Chittaranjan Kole *Editor*

# Genomic Designing for Biotic Stress Resistant Cereal Crops

# Genomic Designing for Biotic Stress Resistant Cereal Crops

Chittaranjan Kole
Editor

# Genomic Designing for Biotic Stress Resistant Cereal Crops

*Editor*
Chittaranjan Kole
Raja Ramanna Fellow
Department of Atomic Energy
Government of India
ICAR-National Institute for Plant
Biotechnology
New Delhi, India

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

© The Editor(s) (if applicable) and The Author(s), under exclusive license to Springer Nature Switzerland AG 2021
This work is subject to copyright. All rights are solely and exclusively licensed by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, expressed or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

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

*Dedicated to*

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

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

# Preface

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

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

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

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

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

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

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

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

This volume on "*Genomic Designing for Biotic Stress Resistant 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

**1 Genomic Designing for Biotic Stress Resistant Rice** . . . . . . . . . . . . . 1
Deepti B. Sagare, Nitika Sandhu, Shailesh Yadav, Uma Maheshwar Singh, Shamshad Alam, Shilpi Dixit, Vikas Kumar Singh, and Arvind Kumar

**2 Globally Important Wheat Diseases: Status, Challenges, Breeding and Genomic Tools to Enhance Resistance Durability** . . . . . . . . . . . 59
Sridhar Bhavani, P. K. Singh, Naeela Qureshi, Xinyao He, Akshaya Kumar Biswal, Philomin Juliana, Abdelfattah Dababat, and Amira M. I. Mourad

**3 Resistance to Biotic Stress: Theory and Applications in Maize Breeding** . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 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

**4 Molecular Strategies for Managing Disease Resistance in Barley** . . . 177
Rekha Malik, Pawan Kumar, RPS Verma, Sonia Sheoran, Dinesh Kumar, Lokendra Kumar, Sanjaya Gyawali, and G. P. Singh

**5 Genomic Designing for Biotic Stress Resistance in Sorghum** . . . . . . 213
B. Fakrudin, T. N. Lakshmidevamma, 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

**6 Genomic Designing for Biotic Stress Resistance in Pearl Millet [*Pennisetum glaucum* (L.) R. Br.]** . . . . . . . . . . . . . . . . . . . . . . . . . . 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

# Contributors

**Pooja Rani Aggarwal** Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, Telangana, India

**Shamshad Alam** International Rice Research Institute (IRRI), South-Asia Hub (SAH), Hyderabad, India

**Supriya Ambawat** ICAR-AICRP on Pearl Millet, Jodhpur, Rajasthan, India

**K. A. Apoorva** Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences Campus, Bengaluru, India

**Jayant S. Bhat** Regional Research Centre (ICAR-Indian Agricultural Research Institute), Dharwad, Karnataka, India

**Sridhar Bhavani** Global Wheat Program, International Maize and Wheat Improvement Center (CIMMYT), Mexico

**Akshaya Kumar Biswal** Genetic Resources Program, International Maize and Wheat Improvement Center (CIMMYT), Mexico

**Rashmi Chauhan** G. B. Pant University of Agriculture and Technology, Pant nagar, Uttarakhand, India

**Abdelfattah Dababat** International Maize and Wheat Improvement Center (CIMMYT), Ankara, Turkey

**Abhijit Kumar Das** ICAR-Indian Institute of Maize Research, PAU Campus, Ludhiana, India

**Shilpi Dixit** International Rice Research Institute (IRRI), South-Asia Regional Centre (SARC), Varanasi, India

**M. Doddamani** Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences Campus, Bengaluru, India

**M. Faizan** Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences Campus, Bengaluru, India

**B. Fakrudin** Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences Campus, Bengaluru, India

**R. N. Gadag** Division of Genetics, ICAR-Indian Agricultural Research Institute, PUSA Campus, New Delhi, India

**S. P. Gautham Suresh** Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences Campus, Bengaluru, India

**Robin Gogoi** Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, PUSA Campus, New Delhi, India

**Anurag Gowda** Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences Campus, Bengaluru, India

**Raghavendra Gunnaiah** Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences Campus, Bengaluru, India

**Sanjaya Gyawali** Washington State University Mount Vernon NWREC, Mount Vernon, WA, USA

**A. Hadimani** Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences Campus, Bengaluru, India

**Xinyao He** Global Wheat Program, International Maize and Wheat Improvement Center (CIMMYT), Mexico

**Philomin Juliana** Global Wheat Program, International Maize and Wheat Improvement Center (CIMMYT), Mexico

**S. Kadam** Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences Campus, Bengaluru, India

**B. Kalyana Babu** ICAR—Indian Institute of Oil Palm Research, Pedavegi, Andhra Pradesh, India

**J. Khan** Center for Cellular and Molecular Platforms, National Center for Biological Sciences, Bengaluru, India

**Arvind Kumar** International Rice Research Institute (IRRI), South-Asia Regional Centre (SARC), Varanasi, India

**Dinesh Kumar** ICAR-Indian Institute of Wheat and Barley Research (IIWBR), Karnal, Haryana, India

**Lokendra Kumar** ICAR-Indian Institute of Wheat and Barley Research (IIWBR), Karnal, Haryana, India

**Pawan Kumar** ICAR-Central Soil Salinity Research Institute (CSSRI), Karnal, Haryana, India

**T. N. Lakshmidevamma** Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences Campus, Bengaluru, India

**Charu Lata** CSIR-National Institute of Science Communication and Information Resources, New Delhi, India

**Rekha Malik** ICAR-Indian Institute of Wheat and Barley Research (IIWBR), Karnal, Haryana, India

**M. N. Mamathashree** Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences Campus, Bengaluru, India

**Amira M. I. Mourad** Agronomy Department, Faculty of Agriculture, Assiut University, Assiut, Egypt

**Ganapati Mukri** Division of Genetics, ICAR-Indian Agricultural Research Institute, PUSA Campus, New Delhi, India

**Mehanathan Muthamilarasan** Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, Telangana, India

**Gopalakrishna K. Naidu** All India Coordinated Research Project on Maize, University of Agricultural Sciences, Dharwad, India

**M. L. Nithyashree** Division of Agricultural Economics, ICAR-Indian Agricultural Research Institute, PUSA Campus, New Delhi, India

**K. Omkar Babu** Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

**Gopalareddy Prakash** Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences Campus, Bengaluru, India

**Lydia Pramitha** School of Agriculture and Biosciences, Karunya Institute of Technology and Sciences, Coimbatore, Tamil Nadu, India

**Naeela Qureshi** Global Wheat Program, International Maize and Wheat Improvement Center (CIMMYT), Mexico

**Sumi Rana** Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, Telangana, India

**K. Rashmi** Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences Campus, Bengaluru, India

**Deepti B. Sagare** International Rice Research Institute (IRRI), South-Asia Hub (SAH), Hyderabad, India

**Nitika Sandhu** Punjab Agricultural University (PAU), Ludhiana, India

**Sonia Sheoran** ICAR-Indian Institute of Wheat and Barley Research (IIWBR), Karnal, Haryana, India

**K. Shilpa** Division of Genetics, ICAR-Indian Agricultural Research Institute, PUSA Campus, New Delhi, India

**Chandra Nayaka Siddaiah** Pearl Millet Pathology Laboratory, University of Mysore, Manasagangotri, Mysore, India

**G. P. Singh** ICAR-Indian Institute of Wheat and Barley Research (IIWBR), Karnal, Haryana, India

**P. K. Singh** Global Wheat Program, International Maize and Wheat Improvement Center (CIMMYT), Mexico

**Subaran Singh** College of Horticulture, Maharana Pratap Horticultural University, Karnal, Haryana, India

**Uma Maheshwar Singh** International Rice Research Institute (IRRI), South-Asia Regional Centre (SARC), Varanasi, India

**Vikas Kumar Singh** International Rice Research Institute (IRRI), South-Asia Hub (SAH), Hyderabad, India

**S. B. Suby** ICAR-Indian Institute of Maize Research, PAU Campus, Ludhiana, India

**C. Tara Satyavathi** ICAR-AICRP on Pearl Millet, Jodhpur, Rajasthan, India

**Shalini Tiwari** CSIR-National Botanical Research Institute, Lucknow, India

**J. Ugalat** Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences Campus, Bengaluru, India

**RPS Verma** ICAR-Indian Institute of Wheat and Barley Research (IIWBR), Karnal, Haryana, India

**Pranjal Yadava** Division of Plant Physiology, ICAR-Indian Agricultural Research Institute, PUSA Campus, New Delhi, India

**Sarita Yadav** ICAR-National Institute for Plant Biotechnology, PUSA Campus, New Delhi, India

**Shailesh Yadav** International Rice Research Institute (IRRI), South-Asia Regional Centre (SARC), Varanasi, India

**Sunil Kumar Yadav** Department of Biophysics, University of Delhi, South Campus, New Delhi, India

# 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 | *Bacillus thuringiensis* |
| 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 |
| *cry1Ab* | Delta-endotoxin of *Bacillus thuringiensis* gene (*1Ab*) |
| *cry1Ac* | Delta-endotoxin of *Bacillus thuringiensis* gene (*1Ac*) |
| 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 × environment |
| GAB | Genomics-assisted breeding |

| | |
|---|---|
| GBS | Genotyping-by-sequencing |
| GC | Genomic control |
| GCA | General combining ability |
| GCP | Generation Challenge Program |
| GE | Genome editing |
| GEBVs | 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 |
| $H_2O_2$ | 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 | *Septoria Nodorum* 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 | *Septoria tritici* 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 | Thaumatin-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, Vikas Kumar Singh, and Arvind Kumar**

**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

D. B. Sagare · S. Alam · V. K. Singh
International Rice Research Institute (IRRI), South-Asia Hub (SAH), Hyderabad, India

N. Sandhu
Punjab Agricultural University (PAU), Ludhiana, India

S. Yadav · U. M. Singh · S. Dixit · A. Kumar (✉)
International Rice Research Institute (IRRI), South-Asia Regional Centre (SARC), Varanasi 221006, India
e-mail: a.kumar@irri.org

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021 
C. Kole (ed.), *Genomic Designing for Biotic Stress Resistant Cereal Crops*,
https://doi.org/10.1007/978-3-030-75879-0_1

## 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 bast. 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, Carbendszim 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 is 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 spikletsinto 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-graylesion. As the disease progress the lesions coalesce with each other forming larger lesion which cover the entire tillers. Infection to the inner sheath interrupt the movement of water and nutrient resulting in the death of the entire plant. The fungus survives between crops as 'sclerotia' that can remain dowarment 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 resistance variety along with cultural practice 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 are 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 condition 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 thegall midge larvae, phytoseiid mites which feeds on eggs, and spidersfeeds 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 promisingapproaches 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 $F_1$ 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

yield-enhancing traits/genes from AA genome wild species have been identified and mapped with molecular markers for their integration into *O. sativa* genome (Salgotra and Sajad 2020). Out of the more than 40 genes identified for bacterial blight resistance, ten genes were identified from the wild rice species including *Xa21* from *O. longistaminata* (Song et al. 1995), Xa23 from O. rufipogon (Zhang et al. 1998), *Xa27* and *Xa35* from *O. minuta* (Guo et al. 2010), *Xa29* from *O. officinalis* (Tan et al. 2004), *Xa30, Xa33* and *Xa38* from *O. nivara* (Natarajkumar et al. 2010; Cheema et al. 2008), *Xa32* from *O. australianesis* (Zheng et al. 2009) and *Xa41(t)* from *O. barthii* and *O. glaberrima* (Hutin et al. 2015). Some important blast resistance genes such as *Pi40* from *O. australiensis* (Jena et al. 1991), *Pi9* from *O. minuta* (Liu et al. 2002), and *Pi54rh* (Das et al. 2012) have been successfully identified from the wild species and transferred into the elite cultivars. Interestingly, approximately 19 brown planthopper resistance genes viz. *bph11, Bph10, bph12, Bph12(t), Bph13(t), Bph13, Bph14, Bph15, Bph18, bph20(t), Bph20 (t), bph21(t), Bph21(t), bph22(t), Bph23(t), bph23(t), bph24(t), Bph27* and *Bph34* have been originated from seven wild rice species (*O. officinalis, O. australiensis, O. rufipogon, O. minuta, O. eichingeri, O. folia* and *O. nivara*) (Yang et al. 2012; Huang et al. 2013; Lv et al. 2014; Kumar et al. 2018).

## 1.4 Classical Genetics and Traditional Breeding for Biotic Stress Resistance

Genetic resources are crucial for any plant breeding program and plant breeding efforts in collection, induction, and rearrangement of genetic diversity followed by selection will help achieve the desired breeding goals. Keeping a balance between diversity enhancing and reducing forces determines either gain or loss of genetic diversity in the process of breeding (Louwaars 2018). Plant breeding using artificial selection develop plants with higher economical values from the last 10,000 years (Moose and Mumm 2008). The positive selection is also called Darwinian selection where, the variants with increase in desired alleles due to selective pressure had been created until they fix in the relevant population. While applying a negative selection (purifying selection) removes the unwanted/deleterious alleles from the population. Traditional plant breeding was not enough to achieve the targeted traits at the desired level; however, the recent scientific innovations provide an opportunity to achieve the desired phenotypes in plant breeding (Varshney et al. 2006). Most of the food crops has been shown currently about 0.8–1.2% annual yield enhancement rate and it must be doubled to feed the overgrowing population sustainably (Li et al. 2018).

### *1.4.1 Classical Markers in Plant Breeding*

Initially, plant breeders had used classical markers for ease in the selection process and later replaced them with molecular markers. The examples of classical markers are morphological markers, cytological markers, and biochemical markers.

#### 1.4.1.1 Morphological Markers

Breeders have used markers from many years ago as a selection tool in order to find a plant having desired traits in a breeding program. These markers consist of visible traits, like stem characters, leaf anatomy such as leaf shape, angle, color, pod color, flower color, seed color, seed shape etc. during the initial period of plant breeding. However, the following drawbacks of morphological markers such as (i) limited in number and (ii) influenced by environment and crop growth stages (Eagles et al. 2001) makes it unfit to use in modern plant breeding.

#### 1.4.1.2 Cytological Markers

The physical parameters of the chromosomes such as variations present in the size, shape, numbers, banding patterns, order and position are known as cytological markers. Cytological markers had been widely used in physical mapping and linkage group identification (Jiang 2013).

#### 1.4.1.3 Biochemical Markers

Biochemical markers which is also called as isozymes are structural variants of an enzyme differ in their molecular weights or electrophoretic mobility. The utility of biochemical markers has been demonstrated in exploring the genetic diversity, population structure/distribution, and gene flow (Mateu-Andres and De Paco 2005). However, limitations of biochemical markers are also there, such as; limited in number, less polymorphic, and affected by spatial and temporal expression (Mondini et al. 2009).

#### 1.4.1.4 Limitations of Classical Markers

Nowadays, molecular markers have replaced the use of classical markers due to the advantages over the classical markers such as, (a) highly polymorphic, (b) wide and uniform distribution across the genome, (c) co-dominant, (d) clear allelic differences (e) single copy number and no pleiotropic effect, (f) cost effective (g) easy detection and automation.

## *1.4.2 Breeding Objectives and Important Traits*

### 1.4.2.1 Higher Yield

The ultimate goal of plant breeding is to improve the grain yield of food crops and other economical yield of various crops. It can be tuber yield, fiber yield or oil yield depending upon the crop species. The goal can be achieved by involving the high yielding diverse parents in crossing programme or through the development of vigorous hybrids.

### 1.4.2.2 Improved Nutrition/Quality

Nutritional quality is also the most desirable trait in plant breeding. The quality traits vary from crop to crop. For example, grain shape, size, cooking quality in rice, malting quality in barley, oil content in oilseed crops, and protein content in pulses.

### 1.4.2.3 Abiotic Resistance

Crop improvement for abiotic stresses viz; soil salinity, drought, extreme temperatures such as heat, cold, wind speed and frost are must added traits in the plant genotypes keeping in view the ongoing climate change.

### 1.4.2.4 Resistance to Diseases and Insect Pests

A tremendous yield loss in crop plants by various diseases and insects has prevailed continuously due to current climate change. The use of genetic resources/resistance genes is one of the cheapest and effective control methods of minimizing such losses. Genetic variation is the prerequisite to implement any successful breeding programme including disease and insect/pest resistance breeding. It is imperative to continuously search for diverse sources of resistance including the wild/weedy relatives and land races may be transferred for resistant genes through backcross breeding approach.

### 1.4.2.5 Change in Maturity Duration

Development of varieties with shorter duration in plant breeding has several advantages such as requiring less crop management period, less input use of water, insecticide, and nutrients, etc.

#### 1.4.2.6 Desirable Agronomic Traits

The desired plant type which includes plant height, branching, tillering, growth habit, etc. is required in order to release as a variety. Introduction of dwarfness in cereals leads to lodging resistance and better fertilizer responsive varieties.

#### 1.4.2.7 Elimination of Toxins

Crop varieties have been improved to make them free from toxic compounds in order to make safer for human consumption. One of the potent examples for this is the removal of neurotoxin in Khesari (*Lathyruys sativus*).

#### 1.4.2.8 Non-shattering Characteristics

Seed shattering is also an important trait for breeding in cereal as well as pulses.

#### 1.4.2.9 Photo and Thermo Insensitivity

Development of photo and thermo insensitive varieties helps in crossing and release of the cultivars in new areas.

### *1.4.3 Selection Under G × E Interaction*

Significant genotype by environment interaction (G × E) effects makes it difficult to predict genotypic performance across changing environments and to explore genotypic and environmental precisely (Allard and Bradshaw 1964). Strategies for optimum selection in the presence of G × E were described successfully by McKeand et al. (1997), and Lin and Togashi (2002). Through, conventional breeding programs, superior plants carrying desired alleles must select from a large segregating population in the field which is a very tedious job. Conventional breeding procedures are laborious, difficult, and time-consuming; it may consist of several crosses, several generations, several rounds of costly phenotyping, and phenotypic selections. The linkage drag may also create further difficulty to achieve the targeted trait at the expected level. Modern plant breeding programs have engaged interdisciplinary teams with expertise in the fields of molecular biology, statistics, biochemistry, physiology, bioinformatics, and agronomy. The current DNA sequencing technologies have revolutionized crop breeding and research on the advancement has now shifted in the modern 'genomics era' of plant breeding.

## 1.5 Diversity Analysis

Rice accessions (representing rice diversity in terms of genetic groups—aus, indica, tropical japonica, temperate japonica, and aromatic, and crop duration cycles of the accessions) from IRRI's (*Oryza sativa*) germplasm bank, assessed for their level of susceptibility to sheath blight in a field experiment revealed a strong association between morphological traits (Plant height in particular) and disease intensity, and effect of morphological traits was larger than that of genetic groups, further, the ranking of genetic groups with sheath blight susceptibility was observed as, aus < indica < japonica (Willocquet et al. 2012). Similarly, many of the QTL mapping studies have reported a strong correlation of plant height (PH) and heading date (HD) traits with ShB resistance (Nelson et al. 2012; Eizenga et al. 2013; Liu et al. 2014). There are few studies reporting no association of sheath blight resistance with morphological traits (Taguchi-Shiobara et al. 2013; Zeng et al. 2014). Increase in plant height can be correlated with sheath blight resistance as, disease rating system is based on the lesion height. Similarly, plant height plant compactness and leaf angle (Hossain et al. 2016) were found to be significantly correlated with sheath blight resistance. The false smut, indica type, and late-maturing cultivars are more resistant than japonica type and early maturing cultivars. Singh and Singh (2015) evaluated and screened 27 rice genotypes for their resistance to false smut from 98 rice germplasm. Based on the false smut score, Lore et al. (2013) classified 25 rice hybrids into five groups. There are several studies to identify diversity among genotypes/hybrids for false smut resistance (Kaur et al. 2015). A subset of rice 2 K panel consisting of 216 diverse rice germplasm lines (indica, tropical and temperate japonica, aromatic, aus and admixed) screened against false smut at two different locations in Punjab, India, and was categorized as resistant (112), moderately resistant (51), moderately susceptible (35) and susceptible (18); further, the early flowering (70–90 days) was reported to be associated with resistance against false smut (Hiremath 2018). Genetic diversity analysis of 28 yellow stem borer populations collected from different hotspots of India, carried out using ISSR markers revealed no geographical bias to the clustering, and the gene flow between populations was appeared to be relatively unrestricted. Whole-genome sequencing of 100 *Xoo* strains collected from different states in India was performed by Midha et al. (2017). To place Xoo from China into a global context, a phylogenomic analysis was performed 167 on the core genome SNPs of 109 Xoo genomes available in Genbank (including 100 from India, 8 from the Philippines, and 1 from Japan), and the 247 sequenced ones in 169 this study.

## 1.6 Molecular Mapping of Resistance Genes and QTLs

In QTL mapping, the phenotypic data generated on mapping populations (developed using distinct parent for trait of interest), and genotypic data of the population (generated using equally distributed genome-wide polymorphic markers) are analyzed to identify the association, if any exist, between these two. There are various kinds of mapping populations used in QTL mapping studies viz., biparental mapping populations ($F_2$, $F_2$ derived $F_3$ ($F_{2:3}$), backcross (BC), doubled haploids (DHs), recombinant inbred lines (RILs), near-isogenic lines (NILs), and chromosomal segment substitution lines (CSSLs) (Collard and Mackill 2008). Also different molecular markerssuch as, restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs), single-nucleotide polymorphism (SNP), and diversity arrays technology (DArT) markers (Jiang 2013), and QTL mapping methods viz., (SMA (single marker analysis), IM (Interval mapping), CIM (Composite interval mapping), ICIM (Inclusive composite interval mapping), MIM (Multiple interval mapping), MQM (Multiple QTL mapping), Bayesian mapping), along with several computer packages (JoinMap, MapMaker/QTL, Map Manager QTX, R/qtl, WinQTLCart) areused for QTL mapping.

### *1.6.1 Resistant Genes and QTLs for Major Biotic Stresses in Rice*

Recent advances in genomic research advances have led to the identification of potential donors' QTLs, and genes for major biotic stresses in rice, and the imported QTLs/genes to be targeted to develop multiple disease-resistant rice are depicted in Fig. 1.2 and Table 1.1.

#### 1.6.1.1 Blast Resistance

Rice blast (leaf, neck, or collar blast) is one of the major diseases affecting the yield potentiality of the crop (Kreye et al. 2009). More than 100 major genes including *Pi36*, *Pib*, *Pi10*, *Pi21*, *Pi2*, *Pi9*, *Pi22*, *Pi25(t)*, *Pi40(t)*, *Pid2*, *pigm*(t), *Piz*, *Pi17(t)*, *Pi37*, *Pi5(t)*, *Pi15*, *Pi28(t)*, *Pi1(t)*, *Pi44*, *Pi54*, *Pi60(t)*, *Pil*, *Pik*, *Pilm2*, *Pikh*, *Pi6(t)*, *Pi39*, *Pi51(t)*, *IPi(t)*, *Pita*, *Pita2*, and over 350 QTLs for blast resistance have been identified (Chen et al. 2020). Total of 27 blast resistant genes (*Pib*, *Pita*, *Pik-h*, *Pi9*, *Pi2*, *Piz-t*, *Pid2*, *Pi36*, *Pi37*, *Pik-m*, *Pit*, *Pi5*, *Pi33, Pi-CO39, Pi64, Pid-A4*, *Pb1*, *Pish*, *Pik*, *Pik-p*, *Pi1*, *Pi25*, *Pi54rh*, *Pi64, LABR_64-1, LABR_64-2 and Pigm*) have been cloned (Deng et al. 2017). The *Pita* and *Pita2* genes are linked closely and have broad resistance (Shikari et al. 2013). The *indica* landrace cultivars, Tadukan, TeQing, and Tetep are the widely used sources of *Pita* gene to manage blast. The

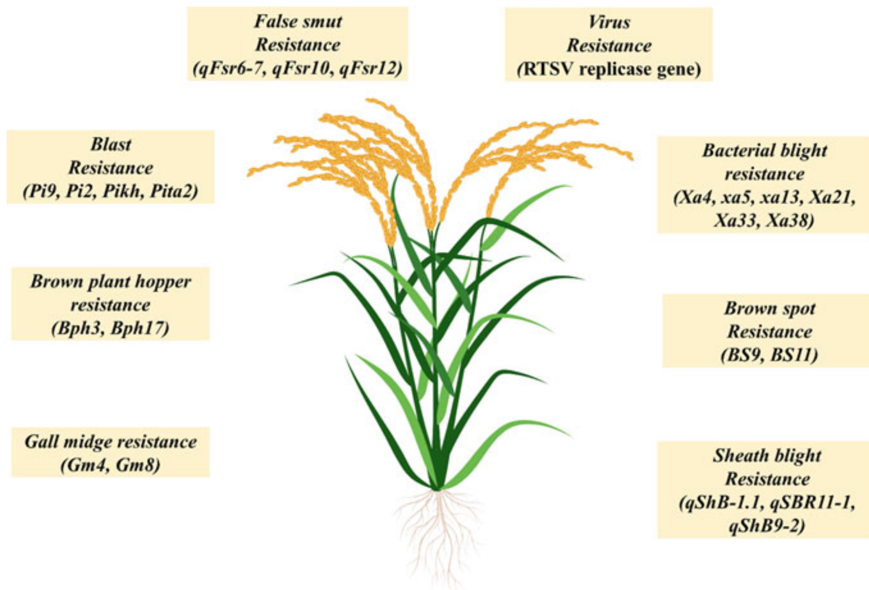



**Fig. 1.2** Genes/QTLs to be targeted to develop multiple disease resistance in rice

*Pi9* gene is reported to have broad-spectrum resistance to blast races from 43 different countries and thus, most favoured in a blast-resistant breeding program (Qu et al. 2006).

#### 1.6.1.2 Bacterial Blight Resistance

Bacterial blight (BB) is one of the devastating diseases in rice-growing countries of Asia and is caused by *X. oryzae* pv. *oryzae* (*Xoo*). So far more than 44 (dominant and recessive) genes for BB resistance have been identified including, *Xa1*, *Xa2*, *xa3*, *Xa4*, *xa5*, *Xa6*, *Xa7, Xa8*, *Xa9, Xa10*, *Xa11*, *Xa12, xa13*, *Xa14*, *Xa15*, *Xa16*,*Xa17*,*Xa18*, *Xa19*, *Xa20*, *Xa21*, *Xa22*(t), *Xa23*, *Xa25*, *Xa26*, *Xa27*, *Xa29*(*t*), *Xa30*(*t*), *Xa31(t)*, *Xa32(t), Xa33, Xa35(t), Xa38(t), Xa39, Xa40* (Kim and Reinke 2019). Seven dominant (Xa1, *Xa3/Xa26, Xa4, Xa10, Xa21, Xa23*, and *Xa27)* and four recessive (*xa5, xa13, xa25,* and *xa41*) genes have been cloned. As the BB resistant govern by single gene is easily breakable, pyramiding multiple BB resistant genes in the background of high yielding popular varieties is a substantial strategy. The *Xa4/Xa7/Xa21* combination*s* in IRBB62 have shown high resistance, followed by *Xa4/xa5/xa13/Xa21* in IRBB60 (Loan et al. 2006).

**Table 1.1** Important QTLs/genes and donors for biotic stress tolerance in rice

| Trait | Gene/ QTL | Donor | Chromosome | References |
|---|---|---|---|---|
| Blast resistance | *Pi9* | IRBL9 | 6 | Qu et al. (2006) |
| | *Pita2* | IRBLTA2-PI | 12 | Shikari et al. (2013) |
| Bacterial blight resistance | *Xa4* | IRBB60 | 11 | Loan et al. (2006) |
| | *xa5* | | 5 | |
| | *xa13* | | 8 | |
| | *Xa21* | | 11 | |
| | *Xa33* | IRGC105710 | 7 | Kumar et al. (2012) |
| | *Xa38* | IRGC81825 | 4 | Bhasin et al. (2012) |
| BPH resistance | *Bph3* | Rathuheenati | 6 | Jairin et al. (2007) |
| | *Bph17* | | 4 | Sun et al. (2005) |
| | *Bph18* | IR65482-7-216-1-2 | 12 | Jena et al. (2006) |
| | *Bph20* | IR71033-121-15 | 4 | Rahman et al. (2009) |
| | *Bph21* | | 12 | |
| Gall midge resistance | *Gm4* | Abhaya | 12 | Sama et al. (2014) |
| | *Gm8* | Aganni | 8 | |
| Brown spot resistance | *qBS9* | Tadukan | 9 | Sato et al. (2008, 2015) |
| | *qBS11* | | 11 | |
| | *qBSR9-kc* | CH45 | 9 | Matsumoto et al. (2017) |
| | *qBSR11-kc* | | 11 | |
| Sheath blight resistance | *qShB9-2* | Jasmine 85 and Teqing | 9 | Liu et al. (2014), Silva et al. (2012), Yadav et al. (2015), Al-Bader et al. (2019) |
| | *qShB1.1* | CR1014 | 1 | Bal et al. (2020) |
| | qSBR11-1 | Tetep | 11 | Channamallikarjuna et al. (2010), Richa et al. (2017) |
| False smut resistance | qFSR-*6-7* | Lemont | 6 | Zhou et al. (2014) |
| | qRFSr-5.2 | IR28 | 5 | Andargie et al. (2018) |

#### 1.6.1.3 Gall Midge Resistance

The rice pest gall midge (GM), *Orseolia oryza* (Asian GMs), and *Orseolia oryzivora* (African GMs) have become prevalent in rice-growing countries. Many gall midge resistance QTLs and 11 genes have been identified from different donors including *Gm1* (Samridhi and Asha); *Gm2* (Phalguna); *Gm4* (Abhaya); *Gm6* (Duokang #1); *Gm7* (RP2333-156-8); *Gm8* (Jhitpiti and Aganni); *Gm9* (Madhuri line 9); *Gm10* (BG380-2); and *Gm11* (CR 57-MR1523). Few GM resistant genes

have been mapped and tagged viz., *Gm1*, *Gm2*, *Gm6* and *Gm7*, *Gm4*, *Gm8*, and *Gm11* (Zhou et al. 2020).

#### 1.6.1.4 Brown Planthopper Resistance (BPH)

Several BPH resistant sources, genes, and number of QTLs have been identified. A total of 31 genes have been genetically mapped including, *Bph1*, *bph2*, *Bph3*, *bph4*, *Bph6*, *Bph9*, *Bph10*(*t*), *bph11*, *bph12*, *Bph13*, *Bph14*, *Bph15*, *bph16*, *Bph17*, *Bph18*(*t*), *bph19*, *Bph20*(*t*), *Bph21*(*t*), *Bph25*(*t*), *Bph26*(*t*), *Bph27* (*t*), *Bph28*(*t*) and *Bph35*. Through map-based cloning, *Bph3, Bph17, Bph14, Bph26,* and *bph29* have been cloned so far (Chen et al. 2020) The cultivar RathuHeenati has the resistance for all four BPH biotypes, and it possesses major resistant gene *Bph17* and two minor genes, *Qbph3* and *Qbph10* (Sun et al. 2005). The cluster loci of BPH resistant genes and the linked markers mapped on different chromosomes are represented in Fig. 1.3 (Du et al. 2020).

#### 1.6.1.5 Brown Spot Resistance

Rice brown spot (BS) is a chronic disease caused by *Bipolaris oryzae* and found in association with physiological stresses. A total of 12 QTLs for BS resistance is reported so far (Mizobuchi et al. 2016). The major effect QTL, *qBS11* in cultivar Tadukan has recently been reported for seedling stage BS resistance (Sato et al. 2008). Further, the QTL *qBSfR11* for field resistance to BS has been found to coincide with *qBS11* (Sato et al. 2015).

#### 1.6.1.6 False Smut Resistance

There are only a few studies on mapping for false smut resistance. A total of 24 QTLs *includingqFsr1, qFsr2, qFsr4, qRFSr-5.2, qFSR-6-7,qFsr8, qFsr8-1, qFsr10, qFsr10a, qFsr10b, qFSR-10-5, qFSR10-2, Fsr11, qFSR-11-2, and qFsr12* on chromosomes 1, 2, 4, 5, 6, 8, 10, 11 and 12 controlling false smut resistance are reported (Andargie et al. 2018; Han et al. 2020). The expression of chitinase genes was highly influenced after the infection by *U. virens*, and these genes were found in close vicinity of the QTLs for false smut resistance (Han et al. 2015).

#### 1.6.1.7 Sheath Blight Resistance

To date there are more than 50 QTLs reported for sheath blight resistance including *qShB-1.1, qSBR11-1, qshb7.1, qSBL7 (E2), qSBPL-7 (E2), qHZaLH3, qHZaLH6, qHZaDR8, qSB-9, qRLL-4, qRLH-4, qSB-11(LE), qRTL6, qRTL3, qRTL6, qShB7, qShB6, qShB6-mc, qsbr_12.1, qsbr_2.2, qsbr_2.1, qSBR1, qSBR11, qSBR2-2,*

**Fig. 1.3** BPH resistance genes on rice chromosomes. (Numbers represent the physical distance on the left. Marker names are shown on the right. The black bars indicate the locations of BPH resistance genes). *Source* Du et al. (2020)

*qSBR4, qSBR5-2, qSBR7, qSBR8, qSBR9, qShB2-1, qSB5, qShB6, qShB9-2*. Among all the ShB QTLs identified, *qShB9-2* and *qSBR11-1* are the major loci confirmed in several studies (Liu et al. 2013; Yadav et al. 2015; Zuo et al. 2014). A physical map of sheath blight resistant QTLs presents on rice chromosomes along with associated markers, and putative candidate genes to major QTLs are represented in Fig. 1.4 (Molla et al. 2020).

## 1.7 Association Mapping Studies

Association mapping (AM) dissects complex traits and identifies QTLs from association panel/diversity panel consisting of varieties, landraces, breeding material etc. (Zhu et al. 2008). In AM, QTL identification is performed based on the marker-trait associations explained by linkage disequilibrium (LD) between marker polymorphism across diverse panels, and the historical recombination events between marker and QTL in a panel are considered (Nordborg and Tavare 2002). The nested association mapping (NAM) population and multi-parent advanced generation intercross (MAGIC) population possess abundant recombinations and therefore can be used for AM studies. Once the location of QTL is identified, using the BLUP (best linear unbiased prediction) effect of individual QTL is estimated, to select the superior lines. The AM involves two categories candidate gene (CG) analysis, and genome-wide association mapping (GWAS). CG analysis is carried out using already studied genes that are related to the trait of interest, whereas for GWAS any prior information about the genetic control of trait of interest is not required and thus, it is a more comprehensive approach (Zhu et al. 2008). The Genotyping by sequencing (GBS) approach is used in GWAS for genotyping and it can generate medium to high marker densities (He et al. 2014), additionally genotyping can be done using SNP chips with different marker densities (Singh et al. 2015). Linkage disequilibrium (LD) is the fundamental basis for QTL detection in AM, and LD is a non-random association of alleles at two or more loci in a diverse panel (Slatkin 2008). LD measures the correlation between markers which is caused by the genetic history that they share (Bush and Moore 2012). Higher mapping resolution is expected when LD decays in a short distance, but it requires a greater number of markers. Whereas, when LD is extended over a long distance, mapping resolution is low and requires less number of markers (Rafalski 2002). Mostly, closely linked, adjacent SNPs/haplotypes rather than single SNP, are used to characterize the QTL region or allele of a gene (Rafalski 2002; Buntjer et al. 2005). In crops, 5-15 SNPs/locus are enough to characterize haplotype (Famoso et al. 2011). The variations in SNP haplotype are used by breeders to identify genomic regions under selection. In rice haplotype blocks (Yamamoto et al. 2010), and SNP haplotype for genes/QTLs (Yonemaru et al. 2015; Abbai et al. 2019) have been investigated recently. In the region around the *xa5* gene in rice, an LD of ~100 kb was estimated (Garris et al. 2003). Similarly, from six genomic regions on chromosome 1 and 4, and unlinked SNPs LD of 75 kb, ~150 kb, and 500 kb

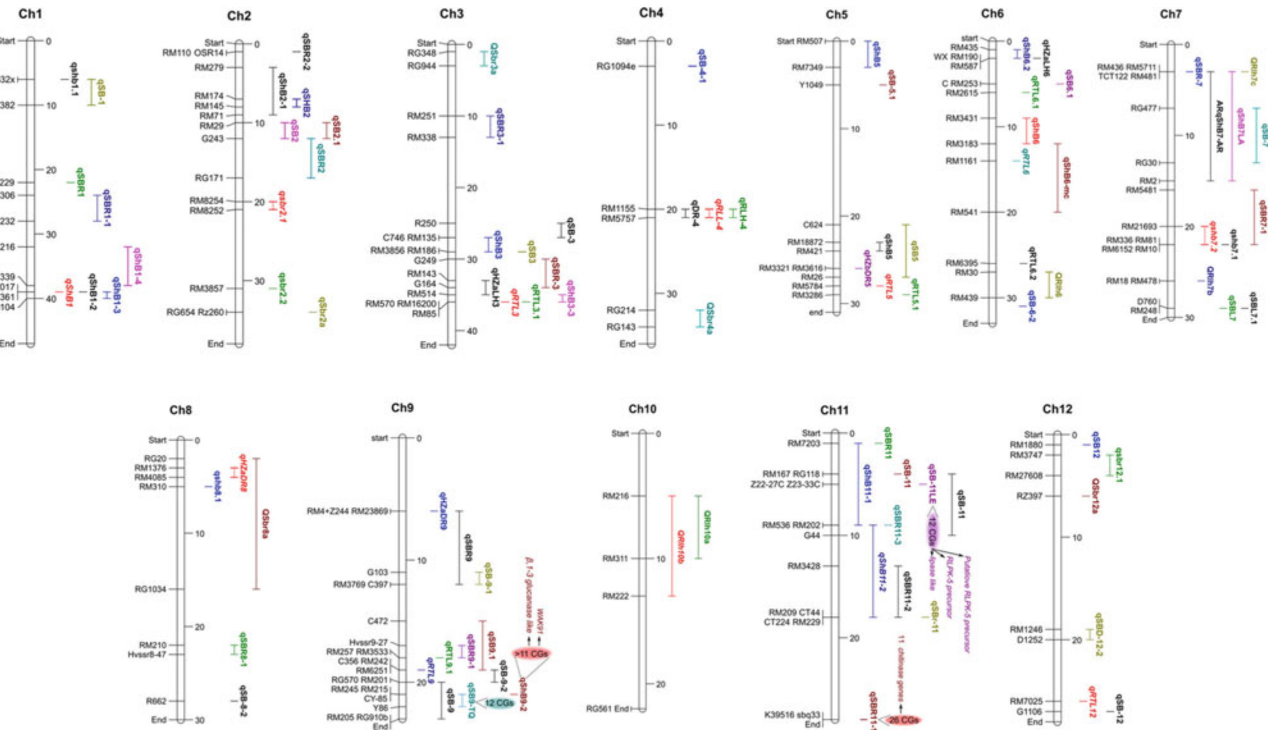



**Fig. 1.4** Physical map of different ShB QTLs on different rice chromosomes. QTLs are depicted on the right side of the bar, while the markers are shown on the left. Putative candidate genes (CGs) assigned to major QTLs are highlighted. Unit: Megabase (Mb). Length of segments on chromosomes represent physical intervals of corresponding QTLs. Abbreviation: RLPK, receptor-like protein kinase; Ch, chromosome. *Source* Molla et al. (2020)

in *indica*, tropical *japonica*, and temperate *japonica*, respectively were reported (Mather et al. 2007). The genome-wide LD rates of ~123 kb and ~167 kb for *indica* and *japonica* subspecies, respectively were estimated by Huang et al. (2010), and these average rates of LD reflect several years of historical recombinations. Based on LD estimated in rice, theoretically, a minimum of 5000 markers are enough to cover the rice genome of 420 Mb as per the estimate of 75 kb proposed by Courtois et al. (2013).

