

Chittaranjan Kole *Editor*

# Genomic Designing for Biotic Stress Resistant Cereal Crops

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ISBN 978-3-030-75878-3      ISBN 978-3-030-75879-0 (eBook)  
<https://doi.org/10.1007/978-3-030-75879-0>

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*Dedicated to*



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

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

# Preface

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

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

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

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

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

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

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

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

This volume on “*Genomic Designing for Biotic Stress Resistant Cereal Crops*” includes eight chapters focused on Rice, Wheat, Maize, Barley, Sorghum, Pearl Millet, Foxtail Millet and Finger Millet contributed by 64 scientists from five countries including Egypt, India, Mexico, Turkey and USA. I remain immensely thankful for their highly useful contributions.

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

New Delhi, India

Chittaranjan Kole

# Contents

<b>1 Genomic Designing for Biotic Stress Resistant Rice</b> . . . . .	1
Deepti B. Sagare, Nitika Sandhu, Shailesh Yadav, Uma Maheshwar Singh, Shamshad Alam, Shilpi Dixit, Vikas Kumar Singh, and Arvind Kumar	
<b>2 Globally Important Wheat Diseases: Status, Challenges, Breeding and Genomic Tools to Enhance Resistance Durability</b> . . . . .	59
Sridhar Bhavani, P. K. Singh, Naeela Qureshi, Xinyao He, Akshaya Kumar Biswal, Philomin Juliana, Abdelfattah Dababat, and Amira M. I. Mourad	
<b>3 Resistance to Biotic Stress: Theory and Applications in Maize Breeding</b> . . . . .	129
R. N. Gadag, Jayant S. Bhat, Ganapati Mukri, Robin Gogoi, S. B. Suby, Abhijit Kumar Das, Sarita Yadav, Pranjal Yadava, M. L. Nithyashree, Gopalakrishna K. Naidu, Sunil Kumar Yadav, and K. Shilpa	
<b>4 Molecular Strategies for Managing Disease Resistance in Barley</b> . . .	177
Rekha Malik, Pawan Kumar, RPS Verma, Sonia Sheoran, Dinesh Kumar, Lokendra Kumar, Sanjaya Gyawali, and G. P. Singh	
<b>5 Genomic Designing for Biotic Stress Resistance in Sorghum</b> . . . . .	213
B. Fakrudin, T. N. Lakshmiddevamma, J. Ugalat, Raghavendra Gunnaiah, J. Khan, S. P. Gautham Suresh, K. A. Apoorva, M. Doddamani, S. Kadam, K. Rashmi, M. N. Mamathashree, K. Omkar Babu, A. Hadimani, M. Faizan, Gopalareddy Prakash, and Anurag Gowda	
<b>6 Genomic Designing for Biotic Stress Resistance in Pearl Millet [<i>Pennisetum glaucum</i> (L.) R. Br.]</b> . . . . .	257
C. Tara Satyavathi, Supriya Ambawat, Subaran Singh, Charu Lata, Shalini Tiwari, and Chandra Nayaka Siddaiah	

**7 Genomic Designing for Biotic Stress Tolerance in Foxtail Millet (*Setaria italica* L.) . . . . . 295**  
Sumi Rana, Lydia Pramitha, Pooja Rani Aggarwal,  
and Mehanathan Muthamilarasan

**8 Genomic Designing for Biotic Stress Resistance in Finger Millet . . . 313**  
B. Kalyana Babu and Rashmi Chauhan

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# Abbreviations

3-DAs	3-Deoxyanthocyanidins
AFLP	Amplified fragment length polymorphism
AICRP	All India Coordinated Research Project
AICSMIP	All India Coordinated Small Millets Improvement Project
AM	Association mapping
ANOVA	Analysis of variance
APR	Adult plant resistance
Avr	Avirelence
BAC	Bacterial artificial chromosome
BBSRC	Biotechnology and Biological Sciences Research Council
BC	Backcross
BC1	First backcross
BC2	Second backcross
BC3	Third backcross
BILs	Backcross inbred lines
BLAST	Basic Local Alignment Search Tool
BLB	Bacterial leaf blight
BMV	Brome mosaic virus
BPH	Brown planthopper
BS	Brown spot
Bt	<i>Bacillus thuringiensis</i>
BVG	Barley virus G
BYD	Barley yellow dwarf
BYDV	Barley yellow dwarf virus
bZIP	Basic leucine zipper
CAD	Cinnamyl alcohol dehydrogenase
CAO	Chlorophyllanoxidase
CAPS	Cleaved amplified polymorphic sequences
Cas	CRISPR associated
Cas9	CRISPR-associated protein 9

