VI. Agron J 50:690–691

Kang S, Lebrun MH, Farrall L et al (2001) Gain of virulence caused by insertion of a Pot3 transposon in a *Magnaporthe grisea* avirulence gene. Mol Plant-Microbe Interact 14:671–674

Korte A, Farlow A (2013) The advantages and limitations of trait analysis with GWAS: a review. Plant Methods 29:9. https://doi.org/10.1186/1746-4811-9-29

Li H, Rodda M, Gnanasambandam A et al (2015) Breeding for biotic stress resistance in chickpea: progress and prospects. Euphytica 204:257–288

Luna MPR, Mueller D, Mengistu A et al (2017) Advancing our understanding of charcoal rot in soybeans. J Integr Pest Manag 8(1):1–8

Ma J, Hill CB, Hartman GL (2010) Production of *Macrophomina phaseolina* conidia by multiple soybean isolates in culture. Plant Dis 94(9):1088–1092. https://doi.org/10.1094/PDIS-94-9-1088

Madden LV, Hughes G, van den Bosch F (2007) The study of plant disease epidemics. APS Press, St. Paul

Mah KM, Uppalapati SR, Tang Y et al (2012) Gene expression profiling of *Macrophomina phaseolina* infected *Medicago truncatula* roots reveals a role for auxin in plant tolerance against the charcoal rot pathogen. Physiol Mol Plant Pathol 79:21–30

Manici LM, Caputo FA, Cerato C (1995) Temperature responses of isolates of *Macrophomina phaseolina* from different climatic regions of sunflower production in Italy. Plant Dis 79:934–938

Mengistu A, Ray JD, Smith JR, Paris RL (2007) Charcoal rot disease assessment of soybean genotypes using a colony-forming unit index. Crop Sci 47:2453–2461

Mengistu A, Arelli PA, Bond JP et al (2011a) Evaluation of soybean genotypes for resistance to charcoal rot. Online Plant Health Prog. https://doi.org/10.1094/PHP-2010-0926-01-RS

Mengistu A, Smith JR, Ray JD (2011b) Seasonal progress of charcoal rot and its impact on soybean productivity. Plant Dis 95:1159–1166

Mengistu A, Bond J, Nelson R et al (2013) Identification of soybean accessions resistant to *Macrophomina phaseolina* by field screening and laboratory validation. Online Plant Health Prog. https://doi.org/10.1094/PHP-2013-0318-01-RS

Mengistu A, Ray JD, Smith JR et al (2014) Maturity effects on colony-forming units of *Macrophomina phaseolina* infection as measured using near-isogenic lines of soybeans. J Crop Improv 28:38–56. https://doi.org/10.1080/15427528.2013.858284

Mengistu A, Ray JD, Smith JR et al (2018) Effect of charcoal rot on selected putative drought tolerant soybean genotypes and yield. Crop Prot 105:90–10

Meyer WA, Sinclair JB, Khare MM (1974) Factors affecting charcoal rot of soybean seedlings. Phytopathology 64:845–849

Moellers TC, Singh A, Zhang J et al (2017) Main and epistatic loci studies in soybean for *Sclerotinia sclerotiorum* resistance reveal multiple modes of resistance in multi-environments. Sci Rep 7:3554. https://doi.org/10.1038/s41598-017-03695-9

Muchero W, Ehlers JD, Close TJ et al (2009) Mapping QTL for drought stress-induced premature senescence and maturity in cowpea [*Vigna unguiculata* (L) Walp]. Theor Appl Genet 118:849–863

Muchero W, Ehlers JD, Close TJ et al (2011) Genic SNP markers and legume synteny reveal candidate genes underlying QTL for Macrophomina phaseolina resistance and maturity in cowpea [Vigna unguiculata (L) Walp.]. BMC Genomics 12:8

Olaya G, Abawi GS, Weeden NF (1996) Inheritance of resistance to Macrophomina phaseolina and identification of RAPD markers linked to the resistance genes in beans. Phytopathology 86:674–679

Paris RL, Mengistu A, Tyler JM et al (2006) Registration of soybean germplasm line DT97-4290 with moderate resistance to charcoal rot. Crop Sci 46:2324–2325

Pastor-Corrales MA, Abawi GS (1988) Reactions of selected bean accessions to infection by *Macrophomina phaseolina*. Plant Dis 72:39–41

Pawlowski ML, Hill CB, Hartman GL (2015) Resistance to charcoal rot identified in ancestral soybean germplasm. Crop Sci 55:1230–1235. https://doi.org/10.2135/cropsci2014.10.0687

Pearson CAS, Schwenk FW, Crowe FJ et al (1984) Colonization of soybean roots by *Macrophomina phaseolina*. Plant Dis 68:1086–1088

Qin J, Song Q, Shi A et al (2017) Genome-wide association mapping of resistance to *Phytophthora sojae* in a soybean (*Glycine max* (L.) Merr.) germplasm panel from maturity groups IV and V. PLoS One 12(9):e0184613. https://doi.org/10.1371/journal.pone.0184613

Radwan O, Rouhana LV, Hartman GL et al (2014) Genetic mechanisms of host-pathogen interactions for charcoal rot in soybean. Plant Mol Biol Rep 32:617–629
Rao DNV, Shinde VK (1985) Inheritance of charcoal rot resistance in sorghum. J Maharashtra Agric Univ 10:54–56
Reznikov S, Chiesa MA, Pardo EM et al (2019) Soybean-*Macrophomina phaseolina*-specific interactions and identification of a novel source of resistance. Phytopathology 109(1):63–73. https://doi.org/10.1094/PHYTO-08-17-0287-R
Ritchie SW, Hanway JJ, Thompson HE et al (1989) How a soybean plant develops. Spec. Rep. No. 53. Iowa State Univ. Sci. Technol. Coop. Ext. Serv., Ames, IA
Saleh AA, Ahmed HU, Todd TC et al (2010) Relatedness of Macrophomina phaseolina isolates from tallgrass prairie, maize, soybean and sorghum. Mol Ecol 19:79–91
Schmitt DP, Shannon G (1992) Differentiating soybean response to *Heterodera glycines* races. Crop Sci 32:275–277
Schneider R, Rolling W, Song Q et al (2016) Genome-wide association mapping of partial resistance to Phytophthora sojae in soybean plant introductions from the Republic of Korea. BMC Genomics 17:607. https://doi.org/10.1186/s12864-016-2918-5
Shivakumar M, Kumawat G, Gireesh C et al (2018) Soybean MAGIC population: a novel resource for genetics and plant breeding. Curr Sci 114:906–908. https://doi.org/10.18520/cs/v114/i04/901-906
Short GE, Wyllie TD, Bristow PR (1980) Survival of *Macrophomina phaseolina* in soil and in residue of soybean. Phytopathology 70:13–17
Silva MP, Klepadlo M, Gbur EE et al (2019) QTL mapping of charcoal rot resistance in PI 567562A soybean accession. Crop Sci 59:1–6
Smith GS, Carvil ON (1997) Field screening of commercial and experimental soybean cultivars for their reaction to *Macrophomina phaseolina*. Plant Dis 81:363–368
Smith GS, Wyllie TD (1999) Charcoal rot. In: Hartman GL, Sinclair JB, Rupe JC (eds) Compendium of soybean disease, 4th edn. American Phytopathological Society, St. Paul, pp 29–31
Smith JR, Ray JD, Mengistu A (2018) Genotypic differences in yield loss of irrigated soybean attributable to charcoal rot. J Crop Improv. https://doi.org/10.1080/15427528.2018.1516262
Su G, Suh SO, Schneider RW et al (2001) Host specialization in the charcoal rot fungus, *Macrophomina phaseolina*. Phytopathology 91:120–126
Sun J, Li L, Zhao J et al (2014) Genetic analysis and fine mapping of RpsJS, a novel resistance gene to Phytophthora sojae in soybean [Glycine max (L.) Merr]. Theor Appl Genet 127:913–919. https://doi.org/10.1007/s00122-014-2266-2
Talukdar A, Verma K, Gowda DSS et al (2009) Molecular breeding for charcoal rot resistance in soybean I. Screening and mapping population development. Indian J Genet 69:367–370
Tenkouano A, Miller FR, Frederiksen RA et al (1993) Genetics of non senescence and charcoal rot resistance in sorghum. Theor Appl Genet 85(5):644–648
Todd TC, Pearson CAS, Schwenk FW (1987) Effect of *Heterodera glycines* on charcoal rot severity in soybean cultivars resistant and susceptible to soybean cyst nematode. Ann Appl Nematol 1:35–40
Tooley PW, Grau CR (1984) Field characterization of rate reducing resistance to *Phytophthora megasperma* f. sp. *glycines* in soybean. Phytopathology 74:1201–1208
Twizeyimana M, Hill CB, Pawlowski M et al (2012) A cut stem inoculation technique to evaluate soybean for resistance to *Macrophomina phaseolina*. Plant Dis 96:1210–1215
Vinholes P, Rosado R, Roberts P et al (2019) Single nucleotide polymorphism-based haplotypes associated with charcoal rot resistance in Brazilian soybean germplasm. Agron J 111:182–192
Vuong TD, Sonah H, Meinhardt CG et al (2015) Genetic architecture of cyst nematode resistance revealed by genome wide association study in soybean. BMC Genomics 16:593. https://doi.org/10.1186/s12864-015-1811-y
Wei W, Mesquita ACO, Figueiró AA (2017) Genome-wide association mapping of resistance to a Brazilian isolate of Sclerotinia sclerotiorum in soybean genotypes mostly from Brazil. BMC Genomics 18:849. https://doi.org/10.1186/s12864-017-4160-1

Weiss MG, Stevenson TM (1955) Registration of soybean varieties: V. Agron J 47:541–543

Wen Z, Tan R, Yuan J et al (2014) Genome-wide association mapping of quantitative resistance to sudden death syndrome in soybean. BMC Genomics 15:11. https://doi.org/10.1186/1471-2164-15-809

Williams A, Hector PQ, Victor MG (2009) Grain sorghum varieties reaction [*Sorghum bicolor* (L.) Moench] to *Macrophomina phaseolina* (Tassi) Goid. Revista Mexicana de Fitopatología 27:148–155

Wrather JA, Anderson TR, Arsyad DM et al (2001) Soybean disease loss estimates for the top ten soybean-producing countries in 1998. Canadian J Plant Pathol 23:115–121

Wrather JA, Shannon JG, Carter TE (2008) Reaction of drought-tolerant soybean genotypes to *Macrophomina phaseolina*. Plant Health Prog. https://doi.org/10.1094/PHP-2008-0618-01-RS

Young PA (1949) Charcoal rot of plants in east Texas. Bulletin Texas Agricultural Experimental Station No. 33

Zhang J, Singh A, Mueller DS et al (2015) Genomewide association and epistasis studies unravel the genetic architecture of sudden death syndrome resistance in soybean. Plant J 84:1124–1136. https://doi.org/10.1111/tpj.13069

Zhang H, Li C, Davis EL et al (2016) Genome-Wide Association Study of resistance to soybean cyst nematode (*Heterodera glycines*) HG type 2.5.7 in wild soybean (*Glycine soja*). Front Plant Sci 7:1214. https://doi.org/10.3389/fpls.2016.01214

Zhao X, Han Y, Li Y et al (2015) Loci and candidate gene identification for resistance to *Sclerotinia sclerotiorum* in soybean (*Glycine max* L. Merr.) via association and linkage maps. Plant J 82:245–255. https://doi.org/10.1111/tpj.12810

Zveibil A, Mor N, Gnayem N et al (2012) Survival, host–pathogen interaction, and management of *Macrophomina phaseolina* on strawberry in Israel. Plant Dis 96:265–272

Chapter 11
Barley, Disease Resistance, and Molecular Breeding Approaches

Baljinder Singh, Sahil Mehta, Sumit Kumar Aggarwal, Manish Tiwari, Shafiqul Islam Bhuyan, Sabhyata Bhatia, and Md Aminul Islam

11.1 Introduction

Next to the control of fire, the fermentation technology is considered as mankind's most important invention. This opinion truly relates to beer production and barley malt, a technology which is enjoyed over many centuries in the world. The first evidence of barley beer usage date back to the ca. 3350–3000 BC. With the increase in human settlement around the globe, many cereal sources were domesticated by mankind for livelihood. Barley (*Hordeum vulgare L.*) is one of the earliest versatile domesticated cereals (Badr et al. 2000; Wang et al. 2015; Harwood 2019). The cultivated barley (family Poaceae) is an annual, self-pollinating temperate grass, which requires a low level of fertilization unlike counterparts like rice and wheat. It grows in both winter and spring season around the globe with variation in spike morphology and hull types (Harwood 2019). According to the Food and Agriculture Organization Corporate Statistical Database, it is among the four major crops produced worldwide (FAOSTAT 2016) with global production of 145.96 million metric tons. The comparison of region-wise barley production globally and other parameters is given in Table 11.1. The major barley-producing areas are in the Russian Federation, Europe, Australia, Canada, North America, UK, and Asia (Harwood 2019) (Fig. 11.1). It is cultivated for stews, cattle feed, brewing, human

B. Singh · M. Tiwari · S. Bhatia · M. A. Islam (✉)
National Institute of Plant Genome Research, New Delhi, India

S. Mehta
International Centre for Genetic Engineering and Biotechnology, New Delhi, India

S. K. Aggarwal
ICAR- Indian Institute of Maize Research, Ludhiana, Punjab, India

S. I. Bhuyan
Department of Botany, Pandit Deendayal Upadhayay Aadarsha Mahavidyalaya, Behali, Biswanath, Assam, India

S. H. Wani (ed.), *Disease Resistance in Crop Plants*,
https://doi.org/10.1007/978-3-030-20728-1_11

Table 11.1 Comparison of region-wise barley production, area harvested, and yield for the year 2017

Region	Production (tonnes)	Area harvested (ha)	Yield (hg/ha)	Production rank
Europe	89,052,689	22,991,499	38,733	1.
Asia	21,153,037	9,484,951	22,302	2.
America	16,785,157	4,695,316	35,749	3.
Oceania	13,803,589	4,876,069	28,309	4.
Africa	6,609,790	4,961,340	13,323	5.

The data have been adapted from FAOSTAT (accessed on 31st January 2019)

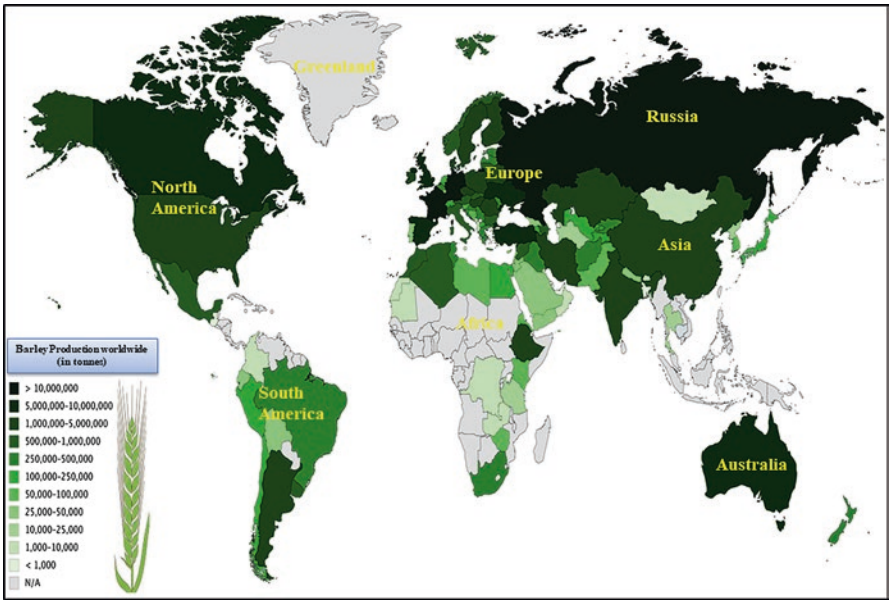

Fig. 11.1 A choropleth map representing the global barley production in tonnes for the year 2016. (Source: Food and Agriculture Organization Corporate Statistical Database (FAOSTAT). Accessed on 31st January 2019)

food, and malt production (Harwood 2019). However, only a small portion of total productivity is directly utilized in the human diet. In the last two decades, the barley yields have seen an increase of about 55% globally, especially in Europe, UK, and Turkey. It has been possible largely, due to the breeding of elite, susceptible cultivars for disease resistance as well as good agricultural practices (Friedt et al. 2011; Harwood 2019).