In rice AM studies various statistical methods were used viz., nucleotide diversity measure, discriminant analysis, nested clade analysis, elliptic fourier analysis, principal component analysis (PCA), generalized linear model (GLM), mixed linear model (MLM), logistic regression (LR), and simple linear model (SLM) Yu and Buckler (2006). MLM is one of the most appropriate and popular methods used for AM, and it takes population structure (Q) and kinship (K) into account for the reduction of false positives (Listgarten et al. 2012). A new statistical method, the Anderson-Darling (A-D) test has been reported to control rare alleles in GWAS (Yang et al. 2014). A stringent statistical significance level (*P*-value), and higher marker density can avoid/minimize false positives. In the last decade several AM studies have been reported in rice and associations with agronomic traits, morphological traits, yield-related traits were the focus of most of the studies. SSR markers were used in most of the earliest AM studies in Rice (Borba et al. 2010). Now, SNPs are being widely used for GWAS in rice (McCouch et al. 2010). The AM studies for biotic stress in rice are summarized in Table 1.2. Many of the AM studies were conducted for blast resistance (Mgonja et al. 2016, 2017; Lin et al. 2018; Liu et al. 2020). Also, studies on other biotic stresses are reported viz., sheath blight disease resistance (Chen et al. 2019); Bakanae disease resistance (Chen et al. 2019a), rice black-streaked dwarf virus resistance (Xiao et al. 2019), BPH resistance (Pan et al. 2019), and false smut resistance (Long et al. 2020). Several genetic signals associated with biotic stress resistance in rice have been discovered through GWAS.

## 1.8 Marker-Assisted Breeding for Resistance Traits

For many centuries, the breeders have been putting efforts and are engaged in the quest for beneficial traits to introduce into high yielding varieties from related species using conventional breeding strategies. Inconventional breeding methods selections are carried out based on phenotypic evaluations and are thus time consuming; also, it is very difficult to transfer polygenic traits using conventional breeding methods (Crossa et al. 2017). Marker-assisted breeding (MAB) is an alternate, faster, and accurate approach to overcome limitations of conventional breeding and is being practiced to accelerate crop breeding (Balachiranjeevi et al. 2018). Recent advances in genomics, sequencing platforms, availability of genome sequences, bioinformatics tools, and several online databases, are boosting MAB to accelerate breeding efforts. In recent past MAB has transpired as promising and an irreplaceable tool for improving yield stability, various traits such as biotic/abiotic

**Table 1.2** Genome wide association mapping studies for biotic stress tolerance in rice

| Trait | Sample size | Population | No. of subgroups | Significant level (*p*-value) | Genotyping method | Association method | Reference |
|---|---|---|---|---|---|---|---|
| Blast resistance | 366 | Indica landraces | | $<10^{-8}$ | 800,000 SNPs | EMMAX | Wang et al. (2014) |
| | 413 | Rice diversity panel 1 (Indica, tropical japonica, temperate japonica, aromatic, aus and admixed varieties) | 6 | $\leq$ 1E-5 | 44 K SNPs | MLM | Zhu et al. (2016) |
| | 162 | Rice diversity panel 1 (indica and temperate japonica) | 2 | <1E-16 | 44,000 SNPs | MLM | Mgonja et al. (2016) |
| | 190 | *O. glaberrima*, *O. sativa*, and NERICA cultivars | 3 | | 184 K SNPs | MLM | Mgonja et al. (2017) |
| | 314 | Rice diversity panel 1 (Indica, tropical japonica, temperate japonica, aromatic, aus and admixed varieties) | 6 | $< 3.1 \times 10^{-4}$ | 44 K SNPs | GLM + MLM | Lin et al. (2018) |
| | 584 | Rice diversity panel 1 (Indica, tropical japonica, temperate japonica, aromatic, aus and admixed varieties) | 6 | <1E-04 | 700,000 SNPs | MLM + Q | Liu et al. (2020) |
| Sheath blight resistance | 217 | USDA mini-core collection | 5 | $<5 \times 10^{-2}$ | 154 SSRs and 1 InDel | MLM | Jia et al. (2012) |
| | 285 | breeding lines, varieties, and landraces | 3 | $< 1 \times 10^{-5}$ | 40,840 SNPs | MLM | Dilla-Ermita et al. (2017) |
| | 563 | 3 K RGP subset (Indica, tropical japonica, temperate japonica, aromatic, aus and admixed varieties) | 6 | | 220,335 SNPs | LMM | Zhang et al. (2019) |
| | 299 | Rice diversity panel 1 (Indica, tropical japonica, temperate japonica, aromatic, aus and admixed varieties) | 6 | $1 \times 10^{-4}$ | 44 K SNPs | MLM | Chen et al. (2019) |

(continued)

**Table 1.2** (continued)

| Trait | Sample size | Population | No. of subgroups | Significant level (*p*-value) | Genotyping method | Association method | Reference |
|---|---|---|---|---|---|---|---|
| Bakane disease resistance | 138 | Tropical *japonica* and temperate *japonica* | 2 | | 166,418 SNPs | MLM | Volante et al. (2017) |
| | 231 | Rice diversity panel 1 (Indica, tropical japonica, temperate japonica, aromatic, aus and admixed varieties) | 6 | $< 10^{-3}$ | 44 K SNPs | GLM + MLM | Chen et al. (2019a) |
| Black-streaked dwarf virus resistance | 420 | Rice diversity panel 1 (Indica, tropical japonica, temperate japonica, aromatic, aus and admixed varieties) | 6 | $<1 \times 10^{-4}$ | 44 K SNPs | MLM | Feng et al. (2019) |
| | 1070 | 3 K RGP subset (Indica, tropical japonica, temperate japonica, aromatic, aus and admixed varieties) | 6 | $<1 \times 10^{-6}$ | 3,069,943 SNPs | MLM | Xiao et al. (2019) |
| False smut resistance | 159 | 2 K panel subset (Indica, tropical japonica, temperate japonica, aromatic, aus and admixed varieties) | 6 | $<10^{-6}$ | 700,000 SNPs | CMLC + MLM | Hiremath (2018) |
| | 315 | 3 K RGP subset (Indica, tropical japonica, temperate japonica, aromatic, aus and admixed varieties) | 6 | $1.84 \times 10^{-7}$ | 271,360 SNPs | GLM | Long et al. (2020) |
| BPH resistance | 83 | F9 lines (14CF2426/TN1) | | | 108,018 SNPs | EMMAX | Pan et al. (2019) |

stress tolerance, grain quality, and acceptance by farmers (Nelson et al. 2018; Balachiranjeevi et al. 2018). In MAB, trait linked markers are used for methodical phenotyping (Jiang 2013), and MAB can transfer complex polygenic traits, accelerates backcross breeding, minimizes linkage drag, allows pyramiding multiple traits together, and assembling of desired traits precisely in a single genotype within less time (Crossa et al. 2017). This approach has been used widely in rice breeding programs to breed the rice against various pathogens, and insect- pests (Reinke et al. 2018; Li et al. 2019a, b; He et al. 2020), and due to pervasiveness of mega varieties, it may persist as a prominent and sustained approach.

### *1.8.1 MAB for Rice Blast Disease Resistance*

To enhance blast resistance, MAB approach was used very extensively (Reinke et al. 2018). The first effort to combine three R genes, *Pi1*, Pita, and *Piz-5* from three different donors in the background of O39 to enhance blast disease tolerance through MAB was carried out using STMS markers by Hittalmani et al. (2000). Subsequently, *Piz5*, *Piz54, Pid(t), Pita2, Pib, Pik, Pi2, Pi1, Pi5, Pi9, Pi40, Pi33, Pi46, Pizt, Pish* genes from different donors were successfully introgressed either single or in combinations in various genetic backgrounds. Also, QTLs for blast disease resistance were used in MAB to enhance disease resistance (Sreewongchai et al. 2010) In several studies lines introgressed with *Pi1, Pi2, Pi-9(t)*, and *Pi54* genes showed blast disease resistance without yield penalty (Hua et al. 2015; Kumar et al. 2016; Wu et al. 2016).

### *1.8.2 MAB for Bacterial Blight Disease Resistance*

Many of the bacterial blight susceptible rice cultivars and landraces were pyramided with resistant genes such as, *Xa1, Xa4, xa5, xa13, Xa21, Xa26, Xa27* (Yap et al. 2016). In the early 1990s the transfer of resistance genes to improve BB tolerance in rice was carried out for the first (Ronald et al. 1992). Several resistant genes were subsequently used to introgress into the various genetic backgrounds resulted in enhanced bacterial blight resistance. Breeding for bacterial blight resistance gene through pyramiding approach has been taken up widelysuch as, *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. 2016), *Xa21* + *Xa27* (Luo et al. 2017), *xa13* + *Xa21* (Arunakumari et al. 2016), *Xa7* + *xa13* + *Xa21* + *Xa4* (Yap et al. 2016), *xa5* + *xa13* + *Xa21* (Baliyan et al. 2018), *Xa4* + *Xa21* + *xa5* + *xa13* (Chukwu et al. 2019). In other instance, the introgression of other R genes viz., *Xa23*, *Xa21*, *Xa33*, *Xa7*, and *Xa40* showed improved bacterial blight disease resistance in different genetic backgrounds worldwide (Ni et al. 2015; Kumar et al. 2016; Nguyen et al. 2018; Reinke et al. 2018).

### 1.8.3 MAB for Sheath Blight (ShB) Disease Resistance

To enhance ShB tolerance, QTLs/genes were transferred/pyramided in the genetic background of elite cultivars. The transfer of QTLs, *qSB-9Tq* (donor-TeQing) into the different japonica rice cultivars, qSB*12-1* and *qSB9-2 into* the Lemont, *qSB-7* and *qSB-9* into the elite variety WLJ1, *qSBR11-1, qSBR11-1, qSBR11-2,* and *qSBR7-1* into the Improved Pusa Basmati and Pusa 6B, are reported to improve ShB disease resistance (Zuo et al. 2008; Wang et al. 2012a, b; Chen et al. 2014).

### 1.8.4 MAB for Gall Midge (GM) Resistance

To develop gall midge resistance first attempt was made by Katiyar et al. (2001) using RFLP and AFLP markers, where $F_3$ populations developed by making a cross between Duokang #1 (donor of *Gm-6(t)*) and Phalguna (donor of *Gm2*), and lines possessing favorable alleles at both loci showed resistance against biotype 4, and biotype 1. This was the first ever report on pyramiding two closely located gall midge resistance loci having dissimilar effects. Further, using linked STS and SSR markers, and functional markers Gm resistant genes viz., *Gm3, Gm6*, *Gm8, Gm11t* were introgressed into the various genetic backgrounds and enhanced resistance to GM biotype 1 and biotype 4 were observed (Himabindu et al. 2010; Sama et al. 2014). Divya et al. (2015) and Kumar et al. (2017) reported successfully pyramiding of GM resistant genes (*Gm1* and *Gm4*) and (*Gm4* and *Gm8*) into the background of improved Samba Mahsuri and DRRH3 (elite rice hybrid), respectively.

### 1.8.5 MAB for Brown Planthopper (BPH) Resistance

The BPH resistant genes *Bph3* (donor-RathuHeenati), and *Bph18* (donor-*indica* line IR65482-7-216-1-2) were incorporated into the most popular Thai rice variety Khao Dawk Mali 105 and Junambyeo, an elite *japonica* cultivar employing MAB (Jairin et al. 2009; Suh et al. 2011). A series of NILs with a single BPH resistance gene/QTL, *Bph3, bph4, Bph6, Bph9, Bph10, Bph14, Bph15, Bph17, Bph18, Bph20, Bph21, Bph24, Bph26, Bph32, qBph3,* and *qBph4* developed in the background of BPH susceptible cultivars 9311 and IR24 showed enhanced resistance (Jena et al. 2017). Further, Pyramiding of *Bph6* + *Bph9* and *Bph14* + *Bph15* into the elite cultivars resulted in enhanced resistance to BPH (He et al. 2019).

### 1.8.6 Pyramiding Multiple Biotic Stress Resistance

With the precision in current era marker technology, developing varieties with multiple biotic and abiotic stress tolerance along with other grain yield and quality traits has become practically possible. And such varieties possessing superior multi-traits can help farmers to minimize yield losses, produce quality grain, and higher farm income under changing climatic conditions. Multiple trait breeding/ pyramiding scheme involves three main steps, assemble first, line fixation, and line evaluation. In the first step, simple and complex crossing are involved to transfer desirable traits/alleles from multiple parents and to accumulate all targeted genes/ QTLs in a single genetic background. In the fixation step, gene-based/linked/SSRs/ other markers are used in each generation from $F_2$ to $F_6$ generation to track the desired allele of each gene/QTL for fixing the target genes into a homozygous state. Also, gene pyramiding can be achieved through marker-assisted backcrossing schemes namely, Stepwise transfer, Simultaneous transfer, and Simultaneous and stepwise transfer. Recently, the pyramiding of *Bph36, Bph3*, *Bph27*, and *Bph29* into the elite cultivars 9311 and MH511 (harboring *Xa23*), exhibited strong resistance to BPH and BLB (Li et al. 2019a, b). Similarly, Dixit et al. (2020) pyramided biotic stress resistant genes viz., *Gm4*, *Gm8, xa5, Xa21, Pi9* along with submergence tolerance gene *Sub1*, and yield under drought QTLs, $qDTY_{12.1}$ and $qDTY_{1.1}$ in the background of Swarna through marker-assisted forward breeding. Some of the studies on gene/QTL pyramiding for biotic stress tolerant genes/QTLs are summarized in Table 1.3.

### 1.8.7 Genomic Selection—A Scientific Advancement and Its Role in Multiple Trait Breeding

Genomic selection (GS) is an approach of genomics-assisted breeding, enables the meteoric selection of superior genotypes and accelerates breeding cycles for higher genetic gain. In GS the genetic values of selected candidates are predicted based on genomic estimated breeding values (GEBVs). In GEBV prediction model phenotypic data, marker data and pedigree data are combined to increase the prediction accuracy. In GS, genome-wide markers are chosen and used appropriately to have all QTLs in LD with minimum a single marker. The breeding lines with high GEBVs could serve as potential material in breeding programs. A potential application of GS to enhance the selection for yield and related traits has been reported in many cereal crops (Michel et al. 2016; He et al. 2016). Using GS parental combinations was predicted for the development of superior hybrids in rice (Xu et al. 2018). Multi-trait GS can be implemented on phenotypic data of different traits such as grain yield, quality, reactions to abiotic and biotic stresses. For effective implementation of genomic prediction models, it is very much important to exist favourable genetic correlations between traits (Schulthess et al. 2016). In

**Table 1.3** Marker assisted selection for pyramiding biotic stress tolerance in rice

| Targeted traits | QTL Combination in pyramided lines/NILs | Reference |
|---|---|---|
| Rice blast resistance | *pi21 + Pi34 + qBR4-2 + qBR12-1* | Fukuoka et al. (2014) |
| Bacterial blight and Rice blast resistance | *Xa4 + xa5 + xa13 + Xa21 + Pi9 + Pi2 + Piz* | Chukwu et al. (2019) |
| Brown plant hopper resistance | *Bph1 + Bph2* | Sharma et al. (2004) |
| Bacterial blight and Rice blast resistance | *Xa21 + xa13 + Pi54 + Pi1* | Jamaloddin et al. (2020) |
| Bacterial blight resistance | *xa5 + xa13 + Xa21* | Pradhan et al. (2015) |
| Bacterial blight resistance | *xa5 + xa13 + Xa21* | Dokku et al. (2013) |
| Bacterial blight and Rice blast resistance | *xa13 + Xa21 + Pi54* | Arunakumari et al. (2016) |
| Brown plant hopper resistance | *BPH15 + BPH26* | Myint et al. (2012) |
| Brown plant hopper resistance | *Bph14 + Bph15 + Bph18* | Hu et al. (2012) |
| Rice blast resistance | *Pi9, Piztt, Pi54; and Pi9 + Pizt* | Xiao et al. (2017) |
| Gall midge resistance | *Gm-2 + Gm-6(t)* | Katiyar et al. (2001) |
| Rice blast resistance | *Pigm + Pi1, Pigm + Pi54 and Pigm + Pi33* | Wu et al. (2019) |
| Brown plant hopper resistance and Rice stripe disease resistance | *Bph14 + Bph15 + Stv-bi* | Xu (2013) |
| Brown plant hopper resistance | *Bph3 + Bph27 (t)* | Liu et al. (2016) |
| Rice blast, bacterial blight and brown planthopper resistance | *Pita + Xa23 + Bph3; Pita + Xa23; Pi1 + Pi2 + Xa23 + xa5; Pi1 + Pi2 + xa5* | Ji et al. (2016) |
| Bacterial blight resistance | *Xa4 + xa5 + Xa4 + xa5 + Xa21* | Sabar et al. (2019) |
| Brown plant hopper resistance | *Bph3 + Bph14 + Bph18 + Bph32* | He et al. (2020) |
| Bacterial blight resistance | *Xa21 + xa13 + xa5* | Baliyan et al. (2018) |
| Brown planthopper, bacterial blight, rice blast, and rice stripe virus resistance | *Bph18 + Xa40 + qSTV11SG + Pib + Pik* | Reinke et al. (2018) |
| Bacterial blight and brown planthopper resistance | *Xa21 + Bph14 + Bph15* | He et al. (2019) |
| Bacterial blight resistance | *Xa21 + Xa33* | Balachiranjeevi et al. (2018) |

the current era of molecular breeding, with the available information from NGS, genotyping with a huge number of markers can be done, and that will help more efficiently to develop improved breeding lines with multiple traits with GS within a short time with higher prediction accuracy compared to MAB approaches.

## 1.9 Map-Based Cloning of the Resistance Genes

Map-based cloning or positional cloning involves the following steps:

- Identification of a marker that is tightly linked to gene of interest in a 'large' mapping population of size 300–500 individuals.
- The next step would be a screening of a large insert genomic library (YAC or BAC) to which the marker probe hybridizes.
- To develop new markers (that map near to the gene of interest or flank the gene) from large-insert clone and to determine if they are co-segregating with the gene of interest. The co-segregation means when one allele of the gene of interest is expressed and the markers associated with that particular allele are always present. It means that the recombination between the gene of interest and the markers is not seen.
- If required meaning if the markers do not cosegregate, then re-screen large-insert genomic library again for other clones and explore other co-segregating markers. To accelerate the process of cloning, it is better, to begin with, a marker that is in tight association with the gene of interest to avoid the additional screening.
- To identify a candidate gene from the large insert clone, whose markers are co-segregating with the gene of interest.
- To perform transformation/genetic complementation to rescue wild-type phenotype.
- To sequence the gene and to determine if the function of the gene of interest is known.
- Chromosomal landing is a genetic technique that is being used to identify and isolate the clones in a genomic library.

Chromosome walking involves positional cloning to find, isolate, and clone a specific allele in a genomic library. Chromosomal landing is advantageous over chromosomal walking as it reduces the problem of analyzing large, and/or highly repetitive genomes by minimizing the necessity for chromosome walking (Tanksley et al. 1995). Chromosomal landing is based on the principle that the expected average distance between the marker associated with the target trait can be lesser than the average insert length of the clone library containing that gene of interest. Chromosome landing has now become the key strategy to isolate genes underlying the quantitative traits in plant species using map-based cloning.

Map-based cloning and complementation test of the *BPH18 gene* for brown planthopper resistance revealed that the BPH18 encodes CC-NBS-NBS-LRR protein. Out of these 34 genes identified for BPH resistance in rice, 20 genes were fine mapped; only the 8 genes (*Bph3*, *Bph18, Bph9, Bph14, Bph29, Bph17, Bph32*and *Bph26S*) have been cloned and also functionally characterized (Guo et al. 2018; Ren et al. 2016; Zhao et al. 2016). The eight out of the eleven gall midge resistance genes viz, *Gm2, Gm1, Gm4, gm3, Gm6, Gm8, Gm11* and *Gm7* have been mapped successfully (Yasala et al. 2012; Sama et al. 2014). The four gall midge resistance genes *Gm2 (NB-ARC),* (*Gm1, gm3 (NB-ARC),* and *Gm4 (NB-LRR)*) have been successfully validated functionally and the linked markers can further be used in marker-assisted introgression program (Bentur et al. 2016; Venkanna et al. 2018). To date, more than 100 genes conferring rice blast resistance have been identified and 30 of them have been successfully cloned and functionally characterized (Wang et al. 2017; Zhao et al. 2018). Considering the success story of the mapping of BLB resistance gene in rice, a total of 45 BLB resistant genes have been identified, and 11 of them have been fine mapped and cloned utilizing modern biotechnological approaches (Zhao et al. 2018; Neelam et al. 2020).

## 1.10 Genomics-Aided Breeding for Resistance Traits

Genomics-assisted breeding is the application of biotechnological tools and techniques in crop improvement. This has been considered as a collection of tools and efforts improving trait phenotypes involving direct manipulation of genotype at the DNA level (Varshney and Tuberosa 2007). Genomics-assisted breeding includes genomic analysis, structural-functional genomics, proteomics, and metabolic profiling (Tyagi et al. 2004). The availability of a high-quality genome sequence of cereal crops has provided an important resource to mine the information about the diversity of genes/alleles contributing to the improvement of valuable agronomic traits (Agarwal et al. 2016). Among cereals, the rice genome with a 430 megabase pairs (Mbp) size was reported as the smallest genome compared to other crops such as sorghum (750 Mbp), maize (3000 Mbp), barley (5000 Mbp), and wheat (16,000 Mbp). The rice genome sequencing involving three BAC (*EcoRI, HindIII, MboI*), one PAC (*Sau3A1*) and the two plasmid libraries (*HaeIII, Sau3A1*) (Baba et al. 2000; Chen et al. 2002; Yang et al. 2003) led to the generation of genetically anchored sequence intending to provide the whole genome sequence in the public domain with an accuracy of more than 99.9%. Following the participation in IRGSP, India has continued the contribution in the areas of germplasm evaluation, diversity analysis, improved donor identification, marker development, QTL mapping, genomics assisted breeding, transcriptomics, and functional genomics through various national and multi-national research programs (Huang and Han

2014). These efforts have helped to generate resources improving rice resistance to various abiotic and biotic stresses, rice production, and quality. The Genoplasmics or GPGR (Genomics-based plant germplasm research) is a novel cross-disciplinary research field seeking the application of the genomics principles and techniques to germplasm research (Jia et al. 2017). GPGR can be divided into the identification of existing genomic diversity in the germplasm, conservation, and protection of the germplasm, designing of a representative core collection utilizing genetic diversity, germplasm enhancement using the developed core collections, and discovery of new genes/alleles in the core collection.

The techniques such as RNA sequencing, microarrays, microRNA sequencing, and downstream analyses have been proven useful to study the transcriptomes, and their regulation involves miRNA in diverse developmental and stress conditions related to rice. The differentially regulated genes have been selected as an important target for functional validation with the aim to raise improved rice plants (Agarwal et al. 2014). Recently, the IRFGC (International Rice Functional Genomics Consortium, http://www.iris.irri.org/IRFGC/) platform has provided potential knowledge for sharing materials, seeking partnerships, database integration, implementation of the cooperative initiatives, and fast-tracking delivery of the research results to improve rice production. Various national programs have been developed to extract the rice genome information (Tyagi and Khurana 2003).

Genomics/marker-assisted QTL/gene pyramiding reported as an effective breeding strategy to transfer multiple genomic regions conferring tolerance/ resistance genes to various abiotic and biotic stresses into a single rice variety to achieve broader and durable impact and high genetic gain (Collard and Mackill 2008; Sandhu et al. 2019; Sagare et al. 2020). Pyramiding of genes providing resistance to BLB viz., *Xa4* + *xa5* + *Xa21* (Suh et al. 2013), *xa5* + *xa13* + *Xa21* (Dokku et al. 2013; Pradhan et al. 2015), *Xa4* + *xa5* + *xa13* + *Xa21* (Stahle et al. 2016), and blast viz., *Pi9* + *Pita* has proven very effective in combating the biotic stress incidence with improvement in the durability of biotic stress resistance genes (Khanna et al. 2015). Multi-disease resistance improved rice breeding lines conferring resistance to blast, BLB, and brown planthopper (*Pi40, Xa4, Xa21*, *xa5,* and *Bph18*) have been developed (Suh et al. 2013). Among abiotic stresses tolerance/resistance, recently, drought (*qDTY1.1* + *qDTY2.1* + *qDTY3.1*) and flood tolerance (*Sub1*) QTLs were combined in the popular rice variety, Swarna using genomics assisted breeding (Sandhu et al. 2019). In recent years there has been a revolution in crop genomics. International Rice Research Institute (IRRI) has re-sequenced 3000 rice accessions in 2014, representing all 5 varietal groups, *indica*, aus/boro, basmati/sadri, tropical *japonica*, and temperate *japonica* (Alexandrov et al. 2015).

## 1.11 Recent Concepts and Strategies Developed

### 1.11.1 Gene Editing

In rice, new breeding technologies have been established, including genome editing, expanding the potential for crop improvement. The TALENs (transcription activator-like effector nucleases), SSNs (Sequence-specific nucleases), including ZFNs (zinc finger nucleases), and the CRISPR (clustered regularly interspaced short palindromic repeats) have been demonstrated to be very useful tools for the plant genome editing (Baltes et al. 2015; Cohn et al. 2014; Wang et al. 2016a, b; Antony et al. 2010; Li et al. 2012; Zhou et al. 2015; Blanvillain-Baufum et al. 2017; Cai et al. 2017). Genome editing technology (GET) exploiting agrobacterium/biolistic mediated transformation method and/or nanoparticles as carriers has immense potential for crop improvement. The reverse genetics based CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated Cas9 endonuclease) technology can be used as a powerful tool to understand the role of wheat gene homoeologs in controlling tillering ability. Genome editing takes full advantage of the accessible genomic information and help the researchers to rapidly validate the candidate genes and their function. Use of nanoparticles for delivery of CRISPR/CAS machinery in the cell can hasten the success rate of genome editing of the target genes. Over the past few decades, remarkable progress has been made in genome sequencing tools. Despite several advancements, most plant species are still difficult to transform. The examples of the recent advancements in developing targeted mutations in the genes of interest involving genome editing technologies include anti-browning apples and mushrooms. CRISPR/CAS based editing of tomato for *polygalacturonase* genefor shelf life has also been attempted in different laboratories including PAU. In crops such as wheat delivery of the CRISPR/cas-sgRNA cassette or RNP complex poses a major limitation as genetic transformation is difficult. Nanoparticle based of delivery of CRISPR/Cas cassette has been successfully accomplished in crops such as *Nicotiana benthamiana*, arugula (*Eruca sativa*), wheat (*Triticum aestivum),* and cotton (*Gossypium hirsutum*). Such delivery methods based on nanomaterial now have tremendous potential for application of genome editing in complex crops such as wheat. Genome editing has the potential to generate novel alleles as well as can be applied for functional validation of the targeted genes/alleles. Gene identification and cloning has progressed much faster in rice as compared to wheat due to smaller genome size and availability of the whole genome sequence much earlier than wheat. A number of genes for very important agronomically desirable traits such as grain size, grain number/panicle, tiller number, etc. have been cloned and characterized in rice.

### 1.11.2 Nanotechnology

Several novel and highly potential concepts and products are developed in nanotechnology to manage the plant pest and pathogen threats in agriculture. Currently, studies on applications of nanotechnology in agriculture are immensely being carried out viz., to deliver plant hormone, to enhance seed germination ability, for water management, precised transfer and tracking of genes, nanobarcoding, nanosensors, and controlled release of agrichemicals (nanopesticides, nanofertilizers, nanoherbicides) for disease-pests and weed management (Hayles et al. 2017). The nanoparticles act as carriers for pesticides, insecticides, fertilizers or other components, such as double-stranded RNA (dsRNA), herbicides etc., and can be applied as a spray or imbibed on seeds, leaves, and roots for protection against insect-pests and pathogens (Yang et al. 2008a, b). Metal nanoparticles such as silver, copper, zinc oxide, and titanium dioxide, etc., possess antibacterial, antifungal, and antiviral properties (Kim et al. 2018). Chitosan is one of the popular nanoparticles with favorable biological properties and has been extensively used for controlling *Phyricularia grisea* in rice and root-knot nematodes (*Meloidogyne javanica*) (Kashyap et al. 2015). Also, PEG nanoparticles were used to control red flour beetles (Yang et al. 2009). Nanoparticles acting as carriers of RNAi-inducing molecules have been targeted against viruses, and aphids. Li-Byarlay et al. (2013) used the perfluorocarbon-siRNA nanoparticles to target get three different aphid species; *Aphis glycines*, *Acyrthosiphonpisum* and *Schizaphisgraminum*, and reported that the nanoparticle-loaded siRNA had a significantly higher gene knockdown. There are very few reports on the delivery of nanoparticle coated RNAi molecules into plant cells for crop protection, but considering its benefits and precision over conventional methods, it has a potential to hold promise for crop protection (Worrall et al. 2018).

## 1.12 Genetic Engineering for Resistance Traits

### 1.12.1 Target Traits and Alien Genes

The impact of climate change on plant growth and crop yield is significant and poses a threat to overall food security. The climatic changes of extreme temperature stress, salinity, drought, and flooding, as well as incidence and severity of biotic stresses such as the emergence of new pests, diseases, and the invasion of alien weed species, will also increase due to climate change. Development of crop resilient through conventional breeding is difficult due to limited variability exists in the crop germplasm for use in the breeding, however, the use of molecular markers and biotechnological interventions along with good crop management may provide solutions for these complex problems of crop plants.

### 1.12.2 Genetic Engineering for Disease Resistance

Plant diseases have been a critical challenge to the farmers and have played a significant negative impact in achieving sustainable crop yield. Global losses due to various diseases in crop plants have been reported from 10 to 30% (Savary et al. 2019) and pose a significant impact on food security. Chemical control for diseases has proven effective from long back in preventing the crop losses including use of various fungicides. However, use of chemical pesticides is not environment friendly and also losing its efficacy rapidly due to new emerging races of pathogens under the ongoing climate change (Berger et al. 2017). In the recent past, use of transgenic technology has been demonstrated as one of the most effective and sustainable way of controlling various crop diseases caused by fungus, bacteria and viruses (van Esse et al. 2019). Crop plants can be modified through genetic transformation to synthesize antimicrobial peptides or compounds that directly restrict the colonization of microbes (Osusky et al. 2000). Transgenes can also be introduced that can inhibit the cell wall degrading enzymes, encode various proteins capable of breaking various mycotoxins (Karlovsky 2011). RNAi approach can be deployed in order to provide robust viral immunity by targeting viral RNA (Wang et al. 2012a). Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated (Cas) technology has provided an excellent opportunity in recent years for engineering resistance to deal the plant viruses (Wright et al. 2016).

### 1.12.3 Genetic Engineering for Insect Resistance

Genetically engineered inherent crop resistance to insect pests is one of the most potent methods of crop protection to fulfill the demands of sustainable agriculture. Chemical control of insect pests has proven harmful to human health and the environment and needs to be replaced with safer methods of insect control. Genetic transformation through *Agrobacterium*-mediated transformation or particle bombardment to introduce specific DNA sequences or genes plays a significant role in conferring resistance against insects/pests (Birkett and Pickett 2014). Bt endotoxin lyses the midgut epithelial cells of the target insect by creating the pores in the membrane of epithelial cells, resulting in the ion leakage and insect mortality (Whalon and Wingerd 2003). Bt cotton (Bollgard I—BG I) expressing *Cry1Ac* was first commercialized and released in 2002 in India and further Bollgard II expressing *Cry1Ac* and *Cry2Ab* (MON15985 event) was developed and approved in 2006, and, currently occupies most of them (95%) cotton growing area in India. Pyramiding of *Cry1Ac* and *Cry2Ab* toxins together had shown broader and durable resistance than the cotton expressing the single gene event with *Cry1Ac*. Transgenic rice lines expressing *Cry2A*, the insecticidal protein, conferred 80% mortality against the rice leaf folder (Gunasekara et al. 2017). Insect resistance through lectins which are carbohydrate-binding proteins and have a high binding affinity for

glycoproteins and glycolipids in cell membranes has shown effectiveness against sucking pests. Transgenic rice line with GNA (Galanthus nivalis lectin), showed the insect resistance against major sap-sucking pests including BPH (Bharathi et al. 2011). Through RNA interference (RNAi) approach, gene knockdown in piercing and sucking insects including stem borers and high insect mortality rates has been demonstrated in maize and rice.

### *1.12.4 Improving Crop Yield and Nutritional Value*

Malnutrition is a serious health concern particularly among children and women in developing and underdeveloped countries due to poor access to nutritious food. The development of biofortified foods through genetic engineering of stable foods for making it more nutritious is a strategic solution to malnutrition (Perez-Massot et al. 2013). Golden rice has been developed by inserting two beta-carotene synthesis genes: phytoene synthase (psy) and lycopene β-cyclase (β-lcy) through the genetic transformation to biosynthesize beta-carotene is a unique example of such effort (Beyer et al. 2002).

### *1.12.5 Herbicide-Resistant Crops*

Herbicide-resistant crops, particularly glyphosate-resistant had provided an effective approach incontrolling the weeds among the growers of various crops. Transgenic lines with transgenes glyphosate-insensitive5-enolpyruvylsh ikimate-3-phosphate synthase (EPSPS) the *cp4 epsps* gene from *Agrobacterium tumefaciens* strain CP4 and herbicide resistance traits are used on more than 80% of the total estimated area of transgenic crops grown annually in more than 25 countries (Dill et al. 2008).

### *1.12.6 Biofuel and Transgenic Plants as Bioreactors for Recombinant Proteins*

The application of the modern metabolic engineering tools in photosynthetic microalgae has the great potential for enhanced biofuel production, an important source of renewable fuel (Radakovits et al. 2010). Transgenic plants had been used as a bioreactor to produce antibodies, metabolites, proteins, and vaccines through recombinant DNA technology (Fischer et al. 2013).

### 1.12.7 Methods to Transfer Transgenes into the Plants

First plant transformation was described in tobacco reported in 1984, Since then, rapid development in transformation technology has happened in various crops. The most preferred gene delivery system by plant biotechnologists is *Agrobacterium*-mediated due to ease in transformation, the tendency to transfer single copy number carrying the genes of interest at lower cost with minimal rearrangement (Shibata and Liu 2000; Gelvin 2003). The other method frequently used for transformation is the gene gun/particle bombardment technique (Sanford et al. 1987) which has certain advantages over other techniques like rapid gene transfer, efficient, non-specific to tissue, no-host limitations, and simultaneous multiple gene transfer. The particle bombardment is also most widely used in achieving plastid transformation in plants and is the only effective method in achieving mitochondrial transformation (Johnston et al. 1988).

### 1.12.8 Plastid Transformation

Plastid transformation is a gene transfer technology where the chloroplast genome was targeted instead of the nuclear genome. Foreign genes had been transformed into chloroplast through biolistic approach or polyethylene glycol (PEG) method. Maternal inheritance of genes and plants can stably produce protein without transgene outcrossing via pollen as well as a high level of transgene expression makes plastid transformation an efficient method of gene transfer.

### 1.12.9 Gene Stacking

The transfer of two or more transgenes of agronomic interest in the same plant refers to Gene stacking. The multigene transfer technology allows researchers to achieve an enhanced level of the phenotype through additive gene action, and expressing entire multi-protein complexes that were impossible by incorporating a single gene (Naqvi et al. 2010). There are two methods of gene stacking (1) simultaneous introduction; (2) sequential introduction. The simultaneous introduction further divided into (a) co-transformation with single plasmid: all the transgenes are present on the same plasmid and (b) co-transformation with multiple plasmids: all the transgenes present on the separate plasmids (Francois 2002). The example of the co-transformation strategy is golden rice, where two T-DNAseach harboring the two genes, were introduced into cereal crop (rice), enabling endosperm to express the carotenoid biosynthetic pathway leads to synthesis of β-carotene (Ye et al. 2000). The sequential introduction of the genes can be obtained by (a) by the sexual crossing between two or more transgenic events or (b) re-transformation processes.

### 1.12.10 Gene Silencing

A major concern in the development of transgenic events is undesired transgene silencing. Gene silencing is the regulation of gene expression where interruption or suppression of the expression of a transgene happened at the transcriptional or translational level. The mechanism behind inactivation/silencing of transgene activity at the transcriptional level was due to promoter methylation and chromatin condensation or transcript degradation (Fagard and Vaucheret 2000). Post-transcriptional gene silencing is the inactivation of a transgene, where transcripts do not accumulate despite continuous transcription (Vaucheret and Fagard 2001). Silencing can also happen if transgenes and endogenous genes both are homologous. Strategies to avoid transgene silencing (Depicker et al. 2005) are, (1) Selection of single-copy transgenic line (2) chloroplast transformation, (3) Selection of the favorable/unique integration sites, (4) Silent transgenes reactivation, (5) Use of SMAR (Scaffold Matrix Attachment Regions) in silencing the mutant host system to prevent gene silencing.

### 1.12.11 Prospects of Cisgenics

Cisgenes from crossable sexually compatible plants are used in gene introduction whereby the problem of linkage drag of other genes can be overcome. The gene used in cisgenic technology is like the introduction of a gene through conventional crossing used in classical breeding. The Cisgenesis a kind of genetic modification with ‘natural genes’ that have been present in the species or in crossable relatives can be transferred through traditional breeding approaches (Schouten et al. 2006). Hence, the cisgenic plants should be treated as non-transgenic plants and exempt from GMO regulations. Cisgenics can play a significant role in sustainable development in the genetic improvement of crops (Schouten et al. 2006).