CCN	Cereal cyst nematode
CDD	Conserved domains database
cDNA	Complementary DNA
CIM	Composite interval mapping
CIMMYT	International Maize and Wheat Improvement Center
CISP	Conserved intron spanning primer
CMS	Cytoplasmic male sterility
CNP	Chitosan nanoparticles
CR	Crown rot
CRISPR	Clustered regularly interspaced short palindromic repeats
CRISPRi	CRISPR interference
<i>cryIAb</i>	Delta-endotoxin of <i>Bacillus thuringiensis</i> gene ( <i>IAb</i> )
<i>cryIAc</i>	Delta-endotoxin of <i>Bacillus thuringiensis</i> gene ( <i>IAc</i> )
CSH	Coordinated sorghum hybrid
CSSLs	Chromosomal segment substitution lines
CV	Cross-validation
CWANA	Central and West Asia and North Africa
CYDV	Cereal yellow dwarf virus
DALP	Direct amplification of length polymorphism
DArT	Diversity array technology
DB	Database
DEG	Differentially expressed gene
DfID	Department for International Development
DH	Doubled haploid
DM	Downy mildew
DMI	Demethylation inhibitor
DMR	Downy mildew resistance
DON	Deoxynivalenol
DSB	Double-stranded break
DUS	Distinctness, uniformity, and stability
epsps	Enol pyruvyl shikimate-3-phosphate synthase
ETL	Economic threshold level
F2	Second filial generation
F3	Third filial generation
F5	Fifth filial generation
FAO	Food and Agriculture Organization
FAOSTAT	FAO-Corporate Statistical Database
FAW	Fall army worm
FCR	Fusarium crown rot
FDK	Fusarium damaged kernel
FDR	False discovery rate
FHB	Fusarium head blight
FoMV	Foxtail mosaic virus
G X E	Genotype $\times$ environment
GAB	Genomics-assisted breeding

GBS	Genotyping-by-sequencing
GC	Genomic control
GCA	General combining ability
GCP	Generation Challenge Program
GE	Genome editing
GEVVs	Genomic estimated breeding values
GFP/gfp	Green fluorescent protein
GLM	Generalized linear model
GM	Gall midge
GP-1	Gene pool 1
GP-2	Gene pool 2
GP-3	Gene pool 3
GS	Genomic selection
GSDS	Gene structure display server
GWAMS	Genome-wide association mapping studies
GWAS	Genome-wide association study/studies
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
HCN	Hydrocyanide
HDR	Homologous-directed repair
HPR	Host plant resistance
HSP	Heat shock protein
HST	Host-selective toxin
IBERS	Institute of Biological, Environmental & Rural Sciences
IBGSC	International Barely Genome Sequencing Consortium
IBSC	Institutional Biosafety Committee
ICAR	Indian Council of Agricultural Research
ICIM	Inclusive composite interval mapping
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IDM	Integrated disease management
ILs	Introgression lines
IM	Interval mapping
IPM	Integrated pest management
ISSR	Inter-simple sequence repeat
ITMI	International Triticeae Mapping Initiative
IWGSC	The International Wheat Genome Sequencing Consortium
KASP	Kompetitive allele-specific PCR
KB	Karnal bunt
KEGG	Kyoto Encyclopedia of Genes and Genomes
LD	Linkage disequilibrium
LG	Linkage group
LM	Interval mapping
LOD	Logarithm or likelihood of odd
LR	Leaf rust
LR	Logistic regression
LRR	Leucine-rich repeat

LTN	Leaf tip necrosis
MAB	Marker-assisted breeding
MABB	Marker-assisted backcross breeding
MABC	Marker-assisted backcrossing
MAGIC	Multiparent advanced generation intercross
MAGP	Marker-assisted gene pyramiding
MARS	Marker-assisted recurrent selection
MAS	Marker-assisted selection
MDA	Malondialdehyde
MEGA	Molecular evolutionary genetics analysis
MIM	Multiple interval mapping
MLM	Mixed linear model
MN	Meganuclease
MQM	Multiple QTL mapping
MSP	Minimum support price
NAM	Nested association mapping
NB	Net blotch
NB	Nucleotide binding
NBPGR	National Bureau of Plant Genetic Resources
NBS	Nucleotide-binding site
NE	Necrotrophic effector
NGS	Next-generation sequencing
NHEJ	Non-homologous end joining
NILs	Near-isogenic lines
NPLS	Non-parasitic leaf spots
NSKE	Neem seed kernel extract
NUC	Nuclease
PA	Proanthocyanidin
PAC	P1-derived artificial chromosome
PAL	Phenylalanine ammonia lyase
PAM	Protospacer adjacent motif
PAS	Prediction accuracies
PCA	Principal component analysis
PCR	Polymerase chain reaction
PDS	Phytoene desaturase
pegRNA	Prime editing guide RNA
PMiGAP	Pearl millet inbred germplasm association panel
POD	Peroxidase
POX	Peroxidase
PPO	Polyphenol oxidase
PPP	Precision phenotyping platforms
PR	Pathogenesis-related
QDR	Quantitative disease resistance
QoI	Quinone outside inhibitors
QTL	Quantitative trait locus