Apart from use in animal feed, human nutrition, and modern agriculture, it has emerged as an important experimental model plant to study and understand the dynamics of genetics, molecular biology, biochemistry, physiology, development, biotechnology, and plant-pathogen interactions (Holzberg et al. 2002; Hein et al. 2005; Saisho and Takeda 2011; Lawrenson et al. 2015; Harwood 2016, 2019; Jost

et al. 2019). This is possible due to the diploid genome ($2n = 14$), easy cultivation, easy pollination techniques, availability of extensive genetic resources, and available high-quality reference genome sequence. This is evidently supported by the protocols available for *Agrobacterium*-mediated genetic transformation, tissue culture regeneration protocols, haploid culture, TILLING, gene microarrays, TALENS, VIGS, and CRISPR-Cas9 (McCallum et al. 2000b; Holzberg et al. 2002; Hein et al. 2005; Travella et al. 2005; Bartlett et al. 2008; Talamè et al. 2008; Budhagatapalli et al. 2015; Lawrenson et al. 2015; Russell et al. 2016; Horler et al. 2017; Mascher et al. 2017; Harwood 2019; Jost et al. 2019).

Apart from developing such biotechnology tools and protocols, humans have also overexploited the earth's resources (Bellard et al. 2012; Boivin et al. 2016; Montanari et al. 2017; Cazalis et al. 2018; Lindley et al. 2019). According to the reports, the whole scenario will get worse in the upcoming three decades and this will highly affect the total food production. As a result, the disease incidence will increase in crop production especially cereals, pulses, oil-yielding plants, spices, etc.

The plant diseases are the most serious biological constraint to the crop's productivity. This is evident from the fact that the phytopathogens account for about 20–45% of total global agricultural losses (Pathak and Khan 1994; Oerke 2005; Bellard et al. 2012; Savary et al. 2012). Therefore, it is essential to improve the disease resistance in the previously existing elite crop varieties for higher yield and durable agriculture. Furthermore, the breeders have already improved many crops including barley using classical as well as improved molecular breeding approaches (Jena and Mackill 2008; Miedaner and Korzun 2012; Mohamed et al. 2014; Mundt 2014; Ashkani et al. 2015; Stenberg et al. 2015; Wiesner-Hanks and Nelson 2016; Shakoor et al. 2017; Nelson et al. 2018).

11.2 Diseases in Barley

Next to grain yield, the disease resistance has been the topmost priority for all barley breeders worldwide (Ceccarelli et al. 1992; Francia et al. 2011; Barati et al. 2018). Unlike rice and wheat, barley is a more adaptable cereal which grows in saline, moist, and drier environments as well as at higher altitudes (Srivastava and Damania 1989; Ceccarelli et al. 1992; Dai and Zhang 2016; Hecht et al. 2016; Serna-Saldivar 2016). However, this adaptability also increases the range of phytopathogens attacking barley. These phytopathogens include fungi, bacteria, and viruses (Pessarakli 2016). Each type of pathogen targets only a specific developmental stage or organ, be it ear, leaf, root, stem, rachis, head, and midveinor grain and lead to specific symptoms (https://cereals.ahdb.org.uk/). Generally, the leaf diseases have symptoms like yellowing of leaves with dark necrotic patches/spots and interveinal chlorosis (https://cereals.ahdb.org.uk/). Infection of stems causes weakened stems which ultimately lead to collapse (Pessarakli 2016). On the contrary, root infection results in stunted growth, photosynthetic arrest, thinning of stems,

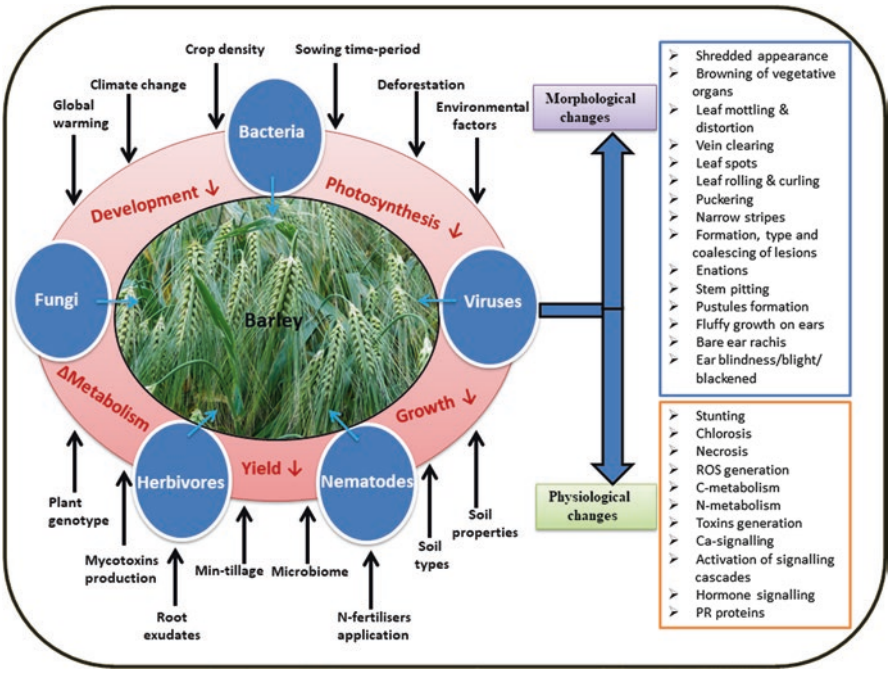

Fig. 11.2 Impact of various biotic factors and their interaction on barley crop physiology

and root rotting. Furthermore, the head infection leads to discolored, shriveled grains (Fig. 11.2) (Oliver 2019; Perovic et al. 2019). The disease incidence depends directly on local climate, geography, soil type, prevailing agricultural practices, plant age, and phytotoxin synthesis (Fig. 11.2).

Although the cultivated barley is a host to more than 250 different phytopathogens, so far the economically relevant diseases are powdery mildew (*Blumeria graminis*); smut (*Ustilago sp.*); head blight (*Fusarium sp.*); speckled leaf blotch (*Septoria passerinii*); leaf spot (*Ramularia sp.*); scald/leaf blotch (*Rhynchosporium secalis*); barley rusts like brown rust, black rust, and yellow rust (*Puccinia* sp.); net blotch (*Pyrenophora teres*); *barley yellow mosaic disease* (BaYMV); *barley yellow dwarf virus* (BYDV); *barley yellow mild mosaic disease* (BaMMV); *cereal yellow dwarf virus disease* (CYDV); etc. (Table 11.2). In addition, it is also infested by a number of pests, particularly aphids, beetles, and worms. Furthermore, the agroclimatic zone-wise barley disease incidence of India is reflected in Fig. 11.3.

Table 11.2 List of all major barley diseases, pathogens, and related information

Sl. no.	Disease name	Pathogen name	Family	Parts affected	Symptoms	Yield loss (%)	Environment
Fungal diseases							
1.	Anthracnose	*Glomerella graminicola*	Glomerellaceae	Leaf, stem	Dark, yellow water-soaked lesions on stems and leaves	5–20	Temperature ranging between 15 and 30 °C and relative humidity of around 90%
2.	Common root rot	*Cochliobolus sativus*	Pleosporaceae	Crown, root, kernel	Dark brown spots on subcrown internode and stem with premature whitening of root-rotted plants and shriveled kernels	2–25	Temperature ranging between 15 and 25 °C with dry, compacted soil with high N-fertilization
3.	Powdery mildew	*Erysiphe graminis f. sp. hordei*	Erysiphaceae	Leaf	White to gray powdery spore masses scattered or joined on the leaf blade	10–40	Temperature ranging between 12 and 20 °C, relative humidity of more than 85% with high N-fertilization
4.	Scab/head blight	*Fusarium graminearum*	Nectriaceae	Glumes, rachis, seed	Brown lesions on the glumes and rachis with the bleached head of the cream-colored plant	5–15	Temperature ranging between 12 and 20 °C, relative humidity of about 80%
5.	Covered smut	*Ustilago hordei*	Ustilaginaceae	Awns, floral bracts, grains	Dark brown spore masses on the head with reduced bracts and awns	15–35	Temperature ranging between 12 and 18 °C with moist, acidic soil
7.	Loose smut	*Ustilago nigra*	Ustilaginaceae	Head, ear, seed, awns, floral bracts	Olive brown spore masses on the head with reduced bracts	10–35	Temperature ranging between 16 and 22 °C for spore dispersal
8.	Take-all	*Gaeumannomyces graminis* var. *tritici*	Magnaporthaceae	Crown, root, grain	Blackening of crown and stem bases with premature shriveled grains	5–15	Temperature ranging between 10 and 25 °C with warm soil

(continued)

Table 11.2 (continued)

Sl. no.	Disease name	Pathogen name	Family	Parts affected	Symptoms	Yield loss (%)	Environment
9.	Spot blotch/leaf blight	*Bipolaris sorokiniana*	Pleosporaceae	Leaf, glumes	Dark brown round spots that join to make irregular patches with yellowing around the net	10–25	Temperature ranging between 15 °C to 25 °C with a relative humidity of about 85%
10.	Scald/leaf blotch	*Rhynchosporium secalis*	Incertae sedis	Leaf	Oval-shaped bluish-green or water-soaked lesions with tan margins	2–45	Temperature ranging between 10 and 15 °C, relative humidity of more than 85%
11.	Stem rust/black rust	*Puccinia graminis f. sp. tritici*	Pucciniaceae	Awns, leaf sheaths, glumes, stem	Dark reddish-brown spore masses on leaves	10–50	Temperature ranging between 10 and 17 °C, relative humidity of more than 80% and soil with high nitrogen content
12.	Brown rust/leaf rust	*Puccinia hordei*	Pucciniaceae	Leaf, leaf sheaths, neck, awns	Small orange-brown circular spore masses on the upper surface of leaves	15–50	Temperature ranging between 15 and 25 °C, relative humidity of more than 92% and soil with high nitrogen content
13.	Yellow rust/ stripe rust	*Puccinia striiformis f. sp. hordei*	Pucciniaceae	Leaf sheath, neck, glumes	Orange-yellow color stripes	25–55	Temperature ranging between 10 and 18 °C, relative humidity of more than 85%, and soil with high nitrogen content
14.	Net blotch	*Pyrenophora teres*	Pleosporaceae	Leaf, stem, glumes, seed	Chocolate brown like-patterns on leaves, leaf sheaths, and glumes with yellowing around the net	10–40	Temperature ranging between 15 and 23 °C and relative humidity of more than 75%
15.	Ramularia leaf spot	*Ramularia collo-cygni*	Mycosphaerellaceae	Leaf	Small, brown rectangular black spots on leaves surrounded by a yellow halo	10–35	Temperature ranging between 17 and 23 °C and relative humidity of more than 80%

Bacterial diseases							
16.	Black bacterial streak	*Xanthomonas translucens pv. Translucens*	Xanthomonadaceae	Leaf, glumes	Dark red-brown transparent water-soaked lesions on leaves with the browning of glumes	2–30	Temperature ranging between 18 and 25 °C and relative humidity of about 80%
17.	Bacterial stripe disease of barley	*Pseudomonas syringae pv. Striafaciens*	Pseudomonadaceae	Leaf	Small, water-soaked coalescing lesions with expanded narrow, yellowish margins	1–3	Temperature ranging between 8 and 25 °C and relative humidity of about 80%
18.	Bacterial blight	*Xanthomonas campestris pv. Translucens*	Xanthomonadaceae	Leaf	Linear water-soaked lesions which elongate to irregular glossy brown stripes	5–35	Temperature ranging between 15 and 25 °C with a relative humidity of about 85%
19.	Basal glume rot/ spikelet rot	*Pseudomonas syringae pv. atrofaciens*	Pseudomonadaceae	Glume, spike, rachis, grains	Small, dark green, water-soaked translucent streaks on glume with charcoal black grains at the germ end	1–3	–
Viral diseases							
20.	Barley mild mosaic	*Barley mild mosaic virus*	*Potyviridae*	Leaf	Irregular chlorotic streaks or necrotic patches on leaves with upward rolling of leaf margins and yellow discoloration	20–65	Temperature ranging between 10 and 15 °C with high soil moisture conditions
21.	Barley stripe mosaic	*Barley stripe mosaic virus*	*Virgaviridae*	Leaf	White mottling with mild stripes, mosaic on leaves, and lethal necrosis on stunted plants	5–20	Temperature ranging between 13 and 25 °C and relative humidity of more than 80% with high light intensity
22.	Barley yellow dwarf	*Barley yellow dwarf virus*	*Luteoviridae*	Leaf, head, root	Bright yellowing in tips, water-soaked margins of mature serrated leaves with stunted plant growth	5–15	Temperature ranging between 12 and 23 °C and relative humidity of more than 80%

(continued)

Table 11.2 (continued)

Sl. no.	Disease name	Pathogen name	Family	Parts affected	Symptoms	Yield loss (%)	Environment
23.	Barley yellow mosaic	*Barley yellow mosaic virus*	*Potyviridae*	Leaf, tillers	Elongated, pale green-yellow flecks on leaves with spiky appearance and necrotic patches on the infected plants	40–50	Temperature ranging between 15 and 23 °C and relative humidity of more than 85%
24.	Barley yellow streak mosaic disease	*Barley yellow streak mosaic virus*	*Rhabdoviridae*	Leaf	Pale yellow streaks and stripes parallel to the mid-rib leading to mosaic pattern and stunting plant growth	5–10	–
Other diseases							
25.	Aster yellows	*Aster yellows phytoplasma*	Acholeplasmataceae	Leaf, Tillers	Phyllody, deformation, chlorosis, stunting, virescence, and formation of sterile flowers	5–10	Temperature ranging between 20 and 25 °C
26.	Cereal cyst nematode	*Heterodera avenae*	Heteroderidae	Roots	Knots in roots, stunting, early senescence, and uneven appearance of infected plants	5–15	Temperature ranging between 18 and 20 °C with relative soil humidity of about 60% and sandy loamy soil
27.	Cereal root-knot nematode	*Meloidogyne naasi*	Heteroderidae	Roots	Cylindrical, spindle-shaped galls with hyperplasia and hypertrophy of cortex	2–10	Temperature ranging between 18 and 20 °C with relative soil humidity of about 60% and sandy loamy soil

The data have been adapted from the American Phytopathological Society (https://www.apsnet.org/Pages/default.aspx), Krishisewa (http://www.krishisewa.com/), Bayer Crop Science (https://cropscience.bayer.co.uk/), Descriptions of Plant Viruses (http://www.dpvweb.net/index.php), and barley disease management guide (https://cereals.ahdb.org.uk/) (accessed on 31st January 2019)

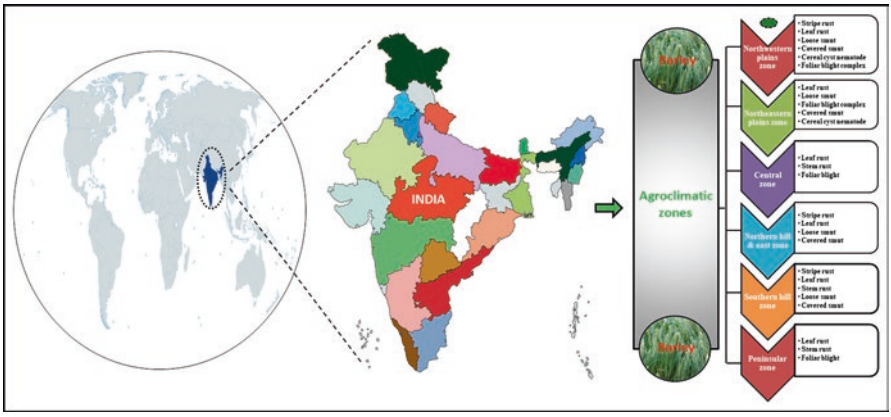

Fig. 11.3 Agroclimatic zone-wise occurrence of various barley diseases in India

11.3 Towards Breeding Barley for Disease Resistance

For the past few decades, disease management in barley relied heavily on pesticide application around the globe (Maguire et al. 2018; Oliver 2019). Seed treatments, crop rotation, and improving agronomic cultivation practices are other approaches used by farmers around the globe (Harwood 2016, 2019; Rehman 2018; Oliver 2019). Commonly in the fields, the combination of more than one approaches are practiced based on disease severity, which ultimately increases farmer's effort. Furthermore, pesticides increase fuel costs, wear-tear costs, and processing costs and require a time-to-time application. In addition, the effectiveness of pesticides is often overcome by developing resistant mutations which lead to the emergence of new virulent races and pathovars (Brown 1994; Frantzeskakis et al. 2018; Sánchez-Vallet et al. 2018; Burdon and Laine 2019). Hence, the most important approach to control various barley diseases is breeding the resistant varieties (Rehman 2018).