## 1.13 Role of Bioinformatics as a Tool

Bioinformatic platforms and their databases play a significant role in understanding the biological processes. With the advance in the sequencing project of various crops, bioinformatics continues to make significant progress in biology by providing the plant breeders with access to genetic information linked with complex economically important traits. Genome sequencing of crop plants has progressed significantly in the present era of molecular biology and opened tremendous opportunities for crop improvement. A high degree of synteny exists in cereal crops and the availability of sequence information has enabled the discovery of various useful traits for crop improvement. Comparative genomics and the availability of high resolution physical and genetic maps of plants has been proven the great applications of bioinformatics tools.

### 1.13.1 Biological Databases

Three kinds of biological databases have been established: (a) large scale public repositories (b) community-specific databases, and (c) project-specific databases. Most commonly used large-scale public repositories are usually developed and maintained by several national/government agencies or international consortia such as GenBank for sequences (Wheeler et al. 2005), UniProt (Schneider et al. 2005) for protein information, Protein Data Bank (Deshpande et al. 2005) for protein structure information, and ArrayExpress (Parkinson et al. 2005) and Gene Expression Omnibus (GEO) (Edgar et al. 2002) for microarray data.

### 1.13.2 Sequence Analysis

Biological sequence such as DNA, RNA, and protein sequence are the most desirable objects to understand any phenomena at the molecular, physiological and biochemical level. Expressed sequence tags (ESTs) from many plants including rice, soybean, cotton, wheat, and sorghum have been generated from sequencing data of various plant genomes (http://www.ncbi.nlm.nih.gov/dbEST/). Numerous software packages exist for sequence assembly (Gibbs and Weinstock 2003), including Phred/Phrap/Consed (http://www.phrap.org), Arachne (http://www.broad.mit.edu/wga/), and GAP4 (http://staden.sourceforge.net/overview.html).

### 1.13.3 Gene Finding and Genome Annotation

The prediction of total introns and exons in a stretch of the DNA sequence is gene finding. Several computer programs are available for identifying protein-coding genes such as Genscan (http://genes.mit.edu/GENSCAN.html), Genie (http://www.fruitfly.org/seqtools/genie.html), and Glimmer (http://www.tigr.org/softlab/glimmer).

### 1.13.4 Computational Proteomics

Proteomics is the qualitative and quantitative characterization of proteins and their interactions on a genome level. Proteomics leads to identification and quantification

of all protein within a cell or tissue on a large scale, analysis of post-translational modification and protein-protein interaction, and characterization of protein functions and structure (Canovas et al. 2004). The various databases exist for studying the proteomics such as SWISS-PROT-database of annotated protein sequences, protein function, protein domains, post-translational modifications, Tr EMBL—a computer-annotated supplement of Swiss-Prot that contains all the translations of EMBL nucleotide sequence entries not yet integrated with Swiss-Prot. NCBInr—translated GenBank DNA sequences, Swiss-Prot, PIR.

### *1.13.5 Metabolomics*

The metabolome is the total metabolite content and identification and quantification of metabolites responsible for the various biological processes of an organism are called metabolomics (Deborde et al. 2017). It plays an important role in studying gene-environment interactions, phenotyping, mutant characterization, identification of various biomarkers, and drug discovery. Metabolome databases exists such as METLIN, NIST, GOLM for the identification of metabolites (Johnson and Lange 2015). Further, by using statistical analysis tools and MetaboAnalyst, Cytoscape software can be used to analyse the identified metabolites (Xie et al. 2015).

## 1.14 Brief Account on Social, Political and Regulatory Issues

Safeguarding new inventions, ideas, pieces of knowledge or product from being misused has been felt necessary for a long time. Law and order came into enforcement as and when required for safeguarding social, political and regulatory issues, to provide maximum benefit to inventor, designer, publisher etc.

### *1.14.1 Patent and IPR Issues*

Innovation in the form of an idea, manuscript, design, composition of a product, hardwares which might benefit the society as a product or application is known as intellectual property. But with this comes the fear/risk of copying, imitating, or reproducing the innovation. Therefore, in order to minimize this and give an economic incentive to inventors, Intellectual property rights (IPR) came into existence. IPR includes copyrights, trademarks, patents, geographical indications, trade secrets, plant selection rights. Rights issued by the government over a period of tenure provides protection in safeguarding invention from being misused by

copying or making commercial use of it. The basic requirement to grant a patent includes four important features viz-novelty, distinctiveness, uniqueness, and stability. Indian Patent Act (1970) protects for 7–14 years, but this did not apply to agrochemicals, foods, pharmaceuticals, Indian Patent (Amendment) Act (1999) allows patent for all products except medicines/drugs. IPR although encourages and safeguards artistic creation, it may harm a company for a firm who might approach a firm for knowing trade secrets/patents, this could severely affect the person. Also, since these rights are obtained by paying huge amount of money, this tends to increase the price of objects, which makes it difficult for common people survival, especially in developing countries. Because of monopoly this is a serious threat in the food supply chain of agriculture.

### *1.14.2 Disclosure of Sources of GRs, Access, and Benefit-Sharing*

In order to get details for access of genetic resources (GRs), traditional knowledge (TK) disclosure of the source through proper agreement is must, to avoid misuse of information. Likewise, for knowing the geographic origin of any of the crop accessions authorities following proper norms of a country to avoid violation of resources, track of patent applications is must. Also, this would-be mutual benefit to countries sharing GRs. Disclosure of the origin of GRs will also be beneficial in tracking novelty and innovations on the other for TK disclosure of source will result in direct interaction with people for practical knowledge with oral and artistic knowledge rather than looking for it in databases at local or national level.

### *1.14.3 Farmer's Rights (FR)*

The Convention on Biological Diversity (CBD) and the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) signed an international treaty in 1986, this was a long-planned agenda to reward farmers for their contribution to germplasm conservation, variety development. Plant Breeder Rights is a right which benefits plant breeders in monetary terms for the variety developed by breeders together with providing protection through IPR, however the farmers involved in sharing genetic material are not given credit under this right. Farmer's right is a privilege given to farmers to traditionally maintain and develop their crop genetic resources (GR's) together with rewarding them from time to time for their contribution to the GR. Collections from the farmer's field possess vast diversity which aids in food security, therefore to safeguard their varieties and resources FR was felt like a great need of the hour. In 2001, for the first time in history, India became the first country to get the benefit of 'The Protection of Plant Varieties and

Farmers Rights Act (PPVFRA)' as a part of IPR. PPVFRA benefits both farmers and breeders in use of variety for personal or non salable purpose variety registration, saving seed from previous harvest or either sharing it with their neighbour's has greatly helped breeders as well farmers.

### *1.14.4 Traditional Knowledge*

Traditional knowledge (TK) is an indigenous knowledge gained through local techniques and community which is preserved and passed from generation to generation to form an identity of that community. Traditional knowledge can be found in a variety of concepts such as calculation of time, food article, plant properties, spice uses, yoga practices, etc. The essential ingredient of TK being vocal in nature and traditionally developed, as a result suffers from bio-piracy in some cases. Bio-piracy is commercial utilization of TK without seeking proper authorization of indigenous or local people associated with it. Under the Copyright Act, 1957, no specific guidelines for protecting traditional knowledge have been mentioned; but under Sect. 1.31A protection for unpublished Indian work has been included however this does not mention the time period for protection, therefore protection of traditional knowledge doesn't have much scope.

### *1.14.5 Participatory Breeding*

Participatory plant breeding (PPB) allows farmers engagement in breeders program at field level in observing plants at different crop stages throughout the crop cycle. The basic idea being, divergent skills and knowledge of farmers and researchers could be utilised in problem diagnosis and solving (Weltzien and Christinck 2008). Combination of strengths and weaknesses of both groups could aid in better research results through cooperation (Hoffmann 2007). Farmer's role in PPB is multidimensional which includes defining objectives of the study, choice of breeding material, randomization and planning of experiment design, selection of field, administrative approval etc. The main component of PPB being cooperation between farmers and researchers, mutual sharing of local germplasm, variety testing, seed production and distribution through seed system channel. PPB focuses more on particular crops suited for a particular environment. PPB in comparison to participatory varietal selection (PVS) has higher empowerment as here farmers are involved in selection, also this has benefitted in empowering women and uplift of traditionally backward society.

## 1.15 Future Perspectives

The first-generation plant breeding methods for crop improvement such as cross-breeding, mutation breeding is laborious and time-consuming, and the untargeted breeding programs cannot fulfil the increasing global food demand for overgrowing population of the world. The availability of modern technologies such as high throughput phenotyping, use of molecular markers for ease of selection, sequencing-based low cost genotyping, haplotype-based superior allele mining, speed breeding for rapid generation advancement, genomic selection, easy accessibility and analysis of big data, strong bioinformatics platforms and strategic genome editing such as CRISPR/Cas9 has revolutionized modern plant breeding and can play a significant role in achieving higher genetic gain targeted in various crops.

## References

Abbai R, VK Singh, Nachimuthu VV, Sinha P, Selvaraj R, Vipparla AK, Singh AK, Singh UM, Varshney RK, Kumar A (2019) Haplotype analysis of key genes governing grain yield and quality traits across 3 K RG panel reveals scope for the development of tailor-made rice with enhanced genetic gains. Plant Biotechnol J 17(8):1612–1622

Agarwal P, Parida SK, Mahto A, Das S, Mathew IE, Malik N, Tyagi AK (2014) Expanding frontiers in plant transcriptomics in aid of functional genomics and molecular breeding. Biotechnol J 9:1480–1492

Agarwal P, Parida SK, Raghuvanshi S, Kapoor S, Khurana P, Khurana JP, Tyagi AK (2016) Rice improvement through genome-based functional analysis and molecular breeding in India. Rice 9(1):1

Al-Bader N, Meier A, Geniza M, Gongora YS, Oard J, Jaiswal P (2019) Loss of premature stop codon in the Wall-Associated Kinase 91 (OsWAK91) gene confers sheath blight disease resistance in rice. bioRxiv 625509

Alexandrov N, Tai S, Wang W, Mansueto L, Palis K, Fuentes RR, Ulat VJ, Chebotarov D, Zhang G, Li Z, Mauleon R (2015) SNP-Seek database of SNPs derived from 3000 rice genomes. Nucleic Acids Res 43(D1):D1023–D1027

Allard RW, Bradshaw AD (1964) Implications of genotype-environmental interactions in applied plant breeding. Crop Sci 4:503–508

Andargie M, Li L, Feng A, Zhu X, Li J (2018) Mapping of the quantitative trait locus (QTL) conferring resistance to rice false smut disease. Curr Plant Biol 15:38–43

Antony G, Zhou J, Huang S, Li T, Liu B, White F et al (2010) Rice *xa13* recessive resistance to bacterial blight is defeated by induction of the disease susceptibility gene *Os-11N3*. Plant Cell 22:3864–3876

Arunakumari K, Durgarani CV, Satturu V, Sarikonda KR, Chittoor PDR, Vutukuri B, Laha GS, Nelli APK, Gattu S, Jamal M, Prasadbabu A (2016) Marker-assisted pyramiding of genes conferring resistance against bacterial blight and blast diseases into Indian rice variety MTU1010. Rice Sci 23(6):306–316

Ashizawa T, Takahashi M, Arai M, Arie T (2012) Rice false smut pathogen, *Ustilaginoidea virens*, invades through small gap at the apex of a rice spikelet before heading. J Gen Plant Pathol 78(4):255–259

Asibi AE, Chai Q, Coulter JA (2019) Rice blast: a disease with implications for global food security. Agronomy 9(8):451

Baba T, Katagiri S, Tanoue H, Tanaka R, Chiden Y, Saji S et al (2000) Construction and characterization of rice genomic libraries: PAC library of japonica variety, nipponbare and BAC library of indica variety, Kasalath. Bull NIAR 14:41–49

Bal A, Samal P, Chakraborti M, Mukherjee AK, Ray S, Molla KA, Behera L, SamalR Sarangi S, Sahoo P, Behera M (2020) Stable quantitative trait locus (QTL) for sheath blight resistance from rice cultivar CR 1014. Euphytica 216(11):1–19

Bacterial leaf blight, IRRI Rice Knowledge bank, http://www.knowledgebank.irri.org/decision-tools/rice-doctor/rice-doctor-fact-sheets/item/bacterial-blight

Balachiranjeevi Ch, Mahadev Swamy HK, Kale RR, Hajira SK, Koushik MBVN, Bhadana VP, Hariprasad AS et al (2018) Marker-assisted pyramiding of two major, broad-spectrum bacterial blight resistance genes, *Xa21* and *Xa33* into an elite maintainer line of rice, DRR17B. PLoS One 10:

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. Comptes Rendus Biologies 341(1):1–8

Baltes NJ, Hummel AW, Konecna E, Cegan R, Bruns AN, Bisaro DM, Voytas DF (2015) Conferring resistance to geminiviruses with the CRISPR–Cas prokaryotic immune system. Nat Plants 1(10):1–4

Barnwal MK, Kotasthane A, Magculia N, Mukherjee PK, Savary S, Sharma AK, Singh HB, Singh US, Sparks AH, Variar M, Zaidi N (2013) A review on crop losses, epidemiology and disease management of rice brown spot to identify research priorities and knowledge gaps. Eur J Plant Pathol 136(3):443–457

Bentur JS, Rawat N, Divya D, Sinha DK, Agarrwal R, Atray I, Nair S (2016) Rice-gall midge interactions: battle for survival. J Insect Physiol 84:40–49

Berger S, Chazli Y, Babu AF, Coste AT (2017) Azole resistance in Aspergillus fumigatus: a consequence of antifungal use in agriculture? Front Microbiol 8:1024

Beyer P, Al-Babili S, Ye X, Lucca P, Schaub P, Welsch R et al (2002) Golden rice: introducing the beta-carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency. J Nutr 132(3):506S–510S

Bharathi Y, VijayaKumar S, Pasalu IC, Balachandran SM, Reddy VD, Rao KV (2011) Biotechnol J (152):63–71

Bhasin H, Bhatia D, Raghuvanshi S, Lore JS, Sahi GK, Kaur B, Vikal Y, Singh K (2012) New PCR-based sequence-tagged site marker for bacterial blight resistance gene Xa38 of rice. Mol Breeding 30(1):607–611

Birkett MA, Pickett JA (2014) Prospects of genetic engineering for robust insect resistance. Curr Opin Plant Biol 19:59–67

Biswas C, Srivastava SSL, Biswas SK (2010) Effect of biotic, abiotic and botanical inducers on crop growth and severity of brown spot in rice. Indian Phytopathol 63(2):187

Blanvillain-Baufum S, Reschke M, Sol M, Auguy F, DoucoureH Szurek B et al (2017) Targeted promoter editing for rice resistance to *Xanthomonas oryzae* pv. *oryzae* reveals differential activities for *SWEET14*-inducing TAL effectors. Plant Biotechnol J 15:306–317

Borba TCDO, Brondani RPV, Breseghello F, Coelho ASG, Mendonca JA, Rangel PHN, Brondani C (2010) Association mapping for yield and grain quality traits in rice (*Oryza sativa* L.). Genet Mol Biol 33(3):515–524

Brar DS, Khush GS (1997) Alien gene introgression in rice. Plant Mol Biol 35:35–47

Brar DS, Khush GS (2003) Utilization of wild species of genus Oryza in rice improvement. In: Nanda JS, Sharma SD (eds) Monograph on genus Oryza. Oxford & IBH Publishing, New Delhi, pp 283–309

Brar DS, Virk PS, Jena KK, Khush GS (2009) Breeding for resistance to planthoppers in rice. In: Plant hoppers: new threats to the sustainability of intensive rice production systems in Asia. International Rice Research Institute, Los Banos, pp 401–409

Buntjer JB, Sorensen AP, Peleman JD (2005) Haplotype diversity: the link between statistical and biological association. Trends Plant Sci 10(10):466–471

Bush WS, Moore JH (2012) Genome-wide association studies. PLoS Comput Biol 8(12):

Cai L, Cao Y, Xu Z, Ma W, Zakria M, Zou L et al (2017) A transcription activator-like effector *Tal7* of *Xanthomonas oryzae* pv. *oryzicola* activates rice gene *Os09g29100* to suppress rice immunity. Sci Rep 7:5089

Canovas FM, Dumas-Gaudot E, Recorbet G, Jorrin J, Mock HP, Rossignol M (2004) Plant proteome analysis. Proteomics 4:285–298

Channamallikarjuna V, Sonah H, Prasad M, Rao GJN, Chand S, Upreti HC, Singh NK, Sharma TR (2010) Identification of major quantitative traitloci qSBR11-1 for sheath blight resistance in rice. Mol Breeding 25(1):155–166

Chaudhary B (1999) Effect of blast disease on rice yield. Nepal Agri Res J 3:8–13

Chauhan BS, Jabran K, Mahajan G (eds) (2017) Rice production worldwide, vol 247. Springer

Cheema KK, Grewal NK, Vikal Y, Sharma R, Lore JS, Das A, Bhatia D, Mahajan R, Gupta V, Bharaj TS, Singh K (2008) A novel bacterial blight resistance gene from *Oryza nivara* mapped to 38 kb region on chromosome 4L and transferred to *Oryza sativa* L. Genet Res 90(5):397–407

Chen M, Presting G, Barbazuk WB, Goicoechea JL, Blackmon B, Fang G et al (2002) An integrated physical and genetic map of the rice genome. Plant Cell 14:537–545

Chen ZX, Zhang YF, Feng F, Feng MH, Jiang W, Ma YY, Pan CH, Hua HL, Li GS, Pan XB, Zuo SM (2014) Improvement of japonica rice resistance to sheath blight by pyramiding *qSB-9TQ* and *qSB-7TQ*. Field Crops Res 161:118–127

Chen SY, Lai MH, Tung CW, Wu DH, Chang FY, Lin TC, Chung CL (2019a) Genome-wide association mapping of gene loci affecting disease resistance in the rice-*Fusarium fujikuroi* pathosystem. Rice 12(1):85

Chen Z, Feng Z, Kang H, Zhao J, Chen T, Li Q, Gong H, Zhang Y, Chen X, Pan X, Liu W (2019b) Identification of new resistance loci against sheath blight disease in rice through genome-wide association study. Rice Sci 26(1):21–31

Chen S, Wang C, Yang J, Chen B, Wang W, Su J, Feng A, Zeng L, Zhu X (2020) Identification of the novel bacterial blight resistance gene *Xa46(t)* by mapping and expression analysis of the rice mutant H120. Sci Rep 10(1):1–11

Chukwu SC, Rafii MY, Ramlee SI, Ismail SI, Oladosu Y, Okporie E, Onyishi G, Utobo E, Ekwu L, Swaray S, Jalloh M (2019) Marker-assisted selection and gene pyramiding for resistance to bacterial leaf blight disease of rice (*Oryza sativa* L.). Biotechnol Biotechnol Equip 33(1):440–455

Cohn M, Bart RS, Shybut M, Dahlbeck D, Gomez M, Morbitzer R, Hou BH, Frommer WB, Lahaye T, Staskawicz BJ (2014) *Xanthomonas axonopodis* virulence is promoted by a transcription activator-like effector-mediated induction of a SWEET sugar transporter in cassava. Mol Plant Microbe Interact 27(11):1186–1198

Collard BC, Mackill DJ (2008) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Phil Trans R Soc B: Biol Sci 363(1491):557–572

Couch BC, Kohn LM (2002) A multilocus gene genealogy concordant with host preference indicates segregation of a new species, *Magnaportheoryzae*, from *M. grisea*. Mycologia 94 (4):683–693

Courtois B, Audebert A, Dardou A, Roques S, Ghneim-Herrera T, Droc G, Frouin J, Rouan L, Goze E, Kilian A, Ahmadi N (2013) Genome-wide association mapping of root traits in a japonica rice panel. PLoS One 8(11):

Crossa J, Perez-Rodriguez P, Cuevas J, Montesinos-Lopez O, Jarquin D, De los Campos G, Burgueno J, Gonzalez-Camacho JM, Perez-Elizalde S, Beyene Y, Dreisigacker S (2017) Genomic selection in plant breeding: methods, models, and perspectives. Trends Plant Sci 22 (11):961–975

Das A, Soubam D, Singh PK, Thakur S, Singh NK, Sharma TR (2012) A novel blast resistance gene, *Pi54rh* cloned from wild species of rice, *Oryza rhizomatis* confers broad spectrum resistance to *Magnaportheoryzae*. Funct Integr Genom 12:215–228

Dean RA, Talbot NJ, Ebbole DJ, Farman ML, Mitchell TK, Orbach MJ, Thon M, Kulkarni R, Xu JR, Pan H, Read ND (2005) The genome sequence of the rice blast fungus *Magnaporthe grisea*. Nature 434(7036):980–986

Deborde C, Moing A, Roch L, Jacob D, Rolin D, Giraudeau P (2017) Plant metabolism as studied by NMR spectroscopy. Progr Nuclear Magnet Reson Spectros 102:61–97

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

Depicker A, Sanders M, Meyer P (2005) Transgene silencing. In: Meyer P (ed) Plant epigenetics. Oxford Ames. Blackwell Publishing, Iowa, pp 1–32

Deshpande N, Address KJ, Bluhm WF, Merino-Ott JC, Townsend-Merino W et al (2005) The RCSB protein data bank: a redesigned query system and relational database based on the mmCIF schema. Nucleic Acids Re 33:D233–D237

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

Dilla-Ermita CJ, Tandayu E, Juanillas VM, Detras J, Lozada DN, Dwiyanti MS, Cruz CV, Mbanjo EGN, Ardales E, Diaz MG, Mendioro M (2017) Genome-wide association analysis tracks bacterial leaf blight resistance loci in rice diverse germplasm. Rice 10(1):1–17

Divya D, Himabindu K, Nair S, Bentur JS (2015) Cloning of a gene encoding LRR protein and its validation as candidate gall midge resistance gene, Gm4, in rice. Euphytica 203(1):185–195

Dixit S, Singh UM, Singh AK, Alam S, Venkateshwarlu C, Nachimuthu VV, Yadav S, Abbai R, Selvaraj R, Devi MN, Ramayya PJ (2020) Marker assisted forward breeding to combine multiple biotic-abiotic stress resistance/tolerance in rice. Rice 13(1):1–15

Dokku P, Das KM, Rao GJN (2013) Pyramiding of four resistance genes of bacterial blight in Tapaswini, an elite rice cultivar, through marker-assisted selection. Euphytica 192(1):87–96

Du B, Chen R, Guo J, He G (2020) Current understanding of the genomic, genetic, and molecular control of insect resistance in rice. Mol Breed 40(2):24

Eagles HA, Bariana HS, Ogbonnaya FC et al (2001) Implementation of markers in Australian wheat breeding. Crop Pasture Sci 52(12):1349–1356

Edgar R, Domrachev M, Lash AE (2002) Gene expression omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res 30:207–210

Eizenga G, Prasad B, Jackson A, Jia M (2013) Identification of rice sheath blight and blast quantitative trait loci in two different O. sativa/O. nivara advanced backcross populations. Mol Breed 31:889–907

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

Fagard M, Vaucheret H (2000) Transgene silencing in plants: how many mechanisms? Ann Rev Plant Mol Biol Physiol (51):167–194

Famoso AN, Zhao K, Clark RT, Tung CW, Wright MH, Bustamante C, Kochian LV, McCouch SR (2011) Genetic architecture of aluminum tolerance in rice (Oryza sativa) determined through genome-wide association analysis and QTL mapping. PLoS Genet 7(8): e1002221

Feng Z, Kang H, Li M, Zou L, Wang X, Zhao J, Wei L, Zhou N, Li Q, Lan Y, Zhang Y (2019) Identification of new rice cultivars and resistance loci against rice black-streaked dwarf virus disease through genome-wide association study. Rice 12(1):1–13

Fischer R, Schillberg S, Buyel JF, Twyman RM (2013) Commercial aspects of pharmaceutical protein production in plants. Curr Pharm Des 19(31):5471–5477

Francois JA et al (2002) Different approaches for multi-transgene-stacking in plants. Plant Sci (163):281–295

Fukuoka S, Yamamoto SI, Mizobuchi R, Yamanouchi U, Ono K, Kitazawa N et al (2014) Multiple functional polymorphisms in a single disease resistance gene in rice enhance durable resistance to blast. Sci Rep 4:4550

Garris AJ, McCouch SR, Kresovich S (2003) Population structure and its effect on haplotype diversity and linkage disequilibrium surrounding the xa5 locus of rice (*Oryza sativa* L.). Genetics 165(2):759–769

Ge S, Sang T, Lu BR, Hong DY (1999) Phylogeny of rice genomes with emphasis on origins of allotetraploid species. Proc Nat Acad Sci 96(25):14400–14405

Gelvin SB (2003) Agrobacterium-mediated plant transformation: the biology behind the 'Gene-Jockeying' tool. Microb Mol Biol Rev (67):16–37

George MLC, Bustamam M, Cruz WT, Leach JE, Nelson RJ (1997) Movement of Xanthomonas oryzaepv. oryzae in Southeast Asia detected using PCR-based DNA fingerprinting. Phytopathol 87(3):302–309

Gibbs RA, Weinstock GM (2003) Evolving methods for the assembly of large genomes. Cold Spring Harb Symp Quant Biol 68:189–194

Gladieux P, Condon B, Ravel S, Soanes D, Maciel JLN, Nhani A, Chen L, Terauchi R, Lebrun MH, Tharreau D Mitchell T (2018) Gene flow between divergent cereal-and grass-specific lineages of the rice blast fungus *Magnaportheoryzae*. Mol Bio 9(1)

Gunasekara JM, Jayasekera GA, Perera KL, Wickramasuriya AM (2017) Development of a Sri Lankan rice variety Bg 94-1 harbouring Cry2A gene of Bacillus thuringiensis resistant to rice leaffolder [Cnaphalocrocismedinalis (Guenee)]. J Natl Sci Found Sri Lanka 45:2

Guo S, Zhang D, Lin X (2010) Identification and mapping of a novel bacterial blight resistance gene *xa35(t)* originated from Oryza minuta. Scientia Agricultura Sinica 43(13):2611–2618

Guo J, Xu C, Wu D, Zhao Y, Qiu Y, Wang X, Ouyang Y, Cai B, Liu X, Jing S, Shangguan X (2018). Bph6 encodes an exocyst-localized protein and confers broad resistance to planthoppers in rice. Nat Genet 50(2):297–306

Gupta V, Shamas N, Razdan VK, Sharma BC, Sharma R, Kaur K, Singh I, John D, Kumar A (2013) Foliar application of fungicides for the management of brown spot disease in rice (*Oryza sativa* L.) caused by Bipolarisoryzae. African J Agri Res 8(25):3303–3309

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

Han Y, Zhang K, Yang J, Zhang N, Fang A, Zhang Y, Liu Y, Chen Z, Hsiang T, Sun W (2015) Differential expression profiling of the early response to Ustilaginoidea virens between false smut resistant and susceptible rice varieties. BMC Genom 16(1):1–15

Han Y, Li D, Yang J, Huang F, Sheng H, Sun W (2020) Mapping quantitative trait loci for disease resistance to false smut of rice. Phytopathol Res 2(1):1–11

Harlan JR, de Wet JM (1971) Toward a rational classification of cultivated plants. Taxon 20 (4):509–517

Hasan MM, Rafii MY, Ismail MR, Mahmood M, Rahim HA, Alam MA, Ashkani S, Malek MA, Latif MA (2015) Marker-assisted backcrossing: a useful method for rice improvement. Biotechnol Equipment 29(2):237–254

Hayles J, Johnson L, Worthley C, Losic D (2017) Nanopesticides: a review of current research and perspectives. New Pestic Soil Sens:193–225

He J, Zhao X, Laroche A, Lu ZX, Liu H, Li Z (2014) Genotyping-by-sequencing (GBS), an ultimate marker-assisted selection (MAS) tool to accelerate plant breeding. Front Plant Sci 5:484

He S, Schulthess AW, Mirdita V, Zhao Y, Korzun V, Bothe R, Ebmeyer E, Reif JC, Jiang Y (2016) Genomic selection in a commercial winter wheat population. Theor Appl Genet 129 (3):641–651

He C, Xiao Y, Yu J, Li J, Meng Q, Qing X, Xiao G (2019) Pyramiding Xa21, Bph14, and Bph15 genes into the elite restorer line Yuehui9113 increases resistance to bacterial blight and the brown planthopper in rice. Crop Prot 115:31–39

He L, Zou L, Huang Q, Sheng X, Wu W, Hu J (2020) Development of InDel markers of *Bph3* and pyramiding of four brown planthopper resistance genes into an elite rice variety. Mol Breed 40 (10):1–11

Himabindu K, Suneetha K, Sama VSAK, Bentur JS (2010) A new rice gall midge resistance gene in the breeding line CR57-MR1523, mapping with flanking markers and development of NILs. Euphytica 174(2):179–187

Hiremath SS (2018) Identification of potential donors and association mapping for false smut resistance in rice germplasm. Doctoral dissertation, Punjab Agricultural University, Ludhiana

Hittalmani S, Parco A, Mew TV, Zeigler RS, Huang N (2000) Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. Theor Appl Genet 100(7):1121–1128

Hoffmann WH (2007) Strategies for managing a portfolio of alliances. Strat Manag J 28(8):827–856

Hossain MK, Jena KK, Bhuiyan MAR, Wickneswari R (2016) Association between QTLs and morphological traits toward sheath blight resistance in rice (*Oryza sativa* L.). Breed Sci 66:613–626

https://www.statista.com. Accessed on 3 Nov 2020

Hu J, Li X, Wu C, Yang C, Hua H, Gao G, Xiao J, He Y (2012) Pyramiding and evaluation of the brown planthopper resistance genes *Bph14* and *Bph15* in hybrid rice. Mol Breed 29(1):61–69

Hua LX, Liang LQ, He XY, Wang L, Zhang WS et al (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 Equipment 29(3):448–456

Huang X, Han B (2014) Natural variations and genome-wide association studies in crop plants. Annu Rev Plant Biol 65:531–551

Huang X, Sang T, Zhao Q, Feng Q, Zhao Y, Li C, Zhu C, Lu T, Zhang Z, Li M, Fan D (2010) Genome-wide association studies of 14 agronomic traits in rice landraces. Nat Genet 42 (11):961

Huang D, Qiu Y, Zhang Y, Huang F, Meng J, Wei S, Li R, Chen B (2013) Fine mapping and characterization of *BPH27*, a brown planthopper resistance gene from wild rice (*Oryza rufipogon Griff.*). Theor Appl Genet 126(1):219–229

Hutin M, Sabot F, Ghesquiere A, Koebnik R, Szurek B (2015) A knowledge-based molecular screen uncovers a broad-spectrum *OsSWEET14* resistance allele to bacterial blight from wild rice. Plant J 84:694–703

Ishiguro K (2001) Review of research in Japan on the roles of silicon in conferring resistance against rice blast. Studies Plant Sci 8:277–291

Jairin J, Phengrat K, Teangdeerith S, Vanavichit A, Toojinda T (2007) Mapping of a broad-spectrum brown planthopper resistance gene, *Bph3*, on rice chromosome 6. Mol Breed 19(1):35–44

Jairin J, Teangdeerith S, Leelagud P, Kothcharerk J, Sansen K, Yi M, Vanavichit A, Toojinda T (2009) Development of rice introgression lines with brown planthopper resistance and KDML105 grain quality characteristics through marker-assisted selection. Field Crops Res 110 (3):263–271

Jamaloddin M, Durga Rani CV, Swathi G, Anuradha C, Vanisri S, Rajan CPD, Krishnam Raju S, Bhuvaneshwari V, Jagadeeswar R, Laha GS, Prasad MS (2020) Marker assisted gene pyramiding (MAGP) for bacterial blight and blast resistance into mega rice variety 'Tellahamsa'. PloS One 15(6):e0234088

Jena KK, Jeung JU, Lee JH, Choi HC, Brar DS (2006) High-resolution mapping of a new brown planthopper (BPH) resistance gene, Bph18 (t), and marker-assisted selection for BPH resistance in rice (Oryza sativa L.). Theor Appl Genet 112(2):288–297

Jena KK, Kim SM (2010) Current status of brown planthopper (BPH) resistance and genetics. Rice 3(2–3):161–171

Jena KK, Multani GS, Khush GS (1991) Monogenic alien addition lines of Oryza australiensis and alien gene transfer. Rice Genet II:728

Jena KK, Hechanova SL, Verdeprado H, Prahalada GD, Kim SR (2017) Development of 25 near-isogenic lines (NILs) with ten BPH resistance genes in rice (*Oryza sativa* L.): production, resistance spectrum, and molecular analysis. Theor Appl Genet 130(11):2345–2360

Ji ZJ, Yang SD, Zeng YX, Liang Y, Yang CD, Qian Q (2016) Pyramiding blast, bacterial blight and brown planthopper resistance genes in rice restorer lines. J Integr Agric 15(7):1432–1440
Jia Y, Jia MH, Wang X, Liu G (2012) Indica and japonica crosses resulting in linkage block and recombination suppression on rice chromosome 12. PLoS One 7(8):e43066
Jia J, Li H, Zhang X, Li Z, Qiu L (2017) Genomics-based plant germplasm research (GPGR). Crop J 5(2):166–174
Jiang GL (2013) Plant marker-assisted breeding and conventional breeding: challenges and perspectives. Adv Crop Sci Technol 1:
Johnson SR, Lange BM (2015) Open-access metabolomics databases for natural product research: present capabilities and future potential. Front Bioengg Biotechnol 3:22
Johnston SA, Anziano PQ, Shark K, Sanford JC, Butow RA (1988). Mitochondrial transformation in yeast by bombardment with microprojectiles. Sci (240):1538–1541
Kannahi M, Dhivya S, Senthilkumar R (2016) Biological control on rice false smut disease using Trichoderma species. Int J Pure App Biosci 4(2):311–316
Karlovsky P (2011) Biological detoxification of the mycotoxin deoxynivalenol and its use in genetically engineered crops and feed additives. Appl Microbiol Biotechnol 91:491–504
Kashyap PL, Xiang X, Heiden P (2015) Chitosan nanoparticlebased delivery systems for sustainable agriculture. Int J Biol Macromol 77:36–51
Katiyar SK, Tan Y, Huang B, Chandel G, Xu Y, Zhang Y, Xie Z, Bennett J (2001) Molecular mapping of gene *Gm-6(t)* which confers resistance against four biotypes of Asian rice gall midge in China. Theor Appl Genet 103(6–7):953–961
Kaur Y, Lore JS, Pannu PPS (2015) Evaluation of rice genotypes for resistance against false smut. Plant Disease Res 30(1):46–49
Khan AA, Sinha AP (2005) Comparative antagonistic potential of some biocontrol agents against sheath blight of rice. Indian Phytopathol 58(1):41–45
Khan MAI, Ali MA, Monsur MA, Kawasaki-Tanaka A, Hayashi N, Yanagihara S, Obara M, Mia MAT, Latif MA, Fukuta Y (2016) Diversity and distribution of rice blast (*PyriculariaoryzaeCavara*) races in Bangladesh. Plant Dis 100(10):2025–2033
Khanna A, Sharma V, Ellur RK, Shikari AB, Krishnan SG, Singh UD, Prakash G, Sharma TR, Rathour R, Variar M, Prashanthi SK (2015) Development and evaluation of near-isogenic lines for major blast resistance gene (s) in Basmati rice. Theor Appl Genet 128(7):1243–1259
Khush GS (1997) Origin, dispersal, cultivation and variation of rice. Plant Mol Biol 35:25–34
Kim SM, Reinke RF (2019) A novel resistance gene for bacterial blight in rice, *Xa43(t)* identified by GWAS, confirmed by QTL mapping using a bi-parental population. PloS one 14(2): e0211775
Kim DY, Kadam A, Shinde S, Saratale RG, Patra J, Ghodake G (2018) Recent developments in nanotechnology transforming the agricultural sector: a transition replete with opportunities. J Sci Food Agric 98:849–864
Kobayashi N, Telebanco-Yanoria MJ, Tsunematsu H, Kato H, Imbe T, Fukuta Y (2007) Development of new sets of international standard differential varieties for blast resistance in rice (*Oryza sativa* L.). Japan Agri Res Quart: JARQ 41(1):31–37
Kongcharoen N, Kaewsalong N, Dethoup T (2020) Efficacy of fungicides in controlling rice blast and dirty panicle diseases in Thailand. Sci Rep 10(1):1–7
Kreye C, Bouman BAM, Reversat G, Fernandez L, Cruz CV, Elazegui F, Faronilo JE, Llorca L (2009) Biotic and abiotic causes of yield failure in tropical aerobic rice. Field Crop Res 112 (1):97–106
Kumar VA, Balachiranjeevi CH, Naik SB, Rambabu R, Rekha G, Harika G, Hajira SK, Pranathi K, Vijay S, Anila M, Mahadevaswamy HK (2016) Marker-assisted improvement of the elite restorer line of rice, RPHR-1005 for resistance against bacterial blight and blast diseases. J Genet 95(4):895–903
Kumar VA, Balachiranjeevi CH, Naik SB, Rekha G, Rambabu R, Harika G, Pranathi K, Hajira SK, Anila M, Kousik M, Kale R (2017) Markerassisted pyramiding of bacterial blight and gall midge resistance genes into RPHR-1005, the restorer line of the popular rice hybrid DRRH-3. Mol Breeding 37(7):1–14

Kumar K, Sarao PS, Bhatia D, Neelam K, Kaur A, Mangat GS, Brar DS, Singh K (2018) High-resolution genetic mapping of a novel brown planthopper resistance locus, *Bph34* in *Oryza sativa* L. X *Oryza nivara* (Sharma and Shastry) derived interspecific $F_2$ population. Theor Appl Genet 131(5):1163–1171

Ladhalakshmi D, Laha GS, Singh R, Karthikeyan A, Mangrauthia SK, Sundaram RM, Thukkaiyannan P, Viraktamath BC (2012) Isolation and characterization of Ustilaginoidea virens and survey of false smut disease of rice in India. Phytoparasitica 40(2):171–176

Lakshmi PV, Amudhan S, Bindu KH, Cheralu C, Bentur JS (2006) A new biotype of the Asian rice gall midge Orseoliaoryzae (Diptera: Cecidomyiidae) characterized from the Warangal population in Andhra Pradesh, India. Int J Trop Insect Sci 26(3):207–211

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 H, Rasheed A, Hickey LT, He Z (2018). Fast-forwarding genetic gain. Trends Plant Sci (23):184–186