QTLs	Quantitative trait loci
R	Resistance (gene)
RAD	Restriction site-associated DNA
RAD-seq	RAD-sequencing
RAPD	Random amplified polymorphic DNA
RBSDV	Rice black-streaked dwarf virus
RDM	Rajasthan downy mildew
RFLP	Restriction fragment length polymorphism
RGE	RNA-guided genome editing
RGSV	Rice grassy stunt virus
RILs	Recombinant inbred lines
RLN	Root lesion nematode
RNAi	RNA interference
RNA-seq	RNA sequencing
RRSV	Rice-ragged stunt virus
RSV	Rice stripe virus
RVD	Repeat variable di-residue
S/TPK	Serine/threonine protein kinase
SA	South Asia
SB	Spot blotch
SCAR	Sequence characterized amplified region
SDHI	Succinate dehydrogenase inhibitor
SDM	Sorghum downy mildew
SeNP	Selenium nanoparticle
SFR	Shoot fly resistance
sgRNA	Single guide RNA
ShB	Sheath blight
SIM	Single interval mapping
SLM	Simple linear model
SMA	Single marker analysis
SNB	<i>Septoria Nodorum</i> Blotch
SNP	Single nucleotide polymorphism
SR	Stem rust
SSCP	Single strand conformational polymorphism
SSLB	Septoria speckled leaf blotch
SSN	Sequence-specific nuclease
SSR	Simple sequence repeat
STB	<i>Septoria tritici</i> blotch
STITCH	Search tool for interactions of chemicals
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins
STS	Sequence-tagged site
SUPER	Settlement of MLM Under Progressively Exclusive Relationship
TAC	Transformation-competent artificial chromosome
TALEN	Transcription activator-like effector nucleases
TF	Transcription factor

TILLING	Targeted induced local lesion in genomes
TLPs	Thaumatococcus-like proteins
TS	Tan spot
VIGS	Virus-induced gene silencing
WB	Wheat blast
WB1	Wild barley
WDV	Wheat dwarf virus
WGRS	Whole-genome re-sequencing
WGS	Whole-genome sequencing
WSMV	Wheat streak mosaic virus
WUE	Water use efficiency
YAC	Yeast artificial chromosome
YR	Yellow rust
ZFN	Zinc-finger nucleases
ZnNP	Zinc nanoparticle
ZnO	Zinc oxide

# Chapter 1

## Genomic Designing for Biotic Stress Resistant Rice



Deepti B. Sagare, Nitika Sandhu, Shailesh Yadav,  
Uma Maheshwar Singh, Shamshad Alam, Shilpi Dixit,  
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**Abstract** Among major cereal crops, rice plays an important role in global food security as well as to the economic and social stability. Considering the impacts of global warming on agriculture and alarming yield losses due to biotic and abiotic stresses as well as the effect of the climate change on the future insect-pest scenario, effective utilization of advanced tools and techniques of insect-disease biotype/pathotype monitoring and surveillance, identification of stable resistance sources, molecular plant pathology to understand the pathotype/biotype-gene interactions, molecular biology and modern genomics tools to assist crop breeding develop resistant/tolerant varieties shall help researchers find stable solutions. The losses caused by biotic stresses are comparatively high and impart 37–70% yield losses or complete crop failure in many cases. Keeping this in mind, the chapter discusses the importance of rice in global food security, major and emerging biotic stresses in rice, genetic resources of resistant/tolerant genes, map-based gene cloning, trait mapping and major QTLs' identification, conventional and genomic assisted breeding strategies to develop multiple biotic stress resistant rice varieties. Further, the chapter emphasizes on the efforts including genetic engineering, gene editing and nanotechnological approaches in imparting stable resistance to biotic stresses. The chapter also discusses about various available bioinformatics tools and brief account on social, political and regulatory issues.