Earlier, the classical approaches were focused majorly on simple genetics, selection, mutation breeding, and hybridization (Ali et al. 2019). As a result, the development of multiple disease-resistant varieties was a very tedious task. In addition, the classical approaches only provided short-term relief (Harwood 2016, 2019). In order to react in a fast manner to the challenges, the barley breeders shifted their focus to the advance and more integrated molecular approaches in order to develop high-yielding barley varieties with enhanced disease resistance (Grewal et al. 2008a; Hudcovicová et al. 2008; Qi et al. 2012; Harwood 2016; Huang et al. 2018; Leng et al. 2018; Romero et al. 2018; Sayed and Baum 2018; Wang et al. 2018; Yu et al. 2018; Harwood 2019) (Fig. 11.4).

This was even eased by the availability of genetic resources, modern genomic marker technology, and various biotechnology tools (Chutimanitsakun et al. 2011; The International Barley Genome Sequencing et al. 2012; Jones 2016; Mace 2016; Horler et al. 2017; Hamwieh et al. 2018; Stein and Mascher 2018; Szurman-Zubrzycka et al. 2018; Harwood 2019; Jost et al. 2019; Kis et al. 2019). This is also reflected

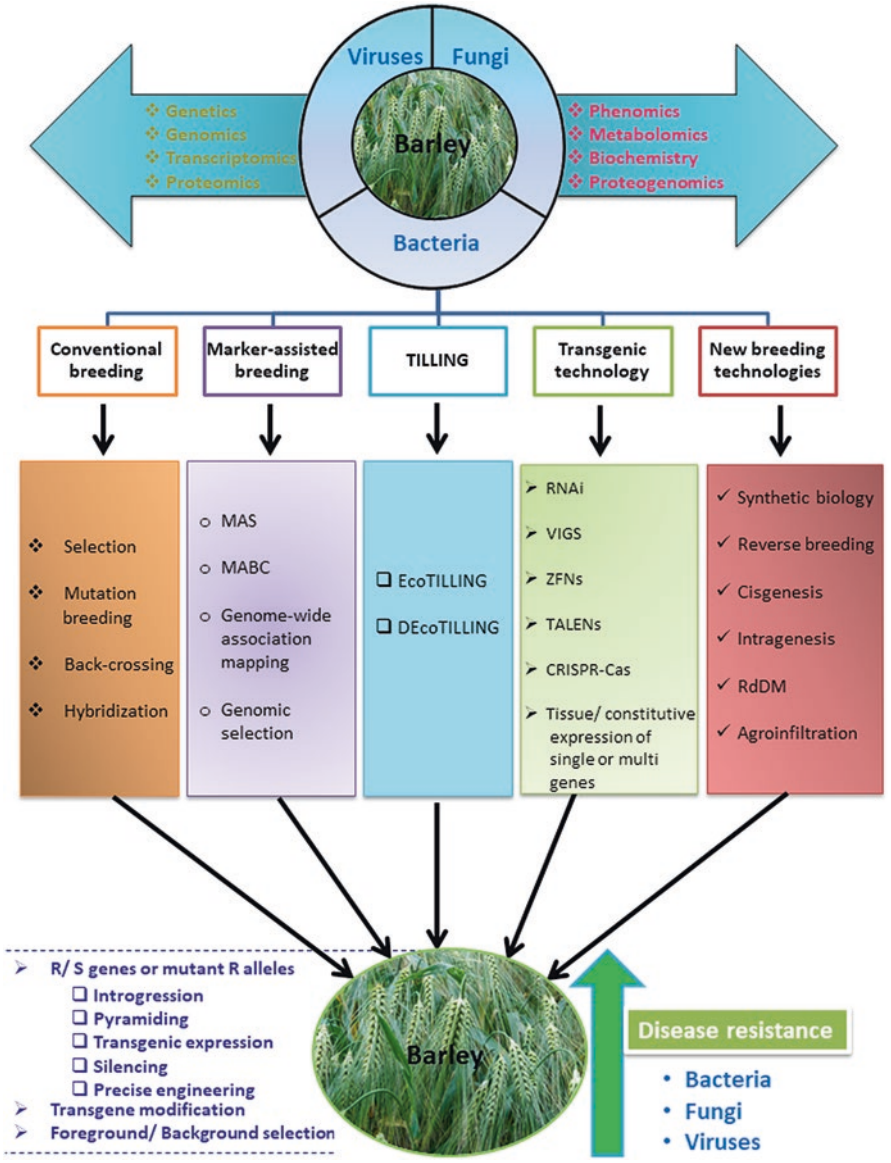

Fig. 11.4 Various molecular approaches for breeding of disease resistance in barley. ∗MAS, marker-assisted selection; MABC, marker-assisted backcrossing; TILLING, targeting induced local lesions in genomes; RNAi, RNA interference; VIGS, virus-induced gene silencing; ZFNs, zinc finger nucleases; TALENs, transcription activator-like effector nucleases; CRISPR-Cas, clustered regulatory interspaced short palindromic repeat-Cas; and RdDM, RNA-dependent DNA methylation

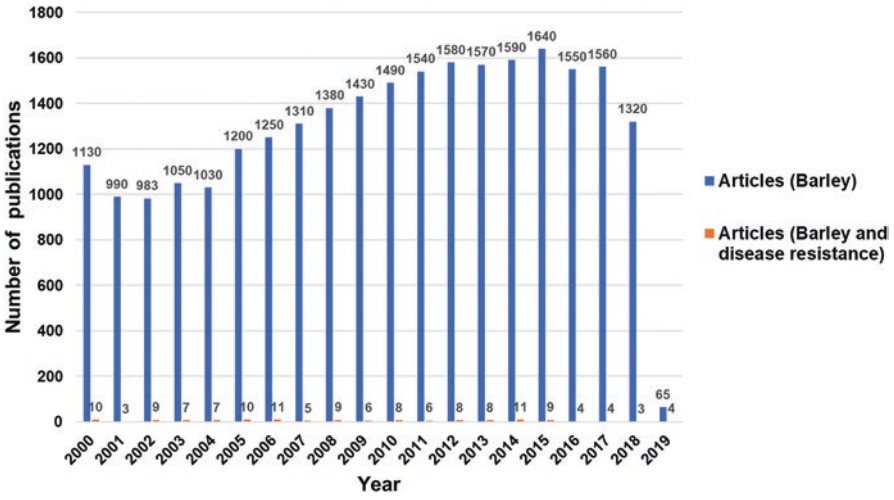

Fig. 11.5 The graph representing the number of publications per year related to barley and disease resistance by years (2000–2019). Keywords used in the search of Google Scholar included *barley* and *disease resistance.* (Accessed on 31st January 2019)

by the surge in the overall number of publications regarding barley and disease resistance (Fig. 11.5).

Furthermore, the barley cultivation practices have been also refined including crop rotation, hygiene management, and continuous surveillance to anticipate variety choice and practices for future years (Sieling and Christen 2015; Harwood 2016; Oliver 2019; Rose et al. 2019; Walls et al. 2019).

11.3.1 QTL Mapping

QTL mapping is a candidate gene approach which is performed in constructed biparental populations with contrasting parents to detect various QTLs and then estimate its position and effect on the plant traits after introgression (Chutimanitsakun et al. 2011; Liu 2017; Hansson et al. 2018; Perovic et al. 2019). This technique maps at a coarser order of 10 to 20 cM of map distance which might contain one or two genes, thus precluding the possibility for cloning the gene associated with the specific trait (Hansson et al. 2018; Perovic et al. 2019). It gives you high statistical power for detecting a QTL; however, it won't point to specific SNPs.

In the disease resistance context, the QTL mapping has been used unambiguously over the last two decades (Toojinda et al. 2000; Arru et al. 2003; Shtaya et al. 2006; Li and Zhou 2011; Chen et al. 2013b; del Blanco et al. 2014; Wonneberger et al. 2017; Case et al. 2018; Huang et al. 2018). In the year 2000, Toojinda and colleagues transferred the QTL 5 (using marker *act8-BMAC213*) to the recurrent background Galena for enhancing tolerance against stripe rust disease (Toojinda et al.

2000). Similarly, the introgression of QTL(2H) in barley cultivars has been also used to confer resistance against diseases such as net blotch (Ma et al. 2004), *Septoria speckled leaf blotch* (St. Pierre et al. 2010), barley leaf blotch (Looseley et al. 2012), and *Fusarium* head blight (Hori et al. 2006). Similarly, the tolerance against *Fusarium* head blight disease was also enhanced in the CIho 4196 background using *Qrgz-2H-8* (using markers ABG461C and BF263615) and *Qrgz-2H-10* (marker ABG459-ABG072) (Horsley et al. 2006). Yu and workers also transferred the QTL, namely, *Qrgz-2H-14* (using markers bPb5755 and bPb1181) from the donor parent Zhenongda 7 to the PI 643302 background to enhance the resistance (Yu et al. 2010). More recently, Huang et al. (2018) reported about a QTL located on chromosome no. 7. In addition, they transferred the QTL (using markers BOPA1_2251-643 and BOPA2_12_31203) to the recurrent PI 383933 background to enhance the resistance in barley.

The first successful report related to the application of QTL mapping for enhancing resistance against *Fusarium crown rot* was by Li et al. (2009). They introgressed *Qcrs.cpi-3H* in the recurrent Franklin background in order to enhance the resistance. Later, in a series of two publications, Chen et al. (2013a) reported about the successful transfer of *Qcrs.cpi-1H, Qcrs.cpi-3H*, and*Qcrs.cpi-4H* to the barley varieties Baudin, Gairdner, and Franklin for enhancing the *Fusarium crown rot* resistance.

In another instance, the introgression of two QTLs, namely, *QRpt6* and *QRpts4*, from the donor parent Dolly to the recurrent parent TR251 enhanced the tolerance against the devastating *Pyrenophora teres* (Grewal et al. 2008b). Similarly, the transfer of *QTL (6H)* (Gupta et al. 2010; St. Pierre et al. 2010), *QTL (3H)* (Gupta et al. 2010), *QTL (3HS)* (Cakir et al. 2011), *QTL (6HS)* (Cakir et al. 2011), *QRpt6* (Grewal et al. 2012), and AL_QRptt5-2 (Wonneberger et al. 2017) in various barley backgrounds from donor parents increased the tolerance against net blotch disease of barley. The similar results have been reported by Cakir et al. (2011) and Grewal and group (2012). Recently, Haas and workers studied the genetic architecture of resistance and performed QTL analysis to identify the spot blotch resistance conferring QTLs, namely, *Rcs-qtl-1H-12_30404, Rcs-qtl-2H-SCRI_RS_233272, Rcs-qtl-4H-SCRI_RS_168399*, and *Rcs-qtl-5H-SCRI_RS_138933* (Haas et al. 2016).

All cereals including barley are also affected by mildew. In order to reduce huge losses, many researchers and breeders have transferred various QTLs into the many susceptible barley cultivars and landraces (Shtaya et al. 2006; Silvar et al. 2010; Li and Zhou 2011; Hickey et al. 2012; Romero et al. 2018). Similarly, QTL mapping has been also used for enhancing disease tolerance against leaf stripe (Arru et al. 2003), nonparasitic leaf spots (Behn et al. 2004), yellow mosaic virus (Miyazaki et al. 2001), cereal yellow dwarf virus (del Blanco et al. 2014), and scald (Shtaya et al. 2006; Wagner et al. 2008; Li and Zhou 2011; Looseley et al. 2012). Similar to powdery mildew, various types of rust have also emerged as most devastating around the globe in the last decades. As a result, there are few successful reports about QTL mapping in the literature related to leaf rust (Li et al. 2006; Cakir et al. 2011; Castro et al. 2012; Li et al. 2013), stripe rust (Esvelt et al. 2016), and stem rust (Case et al. 2018). Table 11.3 summarizes about the successful reports on QTL mapping for enhanced disease resistance in barley.