Li W, Chern M, Yin J, Wang J, Chen X (2019a) Recent advances in broad-spectrum resistance to the rice blast disease. Curr Opin Plant Biol 50:114–120

Li Z, Xue Y, Zhou H, Li Y, Usman B, Jiao X, Wang X, Liu F, Qin B, Li R, Qiu Y (2019b) High-resolution mapping and breeding application of a novel brown planthopper resistance gene derived from wild rice (*Oryza. rufipogon Griff*). Rice 12(1):41

Li-Byarlay H, Li Y, Stroud H, Feng S, Newman TC, Kaneda M, Hou KK, Worley KC, Elsik CG, Wickline SA (2013) RNA interference knockdown of DNA methyl-transferase 3 affects gene alternative splicing in the honey bee. Proc Natl Acad Sci USA 110:12750–12755

Lin CY, Togashi K (2002) Genetic improvement in the presence of genotype by environment interaction. Anim Sci J 73:3–11

Lin HA, Chen SY, Chang FY, Tung CW, Chen YC, Shen WC, Chen RS, Wu CW, Chung CL (2018) Genome-wide association study of rice genes and loci conferring resistance to *Magnaportheoryzae* isolates from Taiwan. BotStudies 59(1):32

Listgarten J, Lippert C, Kadie CM, Davidson RI, Eskin E, Heckerman D (2012) Improved linear mixed models for genome-wide association studies. Nat Methods 9(6):525–526

Liu G, Lu G, Zeng L, Wang GL (2002) Two broad-spectrum blast resistance genes, *Pi9(t)* and *Pi2 (t)*, are physically linked on rice chromosome 6. Mol Genet Genomics 267:472–480

Liu G, Jia Y, McClung A, Oard JH, Lee FN, Correll JC (2013) Confirming QTLs and finding additional loci responsible for resistance to rice sheath blight disease. Plant Dis 97(1):113–117

Liu Y, Chen L, Liu Y, Dai H, He J, Kang H, Pan G, Huang J, Qiu Z, Wang Q, Hu J (2016) Marker assisted pyramiding of two brown planthopper resistance genes, *Bph3* and *Bph27(t)*, into elite rice cultivars. Rice 9(1):27

Liu MH, Kang H, Xu Y, Peng Y, Wang D, Gao L, Wang X, Ning Y, Wu J, Liu W, Li C (2020) Genome-wide association study identifies an NLR gene that confers partial resistance to *Magnaportheoryzae* in rice. Plant Biotechnol J 18(6):1376–1383

Liu Y, Chen L, Fu D, Lou Q, Mei H, Xiong L, Li M, Xu X, Mei X, Luo L (2014) Dissection of additive, epistatic effect and QTL x environment interaction of quantitative trait loci for sheath blight resistance in rice. Hereditas 151(2–3):28–37

Loan LC, Ngan VTT, Du PV (2006) Preliminary evaluation on resistance genes against rice bacterial leaf blight in Can Tho Province-Vietnam. Omonrice 14:44–47

Long W, Yuan Z, Fan F, Dan D, Pan G, Sun H, Zhang Z, Li N, Li S (2020) Genome-wide association analysis of resistance to rice false smut. Mol Breed 40(5)

Lore JS, Pannu PPS, Jain J, Hunjan MS, Kaur R, Mangat GS (2013) Susceptibility of rice hybrids and inbred cultivars to false smut under field conditions. Indian Phytopathol 66(4):397–399

Louwaars NP (2018) Plant breeding and diversity: a troubled relationship? Euphytica (214):114

Luo Y, Zakaria S, Basyah B, Ma T, Li Z, YangJ Yin Z (2014) Marker-assisted breeding of Indonesia local rice variety Siputeh for semi-dwarf phonetype, good grain quality and disease resistance to bacterial blight. Rice 7(1):33

Luo Y, Ma T, Zhang A, Ong KH, Luo Z, Li Z, Yang J, Yin Z (2017) Marker-assisted breeding of Chinese elite rice cultivar 9311 for disease resistance to rice blast and bacterial blight and tolerance to submergence. MolBreed 37(8):106

Lv W, Du B, Shangguan X, Zhao Y, Pan Y, Zhu L, He Y, He G (2014) BAC and RNA sequencing reveal the brown planthopper resistance gene *BPH15* in a recombination cold spot that mediates a unique defense mechanism. BMC Genom 15(1):674

MacManus PS, Stockwell VO, Sundin GW, Jones AL (2002) Antibiotic use in plant agriculture. Ann Rev Phytopathol 40(1):443–465

Magar PB, Acharya B, Pandey B (2015) Use of chemical fungicides for the management of rice blast (Pyricularia grisea) disease at Jyotinagar, Chitwan, Nepal. Intl J Appl Sci Biotechnol 3 (3):474–478

Mateu-Andres I, De Paco L (2005) Allozymic differentiation of the *Antirrhinum majus* and *A. siculum* species groups. Ann Bot 95(3):465–473

Mather KA, Caicedo AL, Polato NR, Olsen KM, McCouch S, Purugganan MD (2007) The extent of linkage disequilibrium in rice (*Oryza sativa* L.). Genetics 177(4):2223–2232

Matsumoto K, Ota Y, Seta S, Nakayama Y, Ohno T, Mizobuchi R, Sato H (2017) Identification of QTLs for rice brown spot resistance in backcross inbred lines derived from a cross between Koshihikari and CH45. Breed Sci 67(5):540–543

McCouch SR, Zhao K, Wright M, Tung CW, Ebana K, Thomson M, Reynolds A, Wang D, DeClerck G, Ali ML, McClung A (2010) Development of genome-wide SNP assays for rice. Breed Sci 60(5):524–535

McKeand SE, Eriksson G, Roberds JH (1997) Genotype by environment interaction for index traits that combine growth and wood density in loblolly pine. Theor Appl Genet 94:1015–1022

Mgonja EM, Balimponya EG, Kang H, Bellizzi M, Park CH, Li Y, Mabagala R, Sneller C, Correll J, Opiyo S, Talbot NJ (2016) Genome-wide association mapping of rice resistance genes against *Magnaportheoryzae* isolates from four African countries. Phytopathol 106 (11):1359–1365

Mgonja EM, Park CH, Kang H, Balimponya EG, Opiyo S, Bellizzi M, Mutiga SK, Rotich F, Ganeshan VD, Mabagala R, Sneller C (2017) Genotyping-by-sequencing-based genetic analysis of African rice cultivars and association mapping of blast resistance genes against *Magnaportheoryzae* populations in Africa. Phytopathol 107(9):1039–1046

Michel S, Ametz C, Gungor H, Epure D, Grausgruber H, Loschenberger F, Buerstmayr H (2016) Genomic selection across multiple breeding cycles in applied bread wheat breeding. Theor Appl Genet 129(6):1179–1189

Midha S, Bansal K, Kumar S, Girija AM, Mishra D, Brahma K, Laha GS, SundaramRM, Sonti RV, Patil PB (2017) Population genomic insights into variation and evolution of *Xanthomonas oryzaepv. oryzae*. Sci Reports 7(1):1–13

Miller DG, Raman A (2019) Host-plant relations of gall-inducing insects. Ann Entomol Soc Am 112(1):1–19

Mittler R (2006) Abiotic stress, the field environment and stress combination. Trends Plant Sci 11:15–19

Mizobuchi R, Fukuoka S, Tsushima S, Yano M, Sato H (2016) QTLs for resistance to major rice diseases exacerbated by global warming: brown spot, bacterial seedling rot, and bacterial grain rot. Rice 9(1):23

Molla KA, Karmakar S, Molla J, Bajaj P, Varshney RK, Datta SK, Datta K (2020) Understanding sheath blight resistance in rice: the road behind and the road ahead. Plant Biotechnol J 18 (4):895–915

Mondini L, Noorani A, Pagnotta MA (2009) Assessing plant genetic diversity by molecular tools. Diversity 1(1):19–35

Moose SP, Mumm RH (2008) Molecular plant breeding as the foundation for 21st century crop improvement. Plant Physiol (147):969–977

MorishimaH Oka HI (1960) The pattern of interspecific variation in the genus *Oryza*: its quantitative representation by statistical methods. Evolution 14:153–165

Muthayya S, Sugimoto JD, Montgomery S, Maberly GF (2014) An overview of global rice production, supply, trade, and consumption. Ann New York Acad Sci 1324(1):7–14

Myint KKM, Fujita D, Matsumura M, Sonoda T, Yoshimura A, Yasui H (2012) Mapping and pyramiding of two major genes for resistance to the brown planthopper (*Nilaparvatalugens* [*Stal*]) in the rice cultivar ADR52. Theor Appl Genet 124(3):495–504

Naqvi S, Farré G, Sanahuja G, Capell T, Zhu C, Christou P (2010) When more is better: multigene engineering in plants. Trends Plant Sci 15(1):48–56

Natarajkumar P, Sujatha K, Laha GS, Viraktamath BC, Reddy CS, Mishra B et al (2010) Identification of a dominant bacterial blight resistance gene from *Oryza nivara* and its molecular mapping. Rice Genet Newsl 25:54–56

Neelam K, Mahajan R, Gupta V, Bhatia D, Gill BK, Komal R, Lore JS, Mangat GS, Singh K (2020) High-resolution genetic mapping of a novel bacterial blight resistance gene *xa-45(t)* identified from *Oryza glaberrima* and transferred to *Oryza sativa*. Theor Appl Genet 133 (3):689–705

Nelson JC, Oard JH, Groth D, Utomo HS, Jia Y, Liu G, Moldenhauer KAK et al (2012) Sheath-blight resistance QTLs in japonica rice germplasm. Euphytica 184:23–34

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

Nessa B, Salam MU, Haque AM, Biswas JK, Kabir MS, MacLeod WJ, D'Antuono M, Barman HN, Latif MA, Galloway J (2015) Spatial pattern of natural spread of rice false smut (*Ustilaginoidea virens*) disease in fields. Am J Agri Biol Sci 10(1):63–73

Nguyen HT, Vu QH, Van Mai T, Nguyen TT, Vu LD, Nguyen TT, Nguyen LV, Vu HTT, Nong HT, Dinh TN, Toshitsugu N (2018) Marker-assisted selection of Xa21 conferring resistance to bacterial leaf blight in indica rice cultivar LT2. Rice Sci 25(1):52–56

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

Noda T, Li C, Li J, Ohiai H, Ise K, Kaku H (2001) Pathogenic diversity of *Xanthomonas oryzaepv. oryzae* strains from Yunnan province, China. Japan Agri Res Quart: JARQ 35(2):97–103

Nordborg M, Tavare S (2002) Linkage disequilibrium: what history has to tell us. Trends Genet 18 (2):83–90

Normile D (2008) Reinventing rice to feed the world. Science 321(5887):330–333

Osusky M, Zhou GQ, Osuska L, Hancock RE, Kay WW, Misra S (2000) Transgenic plants expressing cationic peptide chimeras exhibit broad-spectrum resistance to phytopathogens. Nat Biotechnol 18:1162–1166

Pagliaccia D, Urak RZ, Wong F, Douhan LI, Greer CA, Vidalakis G, Douhan GW (2018) Genetic structure of the rice blast pathogen (*Magnaportheoryzae*) over a decade in North Central California rice fields. Microb Eco 75(2):310–317

Pan Y, Huang L, Song S, Hu M, Chang S, Lv Q, Li Y, Wang T, Ouyang X, Xiao Y, Fu X (2019) Identification of brown planthopper resistance gene *Bph32* in the progeny of a rice dominant genic male sterile recurrent population using genome-wide association study and RNA-seq analysis. Mol Breed 39(5):72

Parkinson H, Sarkans U, Shojatalab M, Abeygunawardena N, Contrino S et al (2005) Array Express—a public repository for microarray gene expression data at the EBI. Nucleic Acids Res 33:D553–D555

Perez-Massot E, Banakar R, Gomez-Galera S, Zorrilla-Lopez U, Sanahuja G, Arjo G et al (2013) The contribution of transgenic plants to better health through improved nutrition: opportunities and constraints. Genes Nutri 8(1):29–41

Plant hopper-IRRI Rice Knowledge Bank http://www.knowledgebank.irri.org/training/fact-sheets/pest-management/insects/item/planthopper

Pradhan SK, Nayak DK, Mohanty S, Behera L, Barik SR, Pandit E, LenkaS Anandan A (2015) Pyramiding of three bacterial blight resistance genes for broad-spectrum resistance in deepwater rice variety, Jalmagna. Rice 8(1):19

Qu S, Liu G, Zhou B, Bellizzi M, Zeng L, Dai L, Han B, Wang GL (2006) The broad-spectrum blast resistance gene *Pi9* encodes a nucleotide-binding site–leucine-rich repeat protein and is a member of a multigene family in rice. Genet 172(3):1901–1914

Radakovits R, Jinkerson RE, Darzins A, Posewitz M (2010) Genetic engineering of algae for enhanced biofuel production Euk Cell (9):486–501

Rafalski A (2002) Applications of single nucleotide polymorphisms in crop genetics. Curr Opin Plant Biol 5(2):94–100

Rahman ML, Jiang W, Chu SH, Qiao Y, Ham TH, Woo MO, Lee J, Khanam MS, Chin JH, Jeung JU, Brar DS (2009) High-resolution mapping of two rice brown planthopper resistance genes, Bph20 (t) and Bph21 (t), originating from Oryza minuta. Theor Appl Genet 119 (7):1237–1246

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

Ray DK, Mueller ND, West PC, Foley JA (2013) Yield trends are insufficient to double global crop production by 2050. PLoS ONE 8(6):

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 Genet Genom 293(6):1565–1575

Ren J, Gao F, Wu X, Lu X, Zeng L, Lv J, Su X, Luo H, Ren G (2016) *Bph32*, a novel gene encoding an unknown SCR domain-containing protein, confers resistance against the brown planthopper in rice. Sci Rep 6:1–14

Richa K, Tiwari IM, Devanna BN, Botella JR, Sharma V, Sharma TR (2017) Novel chitinase gene *LOC_Os11g47510* from indica rice Tetep provides enhanced resistance against sheath blight pathogen Rhizoctonia solani in rice. Front Plant Sci 8:596

Ronald PC, Albano B, Tabien R, Abenes L, Wu KS, McCouch S, Tanksley SD (1992) Genetic and physical analysis of the rice bacterial blight disease resistance locus, *Xa21*. Mol Gen Genet MGG 236(1):113–120

Sabar M, Akhter M, Bibi T, Riaz A, Haider Z, Khan AR, Bibi A (2019) Basmati rice lines development carrying multiple bacterial blight resistance genes pyramided using the marker-assisted backcross breeding approach. Mol Breed 39(11):155

Sagare DB, Abbai R, Jain A, Jayadevappa PK, Dixit S, Singh AK, Challa V, Alam S, Singh UM, Yadav S, Sandhu N (2020) More and more of less and less: Is genomics-based breeding of dry direct-seeded rice (DDSR) varieties the need of hour? Plant Biotechnol J 18(11):2173–2186

Salgotra RK, Sajad MZ (2020) Rediscovery of genetic and genomic resources for future food security. Springer, pp 1–376. ISBN: 978-981-15-0155-5

Sama VS, Rawat N, Sundaram RM, Himabindu K, Naik BS, Viraktamath BC, Bentur JS (2014) A putative candidate for the recessive gall midge resistance gene *gm3* in rice identified and validated. Theor Appl Genet 127(1):113–124

Sandhu N, Dixit S, SwamyBP Raman A, Kumar S, Singh SP, Yadaw RB, Singh ON, Reddy JN, Anandan A, Yadav S (2019) Marker assisted breeding to develop multiple stress tolerant varieties for flood and drought prone areas. Rice 12:8

Sanford JC, Klein TM, Wolf ED, Allen N (1987) Delivery of substances into cells and tissues using a particle bombardment process. Particulate Sci Technol 5(1):27–37

Sardesai N, Rajyashri KR, Behura SK, Nair S, Mohan M (2001) Genetic, physiological and molecular interactions of rice and its major dipteran pest, gall midge. Plant Cell Tissue Organ Cult 64(2–3):115–131

Sato H, Ando I, Hirabayashi H, Takeuchi Y, Arase S, Kihara J, Kato H, Imbe T, Nemoto H (2008) QTL analysis of brown spot resistance in rice (*Oryza sativa* L.). Breed Sci 58(1):93–96

Sato H, Matsumoto K, Ota C, Yamakawa T, Kihara J, Mizobuchi R (2015) Confirming a major QTL and finding additional loci responsible for field resistance to brown spot (*Bipolarisoryzae*) in rice. Breed Sci 5(2):170–175

Savary S, Willocquet L, Elazegui FA, Castilla NP, Teng PS (2000) Rice pest constraints in tropical Asia: quantification of yield losses due to rice pests in a range of production situations. Plant Dis 84(3):357–369

Savary S, Castilla NP, Elazegui FA, Teng PS (2005) Multiple effects of two drivers of agricultural change, labour shortage and water scarcity, on rice pest profiles in tropical Asia. Field Crop Res 91(2–3):271

Savary S, Willocquet L, Pethybridge SJ, Esker P, McRoberts N, Nelson A (2019) The global burden of pathogens and pests on major food crops. Nature Eco Evo 3:430–439

Schneider M, Bairoch A, Wu CH, Apweiler R (2005) Plant protein annotation in the UniProt Knowledgebase. Plant Physiol 138:59–66

Schouten HJ, Krens FA, Jacobsen E (2006) Cisgenic plants are similar to traditionally bred plants: international regulations for genetically modified organisms should be altered to exempt cisgenesis. EMBO Report 7:750–753

Schulthess AW, Wang Y, Miedaner T, Wilde P, Reif JC, Zhao Y (2016) Multiple-trait-and selection indices-genomic predictions for grain yield and protein content in rye for feeding purposes. Theor Appl Genet 129(2):273–287

Shabana YM, Abdel-Fattah GM, Ismail AE, Rashad YM (2008) Control of brown spot pathogen of rice (*Bipolarisoryzae*) using some phenolic antioxidants. Brazilian J Microbiol 39(3):438–444

Sharma NP, Torii A, Takumi S, Mori N, Nakamura C (2004) Marker-assisted pyramiding of brown planthopper (Nilaparvata lugens Stal) resistance genes Bph1 and Bph2 on rice chromosome 12. Hereditas 140(1):61–69

Shibata D, Liu YG (2000) Agrobacterium-mediated plant transformation with large DNA fragments. Trends Plant Sci 5:354–357

Shikari AB, Khanna A, Krishnan SG, Singh UD, Rathour R, Tonapi V, Sharma TR, Nagarajan M, Prabhu KV, Singh AK (2013) Molecular analysis and phenotypic validation of blast resistance genes *Pita* and *Pita2* in landraces of rice (*Oryza sativa* L.). Indian J Genet 73(2):131–141

Silva J, Scheffler B, Sanabria Y, De Guzman C, Galam D, Farmer A, Woodward J, May G, Oard J (2012) Identification of candidate genes in rice for resistance to sheath blight disease by whole genome sequencing. Theor Appl Genet 124(1):63–74

Singh AK, Singh RN (2015) Screening for resistance to false smut (*Ustilaginoidea virens*). Plant Dis Res 30(1):46–49

Singh N, Jayaswal PK, Panda K, Mandal P, Kumar V, Singh B, Mishra S, Singh Y, Singh R, Rai V, Gupta A (2015) Single-copy gene based 50 K SNP chip for genetic studies and molecular breeding in rice. Sci Rep 5:11600

Slatkin M (2008) Linkage disequilibrium-understanding the evolutionary past and mapping the medical future. Nat Rev Genet 9(6):477–485

Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, Fauquet C (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, Xa21. Sci 270(5243):1804–1806

Sparks A, Nelson A, Castilla N (2012) Where rice pests and diseases do the most damage. Rice Today 11(4):26–27

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(2):176–180

Stahle DW, Cook ER, Burnette DJ, Villanueva J, Cerano J, Burns JN, Griffin D, Cook BI, Acuna R, Torbenson MC, Szejner P (2016) The Mexican drought atlas: tree-ring reconstructions of the soil moisture balance during the late pre-Hispanic, colonial, and modern eras. Quat Sci Reviews 149:34–60

Suh JP, Yang SJ, Jeung JU, Pamplona A, Kim JJ, Lee JH, Hong HC, Yang CI, Kim YG, Jena KK (2011) Development of elite breeding lines conferring Bph18 gene-derived resistance to brown planthopper (BPH) by marker-assisted selection and genome-wide background analysis in japonica rice (*Oryza sativa* L.). Field Crop Res 120(2):215–222

Suh JP, Jeung JU, Noh TH, Cho YC, Park SH, Park HS, Shin MS, Kim CK, Jena KK (2013) Development of breeding lines with three pyramided resistance genes that confer broad-spectrum bacterial blight resistance and their molecular analysis in rice. Rice 6(1):5

Sun L, Su C, Wang C, Zhai H, Wan J (2005) Mapping of a major resistance gene to the brown planthopper in the rice cultivar Rathu Heenati. Breeding Sci 55(4):391–396

Suzuki N, Rivero RM, Shulaev V, Blumwald E, Mittler R (2014) Abiotic and biotic stress combinations. New Phytologist 203(1):32–43

Swings J, Van den Mooter M, Vauterin L, Hoste B, Gillis M, Mew TW, Kersters K (1990) Reclassification of the causal agents of bacterial blight (*Xanthomonas campestris pv. oryzae*) and bacterial leaf streak (*Xanthomonas campestris pv. oryzicola*) of Rice as Pathovars of *Xanthomonas oryzae* (ex Ishiyama 1922) sp. nov., nom. rev. Intl J Syst Evol Microbiol 40 (3):309–311

Taguchi-Shiobara F, Ozaki H, Sato H, Maeda H, Kojima Y, Ebitani T, Yano M (2013) Mapping and validation of QTLs for rice sheath blight resistance. Breed Sci 63:301

Tan GX, Ren X, Weng QM, Shi ZY, Zhu LL, He GC (2004) Mapping of a new resistance gene to bacterial blight in rice line introgressed from *Oryza officinalis*. Acta Genetica Sinica 31:724–729

Tanksley SD, Ganal MW, Martin GB (1995) Chromosome landing: a paradigm for map-based gene cloning in plants with large genomes. Trends Genet 11(2):63–68

Thuan NTN, Bigirimana J, Roumen E, Van Der SD, Hofte M (2006) Molecular and pathotype analysis of the rice blast fungus in North Vietnam. Eur J Plant Pathol 114(4):381–396

Tyagi AK, Khurana JP (2003) Plant molecular biology and biotechnology research in the post-recombinant DNA era. Adv Biochem Eng Biotechnol 84:91–121

Tyagi AK, Khurana JP, Khurana P, Raghuvanshi S, Gaur A, Kapur A, Gupta V, Kumar D, Ravi V, Vij S, Khurana P (2004) Structural and functional analysis of rice genome. J Genet 83(1):79–99

Udayashankar AC, Nayaka SC, Reddy MS, Srinivas C (2011) Plant growth-promoting rhizobacteria mediate induced systemic resistance in rice against bacterial leaf blight caused by *Xanthomonas oryzaepv. oryzae*. Biol Control 59(2):114–122

Uppala S, Zhou XG (2018) Field efficacy of fungicides for management of sheath blight and narrow brown leaf spot of rice. Crop Prot 104:72–77

USDA (2018) Economic research service. https://www.ers.usda.gov/topics/crops/rice/

Valent B, Chumley FG (1991) Molecular genetic analysis of the rice blast fungus *Magnaporthe grisea*. Ann Rev Phytopathol 29(1):443–467

Van Esse HP, Reuber TL, Van Does D (2019) Genetic modification to improve disease resistance in crops. New Phytol 225:70–86

Varshney RK, Tuberosa R (2007) Genomics-assisted crop improvement: an overview. In: Genomics-assisted crop improvement. Springer, Dordrecht, pp 1–12

Varshney RK, Hoisington DA, Tyagi AK (2006) Advances in cereal genomics and applications in crop breeding. Trends Biotechnol 24:490–499

Vaucheret H, Fagard M (2001) Transcriptional gene silencing in plants: targets, inducers and regulators. Trends Genet 17:29–35

Venkanna V, Hari Y, Rukminidevi K, Chandra BS, Raju J, Malathi S, Reddy PR (2018) Markers assisted selection for pyramiding of gallmidge resistance genes in kavya, a popular rice variety. Intl J Curr Microbiol Appl Sci 7(4):745–753

Volante A, Tondelli A, Aragona M, Valente MT, Biselli C, Desiderio F, Bagnaresi P, Matic S, Gullino ML, Infantino A, Spadaro D (2017) Identification of bakanae disease resistance loci in japonica rice through genome wide association study. Rice 10(1):1–16

Wang C, Wen G, Lin X, Liu X, Zhang D (2009) Identification and fine mapping of the new bacterial blight resistance gene, *Xa31(t)*, in rice. Eur J Plant Pathol 123(2):235–240

Wang MB, Masuta C, Smith NA, Shimura H (2012a) RNA silencing and plant viral diseases. Mol Plant Microbe Interact 25:275–1285

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

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 18(14):311. https://doi.org/10.1186/s12870-014-0311-6

Wang X, Jia M, Ghai P, Lee F, Jia Y (2015) Genome-wide association of rice blast disease resistance and yield-related components of rice. Mol Plant Microbe Interact 28:1383–1392

Wang F, Wang C, Liu P, Lei C, Hao W, Gao Y, Liu YG, Zhao K (2016a) Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene *OsERF922*. PloS one 11(4):

Wang F, Wang C, Liu P, Lei P, Hao W, Gao Y et al (2016b) Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene *OsERF922*. PLoS One 11:

Wang BH, Ebbole DJ, Wang ZH (2017) The arms race between *Magnaportheoryzae* and rice: diversity and interaction of Avr and R genes. J Integrat Agri 16(12):2746–2760

Weltzien E, Christinck A (2008) Participatory plant breeding: developing improved and relevant crop varieties with farmers. In: Agricultural systems: agroecology and rural innovation for development, pp 211–251

Werner K, Friedt W, Ordon F (2005) Strategies for pyramiding resistance genes against the barley yellow mosaic virus complex (*BaMMV*, *BaYMV*, *BaYMV-2*). Mol Breed 16(1):45–55

Whalon ME, Wingerd BA (2003) Bt: mode of action and use. Arch Insect Biochem Physiol 54:200–211

Wheeler DL, Smith-White B, Chetvernin V, Resenchuk S, Dombrowski SM et al (2005) Plant genome resources at the national center for biotechnology information. Plant Physiol 138:1280–1288

Willocquet L, Noel M, Hamilton RS, Savary S (2012) Susceptibility of rice to sheath blight: an assessment of the diversity of rice germplasm according to genetic groups and morphological traits. Euphytica 183(2):227–241

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

Worrall EA, Hamid A, Mody KT, Mitter N, Pappu HR (2018) Nanotechnology for plant disease management. Agronomy 8(12):285

Wright AV, Nunez JK, Doudna JA (2016) Biology and applications of CRISPR systems: harnessing nature's toolbox for genome engineering. Cell 164:29–44

Wu Y, Yu L, Pan C, Dai Z, Li Y, Xiao N et al (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(2):12

Wu Y, Xiao N, Chen Y, Yu L, Pan C, Li Y, Zhang X, Huang N et al (2019) Comprehensive evaluation of resistance effects of pyramiding lines with different broad-spectrum resistance genes against *Magnaportheoryzae* in rice (*Oryza sativa* L.). Rice 12(1):11

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

Xiao S, Wang B, Liu Y, Miao T, Zhang H, Wen P (2019) Genome-wide association study and linkage analysis on resistance to rice black-streaked dwarf virus disease. Mol Breed 39(5):73

Xie LJ, Chen QF, Chen MX, Yu LJ, Huang L, Chen L et al (2015) Unsaturation of very-long-chain ceramides protects plant from hypoxia-induced damages by modulating ethylene signaling in Arabidopsis. PLoS Genet 11(3):1005143

Xu J (2013) Pyramiding of two BPH resistance genes and *Stv-bi* gene using marker-assisted selection in japonica rice. Crop Breed Appl Biotechnol 13(2):99–106

Xu Y, Wang X, Ding X, Zheng X, Yang Z, Xu C, Hu Z (2018) Genomic selection of agronomic traits in hybrid rice using an NCII population. Rice 11(1):1–10

Yadav S, Anuradha G, Kumar RR, Vemireddy LR, Sudhakar R, DonempudiK, Venkata D, Jabeen F, Narasimhan YK, Marathi B, Siddiq EA (2015) Identification of QTLs and possible candidate genes conferring sheath blight resistance in rice (*Oryza sativa* L.). SpringerPlus 4 (1):175

Yamamoto T, Nagasaki H, Yonemaru JI, Ebana K, Nakajima M, Shibaya T, Yano M (2010) Fine definition of the pedigree haplotypes of closely related rice cultivars by means of genome-wide discovery of single-nucleotide polymorphisms. BMC Genom 11(1):1–14

Yang TJ, Yu Y, Nah G, Atkins M, Lee S, Frisch DA et al (2003) Construction and utility of 10-kb libraries for efficient clone-gap closure for rice genome sequencing. Theor Appl Genet 107:652–660

Yang JH, Liu HX, Zhu GM, Pan YL, Xu LP, Guo JH (2008a) Diversity analysis of antagonists from rice-associated bacteria and their application in biocontrol of rice diseases. J Appl Microbiol 104(1):91–104

Yang W, Peters JI, Williams RO (2008b) Inhaled nanoparticles-a current review. Int J Pharm 356:239–247

Yang FL, Li XG, Zhu F, Lei CL (2009) Structural characterization of nanoparticles loaded with garlic essential oil and their insecticidal activity against *Triboliumcastaneum* (*Herbst*) (*Coleoptera: Tenebrionidae*). J Agric Food Chem 57:10156–10162

Yang L, Li RB, Li YR, Huang FK, Chen YZ, Huang SS et al (2012) Genetic mapping of *bph20(t)* and *bph21(t)* loci conferring brown planthopper resistance to *NilaparvatalugensStal* in rice (*Oryza sativa* L.). Euphytica 183(2):161–71

Yang N, Lu Y, Yang X, Huang J, Zhou Y, Ali F, Wen W (2014) Genome wide association studies using a new nonparametric model reveal the genetic architecture of 17 agronomic traits in an enlarged maize association panel. PLoS Genet 10(9):e1004573

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(3):309–317

Yasala AK, Rawat N, Sama VK, Himabindu K, Sundaram RM, Bentur JS (2012) In silico analysis for gene content in rice genomic regions mapped for the gall midge resistance genes. Plant Omics 5(4):405–413

Yasmin S, Hafeez FY, Mirza MS, Rasul M, Arshad HM, Zubair M, Iqbal M (2017) Biocontrol of bacterial leaf blight of rice and profiling of secondary metabolites produced by rhizospheric *Pseudomonas aeruginosa BRp3*. Front Microbiol 8:1895

Ye X, Al-Babili S, Kloti A, Zhang J, Lucca P, Beyer P, Potrykus I (2000) Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Sci 287 (5451):303–305

Yonemaru JI, Choi SH, Sakai H, Ando T, Shomura A, Yano M, Wu J, Fukuoka S (2015) Genome-wide indel markers shared by diverse Asian rice cultivars compared to Japanese rice cultivar ‘Koshihikari’. Breed Sci 65(3):249–256

Yu J, Buckler ES (2006) Genetic association mapping and genome organization of maize. Curr Opin Biotechnol 17(2):155–160

Yugander A, Sundaram RM, Ladhalakshmi D, Hajira SK, Prakasam V, Prasad MS, Madhav MS, Babu VR, Laha GS (2017) Virulence profiling of *Xanthomonas oryzaepv. oryzae* isolates, causing bacterial blight of rice in India. Eur J Plant Pathol 149(1):171–191

Zanao Junior LA, Rodrigues FA, Fontes RLF, Korndorfer GH, Neves JCL (2009) Rice resistance to brown spot mediated by silicon and its interaction with manganese. J Phytopathol 157 (2):73–78

Zaw M, San Aye S, Matsumoto M (2016) Genetic characteristics of *Pyricularia grisea* and their pathotypes in Myanmar. Bull Inst Trop Agri, Kyushu Univ 39(1):103–111

Zeng Y, Ji Z, Yang C (2014) The way to a more precise sheath blight resistance QTL in rice. Euphytica 203:33

Zhang H, Wu Z, Wang C, Li Y, Xu JR (2014) Germination and infectivity of micro conidia in the rice blast fungus *Magnaportheoryzae*. Nat Commun 5:4518

Zhang Q, Lin SC, Zhao BY, Wang CL, Yang WC, Zhou YL, Li DY, Chen CB, Zhu LH (1998) Identification and tagging a new gene for resistance to bacterial blight (Xanthomonas oryzae pv. oryzae) from O. rufipogon. Rice Genet Newsl 15:138–142

Zhang F, Zeng D, Zhang CS, Lu JL, Chen TJ, Xie JP, Zhou YL (2019) Genome-wide association analysis of the genetic basis for sheath blight resistance in rice. Rice 12(1):1–13

Zhao Y, Huang J, Wang Z, Jing S, Wang Y, Ouyang Y, Cai B, Xin XF, Liu X, Zhang C, Pan Y (2016) Allelic diversity in an NLR gene *BPH9* enables rice to combat planthopper variation. Proc Natl Acad Sci 113(45):12850–12855

Zhao H, Wang X, Jia Y, Minkenberg B, Wheatley M, Fan J, Jia MH, Famoso A, Edwards JD, Wamishe Y, Valent B (2018) The rice blast resistance gene *Ptr* encodes an atypical protein required for broad-spectrum disease resistance. Nat Commun 9(1):1–2

Zheng CK, Wang CL, Yu YJ, Liang YT, Zhao KJ (2009) Identification and molecular mapping of *Xa32(t)*, a novel resistance gene for bacterial blight (*Xanthomonas oryzaepv. oryzae*) in rice. Acta Agronomica Sinica 35:1173–1180

Zhou E, Jia Y, Singh P, Correll J, Lee F (2007) Instability of the *Magnaportheoryzae* a virulence gene AVR *Pita* alters virulence. Fungal Genet Biol 44:1024–1034

Zhou YL, Xie XW, Zhang F, Wang S, Liu XZ, Zhu LH, Xu JL, Gao YM, Li ZK (2014) Detection of quantitative resistance loci associated with resistance to rice false smut (*Ustilaginoidea virens*) using introgression lines. Plant Pathol 63(2):365–372

Zhou J, Peng Z, Long J, Sosso D, Liu B, Eom JS et al (2015) Gene targeting by the TAL effector *PthXo2* reveals cryptic resistance gene for bacterial blight of rice. Plant J 82:632–643

Zhou H, Wang X, Mo Y, Li Y, Yan L, Li Z, Shu W et al (2020) Genetic analysis and fine mapping of the gall midge resistance gene *Gm5* in rice (*Oryza sativa* L.). Theor Appl Genet:1–13

Zhu Q, Ge S (2005) Phylogenetic relationships among A-genome species of the genus Oryza revealed by intron sequences of four nuclear genes. New Phytologist 167(1):249–265

Zhu C, Gore M, Buckler ES, Yu J (2008) Status and prospects of association mapping in plants. The Plant Genome 1(1):5–20

Zhu D, Kang H, Li Z, Liu M, Zhu X, Wang Y, Wang D, Wang Z, Liu W, Wang GL (2016) A genome-wide association study of field resistance to Magnaporthe oryzae in rice. Rice 9(1):1–9

Zuo S, Zhang L, Wang H, Yin Y, Zhang Y, Chen Z, MaY Pan X (2008) Prospect of the QTL-*qSB-9Tq* utilized in molecular breeding program of japonica rice against sheath blight. J Genet Genom 35(8):499–505

Zuo S, Zhang Y, Yin Y, Li G, Zhang G, Wang H, Chen Z, Pan X (2014) Fine-mapping of *qSB-9TQ*, a gene conferring major quantitative resistance to rice sheath blight. Mol Breed 34 (4):2191–2203

# Chapter 2
# Globally Important Wheat Diseases: Status, Challenges, Breeding and Genomic Tools to Enhance Resistance Durability

**Sridhar Bhavani, P. K. Singh, Naeela Qureshi, Xinyao He, Akshaya Kumar Biswal, Philomin Juliana, Abdelfattah Dababat, and Amira M. I. Mourad**

**Abstract** Wheat is an important source of dietary protein and daily calories for majority of the world's population. Although several pests and diseases affect yield potential and quality, the three rusts and powdery mildew fungi have caused major

---

**Learning objectives/goals**: Geographical distribution of wheat diseases, impact, management strategies and briefly address the new molecular tools in the current era to enhance resistance breeding and opportunities for wheat improvement.

---

S. Bhavani (✉) · P. K. Singh · N. Qureshi · X. He · P. Juliana
Global Wheat Program, International Maize and Wheat Improvement Center (CIMMYT), Mexico
e-mail: S.Bhavani@cgiar.org

P. K. Singh
e-mail: Pk.Singh@cgiar.org

N. Qureshi
e-mail: N.Qureshi@cgiar.org

X. He
e-mail: X.He@cgiar.org

P. Juliana
e-mail: P.Juliana@cgiar.org

A. K. Biswal
Genetic Resources Program, International Maize and Wheat Improvement Center (CIMMYT), Mexico
e-mail: A.K.Biswal@cgiar.org

A. Dababat
International Maize and Wheat Improvement Center (CIMMYT), P.K. 39 Emek, 06511 Ankara, Turkey
e-mail: A.Dababat@cgiar.org

A. M. I. Mourad
Agronomy Department, Faculty of Agriculture, Assiut University, Assiut 71526, Egypt
e-mail: amira_mourad@aun.edu.eg

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021

C. Kole (ed.), *Genomic Designing for Biotic Stress Resistant Cereal Crops*,
https://doi.org/10.1007/978-3-030-75879-0_2

epidemics in the past and continue to threaten wheat production despite the widespread use of genetic resistance and fungicides. The evolution and migration of more virulent and aggressive race lineages of rust fungi have rendered varieties vulnerable. Fusarium head blight, leaf spotting diseases, root diseases and, more recently, wheat blast (in South America, Bangladesh and more recently Zambia) have become increasingly important owing to narrow options for resistance diversity. Race-specific and quantitative resistance are well studied for most diseases; their selection and deployment as combinations through phenotyping coupled with molecular strategies offer great promise in achieving resistance durability and enhancing global wheat productivity. Advances in next-generation sequencing (NGS) technologies, functional genomics and bioinformatics tools have revolutionized the area of wheat genomics. Recent advances in sequencing an annotated wheat reference genome with a detailed analysis of gene content among sub-genomes will not only accelerate our understanding of the genetic basis of bread wheat, at the same time wheat breeders can now use this information to identify genes conferring disease resistance. The sequence alignment of the wheat genome has facilitated better identification of marker trait associations, candidate genes and enhanced breeding values in genomic selection (GS) studies. High throughput genotyping platforms have not only reduced the cost, but wider genome coverage and density have enabled better estimation of genetic diversity, construction of the high-density genetic maps, dissecting polygenic traits, understanding their interactions through genome-wide association studies (GWAS), quantitative trait locus (QTL) mapping and isolation of R-genes. Ease of deploying breeder's friendly Kompetitive allele specific polymerase chain reaction (KASP) markers in the recent years has expedited the identification and pyramiding of resistance alleles/genes in elite lines. This review provides the overview of important diseases affecting wheat productivity, considering their geographical distribution, impacts, management strategies and briefly addresses the new molecular/genomic tools in the current era to enhance resistance breeding and deployment opportunities for wheat improvement.