**Keywords** Rice · Biotic stresses · QTLs/genes · Genomic assisted breeding · Genetic engineering · Bioinformatics

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## 1.1 Introduction

Rice (*Oryza sativa* L.), a ‘Global Grain’ is cultivated across the globe and consumed by more than 50% of the world’s population (Chauhan et al. 2017). Rice production is the prime source of employment and the basis of earning for almost 200 million households globally (Asibi et al. 2019). Rice provides more than 500 calories/person/day, and a substantial number of proteins (Muthayya et al. 2014). Rice was grown in around 167.13 million hectares of cultivated area in 2018–19 compared to 161.7 million hectares in 2009–2010 worldwide. Almost 90% of the world’s rice is produced in Asia, and China and India are the largest producers (USDA 2018). In the 2018–2019 crop year, a total of 495.9 million metric tons milled rice was produced worldwide, and highest production was reported china (148.5 million metric tons) followed by India (116.42 million metric tons). The world rice demand is expected to shoot up from 496.1 million metric tons (milled rice) in 2019–2020 to 555 million metric tons in 2035 to feed the ever-growing population (USDA 2018).

The rice yields are either stagnant or increasing with lower genetic gain post green revolution era than required to meet the projected future demand to feed the population. This is primarily happening due to climate change related effects and uncertainties as well as lack of suitable rice genotypes adaptable to the changing climate, and vis-a-vis upsurge of insect-pest and diseases occurrence on rice (Ray et al. 2013; Ramegowda and Senthil-Kumar 2015). The crop yield and grain quality losses caused by major biotic stresses (bacterial blight, blast disease, and insect pests) are comparatively high and reported to impart 37–50% yield losses or can cause complete crop failure (Hasan et al. 2015). Bacterial blight (*Pseudomonas syringae*) and blast (*Magnaporthe oryzae*) are the major diseases and, yellow stem borer (*Scirpophaga incertulas*), gall midge (*Orseolia oryzae*), and brown planthopper (*Nilaparvata lugens*) are the major insect pests of rice causing heavy yield losses. The false smut (*Ustilaginoidea virens*) and brown spot (*Cochliobolus miyabeanus*) which were earlier considered as minor diseases are emerging as major diseases causing severe yield losses and deteriorating grain quality (Nessa et al. 2015). Though the biotic stress and plant species coexist together since their evolution, the continuously changing dynamics make it challenging to manage disease and insect-pests for worldwide farmers.

Global warming and its adverse effects make crops face both abiotic and biotic stresses together in a combination, which affects rice yield and quality severely (Suzuki et al. 2014; Ramegowda and Senthil-Kumar 2015). The ‘stress matrix’ that explains the interaction and combined effect of multiple stress on plant productivity can help to design strategies cope with climate change and minimize the yield losses occurring from biotic and environmental stresses (Mittler 2006; Suzuki et al. 2014). The minor pathogens and pests are turning into a potential threat (e.g. false smut, brown spot, sheath blight of rice) due to a cumulative effect of multiple stresses (Spark et al. 2012). The emergence of potential pathogens and pests necessitates novel approaches to enhance the biotic stress resistance/tolerance of

various rice varieties that can withstand severe pathogens attack as well as unfavourable climate without grain yield and quality penalty.

It is difficult and time-consuming to breed biotic stress-resistant/tolerant varieties using conventional breeding strategies because the strains, races, and pathotypes evolve and mutate rapidly to overcome resistance (Zhou et al. 2007). Moreover, the vertical resistance is easily breakable, and developing horizontal resistance through conventional breeding is difficult. In conventional breeding, linkage drag concerns due to association of several unwanted genes with the desired genes, makes difficulty in achieving yield potential along with stress tolerance (Wang et al. 2015). Though, there are several limitations, conventional breeding approaches are very much important for wild germplasm conservation, hybridization between contrasting parents, identification of novel genetic variants and mutants (Werner et al. 2005). Recent advances in molecular biology and genomics led to identifying major resistant genes and quantitative trait loci (QTLs) for major biotic constraints and subsequent developments in marker technologies pave the way to accelerate biotic stress tolerant breeding.

## 1.2 Description of Different Biotic Stresses

The major biotic stresses in rice are, blast, bacterial leaf blight, brown spot, false smut, sheath blight, gall midge, and brown planthopper, and the emergence of their newer races/pathotype/biotypes with increased virulence is a threat to rice production at the global level. Visible symptoms for different diseases' and insects' infestation are mentioned in Fig. 1.1.