Table 11.3 Summary of studies on QTL mapping for various barley diseases

Sl. no.	Disease	Pathogen	Population	Parental cross	Linked/flanking markers	QTL name/ position	References
1.	Fusarium head blight (FHB)	*Fusarium* species	93 $F_{5:7}$RILs	Rasmusson × PI 383933	BOPA2_12_31203 and BOPA1_2251-643	QTL on 7H chromosome	Huang et al. (2018)
2.	Powdery mildew	*Blumeria graminis*	110 $F_{7:8}$RILs 115 $F_{8:9}$RILs	Vada × SusBgtSC Vada × SusBgtDC	SC_C5-SNP46 and SC_C2-SNP54 DC_C5-SNP52 and DC_C2-SNP57	Rbgnq1 and Rbgnq2	Romero et al. (2018)
3.	Stem rust	*Puccinia graminis f. sp. tritici* *Puccinia graminis f. sp. secalis*	280 F_5 RILs 278 F_5 RILs	PI 382313 × Hiproly Hietpas-5 × Hiproly	S5H_461048085 2H_626235770	*Rpg-qtl-PH-PI38-5H* Rpg-qtl-HH-Hie-2H.3	Case et al. (2018)
4.	Net blotch (NB)	*Pyrenophora teres*	109 DH lines	Arve × Lavrans	SCRI_RS_140499 and SCRI_RS_8410	AL_QRptt5-2	Wonneberger et al. (2017)
5.	Stripe rust	*Puccinia striiformis f. sp. hordei*	156 $F_{5:8}$RILs	Lenetah × Grannelose Zweizeilige	SCRI_RS_188827, SCRI_RS_157611, and SCRI_RS_10818	Qpsh4Hb	Esvelt et al. (2016)
6.	Spot blotch	*Cochliobolus sativus*	244 BC_2F_4	PI 466423 × Rasmusson	SCRI_RS_4891–SCRI_RS_132028 SCRI_RS_233272 BOPA2_12_30655–BOPA1_5611-811 SCRI_RS_168141–SCRI_RS_13320	*Rcs-qtl-1H-12_30404* *Rcs-qtl-2H-SCRI_RS_233272* *Rcs-qtl-4H-SCRI_RS_168399* *Rcs-qtl-5H-SCRI_RS_138933*	Haas et al. (2016)

(continued)

Table 11.3 (continued)

Sl. no.	Disease	Pathogen	Population	Parental cross	Linked/flanking markers	QTL name/ position	References
7.	*Cereal yellow dwarf virus* (CYDV)	*Poleovirus*	184 F_5 RILs	Butta 12 × Madre Selva	12_30872 11_20247	*Qcyd.MaBu-1* *Qcyd.MaBu-2*	del Blanco et al. (2014)
8.	*Fusarium* crown rot (FCR)	*Fusarium* species	125 F_8RILs 117 F_8RILs 125 F_8RILs	Baudin/AWCS079 Gairdner/AWCS079 Franklin/AWCS079	bPb-6065 and bPb-8619 bPb-7278 and bPb-0619	*Qcrs.cpi-1H* *Qcrs.cpi-3H*	Chen et al. (2013b)
9.	*Fusarium* crown rot	*Fusarium* species	132 $F_{7:8}$RILs 131 $F_{7:8}$RILs	Baudin/AWCS276 Fleet/AWCS276	K01150 and WMS6	*Qcrs.cpi-4H*	Chen et al. (2013a)
10.	Leaf rust	*Puccinia hordei*	200 DH lines	Pompadour/Stirling	Hv0963-Bpb-8580	*QTL 5H*	Li et al. (2013)
11.	Leaf rust	*Puccinia hordei*	100 DH lines	BCD47 × Baronesse	Bmag173-Bmag009	*QTL (6H)*	Castro et al. (2012)
12.	Net blotch Spot blotch	*Pyrenophora teres*	233 DH lines	CDC Bold × TR251	HVM62b bPb–9604 and bPb–6127	*QRpt6* *QRcss1* and *QRcs3*	Grewal et al. (2012)
13.	Barley leaf blotch/scald	*Rhynchosporium secalis*	191 DH lines	Cocktail × WB05-13	11_11098 and 11_10169	*QTL (7H)*	Looseley et al. (2012)
14.	Powdery mildew	*Blumeria graminis*	321 DH lines	ND24260 × Flagship	bPb-0837	*QTL (5HS)*	Hickey et al. (2012)
15.	Net type net blotch Spot type net blotch Leaf rust	*Pyrenophora teres f. teres* *P. teres f. maculate* *Puccinia hordei*	178 DH lines	Baudin × AC Metcalfe	HVM0060 and Bmag0173 Bmag0496 HVHOTR0001	*QTL (3HS)* and *QTL (6HS)* *QTL (6HS)* *QTL (2HL)*	Cakir et al. (2011)

16.	Powdery mildew	*Blumeria graminis* f.sp. *hordei*	92 DH lines 177 DH lines	TX9425 × Franklin Yerong × Franklin	bPb-8179 and bPb-7769 bPb-5638	*QPm.TxFr.5H* and *QPm.TxFr.7H* *QPm.YeFr.1H*	Li and Zhou (2011)
	Scald	*Rhynchosporium secalis*			bPb-7356 and Bmag0006	*QSc.YeFr.3H*	
17.	Powdery mildew	*Blumeria graminis f. sp. Hordei*	262 $F_{5:6}$ RILs	SBCC97 × Plaisant	GBM1126 and HvM004 EBmac0755	*QTL (7HS)*	Silvar et al. (2010)
18.	*Septoria* speckled leaf blotch (SSLB) Net form net blotch	*Septoria passerine* *Pyrenophora teres f. teres*	115 F_4 RILs	M120 × Sep2-72	Bmag500 Ebmac787 and Ebmac874	*Rsp4* *QTL (6H)*	St. Pierre et al. (2010)
19.	*Fusarium* head blight	*Fusarium graminearum*	160 $F_{6:7}$ RILs	Zhenongda 7 × PI 643302	bPb5755 and bPb1181	*Qrgz-2H-14*	Yu et al. (2010)
20.	Net type net blotch	*Pyrenophora teres f. teres*	109 DH lines	Pompadour × Stirling	Bmag0807-Bmag0496 Bmac0209–Bmag0841	*QTL (6H)* *QTL (3H)*	Gupta et al. (2010)
21.	Crown rot	*Fusarium* species	92 DH lines	TX9425 × Franklin	bPb-4747 and bpb-6765	*Qcrs.cpi-3H*	Li et al. (2009)
22.	Net blotch	*Pyrenophora teres*	150 DH lines	Dolly × TR251	HVM74 and Bmag496 HVM03 and Bmac181	*QRpt6* *QRpts4*	Grewal et al. (2008a)
23.	Scald	*Rhynchosporium secalis*	135 DH lines	Igri × Triton	GBM1281 and GemS13	*QTL (2HS)*	Wagner et al. (2008)
24.	Leaf rust	*Puccinia hordei*	207 BC_3F_2 lines	Brenda × HS584	GBMS137 and Bmag13	*Qrph2.1* and *Qrph3.1*	Li et al. (2006)
25.	Fusarium head blight	*Fusarium graminearum*	250 $F_{6:7}$ RILs	Foster × CIho 4196	ABG461C and BF263615 ABG459-ABG072	*Qrgz-2H-8* and *Qrgz-2H-10*	Horsley et al. (2006)

(continued)

Table 11.3 (continued)

Sl. no.	Disease	Pathogen	Population	Parental cross	Linked/flanking markers	QTL name/ position	References
26.	Powdery mildew	*Blumeria graminis* f.sp. *hordei*	103 F_9 RILs	L94 × Vada	–	*Rbgq1, Rbgq2,* and *Rbgq3*	Shtaya et al. (2006)
	Scald	*Rhynchosporium secalis*				*Rrsq1, Rrsq2, Rrsq3,* and *Rrsq4*	
27.	Fusarium head blight (FHB)	*Fusarium* species	235 F_{12} RILs	Harbin 2-row × Turkey 6	FXLRRfor_XLRRrev119 – STS_FEgtaMacg677	*QTL (2H)*	Hori et al. (2006)
28.	Net blotch	*Pyrenophora teres f. teres*	147 DH lines	Chevron × Stander	*Xksua3b* and *Xwg719d Xcdo786-Xabc156a*	*Rpt QTL QTL (2H)*	Ma et al. (2004)
29.	Nonparasitic leaf spots (NPLS)	–	430 DH lines	IPZ24727 × Barke	*EBmac0635*	*QNpls.lfl-4H*	Behn et al. (2004)
30.	Leaf stripe	*Pyrenophora graminea*	143 DH lines	Steptoe × Morex	*Pcr 1*	*QTL (2H)*	Arru et al. (2003)
31.	Yellow mosaic virus	*Polymyxa graminis*	120 F_2 RILs	Ko A × Mokusekko 3	*MWG2134*	*QTL (2H)*	Miyazaki et al. (2001)
32.	Stripe rust	*Puccinia striiformis f.sp. hordei*	100 DH lines	Shyri × Galena	*act8-BMAC213*	*QTL 5(1H)*	Toojinda et al. (2000)

11.3.2 Gene Mapping

In gene mapping, the genetic markers are developed and a population is mapped to identify the distances between genes via analysis of co-segregation patterns and specific locus of a gene based solely on their phenotypic effect (Drader and Kleinhofs 2010; Richards et al. 2017; Hamwieh et al. 2018). The genetic map quality depends on the size of the mapping population and genetic markers used. The knowledge of the genetic maps is used to develop crops that are more productive, nutritious, and better resistant against diseases (Bilgic et al. 2006; Richards et al. 2017; Yu et al. 2018).

In the literature, there are many successful examples related to the gene mapping approach and disease resistance in barley (Bulgarelli et al. 2004; Bilgic et al. 2006; Sui et al. 2010; Soldanova et al. 2013; Singh et al. 2015; Dawson et al. 2016; Ziems et al. 2017; Yu et al. 2018). Leaf rust is among the most devastating disease which affects the global productivity and yield. In the 21st century, the first successful report related to gene mapping was by Graner et al. (2000). He transferred the *Rph7* gene to the Cebada Capa background and enhanced the tolerance against the devastating pathogen *Puccinia hordei.* This report opened the way for other publications in the field for enhancing disease resistance in barley. In another report, Mammadov et al. (2003) also used the same approach to increase the tolerance against leaf rust. Other reports in the literature including Hickey et al. (2011), Sandhu et al. (2012), König et al. (2012), Dracatos et al. (2014), Singh et al. (2015), Ziems et al. (2017), and Yu et al. (2018) also support the fact that gene mapping is an essential tool to enhance the barley resistance against leaf rust.

Barley leaf stripe is also considered a major barley disease around the globe. Like QTL mapping, gene mapping has been also used by many researchers and barley breeders to enhance the resistance in various elite, susceptible barley cultivars and varieties. In the year 2001, Tacconi and group published an article in which they reported about the successful transfer of *Rdg2a* (markers *OPQ-9*$_{700}$ and *MWG 2018*) gene to the susceptible *Mirco*barley cultivar (Tacconi et al. 2001). As a result, the resistance against barley leaf stripe increased by multiple folds in the susceptible parent. In another report, Castro and workers also used the same approach of gene mapping for enhancing the tolerance in the barley stripe rust disease (Castro et al. 2003) using gene *Rpsx* (using markers *Ris44* and *ABG461*). Similarly, Bulgarelli and coworkers successfully introgressed the gene *Rdg2a* (using *ssCH4* and *MWG851* markers) in the leaf-stripe-susceptible *Mirco* background (Bulgarelli et al. 2004). In another report, Yan and Chen (2006) transferred the gene *rpsGZ* in the susceptible parent Grannenlose Zweizeilige using SSR marker EBmac0679 to enhance the resistance for the pathogen *Puccinia striiformis f. sp. hordei*. Furthermore, powdery mildew and true loose smut impose high agricultural losses to the farmers, and as a result, the technique of gene mapping have been also used to breed the susceptible cultivars (Soldanova et al. 2013; Zang et al. 2015). Additionally, to breed the crops for virus resistance has been also a major challenge in the past two decades. However, gene mapping has been a choice of many researchers to overcome the

difficult challenge of enhancing virus resistance in various barley varieties and landraces. This approach has been significantly used for barley yellow dwarf virus (Niks et al. 2004) and barley yellow mosaic disease (Ruge et al. 2003; Le Gouis et al. 2004; Kai et al. 2012).

Similarly, gene mapping has been also used to breed the barley against net blotch (Manninen et al. 2006), *Septoria* speckled leaf blotch (Zhong et al. 2006; Lee and Neate 2007; St. Pierre et al. 2010), leaf scald (Hanemann et al. 2009; Hofmann et al. 2013), wheat stripe rust (Sui et al. 2010; Dawson et al. 2016), and spot blotch (Bilgic et al. 2006; Leng et al. 2018; Wang et al. 2018). Table 11.4 summarizes about the various reports regarding gene mapping and enhanced disease resistance.

11.3.3 Marker-Assisted Selection

Marker-assisted selection is a type of indirect selection for the desired plant phenotype which is based on the linked molecular markers banding pattern (Ragimekula et al. 2013). It has been used in the breeding of many crops including barley to speed up the precision of genetic progress (Ordon et al. 1995; Jefferies et al. 2003; Grewal et al. 2008a; Hudcovicová et al. 2008; Sayed and Baum 2018). As noted in the literature, few validated markers associated with resistance genes against various pathogens have been identified and introgressed in barley. In comparison to QTL mapping and gene mapping, the reports in the literature for MAS are few in number (Table 11.5).

For the first time, Ordon et al. (1995) successfully reported the introgression of the gene ym4 (using RAPD marker OP-Z04H660) from the parent Franka into the recurrent Igri background to enhance tolerance for the devastating barley yellow mosaic disease. A few years later, Jefferies et al. (2003) successfully introgressed the gene Yd2 using a marker, YLM, from the parent Franklin into the recurrent Sloop background to enhance tolerance for the viral disease, barley yellow mosaic virus. In another study, Hudcovic and coworkers (2008) transferred genes, rym4, rym11, and Ryd2 (using MWG838, HVM3,andYlp markers), in various susceptible cultivars, namely, Copia, Kamil, Nitran, Luxor, Ludan, and KM-104, to enhance tolerance for barley yellow mosaic virus complex and barley yellow dwarf virus. Similarly, Grewal and coworkers (2008b) reported the transfer of Run8 gene to the recurrent CDC McGwire to enhance the loose smut resistance. In addition, they successfully transferred Ruhq gene to the same recurrent parent to enhance resistance for covered smut using SCAR-type molecular markers aHor 2 and OPO6780.

In addition, the marker-assisted selection has been also used to enhance the tolerance against *Septoria* speckled leaf blotch (Zhong et al. 2006), barley stripe rust (Richardson et al. 2006), and scald (Pickering et al. 2006; Sayed and Baum 2018). Table 11.5 enlists about the various reports regarding marker-assisted selection for enhancement of disease resistance in barley cultivars and varieties.