**Keywords** Disease resistance · Race specific genes · Adult plant resistance genes · Breeding approaches and genomic technologies

## 2.1 Introduction

Wheat (*Triticum* spp.) is globally the most important staple food of about 2.5 billion people (33% of the world population) and provides nearly 20% of the daily proteins and calories consumed globally (Breiman and Graur 1995). In terms of food security, it is the second most important food crop in the developing world after rice, with an estimated 80 million farmers relying on wheat for their livelihoods (Curtis et al. 2002). It is globally the most traded export commodity estimated at US$38.8 billion in 2019 (www.worldstopexports.com). Currently, wheat is the most widely grown cereal crop occupying more than 218 million ha. with a global production of 765 million tons, worth approximately US$150 billion (https://www.statista.com/statistics/267268/

production-of-wheat-worldwide-since-1990/). For future food security, wheat production increase should target over one billion tons to cater to the needs of the rising population of estimated 9.6 billion people by 2050. Increasing consumption of diversified wheat products and quality profiles across countries will demand increased crop production and productivity in different wheat growing environments (Shewry and Tatham 2016). The continuous effort to increase genetic gains can only be possible by overcoming several of the current barriers such as climate change coupled with a variety of abiotic and biotic stresses that pose significant threat to wheat production both locally and globally. Genetic uniformity in the quest of developing high-performing cultivars has also contributed to vulnerability of rapidly evolving pathogens to the point wherein diseases threaten global wheat production.

## 2.2 Impact of Biotic Stresses on Wheat Production

On average, about 20% of the wheat production globally is lost due to pests and diseases every year (Anon 2014). Leaf rust became an increasingly important disease after the wheat variety "Thatcher" became susceptible in 1938, destroying millions of hectares in North America and since then it was considered as a damaging disease in USA, former USSR, and China (Chester 1946). Modern wheat cultivars continue to be affected by this disease worldwide. A cost benefit ratio of 1:27 was attributed for leaf rust resistant cultivar development at International Maize and Wheat Improvement Center (CIMMYT) (Simmonds and Rajaram 1988). The disease causes grain yield loss mainly by affecting both the number and weight of wheat kernels (Huerta-Espino et al. 2011). Yield losses between 2000 and 2004 due to leaf rust was estimated at US$350 million in the USA alone. In Mexico, yield losses to leaf rust accounted for US$32 million from 2000 to 2003, and subsequently US$40 million from 2008 to 2009, In South America (Argentina, Brazil, Chile, Paraguay, and Uruguay) between 1996 and 2003, the reported yield loss amounted to US$172 million. The average annual yield loss of 3 million tons was reported in China, whereas in Pakistan 10% yield loss to leaf rust were reported in 1978, estimated at US$86 million. In Australia, potential yield losses annually to leaf rust is estimated at AU$197 million under susceptible cultivars however, the use of resistant cultivars can minimize the loss to about AU$12 million (Huerta-Espino et al. 2011). In the first half of the 20th century, stem rust damaged 20% of wheat production in the USA with repeated epidemics between 1920 and 1960s. Yield loss ranging from 9% to 33% was recorded in Scandinavian countries in 1951 and records of 5–20% loss in eastern and central Europe were reported in 1932. Severe stem rust epidemics were reported in spring wheat grown in northern China and Inner Mongolia in the 1948, 1951, 1952, and 1956 cropping seasons (Sharma 2012). In Australia, sporadic epidemics of stem rust caused losses of £2–3 million (1889), £400,000 (1903), £2 million(1916), £7 million (1947), and the most severe loss was reported in New South Wales with estimated losses of AU $200–300 million (1973), which led to the establishment of the National Rust

Control Program (Park 2007). A recent study estimated annual yield losses of wheat due to stem rust could reach 6.2 million tons globally, equivalent to US$1.12 billion (Pardey et al. 2013).

Chen (2005) reported 100% yield losses on stripe rust susceptible cultivars such as "PS 279", and yield losses can range from 10 to 70% depending on cultivar susceptibility, time of the initial infection and degree of disease progress. In USA, severe losses by yellow rust (YR) was reported in four years (1958–1961, 1974–1978, 1980–1984, and 1999–2005) with significant damage in 2003 estimated at US$300 million. In South Africa, US$2.25 million loss was reported in 1998 two years post introduction. In 2002, China reported losses of 1.3 million tons of wheat grain to stripe rust. Yield loss of 20–40% between 1990s and early 2000s was also reported from Central Asian countries (Chen 2005). The 2003 epidemic of stripe rust in Australia resulted in damage amounting to AU$40 million (Wellings 2011). Fungal pathogens of diseases like rusts (*Puccinia* ssp.), powdery mildew (*Blumeria graminis*), Septoria leaf blotch (*Septoria* spp.) and *Fusarium* species are ranked among the most important fungal pathogens (Dean et al. 2012) in wheat growing environments where conditions are favorable for pathogen buildup. In low wheat production environments with lack of seed dressing treatments, diseases like smuts and bunts are common (Oerke and Dehne 2004) and in specific wheat-growing areas, fungal pathogens such as *Pyrenophora tritici-repentis* causing tan spot, *Oculimacula* spp. causing eyespot of wheat and *Cochliobolus sativus* causing spot blotchare of significant importance.

## 2.3 Implications of Climate Change

Climate change can create significant impact not only on the wheat production but also on pathogen dynamics both at regional and global scale. In individual wheat growing environments, shift towards warmer regimes and other climatic conditions such as altered precipitation may result in resurgence and adaptation of older and newer pathotypes of wheat diseases. These changes can also affect the seasonal phenology (better synchronization of pathogen life-cycle events with their host plants), the population dynamics (over-wintering and adaptation to warmer/cooler conditions, and the geographic distribution (expansion or retreat of specific pathogens with increased risk of pathogen incursion) (Chakraborty et al. 1998; Chakraborty and Newton 2011). The impact of climate change on wheat diseases has not yet been extensively studied. Some studies of potential impact of climate change on wheat diseases have already been reported (Chakraborty et al. 1998; Juroszek and von Tiedemann 2013). However, there is an increasing number of studies focusing on specific wheat diseases in relation to climate change, e.g. future changes of Fusarium foot rot in Australia (Backhouse and Burgess 2002), future Karnal bunt risk in Europe (Dumalasová and Bartoš 2009), worldwide changes of rust diseases in future (Chakraborty and Newton 2011), impact of climate change on leaf rust in France (Caubel et al. 2017), stripe rust in central and eastern USA

(Lyon and Broders 2017), and very recently with the emergence of new stem rust races (Chakraborty et al. 2011; Juroszek and von Tiedemann 2013; Saunders et al. 2019) and Septoria tritici blotch risk in France (Gouache et al. 2013). These reports suggest that climate change may modify the range of prevalent wheat diseases in some regions that may turn, as a result currently economically less important wheat pathogens into potential threats in the near future (Duveiller et al. 2007).

## 2.4 Important Biotic Stresses Limiting Wheat Production in Different Environments

The following fungal pathogens are major biotic constraints in intensive wheat production systems worldwide. First, there are the obligate fungal pathogens (biotrophs); *Blumeria graminis* causing powdery mildew, *Puccinia graminis tritici* causing stem rust, *Puccinia recondita/Puccina triticina* causing leaf rust, and *Puccinia striiformis* causing stripe rust. Second, there are crop residue-borne necrotrophic pathogens; *Pyrenophora tritici-repentis* causing tan spot, *Mycosphaerella graminicola* causing Septoria tritici blotch, *Phaeosphaeria nodorum* causing Septoria nodorum blotch, *Cochliobolus sativus* causing spot blotch, and *Fusarium graminearum* and other *Fusarium* species causing Fusarium head blight or scab. There are many more fungal pathogens which are causing wheat diseases such as soil-borne root rots (Duveiller et al. 2007). In regions with low productivity and without seed dressing, smuts (e.g. common bunt caused by *Tilletia caries*) and bunts (e.g. Karnal bunt caused by *Tilletiaindica*) can be of significant importance (Oerke 2006). More than 40 viral diseases of wheat *Triticum* species have been documented; however, their significance is limited to specific geographic regions causing substantial yield losses. Viruses belonging to the genus *Bymovirus* (family Potyviridae) or the genus *Furovirus* (family Virgaviridae) are transmitted by the root-infecting plasmodiophorid *Polymyxa graminis Ledingham* (Rao and Brakke 1969) and some are insect-transmitted viruses. Insect transmitted viruses belong to the family Luteoviridae causing Barley Yellow Dwarf (BYD) disease transmitted by aphids, and the leafhopper-transmitted Wheat Dwarf Virus (WDV), a member of the genus *Mastrevirus* within the family Geminiviridae. Furthermore, the mite-transmitted Wheat Streak Mosaic Virus (WSMV) belonging to the genus *Tritimovirus* within the family Potyviridae are important viral pathogens of wheat. A comprehensive summary of wheat pathogens including fungi, viruses, and bacteria (and economically important animal pests) is well documented (Bockus et al. 2010). Economically important diseases in major wheat growing environments are discussed in detail below.

### 2.4.1 Rust Diseases

Cereal rust fungi are ubiquitous pathogens, known to occur in most wheat production environments causing substantial yield losses and very recently are considered a serious challenge to wheat production threatening the global wheat supplies (Bhavani et al. 2019). It is estimated that average annual losses to wheat rust pathogens range between US$4.3 to 5.0 billion globally (Beddow et al. 2015). Documented evidence suggest rust diseases could be one of the earliest pathogens wherein spores of stem rust dating back to 1300 BC were detected in Israel and also reported as serious disease of cereals in Italy and Greece (Kislev 1982; McIntosh et al. 1996). There are three wheat rust diseases, namely stem (black) rust, stripe (yellow) rust and leaf (brown) rust, all belong to the family Basidiomycota, genus *Puccinia*, and named *P. graminis* f. sp. *tritici* (*Pgt*), *P. striiformis* f. sp. *tritici* (*Pst*) and *P. triticina* (*Pt*), respectively (McIntosh 1998).

#### 2.4.1.1 Stem Rust

Stem rust (SR), or black rust is common in warmer environments usually detected at later stages of crop growth (Roelfs et al. 1992). SR has the potential to completely destroy a healthy looking crop under epidemic situations and linear yield losses have been observed, with early infections can result in shriveled or no grain fill (Bhavani et al. 2019; Dean et al. 2012). SR epidemics have been significantly curtailed worldwide using various approaches; through eradication of barberry species between 1918 and 1980 in the USA (Singh et al. 2006) and in the UK, with the deployment of wheat germplasm carrying broad effective SR resistance genes and the use of fungicides. After effective control of SR for over three decades, the recent emergence of SR race "Ug99" in East Africa posed a serious threat to global wheat production (Bhavani et al. 2019; Singh 2006; Singh et al. 2015). The race Ug99 (TTKSK) caused widespread damage in Kenya (Pretorius et al. 2000; Singh et al. 2006, 2008a; Wanyera et al. 2006) carrying unique virulence as it was able to overcome over 50% of the known SR resistance genes including widely deployed genes *Sr31*, *Sr38* and many other genes that were effective in different geographies (Singh 2006; Singh et al. 2008a). Ug99 race TTKSK was first identified in Uganda in 1999 and has spread through Africa and the middle east (Singh et al. 2015). The origin of the TTKSK race is unknown, it is genetically distinct from other stem rust races which indicate that this race did not evolve through mutations from other *Pgt* races (Olivera Firpo et al. 2015; Pretorius et al. 2007; Singh et al. 2015; Visser et al. 2011, 2019). Detection of several new variants within the Ug99 race group with the ability to overcome effective resistance genes substantially increased the vulnerability of varieties not only in East Africa (Jin and Singh 2006; Singh et al. 2008a, 2015; Bhavani et al. 2019) but predicted migration paths threatened production in other wheat growing environments (Singh et al. 2008a). In 2018, another new race with virulence to *Sr8155B* gene was identified in Kenya (S. Bhavani unpublished

data) and currently, seven of the 14 variants within the Ug99 race group have evolved in Kenya, making it the hot spot for evolution of Ug99 race group (D. Hodson pers. communication).

Ethiopia reported devastating localized epidemics of SR on variety "Digalu" in 2013caused byrace TKTTF, a SR race unrelated to the Ug99 race group (Olivera Firpo et al. 2015), also previously reported in Turkey (Mert et al. 2012), Lebanon and Iran (Singh et al. 2015). In addition to Digalu race group, diverse SR races with rare combination of virulence to *Sr9e* and *Sr13* have been found in the central highlands of Ethiopia (Admassu et al. 2009; Olivera Firpo et al. 2012). Unusual SR infections on winter and spring wheat were observed in 2013 season, which triggered concerns if Ug99 had migrated to Europe. Race analyses found six SR races, similar to variants of the Digalu race with additional virulence to *Sr7a, Sr45,* and *SrTt*-3 were identified (Olivera Firpo et al. 2017). A race TKKTP with virulence combination for *Sr24, Sr36, Sr1A.1R* and *SrTmp* genes has also been identified (Jin and Singh 2006). This race and the TRTTF race (from Yemen and Pakistan) are the only two known races that currently carry virulence to *Sr1A.1R* (Olivera Firpo et al. 2012). The re-emergence of common barberry has also accounted for SR epidemics in oats in Sweden (Berlin et al. 2013). The race TKTTF has also been detected in Germany, UK, Sweden and Denmark (Lewis et al. 2018). More recently the Sicily SR epidemic of durum wheat was also caused by the race TTRTF (Bhattacharya 2017) and recent studies reported its presence in Eritrea (Patpour et al. 2020).

#### 2.4.1.2 Stripe Rust

Stripe (yellow) rust (YR) is a common disease in almost all wheat-growing environments. Even though YR is known to be well adapted to temperate areas with humid and cool weather (Rapilly 1979), races that are more aggressive and adapted to warmer temperatures have migrated and spread across geographies since 2000 (Ali et al. 2014; Hovmøller et al. 2010; Singh et al. 2015). Race shifts towards higher rates of mutation for virulence within the *Pst* pathogen (Hovmøller and Justesen 2007) has resulted in the vulnerability of widely deployed cultivars (Milus et al. 2015). Global estimate of yield losses to YR alone is 5.5 million tons per year (Beddow et al. 2015). Production losses in North America alone since 2000 exceeded over one million tons (Wellings 2011) and in China, over 1.8–6.0 million tons yield losses were observed under epidemic conditions (Wan et al. 2007). Similar reports of yield losses to YR in Europe in the recent decade have been attributed largely to the race shifts derived from the Himalayan region (Hovmøller et al. 2016). Historically, impact of newly evolved YR races on wheat productivity have been occasional, however, new incursions have often resulted in widespread damage, e.g. incursion of YR races from Europe into eastern Australia in 1978 (Wellings and McIntosh 1990) and western Australia in early 2002 (Hovmøller et al. 2008; Wellings et al. 2003). Exotic incursions of YR races replaced the existing populations in the USA since 2000 (Markell and Milus 2008; Milus et al.

2009) and race shifts in the European *Pst* populations in 2011 and 2012 by races from the Himalayan region (Hovmøller et al. 2016; Hubbard et al. 2015) are very good examples of exotic races with different genetic *Pst* lineages causing significant impact on host susceptibility. A recent study linking both virulence and race structure with recent YR epidemics in different geographies (Ali et al. 2017) suggested different *Pst* races in distinct genetic lineages, where aggressive strains adapted across diverse environments were spreading across continents, including the more recent outbreak of YR in Argentina (Hovmøller et al. 2008; Carmona et al. 2019).

#### 2.4.1.3 Leaf Rust

Leaf (or brown) rust (LR), is the most common rust disease in both winter wheat and spring wheat growing areas as well as in durum wheat. Yield losses due to LR can be substantial if susceptible varieties are infected at early stages coupled with favorable temperatures and moisture conditions resulting in rapid progress in short time span. Yield losses are largely due to the reduction of kernels per spike and lower kernel weights (Chester 1946). LR shows widespread adaptation from warm to hot weather, such as the great plains of North America, southeast Asia, Russia and central Asia to southeastern US, Mexico, Uruguay, Argentina, Turkey, China and southern Europe. Populations of *Pt* are specifically adapted to either tetraploid durum wheat or hexaploid common wheat (Anikster et al. 1997) and races conferring virulence to several of the *LR* genes are prevalent throughout the world (Roelfs et al. 1992). Since the early 2000s, races of *Pt* that are highly virulent on durum wheat cultivars have spread across South America (Ordoñez and Kolmer 2007), Mexico (Singh et al. 2004a), Europe (Goyeau et al. 2006), the Mediterranean basin, and the Middle East (Kolmer 2001).

On a global scale, most populations of *Pt* are unique in their virulence and molecular genotypes. Even though the most common mode of evolution is through mutation and selection in a given environment, there is evidence for recent migration of *Pt* races between different continental regions. Since the mid-1990s, isolates of *Pt* with virulence to *Lr1, Lr3a*, and *Lr17a* and avirulence to *Lr28* have increased and spread across the US and Canada (Kolmer 1998; Long et al. 2000). These isolates also had a unique molecular genotype, which indicated that these were likely recently introduced to North America (Kolmer and Anderson 2011). Since the early 2000, these isolates with identical or highly similar virulence and molecular genotypes have been found in Europe (Kolmer et al. 2013), South America (Germán et al. 2007), Ethiopia (Kolmer and Acevedo 2016), Turkey (Kolmer et al. 2013) and Pakistan (Kolmer et al. 2017). Similarly isolates of *Pt* with virulence to durum wheat that also have identical or highly related molecular genotypes have been found in the Middle East, South America, Europe, Ethiopia, Tunisia, Mexico and the US (Ordoñez and Kolmer 2007).

## 2.4.2 Non-rust Diseases

### 2.4.2.1 Powdery Mildew

In contrast to rusts, powdery mildew caused by *B. graminis* f. sp. *tritici* is more common in humid rain-fed conditions or irrigated conditions, which favor successful infection. The disease strongly influenced by the amount of nitrogen application, large single doses or excessive multiple applications of N fertilizers can result in serious outbreaks (Chen et al. 2007). Cooler and humid regions of Asia, Japan and North and East Africa, Northern parts of Europe and America are regions where powdery mildew is an important pathogen. Early infection of powdery mildew stimulates non-productive tillers, which reduces food reserves affecting the grain yield and low levels of disease in susceptible varieties can still reduce yield significantly (Bowen 1991; Everts 1992). Reduction in yield was significant when high disease severity was observed at Feekes stage 10 (booting stage) (Large 1954) and at Feekes 9 (expanded flag leaf), susceptibility ratings of the Feekes 1 to 3 leaves were most useful yield predictions. Application of fungicide at Feekes 9 or earlier stages is important if disease has been detected early with faster progress (Royse et al. 1980). Yield losses up to 40% have been observed and losses are related to the reduction in grain size and number per unit area, which largely depend on host resistance/susceptibility (Bowen 1991; Royse et al. 1980). Impact of powdery mildew can result in reduced flour protein but has no significant effect on milling and baking quality (Johnson et al. 1979).

### 2.4.2.2 Fusarium Head Blight

Fusarium head blight (FHB) is one of the most devastating disease of wheat globally, with major epidemic regions in North America, Europe, East Asia and the Southern Cone of South America. Many species in the genus *Fusarium* cause FHB, but it is *F. graminearum* species complex that has global importance and has been found in all major epidemic regions. The disease is favored by warm and humid environment around anthesis, leading to yield reduction and quality deterioration. More importantly, the disease produces a range of mycotoxins, particularly deoxynivalenol (DON, or vomitoxin), which are toxic to humans and animals, raising a serious concern to food and feed safety (Buerstmayr et al. 2020). In many countries, regulations on DON in wheat and its products have been set up, and the market price of wheat grain may be significantly reduced if DON content exceeds a certain threshold. In USA, losses attributable to FHB in wheat and barley between 1993 and 2001 were estimated at US$7.67 billion (McMullen et al. 2012). In China, the epidemic has increased significantly in the last two decades, amounting on average 5.3 Mha and reached 9.9 Mha in the 2012 great epidemic (Zhu et al. 2019). Yield reductions can reach up to 50–70% in Europe and South America (Buerstmayr et al. 2020).

#### 2.4.2.3 Wheat Blast

Wheat blast (WB) caused by the ascomycetes fungus *Magnaporthe oryzae* pathotype *triticum* (MoT) is one of the devastating diseases in warm and humid wheat growing regions. It can infect all the aerial parts of wheat, but completely or partially bleached spike is the typical symptom. WB was initially identified in the Parana state of Brazil in 1985; afterwards, its rapid widespread to the neighboring states in Brazil and other countries of South America raising serious concerns. The recent outbreak in Bangladesh in 2016 raised a major concern on wheat production in South Asia (SA), as nearly 7 Mha of the wheat growing areas in SA are vulnerable to WB. More recently, occurrence of WB has been reported from Zambia which can be a major threat for wheat production and trade in Africa (Tembo et al. 2020). Under favorable temperatures of 25–30 °C and high humidity, the disease can cause high yield loss ranging from 10 to 100% depending upon the level of infection (Ceresini et al. 2016).

The long-distance spread of the pathogen occurs through infected seeds, followed by the air transmission; therefore, seed quarantine and chemical treatment can effectively manage the primary inoculum load. For field WB management, foliar fungicides application such as demethylation inhibitors (DMI), quinone outside inhibitors (QoI) and succinate dehydrogenase inhibitors (SDHI) are suggested to be used in combination/rotation so as to reduce the fungal resistance against the fungicides (Cruz and Valent 2017). Various agronomic practices viz. optimizing planting dates, weed management, crop rotation with non-hosts and avoiding excessive nitrogen application are reported to be effective in WB control. However, all these measures do not work well under high disease pressure, thus they should be used in combination with genetic resistance to achieve a better management.

#### 2.4.2.4 Karnal Bunt

*Tilletia indica* (syn. *Neovossiaindica*) is a hemibiotrophic fungus which was first described to cause disease in the Indian city of Karnal, hence called 'Karnal bunt' (KB). Currently the disease is distributed in parts of Asia (India, Nepal, Pakistan, Iraq, Iran, Afghanistan), Africa (South Africa), and the Americas (USA, Mexico, Brazil). Though the estimated average yield losses due to KB are as low as 0.01–1%, it is an important disease from international trade perspective where many member countries of WTO use it as a non-tariff barrier. KB significantly deteriorates the wheat quality in terms of reduced vitamins, amino acids, weakened dough, and loss in flour recovery, ultimately affecting the human consumption negatively (Bishnoi et al. 2020).

The conducive conditions for disease development are high humidity with cool temperature (<20 °C) favoring teliospore germination. Infected spikes disperse teliospores that become inoculum for the next season, and the teliospores are reported to remain viable for long durations, indicating the spatial and temporal

dispersal capability of the disease (Carris et al. 2006). Identifying the disease in field is difficult due to confounding the symptoms with other bunts, thus, making the laboratory and molecular confirmation essential. Laboratory confirmation includes observing teliospores under the microscope for specific morphological characteristics, and molecular characterization adds precision to teliospore morphology with *T. indica* specific markers (Bishnoi et al. 2020; Kumar et al. 2021).

#### 2.4.2.5 Tan Spot

Tan spot (TS) is caused by the necrotrophic fungus *Pyrenophora tritici-repentis* (Died.) Drechs. The disease frequently appears in the warm and humid growing regions of bread and durum wheat, especially Canada, Australia, USA and South Africa. Yield and quality losses are common under high disease pressure. Reduced or no-till approaches to prevent soil erosion are important reasons for increased disease pressure. Residue from previous crop carrying pathogen inoculum is considered one of the main inoculum sources. Another major reason that corresponds with increased pathogen virulence is acquisition of a host-selective toxin (HST) PtrToxA by *P. tritici-repentis* from *Stagonospora nodorum* via horizontal gene transfer which overcame the resistance of most cultivars carrying *Tsn1* gene (Friesen et al. 2007). Based on type of lesion (chlorosis or necrosis) and HSTs produced, *P. tritici-repentis* is classified into eight races using six differential genotypes (Table 2.1).

**Table 2.1** Reaction of eight characterized races of *Pyrenophora tritici-repentis* on bread and durum wheat differential lines. Resistance and susceptible response are indicated as R and S, respectively

| Race information | Associated toxins | Reaction of differential genotype set | | | | | |
|---|---|---|---|---|---|---|---|
| | | Glenlea | 6B662 | 6B365 | Salamouni | Coulter | 4B1149 |
| 1 | PtrToxA, PtrToxC | S (necrosis) | R | S (chlorosis) | R | S (necrosis) | R |
| 2 | PtrToxA | S (necrosis) | R | R | R | S (necrosis) | R |
| 3 | PtrToxC | R | R | S (chlorosis) | R | S (necrosis) | R |
| 4 | None | R | R | R | R | R | R |
| 5 | PtrToxB | R | S (chlorosis) | R | R | S (necrosis) | R |
| 6 | PtrToxB, PtrToxC | R | S (chlorosis) | S (chlorosis) | R | S (necrosis) | R |
| 7 | PtrToxA, PtrToxB | S (necrosis) | S (chlorosis) | R | R | S (necrosis) | R |
| 8 | PtrToxA, PtrToxB, PtrToxC | S (necrosis) | S (chlorosis) | S (chlorosis) | R | S (necrosis) | R |

#### 2.4.2.6 Septoria NodorumBlotch (SNB)

*Stagonospora nodorum*, a filamentous ascomycetes fungus, causes wheat leaf and glume blotch and affects wheat yield and quality in the warm and humid areas particularly in Australia, USA, parts of Europe and southern Brazil. Short incubation period enables the pathogen for multiple infection cycles within a season. The fungus can reproduce through asexual conidia and frequent sexual reproduction due to availability of both mating types (MAT1-1 and MAT1-2). Sexual reproduction creates large genetic variability and best-fit strains multiply asexually, having great potential to overcome the effects of resistance genes or fungicides. Therefore, focus should be on the enhancement of quantitative/horizontal resistance in the targeted wheat population, from a breeding perspective (Cowger et al. 2002). SNB produces multiple HSTs, of which 15 have been identified so far. The HSTs (e.g. *SnToxA*) interact with the corresponding host sensitivity genes (e.g. *Tsn1*) in an 'inverse gene-for-gene' manner that causes infection in the host, just as in tan spot. So far, nine necrotrophic effectors (NE) and sensitive gene interactions viz. *SnToxA-Tsn1, SnTox1-Snn1, SnTox2-Snn2, SnTox3-Snn3-B1, SnTox3-Snn3-D1, SnTox4-Snn4, SnTox5-Snn5, SnTox6-Snn6,* and *SnTox7-Snn7* have been identified in wheat. Three important NE genes in the pathogen viz. *SnToxA, SnTox1, SnTox3* and one important host sensitivity gene in wheat viz. *Tsn1* have been cloned which has helped in the extensive study of three important interactions viz. *SnToxA-Tsn1, SnTox1-Snn1* and *SnTox3-Snn3-B1* for better understanding the molecular basis of SNB (Ruud et al. 2019). These studies have indicated that one interaction may enhance or suppress the other interactions depending upon the genetic backgrounds of pathogen/cultivar, which is important from a breeding perspective (Ruud et al. 2017). *Tsn1* was identified on chromosome 5BL (Faris et al. 2010), whereas both *Snn1* and *Snn3-B1* were mapped on 5BS (Ruud et al. 2017). Negative selection of host sensitivity genes during the breeding program would accelerate the breeding progress of resistant varieties.

#### 2.4.2.7 Spot Blotch

Spot blotch (SB) caused by *Bipolarissorokiana* (telemorph *Cochliobolus sativus*) is a destructive disease of wheat in the warm and humid growing regions, especially South Asia, Latin America and Southern Africa. The pathogen causes average yield loss of 15–20%, but under favorable environmental conditions yield loss of up to 87% has been detected on the susceptible varieties(Gupta et al. 2018). The pathogen can infect all parts of the wheat plant, but leaf infection is the most typical where infection starts from the older leaves and then progresses upward towards the younger leaves. High temperature (18–32 °C) and humidity (>90%) favors the disease establishment.

#### 2.4.2.8 Septoria Tritici Blotch

Septoria tritici blotch (STB) is caused by the fungal species *Zymoseptoria tritici (teleo. Mycosphaerella graminicola)*. The pathogen is heterothallic with two mating types and thus has frequent sexual reproduction, resulting in a high level of genetic variation (Cowger et al. 2002). Additionally, *Z. tritici* can make multiple infections during a cropping season, greatly accelerating its evolving speed, leading to a series of problems in STB management, such as break down or erosion of host resistance and fungal resistance to fungicide. Losses to STB can range between 30 and 50% only during severe epidemics in areas with extended periods of cool, wet weather, particularly North America (USA, Canada, Mexico), East Africa (Ethiopia, Kenya), South America (Brazil, Chile, Uruguay, Argentina) and the most damage occurs in Europe and CWANA (Central and West Asia and North Africa) region (Van Ginkel et al. 2002).

Because fungicides usually exhibited low efficiencies in STB control, multiple fungicidal applications are often needed under high disease pressure, leading to high costs to wheat farmers. In Europe, about 70% (US$1.2 billion) of the total fungicide application in cereal crops were for controlling STB. Nevertheless, this disease has been compromised by the emergence of fungicide resistance in *Z. tritici*, and the new regulation in EU on reducing fungicide application favors the active utilization of other STB management strategies such as host resistance (Torriani et al. 2015).

#### 2.4.2.9 Root Diseases

The healthy root system of a wheat plant is the key for the water and nutrient uptake. Root rots are among the major diseases of wheat resulting in a significant yield loss throughout the world and are found wherever cereal-based farming system dominates (Cook 2001). Because the roots are not typically visible, symptoms of root rot become apparent only when the disease is advanced. Root rot pathogens in cereals include *Heterodera* species, cereal cyst nematode (CCN), *Pratylenchus* species, root-lesion nematode (RLN) and many fungal species. Among the latter are *Gaeumannomyces*, *Pythium*, *Rhizoctonia*, *Fusarium*, *Bipolaris* genera, and different species of these genera are favored by different soil, cropping system and climate (Cook 2001). The pathogens have a wide host range and can survive in the soil/organic residue for many years. Root rot symptoms are difficult to identify clearly but generally are characterized by discoloration of roots, coleoptiles and stem bases of the infected seedling. Root rot fungi also may attack the upper parts of plants which may result in foliage lesions, head and seedling blight.

Take-all (*Gaeumannomyces graminis*) is the dominant root disease favored by the moist and cool conditions in winter season followed by the moisture stress during anthesis. There is often a build-up of antagonistic microorganisms following one or two take-all outbreaks, turning soils to be suppressive and subsequently

reducing the disease. Registered fungicides might be an option to control the disease (Cook 2001; James Cook 1992). *Pythium* is a pathogen having a wide host range causing root rot and seedling damping off. *Pythium* infects root system via root tips and root hairs and can also penetrate the embryo of germinated seed. Main *Pythium* symptoms are stunting and yellowing of leaf tissue, which sometimes may be misdiagnosed as nitrogen deficiency. Infected roots are stunted and light brown-yellow coloration is seen near the tips. Infected plants develop poorly filled heads and is often misdiagnosed as Rhizoctonia damage. Rhizoctonia disease can prune off the root and causes water and nutrient stress which causes crop damage. It survives in the top of the soil (0–10 cm) on organic matter (Cook et al. 2002). *Fusarium* spp. especially *F. culmorum* and *F. pseudograminearum* cause root diseases on cereals, including foot rot, root rot, and crown rot. Crown rot is the most widely accepted name for this disease, which encompasses symptoms on the lower part of the wheat plant, including the subcrown internode, crown, crown roots and lower stem including nodes and internodes. Diseased plants are characterized by fungal colonization on the wheat stems, crown and root tissues leading to a honey-brown discoloration of the leaf sheaths and lower stem, and necrosis of the crown region (Scherm et al. 2013). *Bipolaris* spp. especially *B. sorokiniana* cause common root rot of wheat worldwide, which produces a brown to black discoloration of the sub-crown internode.

Three major species belonging to CCN viz. *Heteroderaavenae*, *H. latipons*, and *H. filipjevi*, are distributed worldwide and cause severe damage in cereals. The *Pratylenchus* species, especially *P. thornei*, *P. crenatus*, *P. neglectus* and *P. penetrans*, are widely distributed pathogens for RLN (Dababat et al. 2014). CCN is monocyclic as it completes only one cycle per season while RLN is polycyclic due to a higher multiplication rate of three to five generations per year. RLN causes stunted and poorly tillered plants. The badly damaged roots are thin and poorly branched with short and knotted laterals. CCN can be identified easily through patches and stunted plants. Below-ground symptoms are white females on roots which can be seen with naked eyes in springtime. Identifying which root rot pathogen is present in the field by classical and/or molecular tools is the most important point to tackle the disease (Table 2.2).

## 2.5 Prospects of Genetic Control, Types of Resistance, Strategies to Deploy Different Resistance Mechanisms to Attain Resistance Durability

Even though numerous pests and diseases are known to reduce grain yield and quality in wheat, the three rusts, powdery mildew fungi and other head, foliar and root diseases, continue to be economically important in spite of the extensive use of host resistance and fungicides. The evolution and spread of virulent and aggressive race lineages of rust fungi threaten wheat production worldwide. Fusarium head

**Table 2.2** Characteristics of the root rot diseases

| Disease | Causal pathogen | Symptoms | Host(s) | Survival |
|---|---|---|---|---|
| Take-all (GGT) | *Gaeumannomyces graminis* var. *tritici* | Patches, blackening of roots, plant are easy to pull from the soil | Wheat, barley, rye, oat, grasses | Grass, stubble |
| Pythium root rot | *Pythium* spp. | Patches yellow to brown root system | Wheat, barley, triticale, oats, grasses | Resting spores |
| Rhizoctonia bare patch | *Rhizoctonia solani* | Stunting of plants, seedling rots, roots stunted with spear point | Wheat, barley, triticale, grasses | Plant residue, hyphal fragments |
| Crown Rot (CR) | *F. pseudograminearum, F. culmorum* | Scattered plants, browning of stem base, crown, white heads, pinched no grain, pink lower nodes | Wheat, barley, triticale, grasses | Volunteer grass, stubble residue |
| Common root rot (CCR) | *B. sorokiniana* | Patches, Dark brown discoloration on sub-crown internode | Cereals, grasses | Spores in soil, stubble residue |
| Cereal cyst nematode (CCN) | *H. avenae, H. filipjevi, H. latipons* | Patches, stunted yellow plants, multiple short, branched roots, cysts visible on roots in spring | Wheat, barley, oat, triticale, and grasses | Eggs, cysts |
| Root-lesion nematode (RLN) | *Pratylenchus* spp. | Patches, chlorosis of lower leaves, stunting, fewer tillers, and delayed plant growth | Wheat, grasses | Eggs, nematodes |

blight, leaf-spotting diseases, including more recently, wheat blast (in South America and Bangladesh) have become significantly important in recent years. High diversity for race-specific and quantitative resistance is well known for most diseases. Selection through field phenotyping coupled with complementing molecular tools/strategies can offer great promise in achieving durable resistance and enhancing wheat production and productivity. Disease resistance remains a core trait for several plant-breeding programs, and a complete package of high yield, disease resistance, agronomic performance and end-use quality is most preferred in varieties that are released globally.

### 2.5.1 Types of Resistance

There are two main ways to control diseases in wheat viz. incorporating genetic resistance through breeding and chemical control using fungicides. Genetic control has advantages for environmental and economic reasons, particularly for resource poor farmers in the developing world and the possibility that rust pathogens develop resistance to fungicides (Carmona et al. 2020). Genetic resistance deployed by wheat breeders belong to two general classes of genes based on their phenotypic effects, pathogen race-or strain-specific resistance (R-genes) and adult plant resistance (APR) genes. R-genes mostly function at all growth stages whereas APR genes function mainly at the adult stage. Wheat rust resistance genes of both R and APR classes are designated *Lr*, *Sr*, and *Yr* for leaf, stem, and stripe or YR resistance, respectively.

#### 2.5.1.1 Race-Specific/Seedling Resistance

Race specific, or seedling resistance/all stage resistance/qualitative resistance is effective at all growth stages and belongs to the "R-gene" class (Ellis et al. 2014). R-genes are perceived to confer a major resistance effect/complete resistance, However majority of the R-genes conferring rust resistance do not confer clean phenotype (McIntosh et al. 1996) and some are influenced by varying temperature and light regimes (Chen et al. 2015; Chen 2013; Forsyth 1956). The ease of selecting these genes at both seedling and field stages has made it easier to incorporate such resistance in several wheat breeding programs. However, deployment of single R-genes has often resulted in pathogen acquiring virulence post deployment as varieties in a short period leading to "boom and bust cycles" e.g. widespread virulence for *Yr9* and *Yr27* genes (Hovmøller et al. 2008), virulence for *Sr31* gene and other important SR genes *Sr24*, *Srtmp* to the Ug99 race group (Jin et al. 2008; Patpour et al. 2016; Pretorius et al. 2000) and ineffectiveness of LR resistance genes in the United States (Kolmer and Hughes 2015). However, deployment of genes in combination often referred as "pyramiding" can effectively enhance durability of resistance and keep pathogen populations under check.

#### 2.5.1.2 Adult-Plant Resistance (APR) Genes Conferring Pleiotropic Effects

Race-nonspecific resistance often referred as adult plant resistance or partial resistance is effective against wider races of a pathogen species and/or effective against broad range of pathogens. APR is generally quantitative, exhibiting incomplete resistance that is usually expressed at later stages of plant development. These genes help slow the disease progress through increased latency period, reduced infection frequency, reduced pustule size and thus resulting in lower spore

production. The phenotypic effects of such genes is relatively minor or inadequate when alone, however, additive effects of such minor APR genes (4–5) in combinations (Knott 1988; Singh et al. 2004b, 2015) can result in enhanced levels of resistance.