### 1.2.1 Rice Blast (BB)

It is caused by ascomycetes fungus *Magnaporthe oryzae* (Couch and Kohn 2002), and is a major constrain to rice production globally (Gladieux et al. 2018). It causes 10–30% of yield loss annually in different production zones and up to 80–100% yield loss under favourable condition (Pagliaccia et al. 2018). Blast fungus develops spindle to diamond-shaped lesion on leaves surface having an off-white to tan center with a brown margin. At flowering stage, the pathogen infects the neck or node of the rice plants resulting in a 'neck blast' or panicle blast. The pathogen infects all stage of the rice plants but the infection at reproductive stage to neck or node of the rice plant are the most damaging phases of the disease (Dean et al. 2005; Pagliaccia et al. 2018). Favorable conditions for disease development are high humid, cloudy weather, prolong dew periods, frequent light rains. Late seeding date is also one of the causes of increased blast infection. Blast fungus shows a high degree of variability in the field leading to frequent emergence of new races/pathotype knocking down prevalent resistant cultivars (Valent and Chumley 1991).



**Fig. 1.1** Symptoms of **a** leaf blast, **b** brown spot, **c** false smut, **d** bacterial leaf blight, *Source* <http://www.knowledgebank.irri.org/decision-tools/rice-doctor/rice-doctor-fact-sheets/item/bacterial-blight>, **e** sheath blight, *Source* Uppala and Zhou (2018), **f** brown plant hopper, hopper burn, yellowing and drying of plants, *Source* IRRI-Rice knowledge bank <http://www.knowledgebank.irri.org/training/fact-sheets/pest-management/insects/item/planthopper>, **g** silver shoot induced by gall midge insect, *Source* Miller and Raman (2019)

To develop durable resistance variety, knowledge of population structure and effective resistance gene/QTLs are prerequisite for any geographical region (Wang et al. 2017). Race/pathotype is conventionally classified based on its profile of pathogenicity to a panel of cultivars having known resistance genes. In the case of the rice-blast-pathosystem, ten different international differential sets are available which are widely used to classify the *M. oryzae* population into races/pathotypes. To identify the resistance spectra of resistant genes and race classification precisely, a set of 26 differential varieties targeting 24 resistance genes in the genetic background of LTH were developed at IRRI in collaboration with JIRCAS (Kobayashi et al. 2007). Several races/pathotypes of *M. oryzae* were identified from different part of the world Viz., 267 races in Bangladesh (Khan et al. 2016), 39 races from the United States (Wang et al. 2017), 23 pathotypes in Vietnam (Thuan et al. 2006), nine pathotypes from Myanmar (Zaw et al. 2016).

The deployment of broad-spectrum resistance genes is one of the safest and economically feasible ways for the management of blast disease (Deng et al. 2017). Cultural practices such as early planting, field sanitation, crop rotation, nutrient and water management influences the onset and development of rice blast disease. Crop rotation and nutrient management plays a significant role in disease control. Heavy use of nitrogen fertilizer increases susceptibility of rice plants to blast. Application of silicon to soil results in localization in leaf surfaces which act as a physical barrier against blast (Ishiguro 2001). For the better management of disease, two techniques can be employed. First, seed treatments with systemic fungicides to prevent infection at the seedlings stage and the second, foliar sprays of fungicides to prevent infection of leaves and panicles (Chaudhary 1999). Several fungicides were evaluated under field and laboratory conditions and found that Fluopyram + tebuconazole, difenoconazole + propiconazole, flutriafol + azoxystrobin, Tricyclazole 22% + Hexaconazole were highly effective in reducing disease severity (Kongcharoen et al. 2020). Other fungicide which can be used to control blast disease are Benomyl, Carbendazim 12% + Mancozeb 63%, Iprobenfos, Capropamid, Hexaconazole, Tebuconazole etc. (Magar et al. 2015). Seed treatments with systemic fungicides, carbendazim or biocontrol agent *T. viride* or *Pseudomonas fluorescens*, *Bacillus subtilis*, and *Streptomyces sindeneusis* have shown their potential in reducing the blast disease (Yang et al. 2008).

### **1.2.2 Bacterial Leaf Blight (BLB)**

It is caused by *Xanthomonas oryzae* pv. *oryzae* (Swings et al. 1990), and is major foliage disease in rice resulting in 20–50% yield reductions in Asia, Latin America, Australia, and Africa (Yasmin et al. 2017). BLB is a vascular disease thus, causes systemic infection. Lesions on leaf increases in length and width and extend to leaf sheath produces whitish and wavy margin. BLB may occur at all growth stages, but the most common symptom occurs at maximum tillering to maturity stage. In general, the favorable condition for disease development is temperatures ranged

from 25–34 °C with relative humidity more than 70%. A small droplet of bacterial ooze can be observed on the young lesion (Chukwu et al. 2019). The effectiveness of any resistance gene depends on the races/pathotype structure of the pathogens. 30 races/pathotypes of *Xoo* have been reported all over the world (Noda et al. 2001). Pathogenicity and race/pathotype identification have been extensively studied to understand the resistant mechanism, and reported several pathotypes/races viz., six pathotypes/races from Philippine over 17 years, nine pathogenic races from Nepal, 4 races/pathotypes in Iran, and 22 pathotypes in India (Yugander et al. 2017).