Table 11.4 Summary of studies on gene mapping for various barley diseases

Sl. no.	Disease	*Pathogen*	Population	Parental cross	Linked/flanking markers	Marker type	Gene name	References
1	Leaf rust	*Puccinia hordei*	1368 F_2 lines	200A12 × Emir	CM_1194		*Rph26*	Yu et al. (2018)
2	Spot blotch	*Bipolaris sorokiniana*	160 F_8 lines	PI 356741 × PI 235186	M13.06 and M13.37		*Rbs7*	Wang et al. (2018)
3	Spot blotch	*Cochliobolus sativus*	105 DH lines	Bowman × Calicuchima	Bc183711 and Bc13291		*Scs6*	Leng et al. (2018)
			2 F_2 populations	Bowman × ND 5883				
4	Leaf rust	*Puccinia hordei*	160 $F_{2:3}$ lines	ND24260-1 × Gus	3,999,875, 3,265,068, 3,272,559, and 3,272,930		*Rph24*	Ziems et al. (2017)
5	Wheat stripe rust	*Puccinia striiformis f. sp. hordei*	$F_{2:3}$ population	Abed Binder 12 × Russell	FPC 320		*Rps6*	Dawson et al. (2016)
6	Leaf rust	*Puccinia hordei*	138 DH lines	Yerong × Franklin	Ebmac0603		*Rph23*	Singh et al. (2015)
					bPb-8660 and bPb-9601			
7	True loose smut	*Ustilago nuda*	4625 F_4 RILs	TR09398 × TR07728	Un8 SNP4 and		*Un8*	Zang et al. (2015)
					0498L15 F8/R8			
8	Leaf rust	*Puccinia hordei*	258 DH lines	CI 9214 × Stirling	DART4872 and DART7508		*RphC*	Dracatos et al. (2014)
9	Leaf scald	*Rhynchosporium commune*	198 DH lines	SBCC145 × Beatrix	11_0010 and 11_0823		*Rrs1*	Hofmann et al. (2013)
			168 DH lines	SBCC154 × Beatrix				
10	Powdery mildew	*Blumeria graminis*	238 F_2 lines	Tiffany × PI296825	GBM1126 and GBM1060		*Gene (7HS)*	Soldanova et al. (2013)
			498 F_2lines	Tiffany × PI466461	GBMS192 and GBM1060			
11	Leaf rust	*Puccinia hordei*	91 DH lines	MBR1012 × Scarlett	GBS546 and GBMS187	*SNP* and *SSR*	*Rph* $_{MBR1012}$	König et al. (2012)
12	Yellow mosaic virus	*Polymyxa graminis*	61 DH lines	Daisen-gold × PK23-2	ABG070 and Bmag0490	*CAPS* and *SSR*	*Rym17* and *rym18*	Kai et al. (2012)

(continued)

Table 11.4 (continued)

Sl. no.	Disease	*Pathogen*	Population	Parental cross	Linked/flanking markers	Marker type	Gene name	References
15	*Septoria* speckled leaf blotch	*Septoria passerinii*	115 F_4 RILs	M120 × Sep2-72	Bmag500	*SSR*	*Rsp4*	St. Pierre et al. (2010)
16	Wheat stripe rust	*Puccinia striiformis f. sp. tritici*	147 BC_1 plants	Y12 × Y16	XEBmac0755 and XAWBMS0022	*SSR*	*YrpstY1*	Sui et al. (2010)
17	Leaf scald	*Rhynchosporium secalis*	9179 F_2 plants	Atlas × Steffi	693M6_6 and P1D23R		*Rrs2*	Hanemann et al. (2009)
18	*Septoria* speckled leaf blotch	*Septoria passerinii*	100 to 120 F_2	Robust × CIho 14300	bPb-6978 and bPb-9945		*Rsp1*	Lee and Neate (2007)
				Robust × CIho 4780	MWG938 and OPAH5545C		*Rsp2*	
				Robust × CIho 10644	OPBA12314C and OPB17451R		*Rsp3*	
19	Net blotch	*Pyrenophora teres Drechs. f. teres*	119 DH lines	Rolfi × CI 9819	HVM14 and HVM65	*SSR*	*Rpt5*	Manninen et al. (2006)
20	*Septoria* speckled leaf blotch (SSLB)	*Septoria passerinii*	$F_{2:3}$ families	Foster × CIho 4780	Act8 and ksuD14		*Rsp2*	Zhong et al. (2006)
				Foster × CIho 10644	E-ACT/M-CAA-170a		*Rsp3*	
21	Barley stripe rust	*Puccinia striiformis f. sp. hordei*	F_8 RILs	Steptoe × Grannenlose Zweizeilige	EBmac0679	*SSR*	*rpsGZ*	Yan and Chen (2006)
22	Spot blotch	*Cochliobolus sativus*	110 DH lines	Calicuchima-sib × Bowman-BC	cer-yy and Hor2		*Rcs6*	Bilgic et al. (2006)
23	Barley yellow dwarf virus	*Luteoviruses*	103 F_9 RILs	L94 × Vada	HVM22	*SSR*	*Ryd3*	Niks et al. (2004)
24	Leaf stripe	*Pyrenophora graminea*	1400 F_2 Lines	*Thibaut* × *Mirco*	ssCH4 and MWG851		*Rdg2a*	Bulgarelli et al. (2004)

Table 11.5 Summary of successful reports about MAS and disease resistance in barley

Sl. no.	Donor line(s)	Recurrent line(s)	Gene(s)	Disease	Pathogen(s)	Marker type	Marker(s) name	References
1	Sel160 and WI2291	Arta and Tadmor	Rrs1	Scald	*Rhynchosporium commune*	SSR	Bmac209, HVS3, Bmac67, Ebmac871, and Bmag0006	Sayed and Baum (2018)
2	Romanze	Copia, Kamil and Luxor, and KM-104	rym4	*Barley yellow mosaic virus* complex (BaYMV/ BaMMV)	*Barley yellow mosaic virus* *Barley mild mosaic virus*	STS	MWG838	Hudcovicová et al. (2008)
	Russia 57		rym11			SSR	HVM3	
	Sutter and Shannon	Nitran and Ludan and line SK 5104	Ryd2	Barley yellow dwarf virus disease (BYDV)	*Barley yellow dwarf virus*		Ylp	
3	F1 (SH00752 × SH01470)	CDC McGwire	Run8	True loose smut	*Ustilago nuda*	SCAR	Un8-700R	Grewal et al. (2008b)
			Ruhq	Covered smut	*U. hordei*	STS and RAPD	aHor 2 and OPO6780	
4	Sep1-29 and Sep1-44	M110 and M96-46	Rsp2	Septoria speckled leaf blotch (SSLB)	*Septoria passerinii*	SCAR	E-ACT/M-CAA-170a	Zhong et al. (2006)
5	BCD47 and BCD12	Baronesse	QTL 1H	Barley stripe rust	*Puccinia striiformis f.sp. hordei*	SSR	GMS021, k06267, Bmac0213, and Bmac0399	Richardson et al. (2006)
			QTL 4H				EBmac0679, EBmac0788, and HvMLO3	
			QTL 5H				Bmag0337 and GBM1039	

(continued)

Table 11.5 (continued)

Sl. no.	Donor line(s)	Recurrent line(s)	Gene(s)	Disease	Pathogen(s)	Marker type	Marker(s) name	References
6	261B1	Emir	Rrs16Hb	Scald	*Rhynchosporium secalis*	STS and SSR	Xmwg634, Xwg622, and Xiac511	Pickering et al. (2006)
7	Franklin	Sloop	Yd2	Barley yellow dwarf virus disease	*Barley yellow dwarf virus*	PCR	YLM	Jefferies et al. (2003)
8	Franka	Igri	ym4	Barley yellow mosaic disease	*Barley yellow mosaic virus (BaYMV), barley mild mosaic virus (BaMMV)*	RAPD	OP-Z04H660	Ordon et al. (1995)

11.3.4 TILLING

It is a general reverse genetics strategy developed a decade ago which includes mutagenesis of a large plant population followed by identification of point mutations in the gene of interest via high-throughput detection system (McCallum et al. 2000a; Comai et al. 2004; Talamè et al. 2008; Gottwald et al. 2009; Jost et al. 2019). On contrary to QTL mapping, there are very few reports in the literature related to TILLING and disease resistance in barley (Talamè et al. 2008; Gottwald et al. 2009). However, TILLMore (Talamè et al. 2008) and HorTILLUS (Szurman-Zubrzycka et al. 2018) are available public platforms resource developed for forward genetics and reverse genetics in barley.

A 10,279 M2size TILLING population was created using the barley cultivar 'Barke'. Mutations were identified in various genes including HvCIGR2, *HvHox1*, and *Mlo9* by screening the full M2size population. The Mlo9-identified mutants exhibited resistance to powdery mildew. These mutations constituted a link between the gene and the disease resistance (Gottwald et al. 2009). Talame and colleagues (2008) analyzed NaN_3-induced mutations in 3148 M2size barley cultivar 'Morex' TILLING population using LI-COR detection technology. The Rpg1-gene-identified mutants exhibited resistance to barley stem rust. In another report, a10,389 M2size TILLING population was created using the cultivar 'Tamalpais'. The COI1-gene-identified mutants exhibited high resistance to leaf spot and leaf stripe (Qi et al. 2012). Similarly, Hu et al. (2012) generated an M2size population of 2154 using the barley cultivar 'Tamalpais'. They screened the whole M2 population and identified that EDR1 and NPR1 mutants had enhanced multiple disease resistance. Table 11.6 summarizes about the successful reports on TILLING for enhancement of disease resistance in barley.

11.3.5 Transgenics

In the last two decades, various ushered breakthroughs in science have permitted the genes to be identified and manipulated as molecules (Chawla 2009; Gresshoff 2017; Mall et al. 2019). The biotechnology tools have changed the way to address problems in agriculture (Jones 2016). In various crops including barley, biotic stress tolerance is one major area facing changes as a result of this new technology (Chopra and Saini 2014; Harwood 2016; Pessarakli 2016; Harwood 2019). Various diseases in barley significantly cause economic losses to farmers. Additionally, there are very fewer effective chemicals available (Cunniffe et al. 2015; Roberts and Mattoo 2018). In addition, to make the situation worse, conventional breeding techniques consume a large span of time. As a result, the most effective and reliable option to develop resistant plants is through biotechnological interventions, such as genetic (Risk et al. 2013) engineering, RNA silencing technologies, and gene editing (Eichmann et al. 2010; Risk et al. 2013; Hatta et al. 2018). Since the first report on

Table 11.6 Summary of successful reports regarding TILLING and disease resistance in barley

Sl. no.	Genotype used	Mutagen	M2size	Mutation frequency (1/kb)	Traits	Country	Mutation detection technology	Genes screened	References
1	Tamalpais	EMS	10,389	1/673	Leaf stripe and leaf spot resistance	China	NA	COI1	Qi et al. (2012)
2	Tamalpais	EMS	2154	N.A.	Multiple disease resistance	China	CEL I	EDR1 and NPR1	Hu et al. (2012)
3	Barke	EMS	10,279	1/500	Resistance to powdery mildew	Germany	LI-COR	HvHox1, HvCO1, Mlo9, HveIF4E, HvDnaJ-like, and HvCIGR2	Gottwald et al. (2009)
4	Morex	NaN_3	3148	1/374	Virus resistance and resistance to barley stem rust	Italy	LI-COR	HvCO1, Rpg1, eIF4E, and NR	Talame et al. (2008)

the transgenic barley in 1994 (Ritala et al.), enormous progress has been made in this field of barley transgenics.

The most common method for introducing the transgene is cocultivation of tissue cultures with *Agrobacterium tumifaciens* or *A. rhizogenes* containing a transgene(s) (Risk et al. 2013; Hao et al. 2018; Hatta et al. 2018; Mall et al. 2019). Other methods include particle bombardment of tissue cultures, microinjection, and electroporation (Travella et al. 2005; Manoharan et al. 2006; Eichmann et al. 2010; Mall et al. 2019). Once the gene is transferred and integrated into the host genome, various types of methods are used to regenerate the entire transgenic plant. Detailed knowledge of plant gene structure, the regulatory mechanism for particular molecular responses to the pathogen, and the pathogen molecular organization is the most important prerequisite in the development of transgenic plants with increased disease resistance. As a result, many genes have been transferred into the barley for enhancing disease resistance to multiple pathogens (Cejnar et al. 2018) including *Vst1* (Leckband and Lörz 1998), *Rpg1* (Horvath et al. 2003), *Mtk* (Rahnamaeian et al. 2009), *Lr34res* (Risk et al. 2013), *LEMK1* (Rajaraman et al. 2016), *CslD2* (Douchkov et al. 2016), *CSD1* (Lightfoot et al. 2017), and *Sr22* (Hatta et al. 2018). Considerable progress that has been achieved in the transformation of barley for enhancing disease resistance is outlined in Table 11.7.

Next to stable transformation, various researchers have used transient expression assays over the last decades to understand the mechanism of disease resistance (Christensen et al. 2004; Nowara et al. 2010; Pliego et al. 2013; Kis et al. 2016). The different mechanisms include microRNAs, RNAi, virus-induced gene silencing (VIGS), and host-induced gene silencing (VIGS) (Douchkov et al. 2016; Kis et al. 2016). The first report in the literature was by Christensen et al. (2004). They transferred the germin-like proteins to the leaves of barley cultivar Golden Promise using particle bombardment technique and performed the GUS assay. Followed by this report, there was a huge surge in the number of publications regarding transient assays and disease resistance (Hein et al. 2005; Babaeizad et al. 2009; Rahnamaeian et al. 2009; Eichmann et al. 2010; Rajaraman et al. 2016).

As compared to the other genetic engineering methods, gene editing has emerged as the biggest invention in the biotechnology era (Jones 2016; Kis et al. 2019). It is the most important biotechnological tool used which utilizes ZFNs, TALENs, and CRISPR/Cas9 system (Jones 2016). In recent years, CRISPR/Cas-based gene editing has emerged as the novel, efficient, and precise technique which surpasses the limitations of conventional breeding approach (Harwood 2016; Jones 2016). This method has applications including providing higher yield and biofortification and improving stress tolerance in multiple economically important crops (Harwood 2016). To date, the only report of gene editing in barley for enhancing disease resistance is by groups of Kis et al. (2019) (Table 11.8). They used the CRISPR/Ca9 system to show the antiviral effect on the *wheat dwarf virus* in barley cultivar Golden Promise. First, they performed in silico studies to identify the potential sites for sgRNA target sequences. They generated four different sgRNAs which show complementarity to the different genomic regions.

Table 11.7 Summary of successful reports regarding transgenics and disease resistance in barley

Sl. no.	Method	Gene	Compounds	Tissue	Selection	Pathogen	Promoter/vector	Accession name	References
Stable transgenics									
1	*Agrobacterium*	*RepA*	Replication-associated gene	Immature embryos	Hygromycin	*Wheat dwarf virus*	pIPKb002	Golden Promise	Cejnar et al. (2018)
2	*Agrobacterium*	*Sr22*	Stem rust resistance gene	Immature embryos	Hygromycin	*Puccinia hordei*	pVec8	Golden Promise	Hatta et al. (2018)
3	*Agrobacterium*	*ICS*	Isochorismate synthase	Immature embryos	Glufosinate ammonium	*Fusarium graminearum*	PC186	Golden Promise	Hao et al. (2018)
4	*Agrobacterium*	*ADH-1*	Alcohol dehydrogenase 1	Immature embryos	Hygromycin	*Blumeria graminis f. sp. hordei*	pIPKb007	Golden Promise	Käsbauer et al. (2018)
5	*Agrobacterium*	*Lr67res*	Lr67 hexose transporter variant	Immature embryos	Hygromycin	*Puccinia hordei*	pVec8	Golden Promise	Milne et al. (2018)
6	*Agrobacterium*	*CSD1*	Superoxide dismutase	Immature embryos	Hygromycin	*Pyrenophora teres f. teres*	pSTARGATE	Golden Promise	Lightfoot et al. (2017)
7	*Agrobacterium*	*CslD2*	Cellulose synthase-like D2	Immature embryos	Hygromycin	*Blumeria graminis*	pIPKb009	Golden Promise	Douchkov et al. (2016)
8	*Agrobacterium*	*LEMK1*	LRR-malectin domain-containing transmembrane RLK	Immature embryos	Hygromycin	*Blumeria graminis f.sp. tritici*	pIPKb009	Golden Promise	Rajaraman et al. (2016)
9	*Agrobacterium*	*Lr34res*	ABC transporter	Immature embryos	Hygromycin	*P. graminis* f.sp. *tritici*	P6u and pWBVec8	Golden Promise	Risk et al. (2013)
10	Biolistics-mediated transformation	*BI-1*	BAX inhibitor-1	Epidermal cells	GUS	*Blumeria graminis* f.sp. *hordei*	pIPKTA30N	Golden Promise	Eichmann et al. (2010)
11	*Agrobacterium*	*BI-1*	BAX inhibitor-1	Immature embryos	Hygromycin	*Blumeria graminis* f.sp. *hordei*	pLH6000	Golden Promise	Babaeizad et al. (2009)