*Lr34* was first reported in cultivar “Frontana” (Dyck et al. 1966), although it has been a part of wheat improvement since the early 20th century. Wheat cultivars containing *Lr34* are widely present and occupy more than 25 million ha in developing countries and is effective in reducing yield losses in epidemic years (Marasas et al. 2003). The *Lr34* gene has remained durable as virulence for this gene has not been observed for more than 60 years. *Lr34* is located on the short arm of chromosome 7D (Dyck 1987). This gene confers modest levels of resistance and has pleiotropic effects on resistance to multiple diseases such as YR, SR, powdery mildew, barley-yellow dwarf virus and spot blotch (*Lr34*/*Yr18*, *Sr57*, *Pm38*, *Bdv1* and *Sb1*), respectively (Krattinger et al. 2009b; Lagudah et al. 2006, 2009; Lillemo et al. 2007). *Lr34* is associated with a morphological marker expressed as leaf tip necrosis (LTN) on the flag leaves, which can be used as a phenotypic marker (Singh 1992b). *Lr34* was cloned and the gene encodes a full-size ATP-binding cassette (ABC) transporter (Krattinger et al. 2009b). Based on the knowledge of the *Lr34/Yr18* gene sequence, gene-specific markers were developed and have proven to be highly diagnostic for the *Lr34* gene (Lagudah et al. 2009).

*Lr46* was first described in 1998 in cultivar “Pavon 76” (Singh et al. 1998) and is located on chromosome 1BL. The latency period of infected adult plants carrying *Lr46* was significantly lower compared to the controls without the gene (Martínez et al. 2001). The resistance type conferred by *Lr46* is similar to that of *Lr34*, although smaller in effect and is also known to confer partial APR to YR, SR, powdery mildew with corresponding designations *Yr29*, *Sr58* and *Pm39*, respectively (Singh et al. 2015; Bhavani et al. 2019). *Lr46* is also associated withLTN and is very common in both old and new wheat varieties including durum wheat (Lan et al. 2017a).

The *Lr67* gene was identified in the common wheat accession “PI250413” (Dyck and Samborski 1979) and transferred into “Thatcher” to produce the isoline “RL6077” (Thatcher*6/PI250413). *Lr67* shows similar pleiotropic effect as *Lr34* due to the association with resistance to SR (Dyck et al. 1994) and YR (Singh 1992a). However, *Lr67* confers a lower level of LR resistance than that conferred by *Lr34* (Hiebert et al. 2010). It was earlier assumed that the gene in RL6077 could be *Lr34* translocated from chromosome 7D to a different chromosomal location, however later studies showed that *Lr34* is not present in RL6077 (Lagudah et al. 2009). Recent studies mapped *Lr67/Yr46/Pm46* on chromosome arm 4DL (Hiebert et al. 2010; Herrera-Foessel et al. 2014). Cloning elucidated that *Lr67* gene encodes a hexose transporter (Moore et al. 2015). *Lr68* is another APR gene located on chromosome arm 7BL, that confers slow rusting resistance to wheat LR (Herrera-Foessel et al. 2012). This gene was first described in CIMMYT’s spring bread wheat “Parula” (Pedigree: FKN/3/2*Frontana//Kenya 350 AD.9C.2/Gabo 55/4/Bluebird/Chanate). Parula was developed by CIMMYT in 1981 and is also known to carry *Lr34* and *Lr46* (William et al. 2007) and likely origin of *Lr68* is the

Brazilian cultivar "Frontana" (Herrera-Foessel et al. 2012). *Lr68* showed a weaker effect than *Lr34, Lr46* and *Lr67* but combined effect of *Lr34*, *Lr46* and *Lr68* in Parula resulted in near immunity (Lillemo et al. 2011; Herrera-Foessel et al. 2012), however its effect on other diseases could not be determined.

Stem rust gene *Sr2* is one of the most important and widely used gene, confers modest levels of resistance and has been effective until date (over 100 years) even to the more recent and virulent Ug99 and Digalu race groups of SR in East Africa. This gene was transferred from "Hope" and "H-44" into common cultivars (McFadden 1930) and is derived from a tetraploid "Yaroslav" emmer. The *Sr2* gene is located on chromosome arm 3BS. This gene was widely used by Dr. N. E. Borlaug when he initiated wheat breeding in 1944 in Mexico, which resulted in varieties such as "Yaqui 50" and several high yielding semi dwarf varieties that were deployed in different wheat programs (Singh et al. 2015). The *Sr2* gene shows pleiotropic effects with YR resistance gene *Yr30* that also confers moderate resistance. *Sr2* gene is also associated with a morphological marker called pseudo-black chaff (PBC) that is expressed as purplish pigmentation on the glumes, internodes and peduncles under favorable conditions. Efforts to combine *Sr2* with other minor effect genes to enhance SR resistance in breeding materials at CIMMYT has resulted in several resistant or moderately resistant varieties. Several new uncharacterized slow rusting genes, some potentially pleiotropic, have been identified in the recent years (Rosewarne et al. 2013; Li et al. 2014; Yu et al. 2014) suggesting diversity for APR QTL and their potential in breeding resistant varieties.

Other adult plant resistance genes reported to confer partial or slow rusting include *Lr74* (Kolmer et al. 2018b), *Lr75* (Singla et al. 2017), *Lr77* (Kolmer et al. 2018c), and *Lr78* (Kolmer et al. 2018a) for LR, *Yr11, Yr12, Yr13, Yr14, Yr16, Yr36, Yr39, Yr52, Yr59, Yr62, Yr68, Yr71, Yr75, Yr77, Yr78, Yr79, Yr80* and *Yr82* (Chen and Kang 2017; Feng et al. 2018; McIntosh et al. 2017; Nsabiyera et al. 2018; Pakeerathan et al. 2019) for YR and more recently *Sr56* identified in cultivar 'Arina' for SR (Bansal et al. 2014).

Currently, over 220 rust resistance genes viz. 79 LR resistance genes (Qureshi et al. 2018a), 82 YR resistance genes (Pakeerathan et al. 2019) and 72 SR genes (Chen et al. 2020; McIntosh et al. 2017) have been formally cataloged and designated of which majority of them confer race specific resistance and only a few genes confer slow rusting/partial adult plant resistance to the three rust diseases.

### *2.5.2 Enhancing Durable Rust Resistance in Wheat Breeding Germplasm at CIMMYT*

Wheat breeding at CIMMYT focuses on small-holder farmers across wheat growing regions of Asia, Africa and Latin America, and strongly emphasizes selecting high-yielding wheat germplasm that possesses good levels of rust resistance based on diverse combinations of multiple pleiotropic resistance genes and

other QTLs with significant progress made for all three rusts (Singh et al. 2015; Bhavani et al. 2019). Breeding for rust resistance has been a rigorous exercise owing to the continued evolution and selection of pathogen for new virulence to previously effective resistance genes largely through mutation or sexual recombination, or transboundary migration of races to new wheat production environments. In most developing countries, varieties with genetic resistance are preferred by farmers; therefore, resistance is a required trait for release. Even though several race-specific resistance genes have been identified only a handful of genes are used actively in breeding as several genes are only effective in certain environments and majority are easily overcome in few years of deployment, linkage drag associated with genes transferred from secondary and tertiary gene pools or originating from unadapted genetic backgrounds.

One of the best approaches to utilize these race-specific resistance genes is through pyramiding combinations of multiple effective genes in varieties. Molecular markers linked to some of the effective resistance genes have facilitated the selection for multiple resistance genes and releases of varieties that carry them. However, the lack of diagnostic markers to select genes in different genetic backgrounds leaves no option but to use field-based selections under artificial epidemics, which continues to be the most common practice in several breeding programs.

Other approach is to utilize quantitative APR in breeding, although the individual effects of pleiotropic APR genes and other QTLs are small or moderate in their effect when present alone; near-immune levels of resistance have been achieved by combining 4 to 5 of these genes that often have additive effects (Singh et al. 2008b, 2015). Incorporating such type of resistance has been found to enhance durability and significant progress was made for LR resistance, and more recently for resistance to Ug99 race group and stripe rust resistance in CIMMYT germplasm using a single back cross selected bulk scheme (Singh et al. 2015, 2016). Although breeding for APR is cumbersome initially, additive effect of multiple minor APR genes enables combinations of high disease resistance, which can be simultaneously selected together with high yields with appropriate agronomic traits and the frequency of these genes can be increased within the breeding germplasm. Comparison of grain yield performance of 697 EYT lines (Stage II) 2018–19 derived from Mexico Shuttle and Mexico Kenya Shuttle breeding schemes identified similar frequency of lines that combine high yield potential and SR resistance (Fig. 2.1) and significant progress has been achieved in combining yield potential and rust resistance in CIMMYT breeding lines.

One of the prerequisites for enhancing APR is the absence of epistatic race-specific resistance gene interactions in breeding materials, which enables selection of transgressive segregants with low disease severity under high disease pressure to select for combinations of APR based on their additive effects. The progress in breeding APR to the Ug99 race group was facilitated by extending shuttle breeding scheme and testing between field sites in Mexico and Njoro, Kenya. Combinations of diverse multiple minor genes based APR is especially important to curtail the evolution of new virulent races in most wheat growing environments. Majority of the race specific genes also condition intermediate

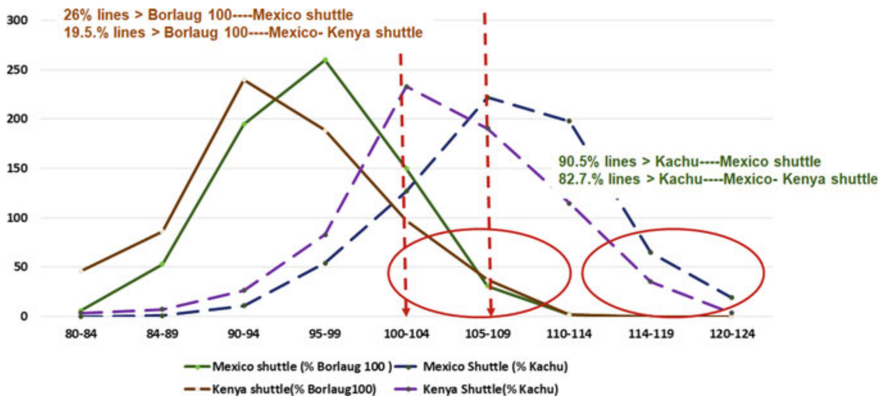



**Fig. 2.1** Performance of grain yield of 697 EYT lines (Stage II) 2018–19 derived from Mexico Shuttle and Mexico Kenya Shuttle breeding schemes

resistance phenotype and interactions of these moderately effective genes in good APR backgrounds have also enhanced the resistance to the rust diseases in CIMMYT germplasm. We have shown that the success in achieving high levels of complex APR to rusts in the CIMMYT high-yielding germplasm has enhanced resistance durability, provide excellent yield protection, and free up resources to focus on much needed, accelerated yield enhancement and make progress toward resistance to other diseases that are gaining importance. Despite significant progress, incursion and evolution of new races in East African region both for SR and YR has rendered some R-genes carrying varieties susceptible, favourable climatic conditions and incursion of new YR races into Europe, Asia and the America's has further compounded the biotic stress constraints in the region especially with dependence on R-genes based resistance (Hovmøller et al. 2008, 2016; Milus et al. 2009; Olivera Firpo et al. 2015; Singh et al. 2015; Ali et al. 2017; Lewis et al. 2018).

### 2.5.3 *Genetics and Breeding of Other Wheat Foliar and Root Diseases*

Many race-specific resistance genes against powdery mildew are deployed in wheat cultivars. Currently, 68 resistance genes conferring resistance to wheat powdery mildew are known (He et al. 2020). However, cultivars in general carry only one or very few resistance genes which results in selective pressure on the pathogen population to acquire virulence and therefore, resistances are of low durability (Parks et al. 2008; Shah et al. 2018). Several of these race-specific resistances have been easily overcome by simple genetic changes in the pathogen, e.g. *Pm8* and *Pm17* were overcome already in the 1980s. *Pm4a* was overcome in some areas of

China and *Pm21* was overcome after extensive use in Europe (Gao et al. 2012). Therefore, deployment of race-nonspecific pleotropic resistances, such as *Lr34 [Syn. = Yr18 = Sr57 = Pm38 = Sb1 = Bdv1 = Ltn1], Lr46 [Syn. = Yr29 = Sr58 = Pm39 = Ltn2]* and *Lr67[Syn. = Yr46 = Sr55 = Pm46 = Ltn3]*, which is also effective against powdery mildew in combination with other resistance genes, can prevent the emergence of new virulent races and enhance durability. In addition, more than 100 QTLs have been identified and can be employed in marker-based selection procedures (Keller et al. 1999; Marone et al. 2012; Asad et al. 2014). Genome editing technology in the recent years has shown great potential to surpass the bottlenecks of conventional resistance breeding. This technology offers the modification of specific target genes in elite varieties, thus bypassing the whole process of crossing. Recent advances in gene-editing technology can also offer avenues to building resistance durability. Genome editing was found to be effective in improving powdery mildew resistance by editing *Mlo* homologs in wheat to produce a triple knockout in hexaploid wheat (Wang et al. 2014c). As gene-editing technology develops, site-specific editing of alleles may become practical in the future.

FHB resistance is a typical quantitative trait, conditioned by numerous genes of minor effects. The complexity of FHB resistance also lies on the different resistance mechanisms, e.g. resistance to initial infection (Type I), resistance to fungal spread in the rachis (Type II), resistance to toxin accumulation (Type III), resistance to kernel infection (Type IV) and resistance/tolerance to yield reduction (Type V). Numerous sources of resistance were reported in literature but only a few have been successfully utilized in breeding programs, such as 'Sumai 3', 'Wuhan 1', 'Frontana' etc. (Buerstmayr et al. 2020). Other than these accessions, many wheat lines carrying so-called 'native' resistance have been reported and utilized in regional breeding programs (Brar et al. 2019). FHB resistance genes/QTL have been mapped on all the 21 wheat chromosomes, though, only seven QTL have formally been designated as Mendelized genes, of which only *Fhb1, Fhb2, Fhb4* and *Fhb5* are from common wheat, whereas *Fhb3, Fhb6* and *Fhb7* are from wild wheat relatives (Bai et al. 2018). So far, only *Fhb1* and *Fhb7* have been cloned, and their functional markers have been developed for marker-assisted selection (MAS) (Su et al. 2018; Wang et al. 2020).

Breeding for FHB resistance per se is not difficult and high level of resistance comparable to the famous resistant source 'Sumai 3' is achievable. However, in breeding practices, FHB resistance is often linked to unfavorable traits such as low yielding, late maturity and high stature (Buerstmayr et al. 2020) and it is often difficult to reconcile FHB resistance and other preferred traits. At CIMMYT, two breeding strategies for FHB are being used, i.e. exploitation of native resistance and introduction of exotic resistance. There is no strong FHB resistance available in the current CIMMYT gene pool, though some moderately resistant lines have been identified and a few QTL with major effects have been mapped. Among those lines are 'Shanghai3/Catbird', 'Mayoor', 'Soru#1', 'IAS20*5/H567.71' etc. It is noteworthy that a major QTL on chromosome 2DL has been consistently identified in the first three genotypes and haplotype analysis of a few FHB Screening Nurseries

of CIMMYT also demonstrated its high frequencies. Apart from this QTL, others are either of low frequencies or of minor effects but higher level of resistance can still be achieved via accumulating those QTL in elite breeding lines, similar to rust resistance breeding (Singh et al. 2016). The limitation of using native resistance is, however, a lack of QTL/gene with strong Type II resistance, which could be compensated via introduction of exotic FHB resistance genes like *Fhb1* and *Fhb7*. The former is the most well-known FHB resistance gene and has been extensively utilized in China, USA and Canada (Zhu et al. 2019), however, its repulsive linkage with the SR gene *Sr2* limited its application in the CIMMYT wheat breeding. To address this problem, several recombinant lines with both *Fhb1* and *Sr2* were introduced from Australia and included in various crosses with elite CIMMYT breeding lines. Many of the progenies exhibited good agronomic traits as well as promising resistance to FHB and other diseases (Xu et al. 2019).

Since no immunity to FHB has been found in wheat and high level of FHB resistance is difficult to achieve, other disease management strategies are also important in wheat production regions where FHB is a limiting factor. Removal of crop residue and rotation with non-host crops are helpful in reducing inoculum concentration. It is well known that maize-wheat rotation greatly increases the risk of FHB and thus should be avoided, otherwise integrated disease management including deep tillage, fungicide application and growing FHB resistant cultivars are recommended (McMullen et al. 2012).

Genetic resistance to wheat blast involves both qualitative and quantitative loci, with the former being reported under greenhouse experiments. Various major resistance genes viz. *Rmg2, Rmg 3, Rmg7, Rmg 8*, and *RmgGR119* are found to be effective against MoT, whereas, several other resistance genes viz. *Rmg1, Rmg4, Rmg5, Rmg6, and RmgTd(t)* are effective against non-MoT species (Ceresini et al. 2016; Kumar et al. 2020). Several avirulence (Avr) genes that interact with the host R genes have been detected in non-MoT species, viz. *PWT1* (MoO, Oryzae isolate), *PWT2* (MoS, Setaria isolate), *PWT3* and *PWT4* (MoA, Avena isolate). Loss of such Avr genes in non-MoT isolates enables them to become virulent to wheat, just as the case of *PWT3*, which was likely responsible for the emergence of WB in Brazil (Inoue et al. 2017). It is important to mention that of the five MoT resistance genes, *Rmg2, Rmg3*, and *Rmg7* have been overcome by new MoT isolates, whereas *Rmg8* and *RmgGR119* exhibited effective resistance in greenhouse but need to be validated in large scale field trials. New technology like Clustered regularly interspaced short palindromic repeats—CRISPR associated protein 9 (CRISPR-Cas9) can also be used in future to silence susceptibility genes for WB when identified, which has already been used in rice blast.

Apart from the *Rmg* genes, the 2NS/2AS translocation has been widely acknowledged as a stable and effective resistance source, although virulent isolates have emerged recently in South America (Ceresini et al. 2016). The translocation was introduced from *Ae. ventricosa* and has been widely utilized in wheat breeding due to its resistance against rusts (*Yr17, Lr37, Sr38*), nematodes (*Cre5, Rkn3*) and WB. Most well-known WB resistant lines have the 2NS/2AS translocation, e.g. ‘Milan’ and ‘Borlaug #100’ in the CIMMYT germplasm, ‘Sausal CIAT’, ‘CD 116’,

'Caninde #1' in South America, 'BARI Gom33' in Bangladesh, 'HD2967' and 'DBW189' in India (He et al. 2020). A recent GWAS involving 1,106 CIMMYT breeding lines identified only one stable QTL on 2NS/2AS, whereas the remaining QTL were of small effects and were detected in only some environments (Juliana et al. 2020). Similar results have been obtained in other germplasm pools too (Singh et al. unpublished data). This highlights the importance of identification and utilization of new WB resistance genes for breeding use, which could alleviate the selection pressure that is being applied to 2NS virulent isolates, to prolong the lifespan of 2NS varieties.

Conventional breeding programs of different countries have succeeded in identifying moderately resistant to resistant lines, but the bottle neck is the reliable WB screening. Early WB resistance breeding in South America depended heavily on natural infection, which was sporadic and unpredictable, with great variation in disease pressure. As for countries being threatened by WB but still do not have the disease (like India), or those have WB but do not have the screening capacity (like Zambia), the request for an international precision phenotyping platform (PPP) is very strong. In collaboration with its national partners, CIMMYT has established three WB PPPs, with one in Jashore, Bangladesh, and two in Bolivia (Quirusillas and Okinawa) to screen germplasm and advanced lines from across the globe (Singh et al. 2016). High quality phenotypic data have been produced from the three PPPs, which greatly facilitated the WB resistance breeding, germplasm screening as well as genetic studies (Juliana et al. 2020). In the early days of KB resistance breeding at CIMMYT, important genetic stocks used were 'Aldan/IAS58' from Brazil, 'Shanghai-7' from China, and native CIMMYT lines 'Roek//Maya/Nac', 'Star', 'Vee#7/Bow' and 'Weaver'. To date, screening programs have resulted in the identification of numerous resistant sources for bread wheat and durum wheat from various countries (Bishnoi et al. 2020). Resistant sources have been identified in primary to tertiary gene pools of wheat, durum and triticale, including numerous dipoid, tetraploid and hexaploid species, especially *T. urartu* (AA) and *Ae. tauschii* (DD) that have high degree of resistance.

Understanding the epidemiology and population dynamics of *T. indica* is important for its effective management. Boot emergence to anthesis is the optimum stage for a germinated teliospore to infect, however, an infection can happen as late as at late dough stage (Carris et al. 2006). Treating seed with chlorothalonil or mixture of carboxin and thiaram and foliar spray with propiconazole, triadimefon and carbendazim are the suggested chemical control measures. The natural populations of *T. indica* have high genetic diversity owing to the sexual recombination occurring between heterothallic fungi generating many recombinants. This results in diversity for virulence of KB strains as well as diversity in the wheat genotypes for resistant/susceptible reaction against the disease (Kumar et al. 2021). The use of a mixture of pathogen isolates as present in the population is advocated as it increases horizontal resistance in the population targeted for breeding resistance (Bishnoi et al. 2020).

Some morphological features have been frequently associated with KB resistance, including presence of pubescence, tight glumes, flat flag leaf angle, low

stomata but high hair counts on rachis, high spike compactness and/or narrow glume opening. These traits can be used in the phenotypic selection for KB resistance, but it should be noted that some of the associations might be dependent on genetic background and associated with disease escape rather than actual genetic resistance (Bishnoi et al. 2020).

Currently, it is widely accepted that genetic resistance against KB is governed by polygenes with quantitative inheritance, although gene for gene interaction may exist to some extent. Many genes with small additive effects acting in an additive and epistatic mode impart KB resistance. Since additive genes respond to selection, stacking additive genes along with an eye for significant epistatic gene interactions can enhance levels of KB resistance (Fuentes-Davila et al. 1995). In QTL mapping studies, as expected, majority of the identified QTL had minor effects and only a few major QTL have been identified on chromosomes 4B, 5B, 6B where the one on 4B associated with simple sequence repeat (SSR) marker *Xgwm538* was the largest one with phenotypic variation ($R^2$) of 25% (Singh et al. 2007). A GWAS study on 339 accessions from Afghanistan led to the identification of a consistent QTL on chromosome 2BL along with some other novel locations (Gupta et al. 2019).

Tan spot (*P. tritici-repentis)* is a necrotroph and follows inverse gene-for-gene relationship where recognition of host sensitivity gene by pathogen produced HST results in a compatible (susceptible) interaction, which is opposite to Flor's classical gene-for-gene model in biotrophic diseases such as mildews and rusts. High level of resistance has been found in several wheat genotypes although immunity is not reported (Faris et al. 2013). Host resistance in wheat against tan spot can be qualitative or quantitative and major/qualitative genes responsive to tan spot are designated as *'Tsr', 'Tsc',* or *'Tsn'* corresponding to identification of genes by phenotyping assay with only fungal conidial cultures, HST containing fungal culture infiltrates inducing chlorosis and HST fungal culture infiltrate inducing necrosis, respectively. Some of the most well-characterized genes are *Tsn1* (interacts with PtrToxA), *Tsc2* (interacts with PtrToxB), and *Tsc1* (interacts with PtrToxC) (Faris et al. 2013). *Tsn1* is the only cloned tan spot resistance gene, which is located on chromosome 5BL and harbors serine/threonine protein kinase (S/TPK), nucleotide binding (NB) and leucine-rich repeat (LRR) domains (Faris et al. 2010). Dominant functional marker *Xfcp623* and co-dominant markers, *Xfcp394* and *Xfcp620* can be used for marker-assisted selection of the resistant allele at *Tsn1* locus (Faris et al. 2010). *Tsc1* is located on chromosome 1A, being surrounded by markers *Xhbd152, XksuM182*, *XksuM104, Xgwm136* in the distal side and *XksuD14* in the proximal side. *Tsc2* is located on chromosome 2BS and a PCR-based diagnostic marker *XBE444541* is available for MAS. In addition to these three major genes, a recent meta-QTL study identified 19 QTL/loci for resistance to tan spot which can be utilized in wheat breeding programs (Liu et al. 2020).

Resistance breakdown is a major concern in R-genes conferring resistance to biotrophic pathogens as the pathogen *Avr* genes mutate rapidly. In case of tan spot resistance, if sensitivity genes are knocked-out of mutated, the pathogen cannot evolve as rapidly as biotrophs, so the resistance is more durable. Additionally, the fungus is saprophytic in nature and selection pressure on host would not be as high

as in mildews or rusts. Molecular markers associated with major loci conferring susceptibility or resistance are very useful to select for tan spot resistant cultivars. Stacking of multiple QTL (including race non-specific) for tan spot resistance is an important and desirable strategy to manage the disease.

Managing root diseases in the modern farming system is a difficult task due to their hidden nature and when compared to leaf diseases. A variety of management strategies have been studied to control root rots (Cook 2001). Better understanding of the pathogen biology is the first step to apply the best management strategy for targeted root rot disease. Sowing healthy and high-quality seeds at the correct depth and sowing time with adequate levels of nitrogen are main agronomy practices. As these pathogens have a wide range of host crop, rotation with non-host crops may help to reduce inoculum level in the soil (Cook et al. 2002). If there is a registered fungicide, its seed treatment may support stand establishment. ‘Green bridge’ must be broken off, since the volunteer plants or weeds helps the fungi/nematode to survive during offseason, and control of “green bridge” at least four weeks before the seeding can help to reduce the multiplication of the fungus and nematodes (Cook 2001; Dababat et al. 2014).

Using resistant crops of high yield potential is the most efficient and economical way to improve the productivity of the crop and manage root rot diseases, especially in dryland areas. The advantage of using a resistant variety is not only in terms of gained crop yields, but also in reducing inoculum for the next season. Tolerant varieties are also effective in reducing the yield losses; however, they may conduce inoculum build-up/increase in the soil. Wheat and its wild relatives have been screened for resistance against the soil-borne pathogens, and several *Cre* genes (*Cre1* to *Cre9, CreX, CreY*) against CCN have been identified, which are reported to follow gene for gene hypothesis. The presence of resistance in wheat progenitors has helped in synthesizing a few synthetic wheat derivatives resistant to a variety of root pathogens including CCN and RLN. International collaborative efforts, viz. distribution and utilization of CIMMYT’s International root disease resistance nurseries in the respective national breeding programs, is important to achieve desired resistance in locally adapted wheat varieties (Dababat et al. 2014).

In a nutshell, weakened plants are more vulnerable to infection by root rot fungi. Recognizing the disease by a grower is the most important point to handle the disease. Integrated disease management are likely to be effective for an extended period. Pathologists and breeders should work synergistically to identify resistant germplasm for specific pathogens and preferably sources with multiple diseases resistant.

## 2.6 Molecular Mapping of Resistance Genes and QTL

In classical breeding programs, selection process is based on the observable phenotypes of the candidate lines but not much knowledge about which genes are going to be selected for what trait. Whereas molecular plant breeding provides

breeder with the opportunity to improve existing cultivars and to develop new cultivars using MAS approach using advanced technologies (Moose and Mumm 2008). MAS involve use of molecular markers linked to specific trait of interest in crops (He et al. 2014b).

### 2.6.1 Molecular Markers

Molecular markers are the specific DNA sequences present at definite locations of the genome and are transferred from one generation to the other by law of inheritance. In contrast to the morphological markers (based on visible traits) and biochemical markers (based on proteins produced by genes), molecular markers are based on DNA assay (Choudhary et al. 2008). DNA markers have been used for the characterization of various traits in wheat over the past two decades (Hoisington et al. 2002).

First plant DNA markers were based on the restriction fragment detection that includes restriction fragment length polymorphism (RFLP) (Botstein et al. 1980). RFLPs were developed about 15 years ago and were used successfully for generating linkage maps of various species but are time consuming and have limited available probes (Bernatzky and Tanksley 1986). With advances in technology, development of PCR-based markers replaced RFLP markers (Collard et al. 2005; Hoisington et al. 2002). Among them, SSR (Litt and Luty 1989; Salimath et al. 1995) were highly useful as genetic markers due to their co-dominant, highly reproducible nature and huge abundance in the genome (Deschamps et al. 2012). Other PCR-based markers included random amplified polymorphic DNA (RAPD) (Williams et al. 1990), amplified fragment length polymorphism (AFLP) (Vos et al. 1995), sequence characterized amplified region (SCAR) (Paran and Michelmore 1993), cleaved amplified polymorphic sequences (CAPS) (Konieczny and Ausubel 1993), sequence tagged site (STS) (Schachermayr et al. 1994) and direct amplification of length polymorphism (DALP) (Desmarais et al. 1998).

With further advancements in the marker technologies, diversity array technology (DArT) and single nucleotide polymorphism (SNP) are considered as the new generation molecular markers and have become main genotyping platforms. DArT is a high-throughput technique with minimum DNA sample requirement that allows the identification of hundreds of markers over the genome in one experiment without any previous DNA sequence information (Jaccoud et al. 2001). SNPs are basically the single base differences in DNA among individuals with alternate nucleotides in a same position (Vignal et al. 2002). SNP loci are present in abundance in the genome which makes it more convenient to develop genetic maps of high density, required for identifying new genes for disease resistance and other valuable traits. By using more modern DNA microarray techniques, thousands of SNPs can be analyzed simultaneously and the analysis is much more effective than any other DNA analysis (Khlestkina and Salina 2006).

Despite some challenges, this high-throughput SNP genotyping platform has been used in wheat research using the Illumina GoldenGate assay (Akhunov et al. 2009). The access to NGS and expressed sequence tags (ESTs) led to the development of new high-throughput non-gel based genotyping methodology, the KBioscience KASP assay (Allen et al. 2011). KASP assay is fast in genotyping a huge set of genotypes for both alleles in a single reaction (He et al. 2014a). Several sequencing platforms have been discovered based on continuous advancement in high-throughput genomic technologies such as development of 90 K SNP iSelect assay by Illumina.

The genome sequence information from other crops has also provided opportunities for comparative mapping. *Brachypodium distachyon* has replaced rice as a model species due to its high level of collinearity and synteny to various cereal genomes (Yu et al. 2009). The reference sequence of wheat is now available on public domain (Mayer et al. 2014; Appels et al. 2018), thereby serving as an important genomic tool for genetic studies. Another method to sequence specific chromosomes using flow cytometry is becoming popular in allopolyploids due to availability of all the genomic resources adding a new perspective to marker development platforms (Doležel et al. 2012; Mourad et al. 2019a; Nsabiyera et al. 2020).

### 2.6.2 Mapping Populations

The mapping populations are assessed for variation for the target trait and are developed by crossing resistant and susceptible lines. The size of the population ranges between 50 and 250 lines across many studies depending on the target trait (Mohan et al. 1997). The populations that are used for mapping in case of self-pollinating crop species are $F_2$ (selfed $F_1$ progenies), single backcross (BC; derived from crossing $F_1$ hybrid to the recurrent parent), recombinant inbred lines (RILs; produced through selfing of filial generation $F_6$ or higher), doubled haploid (DH; produced through doubling of $F_1$ embryos of wheat/maize crosses), near isogenic line (NILs). $F_2$ and BC populations are quick to produce but have high level of heterozygosity for segregating loci. In contrast, RIL and DH populations consist of series of homozygous lines representing recombination events and parental types. For mapping, generally $F_6$ generation of RILs is considered good due to attaining high level of homozygosity. NILs are traditionally developed through backcross introgression method and can be used for validating a putative QTL where there are large genomic intervals associated with QTLs. NIL only differs from its parents in one genomic location, where there will be QTL.

### 2.6.3 Mapping Software

Several mapping software have been used to determine genomic locations of rust resistance genes in wheat such as Map manager QTXb20 (Manly et al. 2001), JoinMap by Kyazma B.V.software from Wageningen University (https://www.kyazma.nl/index.php/JoinMap/) (Van Ooijen 2006), MapDisto 2.0 (http://mapdisto.free.fr/) (Heffelfinger et al. 2017), QTL Cartographer v2.5 (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm) (Wang et al. 2007), QTL IciMapping (http://www.isbreeding.net/) (Meng et al. 2015), MapChart (https://www.wur.nl/en/Research-Results/Research-Institutes/plantresearch/biometris/Software-Service/Download-MapChart.htm) (Voorrips 2002) and Pretzel, a tool to compare genetic and physical maps in wheat (http://plantinformatics.io) (Keeble-Gagnère et al. 2019).

### 2.6.4 Maps of Different Generations

Genetic linkage maps play vital role in any genomic and genetic studies and have been widely used for the identification of trait specific genetic locus. They provide exceptional framework for various studies including QTL localization, MAS and map-based cloning. The developments in the various DNA marker systems over the time has progressed construction of genetic maps in wheat. Efforts towards genetic mapping in wheat started late 1980s with RFLPs (Chao et al. 1989) but more systematic approach was followed during 1990 through coordination of ITMI. Following the development of microsatellites markers map in wheat (Röder et al. 1995, 1998), many other marker technologies have been developed and employed in molecular mapping (Liu et al. 2015) including integrated or composite maps involving more than one type of molecular markers given by (Somers et al. 2004) and International Triticeae Mapping Initiative (ITMI) maps by (Song et al. 2005). Later, the consensus maps were developed where several different maps were merged into single comprehensive such as map of synthetic W7984 (Syn) X OpataM85 doubled haploid (DH) population produced by (Sorrells et al. 2011). The wheat 9K SNP consensus genetic map based on seven mapping populations was reported by (Cavanagh et al. 2013) using 7504 SNPs. (Saintenac et al. 2013) validated available markers (9K Infinium SNP iSelect array, DArT, SSR and GBS markers) on reconstructed Synthetic X Opata DH population. The development of 90K Infinium SNP iSelect array by Illumina allowed mapping of 40,267 SNPs on combination of six hexaploid mapping populations (Wang et al. 2014b). A high density tetraploid consensus genetic map was also released using both wheat 9K and 90K Infinium arrays from 13 independent bi-parental mapping populations (Maccaferri et al. 2015).

Molecular markers have also been used in the construction of physical maps in wheat for developing a high-quality reference sequence for the wheat genome. These maps allow comparisons between genetic and physical distances of marker in

their respective chromosomes. Numerous methods such as deletion mapping (Endo and Gill 1996; Sears 1954), radiation-hybrid mapping (Balcárková et al. 2017; Kalavacharla et al. 2006), in silico (Parida et al. 2006) and bacterial artificial chromosome (BAC) based physical maps have been utilized in wheat. The International Wheat Genome Sequencing Consortium (IWGSC) along with the collaborators have successfully used BAC-based sequencing for the construction of physical maps of individual chromosomes in wheat for generating high quality whole genome sequence of wheat (Appels et al. 2018). These physical maps are providing vital information for improving various traits in wheat breeding programs as well as providing a significant step forward towards cloning of genes.

### *2.6.5 QTL Mapping*

Many genetic studies have showed that most of the important traits in cereals are inherited quantitatively which makes them difficult to detect within the genome. Now with the development of genetic linkage maps, it becomes easier to identify and characterize such quantitative trait loci (QTLs) in many species. QTL mapping is an approach for studying and dissecting quantitative traits that are of complex inheritance i.e. involving minor genes with additive effects and does not follow Mendelian inheritance (Lan et al. 2017b; Semagn et al. 2010). Various QTLs for different traits including disease resistance, grain yield, winter hardiness etc. have been described over the time (Börner et al. 2002). QTL analysis indicates the number of genetic factors involved and their effect in controlling quantitative resistance (Michelmore et al. 1991).

The primary objective of QTL analysis is to restrain quantitative trait loci to narrow down the chromosomal locations as often chromosomal QTL regions are large which may allow the transfer of other undesirable traits that are linked to the desired QTL in plant breeding. QTL mapping requires biparental mapping populations to detect association between a phenotype and a genetic marker. It involves three steps: (i) accessing the phenotypic data across various environments (ii) construction of linkage maps consisting of genetic markers and (iii) to estimate the loci effect affecting the targeted trait using statistical analysis. The linkage maps can be constructed using various platforms i.e. MapMaker (Lander et al. 1987), JoinMap (Van Ooijen 2006) or using the R package ASMap (Taylor and Butler 2017).

QTL analysis can be carried out using different statistical methods to detect associations between phenotypic data and genetic markers. Single marker analysis (SMA) (Soller et al. 1976) based on analysis of variance (ANOVA) was the first simplest method of QTL mapping. Later on, more powerful method for detecting QTL was developed based on maximum likelihood or regression known as interval mapping (IM) or single interval mapping (SIM) (Lander and Botstein 1989). Logarithm or likelihood of odd (LOD) rule was proposed by Lander and Botstein (1989) for providing confidence intervals for QTL positions. SIM is based on the

single QTL model and it can be biased in the presence of multiple QTLs (Haley and Knott 1992). To overcome this problem of mapping multiple QTLs, Zeng (1994) proposed a composite interval mapping (CIM) method where SIM was combined with multiple marker regression analysis having control over QTL effects at various genomic regions independently. CIM remains a method a choice for QTL mapping since more than a decade due to its advantages over other methods for mapping single QTLs significantly but the algorithm used in this method cannot ensure epistatic effect of trait on QTL (Li et al. 2007). In order to map multiple QTLs and to be able to study epistatic effect of QTL, multiple intervals needed to be performed simultaneously (Zeng et al. 1999) and that led to the development of multiple interval mapping (MIM) (Kao and Zeng 1997). Later on inclusive composite interval mapping (ICIM) was developed by (Wang 2009) having all the benefits of CIM and MIM without having any increased sampling variance and background marker selection process (Meng et al. 2015). QTL Cartographer v2.5 (Wang et al. 2007), QTL IciMapping (Meng et al. 2015), R/qtl (Broman et al. 2003) and plabqtl (Utz and Melchinger 1996) have been the most used programs for conducting QTL mapping due to their free access.

The result of the QTL analysis is usually calculated using a test statistic score on linkage map called as logarithms of odd (LOD). LOD signifies the likelihood of the evidence for the presence of a QTL, with higher the LOD score, greater would be the evidence that QTL is real. Various computer simulations estimated the minimum LOD threshold of 3.0 to be considered significant in most cases (Lander and Botstein 1989). To further establish the significant LOD score thresholds in a given analysis, a permutation test is run by repeating the original data analysis e.g. up to 500 or 1,000 times by shuffling the phenotypic data across the genome while leaving the genetic data unchanged to assess any false marker-trait associations (Churchill and Doerge 1994).

Once the QTL is identified, it is characterized as major QTL i.e. environmentally stable or minor QTL i.e. environment sensitive on the basis of its phenotypic variation ($R^2$). If a QTL accounts for >10% of a phenotypic variance, it is described as major QTL and if it is less than 10%, it is called as minor QTL (Collard et al. 2005). QTLs can also be described in terms of their significance to ensure that no QTL is missed and to decrease background effects (Lander and Kruglyak 1995).