A high mutation rate in pathogenic races hinders the development of durable control (George et al. 1997). Because of the presence of toxic residues, the usage of chemicals for the management of BLB has limitations (MacManus et al. 2002). Therefore, host plant resistance is the most effective and environmentally safe way to control the disease (Wang et al. 2009). Cultural practices like field sanitation, judicious use of nitrogen, maintain shallow water in nursery can prevent the onset of disease. Plant growth-promoting rhizobacteria, some strains of *Pseudomonas* spp. and *Bacillus* spp. have been reported to reduce the BLB infection in rice and help to increase the crop yield (Udayashankar et al. 2011; Yasmin et al. 2017).

### 1.2.3 Rice Brown Spot (BS)

It is caused by fungus *Cochliobolus miyabeanus*, anamorph *Bipolaris oryzae*, is a chronic disease affecting yield and quality loss of rice worldwide every year (Zanao Junior et al. 2009). Brown spot is one of the diseases which caused the Bengal famine during 1942 when approximately two million people died from starvation in India. Brown spot disease is prevalent in almost all rice growing region of India and in the South and South-East Asian countries (Savary et al. 2000). The brown spot causes 4–52% yield losses (Barnwal et al. 2013).

The symptom of brown spot disease appears on the areal part of the plants. Initially small brown spots appear on the leaves, sheath, glumes, and grain. A fully developed lesion is circular to oval in shape with brown margin and grey center. Infection at seedling stage result in stunted plants growth and subsequently reduces yield. The disease is primarily seed born in nature infecting at two crop stages, primary infection at the seedling stage and secondary infection at tillering to maturity stage (Barnwal et al. 2013). The favorable conditions for disease development are high relative humidity (>80%) and temperature ranged from 16–36 °C with leaf wetness (wet for 8–24 h). The disease is generally severe in nutrient deficient soil having low pH with deficiency of essential elements. Due to the increase in variability in rainfall, the incidence of brown spot disease has increased because the disease is more common in field where water supply is scarce and drought is more frequent (Savary et al. 2005). Brown spot is increasing over the year particularly in rainfed areas and the higher incidence has been reported on direct-seeded rice.

Identification of resistance genes/QTLs and their deployment in local popular variety is one of promising approach to manage BS disease. Several strategies such as application of suitable cultural practices, use of resistant variety, improving soil fertility, nutrient and fertilizer management, application of calcium silicate, bio-control measures and fungicides are used to manage this disease. Primary infection can be controlled through seed treatment with hot water (53–54 °C) for 10–12 min before seeding, pre-soaking of seeds in cold water for 8 h, treatment with fungicides is recommended. The fungicides, Propiconazole @ 1 ml/l and Hexaconazole @ 2 ml/l are reported to reduce the disease severity from 37.26% to 5.19% and increase the grain yield up to 55.49% (Gupta et al. 2013) Also, the benzoic acid/salicylic acid and benzimidazoles/carbendazim are reported to inhibit the growth of *B. oryzae* completely (Shabana et al. 2008). Whereas, in biological control, seed treatment with *Pseudomonas* spp., *Trichoderma viride*, or *T. harzianum* alone or in combination with fungicides (Propiconazole) was reported to reduce disease severity up to 70% (Biswas et al. 2010).

### 1.2.4 Rice False Smut

Rice false smut, *Ustilaginoidea virens* Cooke (Takahashi) a grain quality and yield deteriorating fungal disease is very difficult to forecast because the symptoms appear after flowering when the fungus transforms infected spiklets into smut ball. Initially the symptom appears as smut balls are white, slightly flattened and covered with thin membrane which gradually change to yellowish-orange, yellowish-green, and finally to greenish-black (Ashizawa et al. 2012). Rice false smut has been reported in several rice growing regions of the world such as India, China and USA. In India, severe yield losses ranging from 7 to 75% due to false smut are reported (Ladhalakshmi et al. 2012). The favorable condition for disease development is average temperature range 25–30 °C, relative humidity >90% and rain at the time of flowering. The fungus produces two toxins, rhizoxin, and ustiloxin (microtubule inhibitor) which are very toxic to humans and animals feeding on rice grain. Cultural management like early planting, recommended of nitrogen, suitable planting space and healthy seed, has been found to reduce the false smut incidences. To date, the control of this disease has relied on fungicide and the efficacy of several fungicides to false smut is widely studied (Ladhalakshmi et al. 2012). Fungicides like tebuconazole, difenoconazole, propiconazole and hexaconazole, are effective to reduce RFS disease incidence (Zhou et al. 2014). In biological control methods, the isolates of *Trichoderma* showing antagonistic activity against *U. virens* have been used to control false smut (Kannahi et al. 2016).