12	*Agrobacterium*	*Mtk*	Metchnikowin	NA	PCR	*Blumeria graminis* f. sp. *hordei*	pLH6000	Golden Promise	Rahnamaeian et al. (2009)
13	Particle bombardment	*FsTri101*	3-O-acetyltransferase	Immature embryos	FINALE	*Fusarium graminearum*	pUBR1	Conlon	Manoharan et al. (2006)
14	*Agrobacterium*	*racb-G15V*	RAC/ROP family G protein	Immature embryos	Hygromycin	*Blumeria graminis* f.sp. *hordei*	pSB181	Golden Promise	Schultheiss et al. (2005)
15	*Agrobacterium*	*Rpg1*	Receptor-like protein	Immature embryos	PCR	*Puccinia graminis* f. sp. *tritici*.	pNRG040	Golden Promise	Horvath et al. (2003)
16	*Agrobacterium*	*ORFs*	Virus resistance genes	Immature embryos	Hygromycin	Barley yellow dwarf virus	pWBVec8 and/or pWBVec5	Schooner	Wang et al. (2001)
17	Particle bombardment	*Vst1*	Phytoalexin, Resveratrol	Microspores	Basta	*Botrytis cinerea*	Stilbene synthase	Igri	Leckband and Lorz (1998)
Transient transgenics									
18	Particle bombardment	*CslD2*	Cellulose synthase-like D2	Leaves (7-day seedling)	GUS	*Blumeria graminis* f. sp. *tritici* Em. Marchal	NA	Maythorpe	Douchkov et al. (2016)
19	*Agrobacterium*	amiRNAs	Artificial microRNAs	Immature embryos	Hygromycin	*Wheat dwarf virus*	pCUbiVirusBuster171	Golden Promise	Kis et al. (2016)
20	Particle bombardment	*LEMK1*	LRR-malectin domain-containing transmembrane RLK	Leaves (7-day seedling)	GUS	*Blumeria graminis* f.sp. *tritici*	NA	Maythorpe	Rajaraman et al. (2016)
21	Particle bombardment	*BEC*	Blumeria effector candidates	Leaves (7-day seedling)	GUS	*Blumeria graminis f. sp. hordei*	pTA30	Golden Promise	Pliego et al. (2013)

(continued)

Table 11.7 (continued)

Sl. no.	Method	Gene	Compounds	Tissue	Selection	Pathogen	Promoter/vector	Accession name	References
22	*Agrobacterium*	*Mtk*	Metchnikowin	NA	GFP	*Blumeria graminis f. sp. hordei*	pGY1-GFP expression vector	Golden Promise	Rahnamaeianand Vilcinskas (2012)
23	*Agrobacterium*	*BI-1*	BAX inhibitor-1	Immature embryos	Hygromycin	*Blumeria graminis* f.sp. *hordei*	pIPKTA30N-BI-1	Ingrid	Eichmann et al. (2010)
24	Particle bombardment; Agrobacterium	*Avra10; GTF1*	Effector gene; 1,3-β-glucanosyl-transferase	Leaves (7-day seedling); immature embryos	GUS; hygromycin	*Blumeria graminis f. sp. hordei*	pIPKTA30N, pIPKb007_BgGTF1	Golden Promise	Nowara et al. (2010)
26	*Biolistic transformation*	*BI-1*	BAX inhibitor-1	Epidermal cells	GFP	*Blumeria graminis f.sp. hordei*	NA	Golden Promise	Babaeizad et al. (2009)
27	*Barley stripe mosaic virus*	*PDS*	Phytoene desaturase	NA	NA	*Blumeria graminis* f.sp. *hordei*	BSMV	Clansman	Hein et al. (2005)
28	Particle bombardment	*GLP4 and TaGLP4*	Germin-like proteins (superoxide dismutase)	Leaves (7-day seedling)	GUS assay	*Blumeria graminis f. sp. Hordei*	pGY1	Golden Promise	Christensen et al. (2004)

Table 11.8 Gene editing study for disease resistance in barley

Sl. no.	Transformation method	Tissue	Gene(s)	Protein	Cultivar	Vector	Pathogen	Selection	References
1	*Agrobacterium tumefaciens*	Immature embryos	*MP* and *CP* coding sequence	Movement protein and coat protein	Golden Promise	pKSE401	*Wheat dwarf virus*	PCR	Kis et al. (2019)
			Rep/RepA	Replication A protein					
			LIR	Long inverted repeat					
			C-terminus of *Rep*	Replication A protein part					

They evaluated the transgenic plants using infection processes, northern blot analyses, and PCR analysis. They confirmed the developed transgenic lines were fully resistant to *wheat dwarf virus* infection. Thus, the use of CRISPR/Cas9 gene editing system will contribute significantly to develop improved barley varieties against more diseases.

Apart from the approaches discussed in the book chapter, there are more new innovative emerging technologies like reverse breeding, cisgenesis, intragenesis, and synthetic biology used by researchers and breeders. Unlike other crops, there is no single report in the literature related to these technologies for enhancing disease resistance in barley. However, in a medium to long run, there will be many successful reports in the near future.

11.4 Concluding Remarks

At present, barley is the fourth important cereal crop worldwide with major uses as infeed, beer production, spirit production, and food value chain. We have observed a relative increase in usage as well as the production of both types of barleys in tropical and temperate climates. Currently, stress resistance, yield stability, and quality characteristics are the top research areas for barley breeders. Recently, there is a positive development in the enhancement of durable resistance against an array of relevant pathogens due to the combination of conventional breeding with DH production, genomic tools, and molecular marker technology during the last two decades. Incorporation of nonclassical technologies has shortened the time between initial cross and release of improved disease-resistant varieties. This is even boosted by the availability of genomic sequences of rice, *Brachypodium*, sorghum, and wheat (more recently released), high-density maps, map-based cloning, genome-wide transcript profiling, genome editing techniques, and various bioinformatics tools to exploit the synteny between barley and these species.

In the near future, more phytopathogen resistance genes, alleles, and QTLs will be identified, isolated, mined, transferred, and introgressed into the elite-susceptible cultivars using molecular breeding strategies to enhance disease resistance. Taking these data altogether, all these advances have improved the disease resistance breeding programs for barley. However, on the medium to long run, the great potential in the integrated system of all these technologies will be tapped to react in a fast and directed manner to all the present situation challenges.

References

Ali MA, Shahzadi M, Zahoor A, Dababat AA, Toktay H, Bakhsh A et al (2019) Resistance to cereal cyst nematodes in wheat and barley: an emphasis on classical and modern approaches. Int J Mol Sci 20:e432

Arru L, Francia E, Pecchioni N (2003) Isolate-specific QTLs of resistance to leaf stripe (*Pyrenophora graminea*) in the'Steptoe'×'Morex'spring barley cross. Theor Appl Genet 106:668–675

Ashkani S, Rafii MY, Shabanimofrad M, Miah G, Sahebi M, Azizi P et al (2015) Molecular breeding strategy and challenges towards improvement of blast disease resistance in rice crop. Front Plant Sci 6:886

Babaeizad V, Imani J, Kogel K-H, Eichmann R, Hückelhoven R (2009) Over-expression of the cell death regulator BAX inhibitor-1 in barley confers reduced or enhanced susceptibility to distinct fungal pathogens. Theor Appl Genet 118:455–463

Badr A, Sch R, Rabey HE, Effgen S, Ibrahim H, Pozzi C et al (2000) On the origin and domestication history of barley (*Hordeum vulgare*). Mol Biol Evol 17:499–510

Barati M, Majidi MM, Mostafavi F, Mirlohi A, Safari M, Karami Z (2018) Evaluation of wild barley species as possible sources of drought tolerance for arid environments. Plant Genet Resour 16:209–217

Bartlett JG, Alves SC, Smedley M, Snape JW, Harwood WA (2008) High-throughput Agrobacterium-mediated barley transformation. Plant Methods 4:22

Behn A, Hartl L, Schweizer G, Wenzel G, Baumer M (2004) QTL mapping for resistance against non-parasitic leaf spots in a spring barley doubled haploid population. Theor Appl Genet 108:1229–1235

Bellard C, Bertelsmeier C, Leadley P, Thuiller W, Courchamp F (2012) Impacts of climate change on the future of biodiversity. Ecol Lett 15:365–377

Bilgic H, Steffenson B, Hayes P (2006) Molecular mapping of loci conferring resistance to different pathotypes of the spot blotch pathogen in barley. Phytopathology 96:699–708

Boivin NL, Zeder MA, Fuller DQ, Crowther A, Larson G, Erlandson JM et al (2016) Ecological consequences of human niche construction: examining long-term anthropogenic shaping of global species distributions. Proc Natl Acad Sci 113:6388–6396

Brown JK (1994) Chance and selection in the evolution of barley mildew. Trends Microbiol 2:470–475

Budhagatapalli N, Rutten T, Gurushidze M, Kumlehn J, Hensel G (2015) Targeted modification of gene function exploiting homology-directed repair of TALEN-mediated double strand breaks in barley. G3 (Bethesda) 5:1857–1863

Bulgarelli D, Collins N, Tacconi G, Dellaglio E, Brueggeman R, Kleinhofs A et al (2004) High-resolution genetic mapping of the leaf stripe resistance gene Rdg2a in barley. Theor Appl Genet 108:1401–1408

Burdon JJ, Laine A-L (2019) Evolutionary dynamics of plant pathogen interactions. Cambridge University Press, Cambridge

Cakir M, Gupta S, Li C, Hayden M, Mather DE, Ablett GA et al (2011) Genetic mapping and QTL analysis of disease resistance traits in the barley population Baudin× AC Metcalfe. Crop Pasture Sci 62:152–161

Case AJ, Bhavani S, Macharia G, Pretorius Z, Coetzee V, Kloppers F et al (2018) Mapping adult plant stem rust resistance in barley accessions Hietpas-5 and GAW-79. Theor Appl Genet 131:2245–2266

Castro A, Capettini F, Corey A, Filichkina T, Hayes P, Kleinhofs A et al (2003) Mapping and pyramiding of qualitative and quantitative resistance to stripe rust in barley. Theor Appl Genet 107:922–930

Castro AJ, Gamba F, German S, Gonzalez S, Hayes PM, Pereyra S et al (2012) Quantitative trait locus analysis of spot blotch and leaf rust resistance in the BCD47× Baronesse barley mapping population. Plant Breed 131:258–266

Cazalis V, Loreau M, Henderson K (2018) Do we have to choose between feeding the human population and conserving nature? Modelling the global dependence of people on ecosystem services. Sci Total Environ 634:1463–1474

Ceccarelli S, Grando S, Hamblin J (1992) Relationship between barley grain yield measured in low-and high-yielding environments. Euphytica 64:49–58

Cejnar P, Ohnoutková L, Ripl J, Vlčko T, Kundu JK (2018) Two mutations in the truncated Rep gene RBR domain delayed the Wheat dwarf virus infection in transgenic barley plants. J Integr Agric 17:2492–2500

Chawla HS (2009) Introduction to plant biotechnology (3/e). CRC Press, Boca Raton

Chen G, Liu Y, Ma J, Zheng Z, Wei Y, McIntyre CL et al (2013a) A novel and major quantitative trait locus for Fusarium crown rot resistance in a genotype of wild barley (*Hordeum spontaneum* L.). PLoS One 8:e58040

Chen G, Liu Y, Wei Y, McIntyre C, Zhou M, Zheng Y-L et al (2013b) Major QTL for *Fusarium crown* rot resistance in a barley landrace. Theor Appl Genet 126:2511–2520

Chopra R, Saini R (2014) Transformation of blackgram (*Vigna mungo* (L.) Hepper) by barley chitinase and ribosome-inactivating protein genes towards improving resistance to *Corynespora* leaf spot fungal disease. Appl Biochem Biotechnol 174:2791–2800

Christensen AB, Thordal-Christensen H, Zimmermann G, Gjetting T, Lyngkjær MF, Dudler R et al (2004) The germinlike protein GLP4 exhibits superoxide dismutase activity and is an important component of quantitative resistance in wheat and barley. Mol Plant-Microbe Interact 17:109–117

Chutimanitsakun Y, Nipper RW, Cuesta-Marcos A, Cistué L, Corey A, Filichkina T et al (2011) Construction and application for QTL analysis of a Restriction Site Associated DNA (RAD) linkage map in barley. BMC Genomics 12:4

Comai L, Young K, Till BJ, Reynolds SH, Greene EA, Codomo CA et al (2004) Efficient discovery of DNA polymorphisms in natural populations by Ecotilling. Plant J 37:778–786

Cunniffe NJ, Koskella B, Metcalf CJE, Parnell S, Gottwald TR, Gilligan CA (2015) Thirteen challenges in modelling plant diseases. Epidemics 10:6–10

Dai F, Zhang G (2016) Domestication and improvement of cultivated barley. In: Exploration, identification and utilization of barley germplasm. Academic Press, pp 1–26. Available at https://www.sciencedirect.com/science/article/pii/B9780128029220000017

Dawson AM, Ferguson JN, Gardiner M, Green P, Hubbard A, Moscou MJ (2016) Isolation and fine mapping of Rps6: an intermediate host resistance gene in barley to wheat stripe rust. Theor Appl Genet 129:831–843

del Blanco IA, Hegarty J, Gallagher L, Falk B, Brown-Guedira G, Pellerin E et al (2014) Mapping of QTL for tolerance to Cereal yellow dwarf virus in two-rowed spring barley. Crop Sci 54:1468–1475

Douchkov D, Lueck S, Hensel G, Kumlehn J, Rajaraman J, Johrde A et al (2016) The barley (*Hordeum vulgare*) cellulose synthase-like D2 gene (HvCslD2) mediates penetration resistance to host-adapted and nonhost isolates of the powdery mildew fungus. New Phytol 212:421–433

Dracatos PM, Khatkar MS, Singh D, Park RF (2014) Genetic mapping of a new race specific resistance allele effective to Puccinia hordei at the Rph9/Rph12 locus on chromosome 5HL in barley. BMC Plant Biol 14:1598

Drader T, Kleinhofs A (2010) A synteny map and disease resistance gene comparison between barley and the model monocot *Brachypodium distachyon*. Genome 53:406–417

Eichmann R, Bischof M, Weis C, Shaw J, Lacomme C, Schweizer P et al (2010) BAX INHIBITOR-1 is required for full susceptibility of barley to powdery mildew. Mol Plant-Microbe Interact 23:1217–1227

Esvelt KK, Gordon T, Bregitzer P, Hayes P, Chen X, Del Blanco I et al (2016) Barley stripe rust resistance QTL: development and validation of SNP markers for resistance to *Puccinia striiformis* f. sp. hordei. Phytopathology 106:1344–1351

FAOSTAT FPAC (2016) Food and agriculture organization of the United Nations, Roma, p 2010