Up to now, numerious QTLs have been reported for rust resistance in wheat. There have been reports for more than 300 and 200 QTLs for stripe rust and LR resistance, respectively (Wang and Chen 2017; Da Silva et al. 2018). Mendalization and detailed characterization of these QTLs is an on going process for formal naming and development of linked markers for their use in marker-assisted selection. Bansal et al. (2008) reported two major APR QTLs; *QSr.Sun-5BL* and *QSr.Sun-7DS* explaining 12% and 26% of phenotypic variation, respectively in Arina/Forno RIL population against SR along with some minor QTLs on chromosome 1AS and 7BL. Later on, a QTL found on chromosome 5BL, *QSr.Sun-5BL* was mendalized and mapped using Arina/Yitpi RIL population and the locus was permanently named as *Sr56* (Bansal et al. 2014).

### 2.6.6 Marker-Assisted Breeding for Resistance Traits

Transfer and introgression of resistance genes in wheat cultivars is often limited by the practical restrictions of selection methods in conventional breeding selection programs (Bariana 2003; Bariana et al. 2007; Ellis et al. 2014). This limitation was initially overcome by a thorough knowledge of host genetics and pathogen variation, a relatively tedious process for breeding programs, also assisted by the use of morphological markers that were indicative of resistance genes and later the development of DNA markers has facilitated selection of resistance genes known as MAS. MAS provides breeders with the opportunity to select combinations of resistance genes and once the DNA is isolated, markers linked with any other trait can also be used to increase selection efficiency (Weeden et al. 1994; Ribaut and Hoisington 1998; He et al. 2014b). Several approaches are currently being used for application of DNA markers in selection procedures such as markers-assisted backcrossing (MABC) and marker-assisted gene pyramiding (MAGP).

Backcrossing method has always been used in wheat breeding to transfer rust resistance genes from a donor plant into an elite cultivar to capture recurrent parental background, but it is a slow process. MABC has accelerated transfer of rust resistance genes and resulted in rapid recovery of recurrent parental genome in as short as 2–3 backcross generations (Ribaut et al. 2002). MAGP is a process where several genes can be combined into a single genotype. The MAGP provides breeders an efficient method to select multiple traits simultaneously in their breeding programs. It has been used for pyramiding of multiple disease resistance genes and/or along with other traits in wheat. Marker linked with many rust resistance genes have been published in the last decade and are being used for MAGP and MABC of rust resistance genes in the breeding programs for pyramiding of these genes in various combinations. Several breeder-friendly markers linked to rust resistance genes are currently available to the wheat breeding programs such as *Yr4* (Bansal et al. 2010), *Yr15* (Ramirez-Gonzalez et al. 2015),*Yr51* (Randhawa et al. 2014), *Lr23* (Chhetri et al. 2017), *Lr48* (Nsabiyera et al. 2016), *Lr49* (Nsabiyera et al. 2020), *Sr2* (Mago et al. 2011), *Sr26* (Qureshi et al. 2018c; Zhang et al. 2019b), *Yr34/Yr48* (Qureshi et al. 2018b), *Lr34/Yr18/Sr57/Pm38* (Lagudah et al. 2009), *Lr46/Yr29/Sr58/Pm39* (ES Lagudah unpublished), *Lr67/Yr46/Sr55/Pm46* (Moore et al. 2015), *Yr47/Lr52* (Qureshi et al. 2017a), *Lr24/Sr24* (Bariana et al. 2016) and many more are routinely used in the breeding programs for pyramiding of these genes in various combinations.

Use of molecular markers in selection of rust resistance genes offers various advantages in wheat breeding programs. As compared to the conventional breeding strategies, MAS can increase selection efficiency through enrichment of positive alleles in early generations of breeding (Ribaut and Hoisington 1998; Collard et al. 2005) allowing breeders to conduct series of selections in one year. The success heavily relies on breeder-friendly markers. MAS, especially for disease resistance is independent of time, environment and plant developmental stage making it much

more feasible. Some of the breeder friendly KASP markers linked with rust resistance genes in wheat are listed in Table 2.3.

But the value and use of such markers in MAS, MABC and MAGP heavily depends upon the degree of linkage between markers and the target gene. The process of validation of a marker across number of genotypes is always required to access the reliability of that marker. In some cases, even the reliable marker cannot be diagnostic because of varying level of polymorphisms in different genetic backgrounds.

### *2.6.7 Map-Based Cloning of Resistance Genes*

The developments in the NGS technologies and the availability of sequenced and assembled genome have greatly improved marker development closely linked with the targeted genes in wheat, a plant species with large, complex and polyploid genome (Appels et al. 2018; Poland et al. 2012a). Many molecular markers linked to rust resistance genes in wheat have been developed but they must be diagnostic and proven to be efficient for their use in marker-assisted breeding. Cloning of the targeted gene(s) allows development of diagnostic molecular markers by isolating the resistance genes from the plants. Map-based cloning, also called as positional cloning is one of the traditional gene cloning methods to clone targeted genes without having any prior knowledge of the gene product. Map-based cloning works best for the targeted genes in plants where phenotypes are easily identified such as disease resistance. It requires various steps to enables us to narrow down to the shortest possible genetic interval of targeted gene (fine genetic mapping) and then to identify the candidate genes within corresponding interval on the DNA sequence (physical mapping) (Salvi and Tuberosa 2005). For map-based cloning, a high-resolution fine mapping population is a pre-requisite, which is used for phenotypic scoring and is genotyped with molecular markers developed using various genomic resources that leads to the construction of precise genetic map signifying targeted gene position (Krattinger et al. 2009a; Bettgenhaeuser and Krattinger 2019).

Positional cloning requires large-insert genomic DNA libraries and the vectors and cloning systems associated with the construction of these libraries are improving with time. The yeast artificial chromosome (YAC) cloning system was the first one to be developed by (Burke et al. 1987) to clone larger DNA fragments of up to 1 Mb. But there are several disadvantages of YAC cloning system such as high level of chimerism (∼40% of whole library) and instability in the yeast host strain that limited its use (Shi et al. 2011; Umehara et al. 1995). In order to overcome these disadvantages, various bacterial mediated cloning systems such as BAC (Shizuya et al. 1992), transformation-competent artificial chromosome (TAC) (Liu et al. 1999) and P1-derived artificial chromosome (PAC) (Loannou et al. 1994) have been developed. BAC vectors were developed to clone DNA sequences in bacterial cells. The BAC system has been widely used and being

**Table 2.3** List of breeder friendly KASP markers linked with rust resistance genes in wheat

| Rust resistant genes | Chromosome | Linked marker | Primer sequences | | | Reference |
|---|---|---|---|---|---|---|
| | | | Allele 1[a] | Allele 2[a] | Common | |
| *Lr13* | 2BS | *Lrseq 302-1* | GAAGGTGACCAAGTTCATGCTGTG | GTGTAATTATTGGGCTCATCACA | CGCTCAAGTTGAAGGTTGAGTGCAA | Qiu et al. (2020) |
| *Lr14a* | 7B | *uwb14* | CTACACTAGTACTACTTTGAGACAATTTTTT | ACACTAGTACTACTTTGAGACAATTTTAA | AACAAACTCCAGTGTAAACACCACAGTTT | Rasheed et al. (2016) |
| *Lr16/Sr23* | 2BS | *2BS-5194460_kwm747* | AATAGCTGGAGGAAAGCTTATCGG | GAATAGCTGGAGGAAAGCTTATCGA | CTTCTTGGTGCACAATGCTTTGAAATGAT | Kassa et al. (2017) |
| *Lr16/Sr23* | 2BS | *2BS-5192454_kwm677* | GTGTGCACCCCAATCCCTGC | AAGTGTGCACCCCAATCCCTGT | GAGTGTTAAGTCGCTTCCAACTTAGATAT | Kassa et al. (2017) |
| *Lr16/Sr23* | 2BS | *2BS-5175914_kwm847* | GTAACCACGGTGAAGCTGGCG | GTAACCACGGTGAAGCTGGCA | TTGTTGTGCCGCCAGCCTCCAT | Kassa et al. (2017) |
| *Lr16/Sr23* | 2BS | *2BS-5175914_kwm849* | TGGCTTCGCGATGTCCACA | GGCTTCGCGATGTCCACG | AGCGCCAAGTAGGCCATGCAGA | Kassa et al. (2017) |
| *Lr21* | 1DS | *Lr21_GQ504819_1346* | CCTTGTTTATTATTTACACTTTAATACTATTTC | CCTTGTTTATTATTTACACTTTAATACTATTTT | CAAATGGGTATGTCTGTCACATGTCTA | Neelam et al. (2013) |
| *Lr22a* | 2DS | *Lr22a* | GCTTGCGGCACATTATAATGGG | GCTTGCGGCACATTATAATGGT | CACAATCCATCAAAAGGCTCCA | Thind et al. (2017) |
| *Lr24/Sr24* | 3DL | *KASP_16434* | CAACATGCCATTACATCTGCCACAT | AACATGCCATTACATCTGCCACAG | CTTGAGACTGAAAGCCGTTAGTCCTT | Bariana et al. (2016) |
| *Lr23-Hexaploid* | 2BS | *sunKASP_16* | CGGTCCGGTGTAATACTTTCG | GACGGTCCGGTGTAATACTTTCT | TACATGGGCCGAGCTAGAAC | Chhetri et al. (2017) |
| *Lr23-Hexaploid* | 2BS | *sunKASP_47* | GAACTCCAGGCAAGCGAAT | GAACTCCAGGCAAGCGAAC | TCATATATAAACTGATCGCACGTAA | Chhetri et al. (2017) |
| *Lr23-Hexaploid* | 2BS | *sunKASP_48* | CCGAGCTAGAACAATGAAAACA | CCGAGCTAGAACAATGAAAACC | GGTGACGGTCCGGTGTAATA | Chhetri et al. (2017) |
| *Lr23-Tetraploid* | 2BS | *KASP_69462* | TTTGCTCAGAAAATAACATCAACG | GTTTGCTCAGAAAATAACATCAACA | GGCAGCAAATTATGATATAGGC | Chhetri et al. (2017) |
| *Lr46/Yr29* | 1BL | *ucw.k31* | ACAGCCTAAATTAGGGAGCGG | ACAGCCTAAATTAGGGAGCGA | AATTTGCGAAGGGGTCTGTTT | Cobo et al. (2019) |
| *Lr46/Yr29* | 1BL | *ucw.k34* | ACTGTGGCCTACTAAGTGGTTA | ACTGTGGCCTACTAAGTGGTTT | GTGCTTGTTGTTCGAATATCTAACA | Cobo et al. (2019) |
| *Lr46/Yr29* | 1BL | *ucw.k18* | CCTCCCATAGCACACGCCT | CCTCCCATAGCACACGCCC | TGAGAAAGAACGATAACCATTGC | Cobo et al. (2019) |
| *Lr46/Yr29* | 1BL | *ucw.k23* | GCAGGATGAAGGGCGACTCA | GCAGGATGAAGGGCGACTCG | GAGGTGAAGAGCGTGAGGAG | Cobo et al. (2019) |
| *Lr47* | 7AS | *Lr47-1* | GCAGCCTGGTAAGTTATCTGAC | GCAGCCTGGTAAGTTATCTGAG | GCCTGGATTCAAGAGAACAT | Rasheed et al. (2016) |
| *Lr48* | 2BS | *IWB70147* | AATCGCCCTACACCCTTATGTACT | CGCCCTACACCCTTATGTACC | TACCAATCTAGAGTAACATTACCGCCAAA | Nsabiyera et al. (2016) |
| *Lr49* | 4BL | *sunKASP_21* | GATTCGAATGTTTTTGTAGGATTTC | TTCAGATCTAAAATCACGGCACT | CTATTAACGTAGAGCCCAGTGC | Nsabiyera et al. (2020) |
| *Lr61* | 6BS | *sunKASP_60* | CACTGAAGCTTGCCGGAT | CACGAAGCTTGCCGGAC | GGATGATATCGTCGGGTAGG | Qureshi et al. (2017b) |
| *Lr67/Yr46* | 4DL | *TM4_67* | TCATCATCGGCAGGATCCTGCTTG | TCATCATCGGCAGGATCCTGCTTC | AACGTACGTAATCTTGCTTACTGA | Moore et al. (2015) |
| *Lr67/Yr46* | 4DL | *TM10_67* | GTAGGTGCACACCATGACCACCAA | GTAGGTGCACACCATGACCACCAC | CGGCGCGCGCTGCTGCTGGAGGCT | Moore et al. (2015) |
| *Lr67/Yr46* | 4DL | *csSNP856* | GCTACTACTATTGGTAGCCTG | GCTACTACTATTGGTAGCCTA | CCAGTAGCTTATGGCACTCAAA | Forrest et al. (2014) |
| *Lr68* | 7BL | *Lr68-2* | CGTGTCTTGGACCTGAGCAAT | CGTGTCTTGGACCTGAGCAAC | TGACCTGAGTCCCGTCAAGA | Rasheed et al. (2016) |
| *Lr77* | 3BL | *IWB73555* | AGATTTTCACCTCTCGCACA | AGATTTTCACCTCTCGCACG | ACCTGTTCTGCTCAACATGT | Kolmer et al. (2018c) |
| *Lr77* | 3BL | *IWB32805* | TCCCAACACCAAGGACAGA | TCCCAACACCAAGGACAGG | GCAACACCGACATCGTGGTA | Kolmer et al. (2018c) |
| *Lr77* | 3BL | *IWB10344* | GTAGCAACATATGGTGAATCATCAT | GTAGCAACATATGGTGAATCATCAG | AATCATGCTCAAACATAAGGCATA | Kolmer et al. (2018c) |
| *Lr78* | 5DS | *IWA6289* | TCTGATTCTCTCTACCACAAGGAT | TCTGATTCTCTCTACCACAAGGAC | CTTGTGTCTTGGCGCAACTG | Kolmer et al. (2018a) |

(continued)

**Table 2.3** (continued)

| Rust resistant genes | Chromosome | Linked marker | Primer sequences: Allele 1[a] | Allele 2[a] | Common | Reference |
|---|---|---|---|---|---|---|
| *Lr79* | 3BL | *sunKASP_256* | TGTAGATCACCATTTGGAGGG | GTTGTAGATCACCATTTGGAGGA | AGGACTTGCATGAAATCACCC | Bansal and Bariana (Personal comm.) |
| *Sr2* | 3BS | *wMAS000005* | GTGCGAGACATCCAACACTCAC | GTGCGAGACATCCAACACTCAT | CTCAAATGGTCGAGCACAAGCTCTA | Toth et al. (2019) |
| *Sr6* | 2D | *IWB26013* | ACAGCAGCAGAACCATCTTCT | ACAGCAGCAGAACCATCTTCC | AGAAGCACGGAGGCTTGTC | Edae et al. (2018) |
| *Sr7a* | 4A | *IAAV3545* | CTGAAGACATCGCCAGAAATG | CTGAAGACATCGCCAGAAATA | TCGGGAAACTCAGCAACCTC | Edae et al. (2018) |
| *Sr8a* | 6AS | *kwh55* | GCCAGTCGGTAAAGCGGCGTT | CCAGTCGGTAAAGCGGCGTC | AGCTCCTGGTACCGGTACCACT | Hiebert et al. (2017) |
| *Sr9b* | 2BL | *IWB28807* | GGCTATAAGAGATGTGAAGTCA | GGCTATAAGAGATGTGAAGTCG | ACCAGTTGTATTCTGTTTTGCAG | Edae et al. (2018) |
| *Sr11* | 6BL | *KASP_6BL_IWB10724* | ATGTAAATGTTGAGATACCTTAGCTGAAAT | GTAAATGTTGAGATACCTTAGCTGAAAC | GGAAAACCGTCATCTCGCGTATGTA | Nirmala et al. (2016) |
| *Sr13* | 6A | 6A | CACAAAACCTTTGTTCTCTAACTATGT | CACAAAACCTTTGTTCTCTAACTATGC | GCGGAGAAGTGCACCATGTA | Zhang et al. (2017a) |
| *Sr15/Lr20* | 7AL | *IWB30995* | TCTACACCTACATGGGAGAACA | TCTACACCTACATGGGAGAACG | TCCAGAGATCGGGTGGCC | Gao et al. (2019) |
| *Sr25* | 7D/7A | *CAP7_c2912*_1387634 | AACCGGTTACAAAGCCAAATCCAGA | CCGGTTACAAAGCCAAATCCAGG | ACTAGTGCTTGGTTTACCAATGTTCCTA | Yu et al. (2017) |
| *Sr26* | 6AL | *sunKASP*_224 | GAGCAGATGAGGAAAAGAGGC | GAGCAGATGAGGAAAAGAGGA | CTTCCGCCCTGTGTATTTCG | Qureshi et al. (2018c) |
| *Sr26* | 6AL | *sunKASP*_225 | CCAAGAATCACACACCATAGGTG | CCAAGAATCACACACCATAGGAT | CCCTACAACTGCACCGATGT | Qureshi et al. (2018c) |
| *Sr33* | 1DS | *Sr33* | GAACCTCTCCGAGTTAATCCTCC | GAACCTCTCCGAGTTAATCCTCA | ATTTCCACGTCCTCCTGCTG | Periyannan et al. (2013) |
| *Sr35* | 3A | *Sr35* | TGCTTTTGTCTCGGTTTCGCA | TGCTTTTGTCTCGGTTTCGCG | TCGTCTATGAGATACTTTTCGTCCC | Saintenac et al. (2013) |
| *Sr36* | 2BS | *Sr36/Pm6_8068* | CATTGTCATTCTCATCATTATACGCATCA | GTCATTCTCATCATTATACGCATCG | AAAGGGCAGTGCTTATGACGACGAT | Rasheed et al. (2016) |
| *Sr45* | 1DS | *Sr45* | GATAAGCATCTCGGCGAG | GATAAGCATCTCGGCGTT | GGAACTCTGCGAACTTGAGA | Toth et al. (2019) |
| *Yr5* | 2BL | *Yr5* | GCGCCCCTTTTCGAAAAAATA | CTAGCATCAAACAAGCTAAATA | ATGTCGAAATATTGCATAACATGG | Marchal et al. (2018) |
| *Yr7* | 2BL | *Yr7*-A | TTAGTCCTGCCCCATAAGCG | TTAGTCCAGCCCCATAAGCC | CAGTGTTAAAACCAGGGAGGA | Marchal et al. (2018) |
| *Yr7* | 2BL | *Yr7*-D | GCTGGAAAGGCTTGACATCA | GCTGGAAAGGCTTGAGATCG | AATGGCGTGGTAAGGACAGA | Marchal et al. (2018) |
| *Yr15* | 1BS | R5 | AGTCAACTTGGATTACACTGAAGTT | AGTCAACTTGGATTACACTGAAGTC | AGATATCACACTGAACATACTGATGAG | Ramirez-Gonzalez et al. (2015) |
| *Yr15* | 1BS | R8 | CAGATCCCCGGTTCTCTCAAG | CAGATCCCCGGTTCTCTCAAA | CCCCCAAATGATCGAGAATA | Ramirez-Gonzalez et al. (2015) |
| *Yr17/Lr37/ Sr38* | 2AS (2NS) | *Yr17/Lr37/Sr38* | GGACGGCGTTTGCTCATGCTA | AGGACGGCGTTTGCTCATGCTG | AGCAGTATGTACACAAAA | Toth et al. (2019) |
| *Yr34/Yr48* | 5AL | *sunKASP*_109 | GGATGTAGTTTGTCACCAGCC | AGGATGTAGTTTGTCACCAGCA | GGATTAACATATTCCTCGAATGC | Qureshi et al. (2018b) |
| *Yr34/Yr48* | 5AL | *sunKASP*_112 | AGCGCGCTCTCTTAGCAG | AGCGCGCTCTCTTAGCAA | AAAGAGGTAATGTGCTGACCTG | Qureshi et al. (2018b) |
| *Yr36* | 5AL | *WKS* | CGATGCTTCTCTCAGAACGA | TTCGATGCTTCTTGTAGAACACA | GATTGGTTCTTGACGTATGTTTT | Fu et al. (2009) |
| *Yr57* | 3BS | *BS00062676* | TGCACGCGTGGAAGATCTA | CTTGCACGCGTGGAAGATCTG | GTTGCAGTCACTGTTACAGATGCGAT | Randhawa et al. (2015) |
| *Yr77* | 6DS | *IWA167* | GTTTTTAGTTTATAGATATATT | GTTTTTAGTTTATAGATATATG | CGCATTTCACTGATCACAAAG | McIntosh et al. (2017) |
| *Yr78* | 6BS | *IWA7257* | AGACCCTACGACGTTAGCGA | AGACCCTACGACGTTAGCGC | ATTGGAATCAGCTGGGTCAT | ( |
| *Yr80* | 3BL | *3B-53113* | TGTACAATGACTCCTCGACTAACA | TGTACAATGACTCCTCGACTAACG | GCCACGCAATATCACCATCG | |

(continued)

**Table 2.3** (continued)

| Rust resistant genes | Chromosome | Linked marker | Primer sequences | | | Reference |
|---|---|---|---|---|---|---|
| | | | Allele 1[a] | Allele 2[a] | Common | |
| | | | | | | Nsabiyera et al. (2018) |
| *Yr81* | 6AS | *IWB3077* | ATTCCAAAGTAATTGGCAACAGGTTCA | CCAAAGTAATTGGCAACAGGTTCG | TGTGGAGCGTGACAATGAGGAAGTT | Gessese et al. (2019) |
| *Yr82* | 3BL | *sunKASP*_300 | CTCGATGTGTGAACAATCTTCACC | CCTCGATGTGTGAACAATCTTCACT | CATTCTCTATTGTAAAGCAGTGCCA | Pakeerathan et al. (2019) |
| *Yr82* | 3BL | *IWB8775* | GACATTGAGGAACCTGAAACCA | GACATTGAGGAACCTGAAACCG | CACCATGAATGCCAAAGGTCG | Pakeerathan et al. (2019) |
| *Yrsp* | 2BL | *YrSP* | GAGAAAATCAGCAGGTGG | GAGAAAATCAGCAGGTGC | AGCGAGTTGAGGACATTGGT | Marchal et al. (2018) |

[a]Allele 1 primer to be labelled with FAM: GAAGGTGACCAAGTTCATGCT; Allele 2 primer to be labelled with HEX: GAAGGTCGGAGTCAACGGATT

instrumental in constructing genomic libraries in plants due to its several advantages such as stability with foreign DNA, cloning inserts of up to 300 kb, relatively easier to purify the plasmid vector and insert DNA from the bacterial host DNA and having higher cloning efficiency (Monaco and Shizuya et al. 1992; Monaco and Larin 1994; Salimath and Bhattacharyya 1999; Ming et al. 2001). In wheat, due to the presence of three highly related homoeologous genomes, chromosome specific BAC library strategy has been successful in sequencing individual chromosomes (Šafář et al. 2004; Paux et al. 2008; Appels et al. 2018).

Once the genomic libraries have been constructed using these vectors, they can be used for chromosome walking or landing approaches. Chromosome walking strategy relies on identifying tightly linked markers to the targeted gene and then taking walking steps ( ~ 100–200 kb at a time) to get to the gene via a series of overlapping clones (Han and Korban 2010). These closet flanking markers are used to screen the BAC library and subclones of the identified BACs for positive clones which are then used to isolate insert-ends (Periyannan 2018). These insert-ends are used for screening additional overlapping clones until the contig spanning the target gene is established and the candidate gene is identified (Krattinger et al. 2009a). On the other hand, chromosome landing approach relies mainly on development of molecular markers that are either tightly linked or co-segregating with the targeted gene. In chromosome landing, the distance between the markers and the gene has to be smaller than the average insert length of a genomic library used for gene isolation (Tanksley et al. 1995). These markers are then used to screen the library and isolate the clone carrying the targeted gene (Han and Korban 2010). For successful cloning approaches, genetic complementation of the mutant phenotype with a wild type allele is required. *Agrobacterium*-mediated genetic transformation and biolistic transformation are the two widely used methods to introduce foreign targeted genes into plant cells (Xia et al. 2012).

## 2.7 Enabling Genomic Tools in Wheat Breeding

### *2.7.1 Association Mapping Studies*

The main objective of genetic mapping is to identify markers in close proximity of genetic factors affecting quantitative traits usually governed by QTL. Genetic mapping can be performed in two ways: (a) developing bi-parental populations, for "QTL-mapping" or "gene tagging" and (b) using diverse panel of lines called "genome-wide association mapping studies," or "association mapping (AM)" or "linkage disequilibrium (LD) mapping". The traditional QTL mapping approach is quite widely used however, it also has some limitations. First, allelic variation in each cross is limited to just two parents used to generate a QTL mapping population. Second, the number of recombination events per chromosome are small when segregating or double haploid populations are used. Third, a typical QTL

detected in a specific cross of few hundred inbred lines can range between a few to tens of centimorgan (cM) interval covering several million basepairs. Such large genome regions contain, typically, hundreds to thousands of genes, making gene identification in a QTL region a tedious exercise through map-based cloning (Price et al. 2006).

Association mapping has emerged as a powerful tool in determining the genetic basis of complex traits where large populations are analyzed to determine marker-trait associations using linkage disequilibrium. This approach has advantages over traditional QTL mapping. Firstly, a larger and more representative gene-pool can be examined. Second, overcomes the cost and time of developing mapping populations and facilitates mapping of several traits on one panel of genotypes. Third, a much finer resolution can be achieved, resulting in shorter confidence intervals of the mapped loci compared to conventional mapping, which also necessitates fine-mapping to develop diagnostic markers. Finally, in addition to identifying and mapping QTL, it helps to identify causal polymorphism within a gene that is responsible for the difference in two alternative phenotypes (Yu et al. 2013). However, AM also has challenges of false positives, especially if the experimental design and quality control is not rigorously implemented. For example, population structure has long been known to induce many false positives and accounting for population structure has become one of the main issues when implementing AM in plants (Breseghello et al. 2005). Also, with an increasing number of genetic markers independent validation of identified associations helps in discriminating false positives. With these limitations, AM still shows great promise in understanding the genetic basis of polygenic traits of agronomic importance.

To increase the power and mapping resolution of marker-trait associations, some specialized populations have been developed using a combination of both QTL mapping and AM. For example, NAM (Nested Association Mapping) populations and MAGIC (Multiparent Advanced Generation Inter Cross) populations have been developed in wheat and other crops (Kover et al. 2009; McMullen et al. 2009; Huang et al. 2012; Cavanagh et al. 2013). NAM populations are generated by crossing a set of diverse lines (5–25) to one reference line. F1's of each cross-are then selfed for multiple generations to develop RIL for each population. MAGIC populations on the other hand are developed by intercrossing for several generations among multiple founder (4–8) lines. Multiple founders are similar to NAM population, which enable capturing more allelic diversity than bi-parental populations, and repeated cycles of intercrossing give greater opportunity of recombination and hence greater precision of QTL mapping. However, generating such specialized populations entails effort, time and investment.

### 2.7.2 Genotyping/Marker Platforms for Genome-Wide Studies

Most commonly used markers in genome wide association studies include AFLP, DArT, SSR and SNP (Crossa et al. 2007; Honsdorf et al. 2010; Adhikari et al. 2012; Upadhyaya et al. 2013; Gupta et al. 2014). AFLP and DArT markers are easily accessible for all organisms even those lacking genomic data. Similarly, the highly polymorphic, multiallelic and co-dominant nature of SSR markers have made them highly suitable for AM studies in many crops including wheat (Peng et al. 2009; Yao et al. 2009; Liu et al. 2010; Reif et al. 2011; Zhang et al. 2011). However, AFLP and DArT markers being dominant can be challenging especially while estimating population structure or during mapping studies (Ritland 2005). Moreover, the three marker platforms (AFLP, DArT and SSR) are rather expensive and time- consuming technologies and the genomic coverage is also limited.

The rapid development of NGS technologies has allowed unprecedented genotyping capabilities, even for large complex polyploid genomes including wheat (Poland et al. 2012b). The current NGS technologies are capable of analyzing tens of millions of DNA molecules and allow the rapid identification of a large numbers of genetic markers, mainly SNPs (Single Nucleotide Polymorphisms) (Imelfort et al. 2009). SNPs are bi-allelic markers that's whythe information content per marker is much lower than SSR markers. This, however, is compensated for by a higher genome coverage. Therefore, SNP markers rapidly becoming the marker of choice for most AM studies. SNP markers are also amenable to high-throughput genotyping enabling options of multiplexing or microarray. Several SNP marker platforms have been established in wheat (Akhunov et al. 2009; Wang et al. 2014b) and genotyping of wheat association panels with up to 90,000 SNP markers is now available (Wang et al. 2014b). The potential of SNP markers in determining marker-trait associations is now being widely used across crops including wheat (Lopes et al. 2015).

With further developments in NGS technologies, sequencing today has extended to entire populations enabling simultaneous genome-wide detection (Elshire et al. 2011). This new approach, called "genotyping-by-sequencing" (GBS), uses data from the genotyped populations, thereby removing bias towards a particular population. GBS is a cost-effective technology producing up to a million SNPs per genotype at a low cost. However, one of the challenges associated with GBS is inadequate genome coverage and incomplete datasets (Fu 2014), sometimes with up to 90% missing observations per line (Elshire et al. 2011; Fu and Peterson 2011). Such data cannot be used for AM and filtration should be done to improve the sequence data (Fu 2014). Several methods for imputation include regression-based methods such as random forest (Stekhoven and Bühlmann 2012) and principal component analysis (PCA)-based tools (Stacklies et al. 2007).

### *2.7.3 Confounding Effects of Population Structure*

One of the challenges in using AM to dissect the genetic architecture of complex traits is the risk of detecting false positives due to population structure (Pritchard et al. 2000). The problem of population structure can arise due to the correlation of phenotypic trait with population structure at neutral loci, which can result in an inflated number of false positive associations resulting in Type I errors. Among several methods used to deal with this problem, the 'genomic control' (GC) method could be considered useful (Devlin and Roeder 1999). GC estimates association using large number of putative neutral markers or markers that are not thought to be associated with the trait of interest. The distribution of the test statistic is then calculated from these associations for trait of interest and a critical value for desired Type I error rate is chosen from this distribution. Another commonly used method is called structured associations (SA) (Pritchard et al. 2000). SA first queries population for closely associated clusters/subdivisions using a Bayesian approach, and then uses clustering matrices (Q) in AM (by a logistic regression) to correct for false associations. Population structure and shared co-ancestry coefficients between individuals of subdivisions of a population can be effectively estimated with the STRUCTURE program (Pritchard et al. 2000) using several models for linked and unlinked markers.

Principal component analysis (PCA) is widely used as a faster and effective way to diagnose population structure (Chengsong and Jianming 2009). The PCA method makes it computationally feasible to handle a large data sets (tens of thousands of markers) and correct for population stratification. Most widely used programs to calculate PCA are DARwin and EIGENSTRAT (Price et al. 2006).

A mixed linear model (MLM) combining both population structure information (*Q*-matrix or PCA) and pairwise relatedness coefficients (kinship-matrix) can be used in the analysis. While the *Q*-matrix explains the structure between groups in a population, the kinship-matrix explains the structure within group. Although MLM approach is computationally intensive, it is very effective in removing the confounding effects of the population in AM (Yu et al. 2006). However, in some cases using MLM + kinship model may result in over correction of the population structure. This could be identified from the QQ-plot when it skews below the reference line. In this case, using generalized linear model and population structure (GLM + PC) will be better in removing the population structure and identifying the markers significantly associated with the studied traits. Similar cases were found in studying disease resistance in wheat and barley indicating the importance of testing both MLM + K and GLM + PC models (Turuspekov et al. 2016; Abou-Zeid and Mourad 2021).

### 2.7.4 Estimates of LD

Linkage Disequilibrium (LD) refers to the correlation between alleles in a population (Flint-Garcia et al. 2003) and Linkage refers to the correlated inheritance of loci through the physical association on a chromosome but not necessarily on the same chromosome. For AM, it is important to understand the patterns of LD for genomic regions of individual plants and the extent of LD among different populations or groups to design unbiased association mapping studies. Two most widely used statistics to measure LD are $r^2$ (square of the correlation coefficient) and D′ (disequilibrium coefficient). The $r^2$and D′ statistics represent different aspects of LD and perform differently under various conditions. The $r^2$ is affected by both mutation and recombination while D′ is affected by more mutational events of the past.

There are several software programs such as GOLD (Abecasis and Cookson 2000), TASSEL (www.maizegenetics.net) or Powermarker (Liu and Muse 2005) to represent the structure and pattern of LD. Average genome-wide decay of LD can be estimated by plotting LD values ($r^2$ values) obtained from a data set adequately covering an entire genome against the genetic (or) physical distance between markers. The decrease in LD within the genetic distance indicates the portion of LD that is conserved with linkage and proportional to recombination events (Gupta et al. 2014). The decay of LD over physical/genetic distance in a population is a determinant of marker density and coverage needed to perform an association analysis. If rapid LD decay is observed, then a higher marker density is needed to capture markers closely linked to functional sites (Flint-Garcia et al. 2003; Gaut and Long 2003). In wheat, depending on the populations used in study, LD decay have been reported to vary from 0.5 to 40 cM (Chao et al. 2007; Crossa et al. 2007; Somers et al. 2007; Tommasini et al. 2007; Yao et al. 2009; Dreisigacker et al. 2012). The higher distance of LD decay was found in wheat genome D followed by genome A and B, respectively which indicated that lower number of markers are required to identify targeted QTLs in genome D compared with genome A, and B (Liu et al. 2017; Ayana et al. 2018; Mourad et al. 2020).

### 2.7.5 Association Analysis Programs

GWAS is a very helpful method in detecting QTLs responsible for different traits (Alqudah et al. 2020). It has been used widely in wheat breeding for disease resistance and helped breeders in identifying genes controlling resistance to different races. There are many softwares which could be used in GWAS analysis. Publicly available software using mixed models for AM studies in plants include TASSEL and EMMA/R. Both can analyze moderate to large datasets but only allow single effects (samples or taxa) to be fit as a random effect and all other effects treated as fixed. EMMA relies on the R for data management and visualization

which is not limited with TASSEL functions. Other commercial software packages for AM studies include ASREML, JMP Genomics, ASREML, SAS and GenStat. General software such as SAS Proc Mixed and GenStat can perform AM analysis requiring more expertise and programming by the user and JMP Genomics are suited specifically for genetic analysis and can handle models that are more complex.

TASSEL on the other hand uses both GUI (graphical user interface) and CLI (command line interface) versions for detailed analysis and use versions depending on their expertise and consistent results can be obtained independent of the interface. In the latest version of TASSEL (TASSEL 5.0), a compressed MLM method has been developed to compute large datasets. GAPIT-R package is also a very useful software in AM and GWAS studies. It could be applied using different methods such as GLM, MLM and Settlement of MLM Under Progressively Exclusive Relationship (SUPER) (Wang et al. 2014a). For disease resistance, SUPER has been reported as a very useful method in detecting the QTLs significantly associated with the resistance of the targeted disease as it conducts GWAS by extracting a small subset of markers and testing their association with resistance by using Fast-LMM method. This method enables the identification of minor genes controlling the resistance (Mourad et al. 2018a).

### 2.7.6 Significance Threshold

Significance threshold is set to declare associations as significant in a particular study. Either FDR (false discovery rate) or 'Bonferroni' correction can be used to correct for multiple comparisons. The correction factor is needed to test multiple hypotheses simultaneously. FDR controls the proportion of false positives among significant results by defining a threshold from the observed *p*-value distribution in the data, whereas Bonferroni corrections detect and control false positives (Benjamini and Hochberg 1995). Given the objective of the study, one may consider a high FDR (e.g. dissection of genetic architecture of a trait) or low FDR (e.g. identifying candidate loci for further characterization and validation).

### 2.7.7 Validation of Association Results

Validation of AM results is an important step before marker information is used for selection decisions, or before identifying causal factors and gene cloning. One way is to compare the AM results with previously published results for the trait; for example, in bi-parental populations, markers in close proximity (<10 cM) to previously reported QTLs/genes, will not only increase the confidence but validate the new genomic target identified for the trait. Secondly, validation can be performed different panels/populations. This is more reliable as the probability of significant

associations are confirmed and false positives discarded when validation is carried in two or more populations. Third, AM results point to alleles with opposite effects (favorable/unfavorable alleles) on a trait of interest, multiple $F_2$ populations can be generated from parents carrying contrasting alleles and determine whether phenotype differences co-segregate with the locus of interest.

In addition, testing the LD between the newly identified markers and previously identified markers will give more power to the results obtained from the association tests. For example, testing the LD between the identified SNP markers located on the same chromosome will give an idea if they are controlling the same QTL or different QTLs. If the studied trait or disease has a published accurate SSR marker, the LD between the identified SNPs and the SSR marker will validate the association between the SNP markers and the studied trait (Mourad et al. 2018b, 2019b). Furthermore, gene models harboring the detected QTLs and their functional annotations could be investigated using IWGSC dataset. If the identified marker is located within or near gene model and annotated to improve the targeted trait, this will give more power to the association results. Once tightly linked markers to the target trait are validated, can enhance the speed and cost efficiency of selection in breeding programs.

### *2.7.8 Genomic Selection in Breeding for Quantitative Disease Resistance in Wheat*

With rapid changes in pathogen races and breakdown of major resistance genes, the benefits of marker-assisted selection in selecting for minor gene based quantitative disease resistance in wheat is limited and hence, the focus has shifted to GS (Rutkoski et al. 2011; Poland and Rutkoski 2016). In GS, dense genome-wide markers and trait phenotypes (disease response in this case) are used to obtain the genomic-estimated breeding values (GEBVs) of individuals for the trait, from which selections are made (Heffner et al. 2009; Meuwissen et al. 2001). Since GS models incorporate all the marker information across the genome to estimate marker effects, they are expected to capture well the total additive genetic variance, including the disease variation resulting from minor effect quantitative trait loci (Heffner et al. 2009; Poland and Rutkoski 2016). The potential of GS for disease resistance in wheat has been explored in several studies that have reported different prediction accuracies (PAs, correlations between the predicted and the true breeding values), some of which are discussed below.

The utility of GS for increasing the gains from selection per unit time (Heffner et al. 2010) was first explored for quantitative APR to SR in wheat, where the authors presented a recurrent GS-based breeding scheme including rounds of intermating and GEBV-based selections, with simultaneous evaluation of lines and model updating (Rutkoski et al. 2011). In 2012, a study by Ornella et al. evaluated genomic predictions for stem and YR in five bi-parental populations from

CIMMYT and observed maximum within-year CV PAs ranging from 0.56 to 0.75 for SR and from 0.63 for YR in the different populations (Ornella et al. 2012). Genomic prediction for rust resistance was also evaluated in a set of landraces from the Watkins collection and five-fold CV PAs of 0.35, 0.27 and 0.44 were obtained for LR, SR and YR, respectively (Daetwyler et al. 2014).