### 1.2.5 Sheath Blight

It is caused by necrotrophic fungus, *Rhizoctonia solani* Kuhn. Sheath blight was first reported in Japan in 1910 and subsequently reported to be widespread. The initial symptom of sheath blight appears on leaf sheath 1–3 cm above the water level as oval or ellipsoidal greenish-gray lesion. As the disease progresses the lesions coalesce with each other forming larger lesions which cover the entire tillers. Infection to the inner sheath interrupts the movement of water and nutrients resulting in the death of the entire plant. The fungus survives between crops as ‘sclerotia’ that can remain dormant in the soil for several years and can also survive in infected rice straw (Singh and Singh 2015). High humidity (>95%), moderate temperature (28–32 °C) and high N application favours the development of sheath blight disease. Sheath blight causes substantial losses in intensive rice production systems worldwide, and its incidence during flowering or panicle initiation causes poor grain quality (Savary et al. 2005). The use of resistant to moderately resistant varieties along with cultural practices and timely application of nitrogen is the most effective and economic way to manage sheath blight disease (Singh et al. 2015). However, there are no highly resistant varieties known, but moderately resistant varieties were identified such as Teqing, Tetep, Jasmine85, and Pecos. Crop rotation is another sound strategy to manage diseases, as sclerotia survive in the soil for several years, rotation may help to control sheath blight (Singh and Singh 2015).

The biocontrol agents such as *Trichoderma*, *Gliocladium*, *Aspergillus*, *Bacillus subtilis*, *B. cereus*, *Enterobacter* sp., *Pseudomonas fluorescens*, *P. putida*, and *P. aureofaciens* are reported as effective biocontrol agents in reducing the sheath blight (Khan and Sinha 2005).

### 1.2.6 Brown Planthopper

Brown planthopper (BPH; *Nilaparvata lugens* Stal.) caused by sap sucking pest *Nilaparvata lugens*, predominant in all rice-growing countries of Asia (Normile 2008). BPH serve as vector for grassy stunt virus (RGSV) and ragged stunt virus (RRSV), that cause secondary damage to rice. Development of BPH and population dynamics is affected by various climatic factors. Temperatures between 25 and 30 °C and relative humidity more than 70% are optimum conditions for egg and nymphal development and subsequent BPH outbreaks.

To date, four biotypes of BPH are known in rice, biotypes 1 and 2 predominant in Southeast and East Asia, and biotype 3 and 4 occurs on the Indian subcontinent and is thus referred to as the South Asian biotype (Jena and Kim 2010). To reduce the pest's incidence, the most durable and environmentally safe strategy is the identification of broad-spectrum resistant genes and their deployment in the resistant breeding program for the target geographical region against the prevalent biotype (Brar et al. 2009).

### 1.2.7 Rice Gall Midge

Rice gall midge (GM) caused by *Orseolia oryzae* (Wood Mason), is a major insect pest in Southern and South-East Asia. Two rice gall midge species have been identified, the Asian rice gall midge, *Orseolia oryzae*, and the African rice gall midge, *O. oryzivora*. The symptom of damage caused by gall midge appears at the base of tillers as tubular gall, resulting in elongation of leaf sheaths called silver shoot. The life cycle between oviposition and adult emergence takes about two to three weeks. The Fly lays elongate, cylindrical, white, or red or pinkish eggs (2–6) at the base of the leaf. After hatching, the larva or maggot is 1 mm long with a pointed anterior end. It creeps down the sheath and form an oval chamber around the feeding site. The pupa wriggles up the tube with the help of the antennal horn to the tip of the silver shoot at the time of emergence and projects halfway out.

So far, seven distinct biotypes of Asian gall midge from India (Lakshmi et al. 2006), four biotypes from China, two biotypes from Sri Lanka, one biotype each from Thailand, and Indonesia have been reported (Sardesai et al. 2001). Mechanical, cultural, and chemical measures and the use of resistant varieties have been recommended to manage gall midge infestation and to keep the pest population below the economic injury level. Ploughing immediately after harvesting, planting early maturing variety, avoiding staggered planting, field sanitation, application of a split dose of nitrogen and potassium are the cultural practices followed to reduce gall midge infestation. In biological control natural enemies of GM viz., *platygaster* sp., eupelmidae and pteromalidae wasps which parasitize the gall midge larvae, phytoseiid mites which feeds on eggs, and spiders feeds on adults) can be used to control GM infestation.