Francia E, Tondelli A, Rizza F, Badeck FW, Nicosia OLD, Akar T et al (2011) Determinants of barley grain yield in a wide range of Mediterranean environments. Field Crop Res 120:169–178
Frantzeskakis L, Kracher B, Kusch S, Yoshikawa-Maekawa M, Bauer S, Pedersen C et al (2018) Signatures of host specialization and a recent transposable element burst in the dynamic one-speed genome of the fungal barley powdery mildew pathogen. BMC Genomics 19:381
Friedt W, Horsley RD, Harvey BL, Poulsen DME, Lance RCM, Ceccarelli S, Grando S, Capettini F (2011) Barley breeding history, progress, objectives, and technology. In: Barley: production, improvement, and uses. Blackwell Publishing, pp 160–220. ISBN 9780813801230, 9780470958636
Gottwald S, Bauer P, Komatsuda T, Lundqvist U, Stein N (2009) TILLING in the two-rowed barley cultivar'Barke'reveals preferred sites of functional diversity in the gene HvHox1. BMC Res Notes 2:258
Graner A, Streng S, Drescher A, Jin Y, Borovkova I, Steffenson B (2000) Molecular mapping of the leaf rust resistance gene Rph7 in barley. Plant Breed 119:389–392
Gresshoff PM (2017) Technology transfer of plant biotechnology. CRC Press. Available at https://www.taylorfrancis.com/books/e/9780203737323
Grewal T, Rossnagel B, Pozniak C, Scoles G (2008a) Mapping quantitative trait loci associated with barley net blotch resistance. Theor Appl Genet 116:529–539
Grewal TS, Rossnagel BG, Scoles GJ (2008b) Validation of molecular markers for covered smut resistance and marker-assisted introgression of loose and covered smut resistance into hulless barley. Mol Breed 21:37–48
Grewal TS, Rossnagel BG, Scoles GJ (2012) Mapping quantitative trait loci associated with spot blotch and net blotch resistance in a doubled-haploid barley population. Mol Breed 30:267–279
Gupta S, Li C, Loughman R, Cakir M, Platz G, Westcott S et al (2010) Quantitative trait loci and epistatic interactions in barley conferring resistance to net type net blotch (*Pyrenophora teres* f. teres) isolates. Plant Breed 129:362–368
Haas M, Menke J, Chao S, Steffenson BJ (2016) Mapping quantitative trait loci conferring resistance to a widely virulent isolate of *Cochliobolus sativus* in wild barley accession PI 466423. Theor Appl Genet 129:1831–1842
Hamwieh A, Alo F, Ahmed S (2018) Molecular tools developed for disease resistant genes in wheat, barley, lentil and chickpea: a review. Arab J Plant Protect 36:50–56
Hanemann A, Schweizer GF, Cossu R, Wicker T, Röder MS (2009) Fine mapping, physical mapping and development of diagnostic markers for the Rrs2 scald resistance gene in barley. Theor Appl Genet 119:1507–1522
Hansson M, Komatsuda T, Stein N, Muehlbauer GJ (2018) Molecular mapping and cloning of genes and QTLs. In: The barley genome. Springer, Cham, pp 139–154. Available at https://link.springer.com/chapter/10.1007/978-3-319-92528-8_10
Hao Q, Wang W, Han X, Wu J, Lyu B, Chen F et al (2018) Isochorismate-based salicylic acid biosynthesis confers basal resistance to *Fusarium graminearum* in barley. Mol Plant Pathol 19:1995–2010
Harwood W (2016) Barley as a cereal model for biotechnology applications. In: Jones HD (ed) Biotechnology of major cereals. CABI, Wallingford, pp 80–87
Harwood WA (2019) An introduction to barley: the crop and the model. Springer, Barley, pp 1–5
Hatta MAM, Johnson R, Matny O, Smedley MA, Yu G, Chakraborty S et al (2018) The wheat Sr22, Sr33, Sr35 and Sr45 genes confer resistance against stem rust in barley. bioRxiv. https://doi.org/10.1101/374637
Hecht VL, Temperton VM, Nagel KA, Rascher U, Postma JA (2016) Sowing density: a neglected factor fundamentally affecting root distribution and biomass allocation of field grown spring barley (*Hordeum vulgare* L.). Front Plant Sci 7:944
Hein I, Barciszewska-Pacak M, Hrubikova K, Williamson S, Dinesen M, Soenderby IE et al (2005) Virus-induced gene silencing-based functional characterization of genes associated with powdery mildew resistance in barley. Plant Physiol 138:2155–2164

Hickey L, Lawson W, Platz G, Dieters M, Arief V, German S et al (2011) Mapping Rph20: a gene conferring adult plant resistance to *Puccinia hordei* in barley. Theor Appl Genet 123:55–68

Hickey LT, Lawson W, Platz GJ, Fowler RA, Arief V, Dieters M et al (2012) Mapping quantitative trait loci for partial resistance to powdery mildew in an Australian barley population. Crop Sci 52:1021–1032

Hofmann K, Silvar C, Casas AM, Herz M, Büttner B, Gracia MP et al (2013) Fine mapping of the Rrs1 resistance locus against scald in two large populations derived from Spanish barley landraces. Theor Appl Genet 126:3091–3102

Holzberg S, Brosio P, Gross C, Pogue GP (2002) Barley stripe mosaic virus-induced gene silencing in a monocot plant. Plant J 30:315–327

Hori K, Sato K, Kobayashi T, Takeda K (2006) QTL analysis of *Fusarium* head blight severity in recombinant inbred population derived from a cross between two-rowed barley varieties. Breed Sci 56:25–30

Horler R, Turner A, Fretter P, Ambrose M (2017) SeedStor: a germplasm information management system and public database. Plant Cell Physiol 59:e5–e5

Horsley RD, Schmierer D, Maier C, Kudrna D, Urrea CA, Steffenson BJ et al (2006) Identification of QTLs associated with *Fusarium* head blight resistance in barley accession CIho 4196. Crop Sci 46:145–156

Horvath H, Rostoks N, Brueggeman R, Steffenson B, Von Wettstein D, Kleinhofs A (2003) Genetically engineered stem rust resistance in barley using the Rpg1 gene. Proc Natl Acad Sci 100:364–369

Hu X, Qi X-l, Lv B, J-j W, D-l F (2012) TILLING-based analysis of disease resistance genes in barley [J]. Journal of Shandong Agricultural University (Natural Science Edition) 1:002

Huang Y, Haas M, Heinen S, Steffenson BJ, Smith KP, Muehlbauer GJ (2018) QTL mapping of fusarium head blight and correlated agromorphological traits in an elite barley cultivar Rasmusson. Front Plant Sci 9:1260

Hudcovicová M, Šudyová V, Šliková S, Gregová E, Kraic J, Ordon F et al (2008) Marker-assisted selection for the development of improved barley and wheat lines. Acta Agronomica Hungarica 56:385–392

Jefferies S, King B, Barr A, Warner P, Logue S, Langridge P (2003) Marker-assisted backcross introgression of the Yd2 gene conferring resistance to barley yellow dwarf virus in barley. Plant Breed 122:52–56

Jena KK, Mackill DJ (2008) Molecular markers and their use in marker-assisted selection in rice. Crop Sci 48:1266–1276

Jones HD (ed) (2016) Biotechnology of major cereals. CABI. Available at https://books.google.co.in/books?hl=en&lr=&id=9AWuDQAAQBAJ&oi=fnd&pg=PR3&dq=Biotechnology+of+major+cereals&ots=NUFuR1QBFd&sig=qvoo2R7x4uVPEpbNGe19CoZ5Ed4#v=onepage&q=Biotechnology%20of%20major%20cereals&f=false

Jost M, Szurman-Zubrzycka M, Gajek K, Szarejko I, Stein N (2019) TILLING in barley. Springer, Barley, pp 73–94

Kai H, Takata K, Tsukazaki M, Furusho M, Baba T (2012) Molecular mapping of Rym17, a dominant and rym18 a recessive barley yellow mosaic virus (BaYMV) resistance genes derived from *Hordeum vulgare* L. Theor Appl Genet 124:577–583

Käsbauer CL, Pathuri IP, Hensel G, Kumlehn J, Hückelhoven R, Proels RK (2018) Barley ADH-1 modulates susceptibility to Bgh and is involved in chitin-induced systemic resistance. Plant Physiol Biochem 123:281–287

Kis A, Hamar É, Tholt G, Bán R, Havelda Z (2019) Creating highly efficient resistance against Wheat dwarf virus in barley by employing CRISPR/Cas9 system. Plant Biotechnol J. https://doi.org/10.1111/pbi.13077

Kis A, Tholt G, Ivanics M, Várallyay É, Jenes B, Havelda Z (2016) Polycistronic artificial miRNA-mediated resistance to W heat dwarf virus in barley is highly efficient at low temperature. Mol Plant Pathol 17:427–437

König J, Kopahnke D, Steffenson B, Przulj N, Romeis T, Röder M et al (2012) Genetic mapping of a leaf rust resistance gene in the former Yugoslavian barley landrace MBR1012. Mol Breed 30:1253–1264

Lawrenson T, Shorinola O, Stacey N, Li C, Østergaard L, Patron N et al (2015) Induction of targeted, heritable mutations in barley and Brassica oleracea using RNA-guided Cas9 nuclease. Genome Biol 16:258

Le Gouis J, Devaux P, Werner K, Hariri D, Bahrman N, Beghin D et al (2004) RYM15 from the Japanese cultivar Chikurin Ibaraki 1 is a new barley mild mosaic virus (BaMMV) resistance gene mapped on chromosome 6H. Theor Appl Genet 108:1521–1525

Leckband G, Lörz H (1998) Transformation and expression of a stilbene synthase gene of *Vitis vinifera* L. in barley and wheat for increased fungal resistance. Theor Appl Genet 96:1004–1012

Lee S, Neate S (2007) Molecular mapping of Rsp 1, Rsp 2, and Rsp 3 genes conferring resistance to Septoria speckled leaf blotch in barley. Phytopathology 97:155–161

Leng Y, Zhao M, Wang R, Steffenson BJ, Brueggeman RS, Zhong S (2018) The gene conferring susceptibility to spot blotch caused by *Cochliobolus sativus* is located at the Mla locus in barley cultivar Bowman. Theor Appl Genet 131:1531–1539

Li C, Gupta S, Zhang X-Q, Westcott S, Yang J, Park R et al (2013) A major QTL controlling adult plant resistance for barley leaf rust. In: Advance in barley sciences. Springer, Dordrecht, pp 285–300

Li H, Zhou M (2011) Quantitative trait loci controlling barley powdery mildew and scald resistances in two different barley doubled haploid populations. Mol Breed 27:479–490

Li H, Zhou M, Liu C (2009) A major QTL conferring crown rot resistance in barley and its association with plant height. Theor Appl Genet 118:903–910

Li J, Huang X, Heinrichs F, Ganal M, Röder M (2006) Analysis of QTLs for yield components, agronomic traits, and disease resistance in an advanced backcross population of spring barley. Genome 49:454–466

Lightfoot DJ, Mcgrann GR, Able AJ (2017) The role of a cytosolic superoxide dismutase in barley–pathogen interactions. Mol Plant Pathol 18:323–335

Lindley J, Techera EJ, Webster D (2019) 11 Extreme human behaviours affecting marine resources and industries. Marine Extremes: Ocean Safety, Marine Health and the Blue Economy 63

Liu BH (2017) Statistical genomics: linkage, mapping, and QTL analysis. CRC press, Boca Raton

Looseley M, Newton A, Atkins SD, Fitt BD, Fraaije B, Thomas W et al (2012) Genetic basis of control of *Rhynchosporium secalis* infection and symptom expression in barley. Euphytica 184:47–56

Ma Z, Lapitan NL, Steffenson B (2004) QTL mapping of net blotch resistance genes in a doubled-haploid population of six-rowed barley. Euphytica 137:291–296

Mace E (2016) Molecular biology support for barley improvement-North. Available at http://era.daf.qld.gov.au/id/eprint/5695/

Maguire K, Charlton W, Yoxall T, Burnett F. (2018) The challenges of managing multiple barley pathogens in winter and spring barley. The Dundee Conference. Crop Production in Northern Britain 2018, Dundee, UK, 27–28 February 2018. The Association for Crop Protection in Northern Britain. p. 73–78

Mall T, Gupta M, Dhadialla TS, Rodrigo S (2019) Overview of biotechnology-derived herbicide tolerance and insect resistance traits in plant agriculture. In: Kumar S, Barone P, Smith M (eds) Transgenic plants: methods and protocols. Springer New York, New York, pp 313–342

Mammadov J, Zwonitzer J, Biyashev R, Griffey C, Jin Y, Steffenson B et al (2003) Molecular mapping of leaf rust resistance gene Rph 5 in barley. Crop Sci 43:388–393

Manninen O, Jalli M, Kalendar R, Schulman A, Afanasenko O, Robinson J (2006) Mapping of major spot-type and net-type net-blotch resistance genes in the Ethiopian barley line CI 9819. Genome 49:1564–1571

Manoharan M, Dahleen LS, Hohn TM, Neate SM, Yu X-H, Alexander NJ et al (2006) Expression of 3-OH trichothecene acetyltransferase in barley (*Hordeum vulgare* L.) and effects on deoxynivalenol. Plant Sci 171:699–706

Mascher M, Gundlach H, Himmelbach A, Beier S, Twardziok SO, Wicker T et al (2017) A chromosome conformation capture ordered sequence of the barley genome. Nature 544:427–433

McCallum CM, Comai L, Greene EA, Henikoff S (2000a) Targeted screening for induced mutations. Nat Biotechnol 18:455–457

McCallum CM, Comai L, Greene EA, Henikoff S (2000b) Targeting induced local lesions in genomes (TILLING) for plant functional genomics. Plant Physiol 123:439–442

Miedaner T, Korzun V (2012) Marker-assisted selection for disease resistance in wheat and barley breeding. Phytopathology 102:560–566

Milne RJ, Dibley KE, Schnippenkoetter WH, Mascher M, Lui AC, Wang L et al (2018) The wheat Lr67 gene of the Sugar Transport Protein family confers multipathogen resistance in barley. Plant Physiol:00945.02018. https://doi.org/10.1104/pp.18.00945

Miyazaki C, Osanai E, Saeki K, Ito K, Konishi T, Sato K et al (2001) Mapping of quantitative trait loci conferring resistance to barley yellow mosaic virus in a Chinese barley landrace Mokusekko 3. Breed Sci 51:171–177

Mohamed A, Ali R, Elhassan O, Suliman E, Mugoya C, Masiga CW et al (2014) First products of DNA marker-assisted selection in sorghum released for cultivation by farmers in sub-saharan Africa. J Plant Sci Mol Breed 3:1–10

Montanari A, Ceola S, Laio F. (2017) Increasing human pressure on freshwater resources threatens sustainability at the global scale. AGU Fall Meeting Abstracts

Mundt CC (2014) Durable resistance: a key to sustainable management of pathogens and pests. Infect Genet Evol 27:446–455

Nelson R, Wiesner-Hanks T, Wisser R, Balint-Kurti P (2018) Navigating complexity to breed disease-resistant crops. Nat Rev Genet 19:21

Niks R, Habekuss A, Bekele B, Ordon F (2004) A novel major gene on chromosome 6H for resistance of barley against the barley yellow dwarf virus. Theor Appl Genet 109:1536–1543

Nowara D, Gay A, Lacomme C, Shaw J, Ridout C, Douchkov D et al (2010) HIGS: host-induced gene silencing in the obligate biotrophic fungal pathogen Blumeria graminis. Plant Cell 22:3130–3141

Oerke EC (2005) Crop losses to pests. J Agric Sci 144:31–43

Oliver R (2019) Integrated disease management of wheat and barley. Burleigh Dodds Science Publishing Limited. Available at https://www.cabdirect.org/cabdirect/abstract/20183367532

Ordon F, Bauer E, Graner A (1995) Marker-based selection for the ym4 BaMMV-resistance gene in barley using RAPDs. Agronomie 15:481–485

Pathak MD, Khan ZR (1994) Insect pests of rice. International Rice Research Institute, Los Banos

Perovic D, Kopahnke D, Habekuss A, Ordon F, Serfling A (2019) Marker-based harnessing of genetic diversity to improve resistance of barley to fungal and viral diseases. In: Applications of genetic and genomic research in cereals. Woodhead Publishing, pp 137–164. Available at https://www.sciencedirect.com/science/article/pii/B9780081021637000077

Pessarakli M (2016) Handbook of plant and crop stress. CRc press, Boca Raton

Pickering R, Ruge-Wehling B, Johnston P, Schweizer G, Ackermann P, Wehling P (2006) The transfer of a gene conferring resistance to scald (*Rhynchosporium secalis*) from Hordeum bulbosum into *H. vulgare* chromosome 4HS. Plant Breed 125:576–579

Pliego C, Nowara D, Bonciani G, Gheorghe DM, Xu R, Surana P et al (2013) Host-induced gene silencing in barley powdery mildew reveals a class of ribonuclease-like effectors. Mol Plant-Microbe Interact 26:633–642

Qi X-l, Xu Z-b, Pei H-c, Hu X, Wu J-j, Li X-b et al (2012) Construction and functional evaluation of an EMS-induced mutant population in barley [J]. J Triticeae Crops 5:008

Ragimekula N, Varadarajula NN, Mallapuram SP, Gangimeni G, Reddy RK, Kondreddy HR (2013) Marker assisted selection in disease resistance breeding. J Plant Breed Genet 1:90–109

Rahnamaeian M, Langen G, Imani J, Khalifa W, Altincicek B, Von Wettstein D et al (2009) Insect peptide metchnikowin confers on barley a selective capacity for resistance to fungal ascomycetes pathogens. J Exp Bot 60:4105–4114

Rahnamaeian M, Vilcinskas A (2012) Defense gene expression is potentiated in transgenic barley expressing antifungal peptide metchnikowin throughout powdery mildew challenge. J Plant Res 125:115–124

Rajaraman J, Douchkov D, Hensel G, Stefanato FL, Gordon A, Ereful N et al (2016) An LRR/malectin receptor-like kinase mediates resistance to non-adapted and adapted powdery mildew fungi in barley and wheat. Front Plant Sci 7:1836

Rehman S (2018) Proceedings of the 2nd international workshop on barley leaf diseases

Richards JK, Friesen TL, Brueggeman RS (2017) Association mapping utilizing diverse barley lines reveals net form net blotch seedling resistance/susceptibility loci. Theor Appl Genet 130:915–927

Richardson K, Vales M, Kling J, Mundt C, Hayes P (2006) Pyramiding and dissecting disease resistance QTL to barley stripe rust. Theor Appl Genet 113:485–495

Risk JM, Selter LL, Chauhan H, Krattinger SG, Kumlehn J, Hensel G et al (2013) The wheat L r34 gene provides resistance against multiple fungal pathogens in barley. Plant Biotechnol J 11:847–854

Ritala A, Aspegren K, Kurtén U, Salmenkallio-Marttila M, Mannonen L, Hannus R et al (1994) Fertile transgenic barley by particle bombardment of immature embryos. Plant Mol Biol 24:317–325

Roberts D, Mattoo A (2018) Sustainable agriculture—Enhancing environmental benefits, food nutritional quality and building crop resilience to abiotic and biotic stresses. Agriculture 8:8

Romero CC, Vermeulen JP, Vels A, Himmelbach A, Mascher M, Niks RE (2018) Mapping resistance to powdery mildew in barley reveals a large-effect nonhost resistance QTL. Theor Appl Genet 131:1031–1045

Rose DC, Sutherland WJ, Barnes AP, Borthwick F, Ffoulkes C, Hall C et al (2019) Integrated farm management for sustainable agriculture: lessons for knowledge exchange and policy. Land Use Policy 81:834–842

Ruge B, Linz A, Pickering R, Proeseler G, Greif P, Wehling P (2003) Mapping of Rym14 Hb, a gene introgressed from *Hordeum bulbosum* and conferring resistance to BaMMV and BaYMV in barley. Theor Appl Genet 107:965–971

Russell J, Mascher M, Dawson IK, Kyriakidis S, Calixto C, Freund F et al (2016) Exome sequencing of geographically diverse barley landraces and wild relatives gives insights into environmental adaptation. Nat Genet 48:1024–1030

Saisho D, Takeda K (2011) Barley: emergence as a new research material of crop science. Plant Cell Physiol 52:724–727

Sánchez-Vallet A, Fouché S, Fudal I, Hartmann FE, Soyer JL, Tellier A et al (2018) The genome biology of effector gene evolution in filamentous plant pathogens. Annu Rev Phytopathol 56:21–40

Sandhu K, Forrest K, Kong S, Bansal U, Singh D, Hayden M et al (2012) Inheritance and molecular mapping of a gene conferring seedling resistance against *Puccinia hordei* in the barley cultivar Ricardo. Theor Appl Genet 125:1403–1411

Savary S, Ficke A, Aubertot J-N, Hollier C (2012) Crop losses due to diseases and their implications for global food production losses and food security. Food Security 4(2):519–537

Sayed H, Baum M (2018) Marker-assisted selection for scald (*Rhynchosporium commune* L.) resistance gene (s) in barley breeding for dry areas. J Plant Protect Res 58:335–344

Schultheiss H, Hensel G, Imani J, Broeders S, Sonnewald U, Kogel K-H et al (2005) Ectopic expression of constitutively activated RACB in barley enhances susceptibility to powdery mildew and abiotic stress. Plant Physiol 139:353–362

Serna-Saldivar SO (2016) Cereal grains: properties, processing, and nutritional attributes. CRC press. Available at https://www.taylorfrancis.com/books/9780429112119

Shakoor N, Lee S, Mockler TC (2017) High throughput phenotyping to accelerate crop breeding and monitoring of diseases in the field. Curr Opin Plant Biol 38:184–192

Shtaya M, Marcel T, Sillero JC, Niks RE, Rubiales D (2006) Identification of QTLs for powdery mildew and scald resistance in barley. Euphytica 151:421–429

Sieling K, Christen O (2015) Crop rotation effects on yield of oilseed rape, wheat and barley and residual effects on the subsequent wheat. Arch Agron Soil Sci 61:1531–1549

Silvar C, Dhif H, Igartua E, Kopahnke D, Gracia MP, Lasa JM et al (2010) Identification of quantitative trait loci for resistance to powdery mildew in a Spanish barley landrace. Mol Breed 25:581–592

Singh D, Dracatos P, Derevnina L, Zhou M, Park RF (2015) Rph23: a new designated additive adult plant resistance gene to leaf rust in barley on chromosome 7H. Plant Breed 134:62–69

Soldanova M, Ištvánek J, Řepková J, Dreiseitl A (2013) Newly discovered genes for resistance to powdery mildew in the subtelomeric region of the short arm of barley chromosome 7H. Czech J Genet Plant Breed 49:95–102

Srivastava J, Damania A (1989) Use of collections in cereal improvement in semi-arid areas. The use of plant genetic resources. Cambridge University Press, Cambridge, pp 88–104

St. Pierre S, Gustus C, Steffenson B, Dill-Macky R, Smith K (2010) Mapping net form net blotch and Septoria speckled leaf blotch resistance loci in barley. Phytopathology 100:80–84

Stein N, Mascher M (2018) Barley genome sequencing and assembly—a first version reference sequence. In: The barley genome. Springer, Cham, pp 57–71. Avaialble at https://link.springer.com/chapter/10.1007/978-3-319-92528-8_5

Stenberg JA, Heil M, Åhman I, Björkman C (2015) Optimizing crops for biocontrol of pests and disease. Trends Plant Sci 20:698–712

Sui X, He Z, Lu Y, Wang Z, Xia X (2010) Molecular mapping of a non-host resistance gene YrpstY1 in barley (*Hordeum vulgare* L.) for resistance to wheat stripe rust. Hereditas 147:176–182

Szurman-Zubrzycka ME, Zbieszczyk J, Marzec M, Jelonek J, Chmielewska B, Kurowska MM et al (2018) HorTILLUS—a rich and renewable source of induced mutations for forward/reverse genetics and pre-breeding programs in barley (*Hordeum vulgare* L.). Front Plant Sci 9:216

Tacconi G, Cattivelli L, Faccini N, Pecchioni N, Stanca A, Vale G (2001) Identification and mapping of a new leaf stripe resistance gene in barley (*Hordeum vulgare* L.). Theor Appl Genet 102:1286–1291

Talamè V, Bovina R, Sanguineti MC, Tuberosa R, Lundqvist U, Salvi S (2008) TILLMore, a resource for the discovery of chemically induced mutants in barley. Plant Biotechnol J 6:477–485

The International Barley Genome Sequencing C, Mayer KFX, Waugh R, Langridge P, Close TJ, Wise RP et al (2012) A physical, genetic and functional sequence assembly of the barley genome. Nature 491:711–716

Toojinda T, Broers L, Chen X, Hayes P, Kleinhofs A, Korte J et al (2000) Mapping quantitative and qualitative disease resistance genes in a doubled haploid population of barley (*Hordeum vulgare*). Theor Appl Genet 101:580–589

Travella S, Ross S, Harden J, Everett C, Snape J, Harwood W (2005) A comparison of transgenic barley lines produced by particle bombardment and Agrobacterium-mediated techniques. Plant Cell Rep 23:780–789

Wagner C, Schweizer G, Krämer M, Dehmer-Badani A, Ordon F, Friedt W (2008) The complex quantitative barley–Rhynchosporium secalis interaction: newly identified QTL may represent already known resistance genes. Theor Appl Genet 118:113–122

Walls J, Rajotte E, Rosa C (2019) The past, present, and future of barley yellow dwarf management. Agriculture 9:23

Wang M-B, Abbott DC, Upadhyaya NM, Jacobsen JV, Waterhouse PM (2001) *Agrobacterium tumefaciens*-mediated transformation of an elite *Australian barley* cultivar with virus resistance and reporter genes. Funct Plant Biol 28:149–156

Wang R, Leng Y, Zhao M, Zhong S (2018) Fine mapping of a dominant gene conferring resistance to spot blotch caused by a new pathotype of *Bipolaris sorokiniana* in barley. Theor Appl Genet 132:41–51

Wang Y, Ren X, Sun D, Sun G (2015) Origin of worldwide cultivated barley revealed by NAM-1 gene and grain protein content. Front Plant Sci 6:803

Wiesner-Hanks T, Nelson R (2016) Multiple disease resistance in plants. Annu Rev Phytopathol 54:229–252

Wonneberger R, Ficke A, Lillemo M (2017) Mapping of quantitative trait loci associated with resistance to net form net blotch (*Pyrenophora teres* f. teres) in a doubled haploid Norwegian barley population. PLoS One 12:e0175773

Yan G, Chen X (2006) Molecular mapping of a recessive gene for resistance to stripe rust in barley. Theor Appl Genet 113:529–537

Yu G, Franckowiak J, Neate S, Zhang B, Horsley R (2010) A native QTL for Fusarium head blight resistance in North American barley (*Hordeum vulgare* L.) independent of height, maturity, and spike type loci. Genome 53:111–118

Yu X, Kong HY, Meiyalaghan V, Casonato S, Chng S, Jones EE et al (2018) Genetic mapping of a barley leaf rust resistance gene Rph26 introgressed from *Hordeum bulbosum*. Theor Appl Genet 131:2567–2580

Zang W, Eckstein PE, Colin M, Voth D, Himmelbach A, Beier S et al (2015) Fine mapping and identification of a candidate gene for the barley Un8 true loose smut resistance gene. Theor Appl Genet 128:1343–1357

Zhong S, Toubia-Rahme H, Steffenson BJ, Smith KP (2006) Molecular mapping and marker-assisted selection of genes for septoria speckled leaf blotch resistance in barley. Phytopathology 96:993–999

Ziems LA, Hickey LT, Platz GJ, Franckowiak JD, Dracatos PM, Singh D et al (2017) Characterization of Rph24: a gene conferring adult plant resistance to *Puccinia hordei* in barley. Phytopathology 107:834–841

Retraction Note to: Chapters

Retraction Note to: Chapter "Impact of Biotic and Abiotic Stresses on Plants, and Their Responses" in: Wani S. (eds) *Disease Resistance in Crop Plants*, https://doi.org/10.1007/978-3-030-20728-1_1

Chapter 1 retraction note

The Editor has retracted this chapter (Ahmad et al., 2019) because of significant overlap with a previously published article by different authors (Pandey et al., 2017). Aamir Raina disagrees with this retraction. Bilal Ahmad and Samiullah Khan have not responded to correspondence from the publisher about this retraction.

Ahmad B., Raina A., Khan S. (2019) Impact of Biotic and Abiotic Stresses on Plants, and Their Responses. In: Wani S. (eds) Disease Resistance in Crop Plants. Springer, Cham.

Pandey, Prachi, et al. "Impact of combined abiotic and biotic stresses on plant growth and avenues for crop improvement by exploiting physio-morphological traits." _Frontiers in plant science_8 (2017): 537.

The retracted version of these chapters can be found at
https://doi.org/10.1007/978-3-030-20728-1_1
https://doi.org/10.1007/978-3-030-20728-1_2
https://doi.org/10.1007/978-3-030-20728-1_9

S. H. Wani (ed.), *Disease Resistance in Crop Plants*,
https://doi.org/10.1007/978-3-030-20728-1_12

Retraction Note to: Chapter "Cloning of Genes Underlying Quantitative Resistance for Plant Disease Control" in: Wani S. (eds) *Disease Resistance in Crop Plants*, https://doi.org/10.1007/978-3-030-20728-1_2

Chapter 2 retraction note

The Editor has retracted this chapter (Shanmugavadivel et al., 2019) because of significant overlap with several previously published articles by different authors, including: Nelson et al. (2018), Yang et al. (2017), French et al. (2016) and Li et al. (2017). P. S. Shanmugavadivel agrees with this retraction. Aravind Kumar, K. R. Soren and Garima Yadav have not responded to correspondence from the publisher about this retraction.

Nelson, R., Wiesner-Hanks, T., Wisser, R. *et al._Navigating complexity to breed disease-resistant crops. Nat Rev Genet* 19, 21–33 (2018).

Yang, Qin, et al. "A gene encoding maize caffeoyl-CoA O-methyltransferase confers quantitative resistance to multiple pathogens." _Nature genetics_9 (2017): 1364.

French, Elizabeth, Bong-Suk Kim, and Anjali S. Iyer-Pascuzzi. "Mechanisms of quantitative disease resistance in plants." *Seminars in cell & developmental biology*. Vol. 56. Academic Press, 2016.

Li, Weitao, et al. "A natural allele of a transcription factor in rice confers broad-spectrum blast resistance." Cell 1 (2017): 114–126.

Shanmugavadivel P.S., Aravind Kumar K., Soren K.R., Yadav G. (2019) Cloning of Genes Underlying Quantitative Resistance for Plant Disease Control. In: Wani S. (eds) Disease Resistance in Crop Plants. Springer, Cham.

Retraction Note to:
Chapter "Molecular Breeding for Resistance to Economically Important Diseases of Fodder Oat" in: Wani S. (eds) *Disease Resistance in Crop Plants*,

https://doi.org/10.1007/978-3-030-20728-1_9

Chapter 9 retraction note

The Editor has retracted this chapter (Saini et al., 2019) because of significant overlap with a previously published chapter by a different author (Martenelli, 2004). Pawan Saini does not agree with this retraction. Mudasir Gani, Pooja Saini, Javaid Akhter Bhat, Rose Mary Francies, Narender Negi and S. S. Chauhan have not responded to correspondence from the publisher about this retraction.

Saini P. et al. (2019) Molecular Breeding for Resistance to Economically Important Diseases of Fodder Oat. In: Wani S. (eds) Disease Resistance in Crop Plants. Springer, Cham.

José Antônio Martinelli (2004) Oat Diseases and Their Control. In: J.M. Suttie and S.G. Reynolds (eds) Fodder Oats: A World Overview. FAO.

Index

S. H. Wani (ed.), *Disease Resistance in Crop Plants*,
https://doi.org/10.1007/978-3-030-20728-1