To control major diseases various cultural, mechanical, biological, and chemical approaches are used. Cultural practices are more economical for resource-poor farmers and are considered as the first line of defense. It involves various strategies such as crop residues management, planting date manipulation, use of recommended/modified dose of nitrogenous, the use of trap crop, establishment of light trap/pheromone trap, and use of resistant varieties. In controlling the pest population below the economic injury level, biological control method is very important. It includes natural enemies such as predators, parasitoids, pathogens, antagonists, or competitors' population to reduce the pest population, rendering it less abundant and less harmful. In the endemic areas where appropriate resistant varieties are not available, use of insecticides is widespread. Breeding resistant varieties is one of the promising approaches to manage biotic stresses in rice. However, because of the evolution of virulent pathotypes/biotypes, knockdown of resistance conferred by single gene has become a major setback to this approach. Several genes/QTLs conferring biotic stress tolerance in rice has been reported and employing novel approaches in molecular biology, breeding, genomics, etc., pyramiding multiple QTLs for single/multiple diseases/pest tolerance is a feasible strategy (Sects. 1.6, 1.7, and 1.8).

### 1.3 Genetic Resources of Resistance Genes

The wild relatives in rice serve as a great store of huge genetic variability and a valuable resource of genes for the biotic stress's resistance such as blast, brown planthopper, bacterial late blight, and grassy stunt virus (Brar and Khush 1997, 2003) and genes for abiotic stress resistance. Harlan and de Wet (1971) proposed a gene pool categorization of the cultivated crops based on the feasibility of gene transfer/gene flow from those species to crop species. The categories defined were primary, secondary, and tertiary gene pools. The primary gene pool comprises the biological species that have no restrictions of gene exchange i.e. that can be intercrossed very easily without any crossing barrier. This primary gene group may contain both wild and progenitors cultivated of the crop species. The secondary gene pool comprises both wild and cultivated relatives of the crop species having crossability issues because of more distant relatedness. However, the hybrids produced are sufficiently fertile allowing successful gene transfer. The F<sub>1</sub> produced from the crossing of crop species from primary and secondary gene pool have fertility issues with more difficulty in success. The tertiary gene pool involves the outer limits of the potential genetic resources. Hybridization involving primary and tertiary gene pools is very challenging, resulting in sterility, lethality, and other abnormalities. The researchers suggested that the breeder should search for the desired genes combination among the genetic materials in the primary gene pool/related species then move to the secondary gene pool and, if required, the tertiary gene pool. The genus *Oryza* constitute 24 species, two cultivated (*O. sativa* and *O. glaberrima*), and the remaining 22 wild species.

The wild *Oryza* species were classified into three main groups/complexes based on the possibility of gene transfer from the wild species into the cultivated rice. These include *O. sativa* complex, *O. officinalis* complex, and the *O. ridleyi* and *O. meyeriana* complex (Morishima and Oka 1960) which were later known as the primary, secondary, and the tertiary gene pools of *Oryza*, respectively (Khush 1997). The *O. sativa* complex comprised of the two cultivated and six (*O. rufipogon*, *O. nivara*, *O. longistaminata*, *O. barthii*, *O. meridionalis*, *O. glumaepatula*) out of the 22 wild species with the AA genome (Zhu and Ge 2005). These primary gene pool species are diploid in nature, show homologous chromosome pairing, and cross-compatible. The secondary gene pool or *O. officinalis* complex comprised of 10 wild species (*O. punctata*, *O. minuta*, *O. officinalis*, *O. rhizomatis*, *O. eichingeri*, *O. latifolia*, *O. alta*, *O. grandiglumis*, *O. australiensis*, *O. brachyantha*) having diploid (BB, CC, EE, FF), and tetraploid (BBCC, CCDD) genomes and are cross incompatible with the *O. sativa*. The *O. meyeriana* complex possessing GG genome comprises two diploid wild species, *O. granulata* and *O. meyeriana* having cross incompatibility with *O. sativa*. Similarly, the *O. ridleyi* complex includes the two tetraploids wild species, *O. longiglumis* and *O. ridleyi* with HHJJ genome and highly cross-incompatible with the cultivated species, *O. sativa*. Further, two more wild species, *O. coarctata*, *O. schlechteri* with the tetraploid genome (HHKK) are similarly included in the tertiary gene pool (Ge et al. 1999). Some of the