One of the first studies on comparison of realized gains from GS and phenotypic selection for quantitative SR resistance in wheat, indicated that while both lead to equal rates of gain in the short-term, GS lead to a significantly greater loss in genetic variance compared to phenotypic selection that could reduce the rates of genetic gain in the long-term (Rutkoski et al. 2015). A comparison between genomic prediction and pedigree-based prediction for rust resistance using CIMMYT's international bread wheat screening nurseries indicated similar accuracies with both the relationship matrices, and the CV genomic PAs ranged between 0.31 and 0.74 for LR seedling resistance, 0.12 and 0.56 for LR APR, 0.31 and 0.65 for SR APR, 0.70 and 0.78 for YR seedling resistance, and 0.34 and 0.71 for YR APR (Juliana et al. 2017a).

Genomic prediction models for FHB resistance in wheat were evaluated using the U.S. cooperative FHB wheat nurseries, and resulted in five-fold within-year cross-validation (CV) PAs of 0.46 and 0.41 for Fusarium damaged kernels (FDK) and deoxynivalenol (DON) content, respectively (Rutkoski et al. 2012). Using a large panel of Central European elite winter wheat lines, Mirdita et al. (2015), reported high five-fold CV PAs of 0.6 for FHB resistance and 0.5 for STB resistance. In another FHB genomic prediction study using breeding lines, high five-fold CV PAs of 0.82 for FDK and 0.64 for DON content were reported (Arruda et al. 2015). A breeding population of spring wheat lines was used to evaluate genomic prediction for FHB and the ten-fold CV PAs for FHB incidence, severity, and DON content were 0.63, 0.43, and 0.42, respectively (Dong et al. 2018). In another study, using elite spring wheat breeding lines from six breeding cycles, Liu et al. (2019) reported genomic PAs ranging from 0.22 to 0.44 for FHB resistance. Genomic prediction for FHB and STB in winter wheat lines indicated PAs of 0.72 and 0.15 for the two traits, respectively (Herter et al. 2019).

Evaluation of genomic prediction for STB, *Stagonospora nodorum* blotch and tan spot in wheat using CIMMYT's international bread wheat screening nurseries indicated that the mean CV PAs for STB APR, *Stagonospora nodorum* blotch seedling resistance, tan spot seedling resistance and tan spot APR were 0.45, 0.55, 0.66 and 0.48, respectively (Juliana et al. 2017b). Comparison of the two whole-genome profiling approaches: genotyping-by-sequencing and diversity arrays technology-sequencing identified that the genotyping-by-sequencing markers performed slightly better than diversity arrays technology sequencing markers and combining markers from the two platforms did not improve the PAs. Another study for the genomic prediction of STB response using European winter wheat varieties, reported a mean five-fold CV PA of 0.44 (Muqaddasi et al. 2019). In a large-scale study involving genomic prediction for several traits, Juliana et al. (2019) reported moderate to high within-panel mean CV PAs of 0.49, 0.5, 0.64 and 0.56 for field resistance to STB, spot blotch, SR and stripe rust. However, when one nursery was

predicted from all other panels, they obtained low mean genomic PAs of 0.28, 0.36 and 0.36 for STB, spot blotch and stripe rust, respectively, but a high genomic PA of 0.61 for SR.

Given the promising PAs obtained from genomic prediction for disease resistance in wheat in most of the aforementioned studies, breeding programs can effectively integrate GS in breeding for resistant varieties. It can be especially useful for traits that have a low heritability and are difficult to phenotype. In the case of disease resistance traits that have a high heritability, where phenotypic selection might be the best method to increase genetic gains, GS can be applied to increase the selection intensity in early generations (Poland and Rutkoski 2016). Overall, further research on developing GS-based breeding strategies for wheat disease resistance and using it in combination with other strategies like rapid generation advancement technologies, high-throughput phenotyping and gene editing are important (Voss-Fels et al. 2019).

## 2.8 Integrating New Tools for Resistance Breeding Presents Opportunities for Wheat Improvement

The proven approach to enhance durability of genetic resistance is the deployment of combinations of multiple effective resistance genes often termed as "pyramiding". A limitation to stack multiple genes is their segregation when parents possessing different genes are crossed. This requires growing large populations to identify multiple gene combinations and the need to have complementing diagnostic markers tagging the R-genes for ensuring that the desired gene combination achieved. However, incomplete/moderate effect R-genes, race-nonspecific APR genes, or their combinations confers enhanced resistance levels due to additive effects, hence have been shown to be effectively selected in the field under high disease pressures (Singh et al. 2008b, 2015, 2016). New research advances have also facilitated options for combining multiple resistance genes in a single line/variety thereby enhancing resistance durability.

In the last two decades several rust resistance genes have been cloned using various approaches (Table 2.4) viz. eleven SR resistance genes: *Sr13* (Zhang et al. 2017a), *Sr21* (Marchal et al. 2018), *Sr22* (Steuernagel et al. 2016), *Sr33* (Periyannan et al. 2013), *Sr35* (Saintenac et al. 2013), *Sr45* (Steuernagel et al. 2016), *Sr46* (Arora et al. 2019), *Sr50* (Mago et al. 2015), *Sr55* (pleiotropic with *Lr67*) (Moore et al. 2015), *Sr57* (pleiotropic with *Lr34*) (Krattinger et al. 2009b) and more recently *Sr60* (Chen et al. 2020); four LR resistance genes *Lr1* (Cloutier et al. 2007), *Lr10* (Feuillet et al. 2003), *Lr21* (Huang et al. 2003), *Lr22a* (Thind et al. 2017) and six YR resistance genes *Yr5* (Marchal et al. 2018), *Yr7* (Marchal et al. 2018), *Yr10* (Liu et al. 2014), *Yr15* (Klymiuk et al. 2018), *YrAS2388R* (Zhang et al. 2019a) and *Yr36* (Fu et al. 2009). In the last decade, R-gene enrichment sequencing (Ren-Seq) approaches have been widely used to clone resistance genes.

Resistance genes from wild relatives can be introgressed to engineer broad-spectrum resistance in domesticated crop species using a combination of association genetics with R-gene enrichment sequencing (AgRenSeq) to exploit pan-genome variation in wild diploid wheat and such approach enabled rapid cloning off our SR resistance genes (Arora et al. 2019) and a relatively new approach called MutRenSeq that combines chemical mutagenesis with exome capture and sequencing has been developed for rapid R-gene cloning and enabled successful cloning of *Sr22* and *Sr45* from hexaploid bread wheat (Steuernagel et al. 2017). Despite these advances, the availability of the currently effective cloned genes remains limited, therefore requiring a responsible strategy for their deployment.

The availability of multiple cloned resistance genes opens the possibility to transform wheat lines with a stack or cassette of multiple cloned effective resistance genes. This transgenic approach can help combine multiple resistance genes in a linkage block with one another on a single translocation thereby reducing the chances of segregation upon further breeding processes and up to eleven cloned genes can be stacked (Wulff and Moscou 2014). However, the current regulatory framework in most countries does not allow the cultivation of transgenic, including cisgenic wheat and if future policy decisions favor approval of transgenic-cassettes such approach can be utilized to enhance durable resistance in wheat varieties.

## 2.9 Gene Editing

R-gene mediated resistance is race-specific though it remains effective in protecting the plant throughout all growth stages. Additionally, mutation in the pathogen *avr*-locus can also lead to break down of resistance. On the other hand, the APR loci show only partial resistance in adult pants while allowing considerable disease development (Ellis et al. 2014). Plant pathogens exploit host genes and machinery such as sugar transporters to draw nutrient from the host plant or to replicate their genome as in case of viruses. Mutations in some of these genes (susceptibility factors) has no impact on the plantgrowth and phenotype but can restrict pathogen growth (Moore et al. 2015).

Despite the tremendous success, marker-assisted breeding can exhaustively take from 7 to 12 years to introduce a new trait and release an improved variety (Acquaah 2007). Considering the recurrent resistance development against pesticides by several pathogens, emergence of new virulent strains or races, and lack of resistant germplasm against some pathogens, the current breeding methods are unlikely to keep pace with the predicted demand for rapid development of improved disease resistant varieties (Scheben et al. 2017). Redundancy of susceptibility factors due to polyploidy in wheat makes it difficult to identify lines that have all copies of these genes mutated. Breeders have been using chemical mutagenesis, gamma irradiation, fast neutron bombardment, and T-DNA insertion to generate artificial mutants. But low frequency, random and undirected nature of these

**Table 2.4** Cloned rust resistance genes in wheat

| Cloned gene | Chromosome arm | Cloning method | Type of resistance | References |
|---|---|---|---|---|
| *Yr5/YrSP* | 2BL | MutRenSeq | Race specific/ major | Marchal et al. (2018) |
| *Yr7* | 2BL | MutRenSeq | Race specific/ major | Marchal et al. (2018) |
| *Yr10* | 1BS | Map based cloning | Race specific/ major | Liu et al. (2014) |
| *Yr15* | 1BS | Map based cloning | Race specific/ major | Klymiuk et al. (2018) |
| *YrAS2388R* | 4DS | Map based cloning | Race specific/ major | Zhang et al. (2019a) |
| *Lr1* | 5DL | Map based cloning | Race specific/ major | Cloutier et al. (2007) |
| *Lr10* | 1AS | Map based cloning | Race specific/ major | Feuillet et al. (2003) |
| *Lr21* | 1DS | Map based cloning | Race specific/ major | Huang et al. (2003) |
| *Sr13* | 6AL | Map based cloning | Race specific/ major | Zhang et al. (2017a) |
| *Sr21* | 2AL | Map based cloning | Race specific/ major | Chen et al. (2018) |
| *Sr22* | 7AL | MutRenSeq | Race specific/ major | Steuernagel et al. (2016) |
| *Sr33* | 1DL | Map based cloning | Race specific/ major | Periyannan et al. (2013) |
| *Sr35* | 3AL | Map based cloning | Race specific/ major | Saintenac et al. (2013) |
| *Sr45* | 1DS | Mut-RenSeq | Race specific/ major | Steuernagel et al. (2016) |
| *Sr46* | 2DS | Ag-RenSeq | Race specific/ major | Arora et al. (2019) |
| *Sr50* | 1RS | Map based cloning | Race specific/ major | Mago et al. (2015) |
| *Sr60* | 5AS | Map based cloning | Race specific/ major | Chen et al. (2020) |
| *Yr36* | 6BS | Map based cloning | APR/partial | Fu et al. (2009) |
| *Lr22a* | 2DS | TACCA | APR/partial | Thind et al. (2017) |
| *Lr34/Yr18/ Sr57* | 7DS | Map based cloning | APR/partial | Krattinger et al. (2009b) |
| *Lr67/Yr46/ Sr55* | 4DL | Map based cloning | APR/partial | Moore et al. (2015) |

mutations has impeded its utility in wheat breeding for disease resistance. Site-specific nuclease mediated editing of target genes offers an excellent alternative to precisely mutate a target gene without disturbing rest of the genome.

Until 2013, the dominant genome editing tools were zinc finger nucleases and transcription activator-like effector nucleases (TALENs) and they have been used successfully in many organisms including plants such as wheat (Wang et al. 2014c). The design of ZFNs is challenging due to the complex nature of the interaction between zinc-fingers and DNA as well as limitations imposed by context-dependent specificity. Though the design of TALEN is relatively simpler, the highly repetitive sequences in the construct promote homologous recombination in vivo making it difficult for wide adoption. The RNA-guided genome editing (RGE) using the CRISPR-Cas9 technology has emerged as a simple, versatile and highly efficient tool for editing of target genes in a wide variety of organisms including plants. The CRISPR-Cas system relies on simple Watson–Crick base pairing of a chimeric single guide RNA (sgRNA) that is partly complementary to the target DNA sequence called proto-spacer element. Thus, only 20 nucleotides (nt) in the gRNA need to be modified to recognize a different target. The target sequence must be followed by a protospacer adjacent motif (PAM) such as NGG or NAG (N: any nucleotide) for target recognition (Biswal et al. 2019). Once the target site is recognized, the Cas9 nuclease makes a double stranded break (DSB), three nucleotides upstream of the PAM (Yin et al. 2017). The DSB is immediately repaired by the host cell by the non-homologous end joining (NHEJ), which is erroneous. The NHEJ may insert, remove or even substitute one or a few nucleotides. Removal or insertion of non-triplets can lead to frameshift mutation that can either result in a complete new protein or may introduce a stop codon downstream of the target site resulting in a truncated protein that can be target of subsequent nonsense-mediated decay of the transcript (Shaul 2015). Multiplexed targeting of two different regions of the genome can result in removal of a chunk of DNA flanked by both targets. The host cell can also follow a homology-directed repair (HDR) mechanism, when a suitable template is supplied with homologous flanking arms. HDR method can be applied to replace a faulty gene, to introduce desired mutations or even to introduce a new coding sequence or promoter. Though the efficiency of HDR method is relatively low, HDR enhancers have also been reported (Song et al. 2016). The CRISPR-Cas based prime editors can also be used to replace, delete or introduce a fragment of DNA at the target site without the special supply of HDR template. The prime editing complex uses a Cas9 nickase (nCas9) fused to a reverse transcriptase and a prime editing guide RNA (pegRNA) that specifies the target site as well as encodes the desired edit without introducing DSBs (Anzalone et al. 2019).

Base editing is a newer genome-editing approach that uses a catalytically inactive Cas9 (dCas9) fused to a nucleotide deaminase enzyme (Gaudelli et al. 2017). Base editors can convert C·G to T·A or A·T to G·C in cellular DNA or RNA without making double-stranded DNA breaks. As SNP is one of the most common form of difference observed between resistant and susceptible alleles, base editors can be of immense importance to wheat molecular breeders to directly improve the

disease resistance in elite wheat lines. It can also be extended to generate artificial alleles of a target gene.

The CRISPR-Cas mediated genome editing is precise, and modifications can be done at specific locus. More importantly the meiotic segregation of the CRSPR tools leaves final product free of transgenic traces that can be released to farmers with minimum regulatory inhibition. Wheat powdery mildew an important wheat disease, caused by an obligate biotrophic ascomycete fungus *Blumeria graminis* f. sp. *tritici*, Bgt, which has a highly selective host range of single-plant genera (Singh et al. 2016). Simultaneous editing of all three homoeoalleles of the MLO locus in hexaploid bread wheat using TALEN and CRISPR-Cas9 has shown heritable resistance to powdery mildew (Wang et al. 2014c). Similarly, simultaneous modification of all homoeologs of *TaEDR1* gene by CRISPR-Cas9 technology generated wheat lines with enhanced resistance to powdery mildew (Zhang et al. 2017b). FHB is another important disease of wheat caused by *Fusarium graminearum* fungus. Deoxynivalenol (DON) is a mycotoxin virulence factor that induces the expression of a transcription factor *TaNFXL1*, a repressor of *F. graminearum* resistance. CRISPR-Cas9 mediated knocking out of *TaNFXL1* demonstrated increased FHB resistance (Brauer et al. 2020).

The CRISPR/Cas system to dissect the pathogen genetics and to diagnose the disease. The lack of genetic tools to analyze and link the pathogen genes to the disease phenotype and progression is a major impediment in developing disease resistant crops. Recent progress in next generation sequencing has helped to predict functions hundreds of pathogen genes that needs functional validation (Levy et al. 2018). The CRISPR-interference (CRISPRi) system uses a catalytically inactive Cas9 protein (dCas9) and programmable single guide RNAs to modulate the pathogen gene expression that can be employed to dissect the functions of essential and non-essential genes in different pathogen species. Recently, a 'Mobile-CRISPRi' system has been developed to analyze antibiotic resistances and host-microbe interactions that uses a modular system and can be transferred to diverse bacterial species by conjugation (Peters et al. 2019). A similar system in plants can be very useful to study plant-pathogen interaction as well as the mechanism of resistance breakdown by new pathotypes.

Rapid and reliable detection of pathogens is important for taking curative measures in order to minimize the crop loss. The CRISPR-Cas technology can provide a versatile tool to detect the pathogen DNA/RNA in plant samples. The SHERLOCK (specific high-sensitivity enzymatic reporter unlocking) system can detect atto-molar concentration of nucleic acids in a solution (Abudayyeh et al. 2019; Gootenberg et al. 2018). The colorimetric lateral flow strips can also be developed to detect certain plant or pathogen genes by using SHERLOCK platform in the field without need of high end instruments or technical expertise (Abudayyeh et al. 2019). The multiplexed SHERLOCK system can also be extended to detect heterozygosity and trait stacking.

## 2.10 Concluding Remarks

Wheat diseases continue to be a significant challenge in several wheat production environments. Major threat is due to the extreme damage these diseases can cause to susceptible varieties. Although severe epidemics have not been reported in the last two decades, lack of genetic diversity in host and constantly evolving and migrating pathogens can pose a significant risk. Genetic resistance through deployment of both race specific genes and APR though quite widely used in breeding programs, however, faster evolution of new races to overcome race specific genes has resulted in wide spread vulnerability of cultivars, and the increasing importance of some diseases due to changes in cropping systems and crop intensification require reinforcing breeding strategies to develop adequate and durable resistance to multiple diseases for enhancing wheat productivity and farmers' income worldwide by reducing crop losses. New genomic tools in conjunction with phenotypic selection provides great promise for harnessing ample genetic diversity for resistance that exists in wheat for a number of important diseases. The impact of cost-effective NGS technologies coupled with new tools of rapidly cloning of rust resistance genes alongside the availability of wheat reference genomes can rapidly accelerate pyramiding strategies into desired wheat backgrounds. Progress in genetic mapping techniques and wheat transformation methods can enhance cloning efforts with the possibility of stacking multiple genes or gene cassettes using functional markers Future policy decisions will determine whether transgenic cassettes can be utilized as a new strategy for durable resistance in various countries.

## References

Abecasis GR, Cookson WOC (2000) GOLD—graphical overview of linkage disequilibrium. Oxford University Press, Oxford

Abou-Zeid MA, Mourad AMI (2021) Genomic regions associated with stripe rust resistance against the Egyptian race revealed by genome-wide association study. BMC Plant Biol 21:42

Abudayyeh OO, Gootenberg JS, Kellner MJ, Zhang F (2019) Nucleic acid detection of plant genes using CRISPR-Cas13. Cris J 2:165–171

Acquaah G (2007) Sexual hybridization and wide crosses in plant breeding. In: Acquaah G (ed) Principles of plant genetics and breeding, 1st edn. Blackwell Publishing Ltd, Oxford, pp 164–180

Adhikari TB, Gurung S, Hansen JM, Jackson EW, Bonman JM (2012) Association mapping of quantitative trait loci in spring wheat landraces conferring resistance to bacterial leaf streak and spot blotch. Plant Genome 5:1–16

Admassu B, Lind V, Friedt W, Ordon F (2009) Virulence analysis of *Puccinia graminis* f.sp. *tritici* populations in Ethiopia with special consideration of Ug99. Plant Pathol 58:362–369

Akhunov E, Nicolet C, Dvorak J (2009) Single nucleotide polymorphism genotyping in polyploid wheat with the Illumina GoldenGate assay. Theor Appl Genet 119:507–517

Ali S, Gladieux P, Leconte M, Gautier A, Justesen AF, Hovmøller MS, Enjalbert J, de Vallavieille-Pope C (2014) Origin, migration routes and worldwide population genetic

structure of the wheat yellow rust pathogen *Puccinia striiformis* f.sp. *tritici*. PLoS Pathog 10: e1003903

Ali S, Rodriguez-Algaba J, Thach T, Sørensen CK, Hansen JG, Lassen P, Nazari K, Hodson DP, Justesen AF, Hovmøller MS (2017) Yellow rust epidemics worldwide were caused by pathogen races from divergent genetic lineages. Front Plant Sci 8

Allen AM, Barker GLA, Berry ST, Coghill JA, Gwilliam R, Kirby S, Robinson P, Brenchley RC, D'Amore R, Mckenzie N, Waite D, Hall A, Bevan M, Hall N, Edwards KJ (2011) Transcript-specific, single-nucleotide polymorphism discovery and linkage analysis in hexaploid bread wheat (*Triticum aestivum* L.). Plant Biotechnol J 9:1086–1099

Alqudah AM, Sallam A, Stephen Baenziger P, Börner A (2020) GWAS: fast-forwarding gene identification and characterization in temperate cereals: lessons from barley—a review. J Adv Res 22:119–135

Anikster Y, Bushnell WR, Eilam T, Manisterski J, Roelfs AP (1997) *Puccinia recondita* causing leaf rust on cultivated wheats, wild wheats, and rye. Can J Bot 75:2082–2096

Anzalone AV, Randolph PB, Davis JR, Sousa AA, Koblan LW, Levy JM, Chen PJ, Wilson C, Newby GA, Raguram A, Liu DR (2019) Search-and-replace genome editing without double-strand breaks or donor DNA. Nature 576:149–157

Appels R, Eversole K, Stein N, Feuillet C, Keller B, Rogers J, Pozniak CJ, Choulet F, Distelfeld A, Poland J, Ronen G, Sharpe AG, Barad O, Baruch K, Keeble-Gagnère G, Mascher M, Ben-Zvi G, Josselin A-A, Himmelbach A, Balfourier F, Gutierrez-Gonzalez J, Hayden M, Koh C, Muehlbauer G, Pasam RK, Paux E, Rigault P, Tibbits J, Tiwari V, Spannagl M, Lang D, Gundlach H, Haberer G, Mayer KFX, Ormanbekova D, Prade V, Šimková H, Wicker T, Swarbreck D, Rimbert H, Felder M, Guilhot N, Kaithakottil G, Keilwagen J, Leroy P, Lux T, Twardziok S, Venturini L, Juhász A, Abrouk M, Fischer I, Uauy C, Borrill P, Ramirez-Gonzalez RH, Arnaud D, Chalabi S, Chalhoub B, Cory A, Datla R, Davey MW, Jacobs J, Robinson SJ, Steuernagel B, van Ex F, Wulff BBH, Benhamed M, Bendahmane A, Concia L, Latrasse D, Bartoš J, Bellec A, Berges H, Doležel J, Frenkel Z, Gill B, Korol A, Letellier T, Olsen O-A, Singh K, Valárik M, van der Vossen E, Vautrin S, Weining S, Fahima T, Glikson V, Raats D, Číhalíková J, Toegelová H, Vrána J, Sourdille P, Darrier B, Barabaschi D, Cattivelli L, Hernandez P, Galvez S, Budak H, Jones JDG, Witek K, Yu G, Small I, Melonek J, Zhou R, Belova T, Kanyuka K, King R, Nilsen K, Walkowiak S, Cuthbert R, Knox R, Wiebe K, Xiang D, Rohde A, Golds T, Čížková J, Akpinar BA, Biyiklioglu S, Gao L, N'Daiye A, Kubaláková M, Šafář J, Alfama F, Adam-Blondon A-F, Flores R, Guerche C, Loaec M, Quesneville H, Condie J, Ens J, Maclachlan R, Tan Y, Alberti A, Aury J-M, Barbe V, Couloux A, Cruaud C, Labadie K, Mangenot S, Wincker P, Kaur G, Luo M, Sehgal S, Chhuneja P, Gupta OP, Jindal S, Kaur P, Malik P, Sharma P, Yadav B, Singh NK, Khurana JP, Chaudhary C, Khurana P, Kumar V, Mahato A, Mathur S, Sevanthi A, Sharma N, Tomar RS, Holušová K, Plíhal O, Clark MD, Heavens D, Kettleborough G, Wright J, Balcárková B, Hu Y, Salina E, Ravin N, Skryabin K, Beletsky A, Kadnikov V, Mardanov A, Nesterov M, Rakitin A, Sergeeva E, Handa H, Kanamori H, Katagiri S, Kobayashi F, Nasuda S, Tanaka T, Wu J, Cattonaro F, Jiumeng M, Kugler K, Pfeifer M, Sandve S, Xun X, Zhan B, Batley J, Bayer PE, Edwards D, Hayashi S, Tulpová Z, Visendi P, Cui L, Du X, Feng K, Nie X, Tong W, Wang L (2018) Shifting the limits in wheat research and breeding using a fully annotated reference genome. Science 361: eaar7191

Arora S, Steuernagel B, Gaurav K, Chandramohan S, Long Y, Matny O, Johnson R, Enk J, Periyannan S, Singh N, Hatta MA, Athiyannan N, Cheema J, Yu G, Kangara N, Ghosh S, Szabo LJ, Poland J, Bariana H, Jones JDG, Bentley AR, Ayliffe M, Olson E, Xu SS, Steffenson BJ, Lagudah E, Wulff BBH (2019) Resistance gene cloning from a wild crop relative by sequence capture and association genetics. Nat Biotechnol 37

Arruda MP, Brown PJ, Lipka AE, Krill AM, Thurber C, Kolb FL (2015) Genomic selection for predicting head blight resistance in a wheat breeding program. Plant Genome 8:1–12

Asad MA, Bai B, Lan C, Yan J, Xia X, Zhang Y, He Z (2014) Identification of QTL for adult-plant resistance to powdery mildew in Chinese wheat landrace Pingyuan 50. Crop J 2:308–314

Ayana GT, Ali S, Sidhu JS, Gonzalez Hernandez JL, Turnipseed B, Sehgal SK (2018) Genome-wide association study for spot blotch resistance in hard winter wheat. Front Plant Sci 9

Backhouse D, Burgess LW (2002) Climatic analysis of the distribution of *Fusarium graminearum, F. pseudograminearum* and *F. culmorum* on cereals in Australia. Australas Plant Pathol 31:321–327

Bai G, Su Z, Cai J (2018) Wheat resistance to fusarium head blight. Can J Plant Pathol 40:336–346

Balcárková B, Frenkel Z, Škopová M, Abrouk M, Kumar A, Chao S, Kianian SF, Akhunov E, Korol AB, Doležel J, Valárik M (2017) A high resolution radiation hybrid map of wheat chromosome 4A. Front Plant Sci 7:2063

Bansal UK, Bossolini E, Miah H, Keller B, Park RF, Bariana HS (2008) Genetic mapping of seedling and adult plant stem rust resistance in two European winter wheat cultivars. Euphytica 164:821–828

Bansal UK, Hayden MJ, Gill MB, Bariana HS (2010) Chromosomal location of an uncharacterised stripe rust resistance gene in wheat. Euphytica 171:121–127

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

Bariana HS (2003) Breeding for disease resistance. In: Thomas B, Murphy DJ, Murray BG (eds) Encycl Appl Plant Sci. Acad Press Harcourt, UK, pp 244–253

Bariana HS, Brown GN, Bansal UK, Miah H, Standen GE, Lu M (2007) Breeding triple rust resistant wheat cultivars for Australia using conventional and marker-assisted selection technologies. Aust J Agric Res 58:576

Bariana H, Forrest K, Qureshi N, Miah H, Hayden M, Bansal U (2016) Adult plant stripe rust resistance gene *Yr71* maps close to *Lr24* in chromosome 3D of common wheat. Mol Breed 36:1–10

Beddow JM, Pardey PG, Chai Y, Hurley TM, Kriticos DJ, Braun H-J, Park RF, Cuddy WS, Yonow T (2015) Research investment implications of shifts in the global geography of wheat stripe rust. Nat Plants 1:15132

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B 57:289–300

Berlin A, Samils B, Djurle A, Wirsén H, Szabo L, Yuen J (2013) Disease development and genotypic diversity of *Puccinia graminis* f. sp. *avenae* in Swedish oat fields. Plant Pathol 62:32–40

Bernatzky R, Tanksley SD (1986) Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics 112:887–898

Bettgenhaeuser J, Krattinger SG (2019) Rapid gene cloning in cereals. Theor Appl Genet 132:699–711

Bhattacharya S (2017) Deadly new wheat disease threatens Europe's crops. Nature Publishing Group

Bhavani S, Hodson DP, Huerta-Espino J, Randhawa MS, Singh RP (2019) Progress in breeding for resistance to Ug99 and other races of the stem rust fungus in CIMMYT wheat germplasm. Front Agric Sci Eng 6:210–224

Bishnoi SK, He X, Phuke RM, Kashyap PL, Alakonya A, Chhokar V, Singh RP, Singh PK (2020) Karnal Bunt: a re-emerging old foe of wheat. Front Plant Sci 11:1

Biswal AK, Mangrauthia SK, Reddy MR, Yugandhar P (2019) CRISPR mediated genome engineering to develop climate smart rice: challenges and opportunities. Semin Cell Dev Biol 96:100–106

Bockus WW, Bowden RL, Hunger RM, Morrill WL, Murray TD, Smiley RW (eds) (2010) Compendium of wheat diseases and pests, 3rd edn. The American Phytopathological Society, pp 1–171

Börner A, Schumann E, Fürste A, Cöster H, Leithold B, Röder MS, Weber WE (2002) Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L.). Theor Appl Genet 105:921–936

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 32:314–331
Bowen KL (1991) Reduction in yield of winter wheat in North Carolina due to powdery mildew and leaf rust. Phytopathology 81:503
Brar GS, Pozniak CJ, Kutcher HR, Hucl PJ (2019) Evaluation of fusarium head blight resistance genes *Fhb1, Fhb2*, and *Fhb5* introgressed into elite Canadian hard red spring wheats: effect on agronomic and end-use quality traits and implications for breeding. Mol Breed 39:1–12
Brauer EK, Balcerzak M, Rocheleau H, Leung W, Schernthaner J, Subramaniam R, Ouellet T (2020) Genome editing of a deoxynivalenol-induced transcription factor confers resistance to *Fusarium graminearum* in wheat. Mol Plant-Microbe Interact 33:553–560
Breiman A, Graur D (1995) Wheat evolution. Isr J Plant Sci 43:85–98
Breseghello F, Finney PL, Gaines C, Andrews L, Tanaka J, Penner G, Sorrells ME (2005) Genetic loci related to kernel quality differences between a soft and a hard wheat cultivar. Crop Sci 45:1685–1695
Broman KW, Wu H, Sen Ś, Churchill GA (2003) R/qtl: QTL mapping in experimental crosses. Bioinformatics 19:889–890
Buerstmayr M, Steiner B, Buerstmayr H (2020) Breeding for fusarium head blight resistance in wheat-progress and challenges. Plant Breed 139:429–454
Burke DT, Carle GF, Olson MV (1987) Cloning of large segments of exogenous DNA into yeast by means of artificial chromosome vectors. Science 236:806–812
Carmona MA, Sautua FJ, Pérez-Hernández O, Grosso C, Vettorello L, Milanesio B, Corvi E, Almada G, Hovmøller MS (2019) Rapid emergency response to yellow rust epidemics caused by newly introduced lineages of *Puccinia striiformis* f. sp. *tritici* in Argentina. Trop Plant Pathol 44:385–391
Carmona M, Sautua F, Pérez-Hérnandez O, Reis EM (2020) Role of fungicide applications on the integrated management of wheat stripe rust. Front Plant Sci 11
Carris LM, Castlebury LA, Goates BJ (2006) Nonsystemic bunt fungi—*Tilletia indica* and *T. horrida*: a review of history, systematics, and biology. Annu Rev Phytopathol 44:113–133
Caubel J, Launay M, Ripoche D, Gouache D, Buis S, Huard F, Huber L, Brun F, Bancal MO (2017) Climate change effects on leaf rust of wheat: implementing a coupled crop-disease model in a French regional application. Eur J Agron 90:53–66
Cavanagh CR, Chao S, Wang S, Huang BE, Stephen S, Kiani S, Forrest K, Saintenac C, Brown-Guedira GL, Akhunova A, See D, Bai G, Pumphrey M, Tomar L, Wong D, Kong S, Reynolds M, Da Silva ML, Bockelman H, Talbert L, Anderson JA, Dreisigacker S, Baenziger S, Carter A, Korzun V, Morrell PL, Dubcovsky J, Morell MK, Sorrells ME, Hayden MJ, Akhunov E (2013) Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. Proc Natl Acad Sci U S A 110:8057–8062
Ceresini PC, Lili V, Rios JA, Aucique-p CE, Moreira SI, Alves E, Croll D, Maciel N (2016) Wheat blast : past, present, and future 1–30
Ceresini PC, Castroagudín VL, Rodrigues FÁ, et al (2018) Wheat Blast: Past, Present, and Future. Annu RevPhytopathol 56:427–456
Chakraborty S, Newton AC (2011) Climate change, plant diseases and food security: an overview. Plant Pathol 60:2–14
Chakraborty S, Murray GM, Magarey PA, Yonow T, O'Brien RG, Croft BJ, Barbetti MJ, Sivasithamparam K, Old KM, Dudzinski MJ, Sutherst RW, Penrose LJ, Archer C, Emmett RW (1998) Potential impact of climate change on plant diseases of economic significance to Australia. Australas Plant Pathol 27:15–35
Chakraborty S, Luck J, Hollaway G, Fitzgerald G, White N (2011) Rust-proofing wheat for a changing climate. Euphytica 179:19–32
Chao S, Sharp PJ, Worland AJ, Warham EJ, Koebner RMD, Gale MD (1989) RFLP-based genetic maps of wheat homoeologous group 7 chromosomes. Theor Appl Genet 78:495–504

Chao S, Zhang W, Dubcovsky J, Sorrells M (2007) Evaluation of genetic diversity and genome-wide linkage disequilibrium among U.S. wheat (*Triticum aestivum* L.) germplasm representing different market classes. Crop Sci 47:1018–1030

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

Chen X (2013) High-temperature adult-plant resistance, key for sustainable control of stripe rust. Am J Plant Sci 04:608–627

Chen X, Kang Z (eds) (2017) Stripe rust. Springer, Dordrecht, pp 1–719

Chen Y, Zhang F, Tang L, Zheng Y, Li Y, Christie P, Li L (2007) Wheat powdery mildew and foliar N concentrations as influenced by N fertilization and belowground interactions with intercropped faba bean. Plant Soil 291:1–13

Chen S, Rouse MN, Zhang W, Jin Y, Akhunov E, Wei Y, Dubcovsky J (2015) Fine mapping and characterization of *Sr21*, a temperature-sensitive diploid wheat resistance gene effective against the *Puccinia graminis* f. sp. *tritici* Ug99 race group. Theor Appl Genet 128:645–656

Chen S, Zhang W, Bolus S, Rouse MN, Dubcovsky J (2018) Identification and characterization of wheat stem rust resistance gene *Sr21* effective against the Ug99 race group at high temperature. PLoS Genet 14:1–21

Chen S, Rouse MN, Zhang W, Zhang X, Guo Y, Briggs J, Dubcovsky J (2020) Wheat gene *Sr60* encodes a protein with two putative kinase domains that confers resistance to stem rust. New Phytol 225:948–959

Chengsong Z, Jianming Y (2009) Nonmetric multidimensional scaling corrects for population structure in association mapping with different sample types. Genetics 182:875–888

Chester KS (1946) The nature and prevention of the cereal rusts as exemplified in the leaf rust of wheat. Chronica Botanica Co., Waltham, pp 1–269

Chhetri M, Bariana H, Wong D, Sohail Y, Hayden M, Bansal U (2017) Development of robust molecular markers for marker-assisted selection of leaf rust resistance gene *Lr23* in common and durum wheat breeding programs. Mol Breed 37:1–8

Choudhary K, Choudhary K, Choudhary OP, Shekhawat NS (2008) Marker assisted selection: a novel approach for crop improvement. J Agron 1:26–30

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

Cloutier S, McCallum BD, Loutre C, Banks TW, Wicker T, Feuillet C, Keller B, Jordan MC (2007) Leaf rust resistance gene *Lr1*, isolated from bread wheat (*Triticum aestivum* L.) is a member of the large *psr567* gene family. Plant Mol Biol 65:93–106

Cobo N, Wanjugi H, Lagudah E, Dubcovsky J (2019) A high-resolution map of wheat QYr. ucw-1BL, an adult plant stripe rust resistance locus in the same chromosomal region as Yr29. Plant Genome 12:1–15

Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. Euphytica 142:169–196

Cook RJ (2001) Management of wheat and barley root diseases in modern farming systems. Australas Plant Pathol 30:119–126

Cook RJ, Schillinger WF, Christensen NW (2002) Rhizoctonia root rot and take-all of wheat in diverse direct-seed spring cropping systems. Can J Plant Pathol 24:349–358

Cowger C, McDonald BA, Mundt CC (2002) Frequency of sexual reproduction by *Mycosphaerella graminicola* on partially resistant wheat cultivars. Phytopathology 92:1175–1181

Crossa J, Burgueño J, Dreisigacker S, Vargas M, Herrera-Foessel SA, Lillemo M, Singh RP, Trethowan R, Warburton M, Franco J, Reynolds M, Crouch JH, Ortiz R (2007) Association analysis of historical bread wheat germplasm using additive genetic covariance of relatives and population structure. Genetics 177:1889–1913

Cruz CD, Valent B (2017) Wheat blast disease: danger on the move. Trop Plant Pathol 42:210–222

Curtis B, Rajaram S, Macpherson H (eds) (2002) Bread wheat: improvement and production. Food and Agriculture Organization of the United Nations (FAO), pp 1–567

Da Silva GBP, Zanella CM, Martinelli JA, Chaves MS, Hiebert CW, McCallum BD, Boyd LA (2018) Quantitative trait loci conferring leaf rust resistance in hexaploid wheat. Phytopathology 108:1344–1354

Dababat AA, Imren M, Erginbas-Orakci G, Ashrafi S, Yavuzaslanoglu E, Toktay H, Pariyar SR, Elekcioglu HI, Morgounov A, Mekete T (2014) The importance and management strategies of cereal cyst nematodes, *Heterodera* spp., in Turkey. Euphytica 202:173–188

Daetwyler HD, Bansal UK, Bariana HS, Hayden MJ, Hayes BJ (2014) Genomic prediction for rust resistance in diverse wheat landraces. Theor Appl Genet 127:1795–1803

Dean R, van Kan JAL, 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

Deschamps S, Llaca V, May GD (2012) Genotyping-by-Sequencing in Plants. Biology (Basel) 1:460–483

Desmarais E, Lanneluc I, Lagnel J (1998) Direct amplification of length polymorphisms (DALP), or how to get and characterize new genetic markers in many species. Nucleic Acids Res 26:1458–1465

Devlin B, Roeder K (1999) Genomic control for association studies. Biometrics 55:997–1004

Doležel J, Vrána J, Šafář J, Bartoš J, Kubaláková M, Šimková H (2012) Chromosomes in the flow to simplify genome analysis. Funct Integr Genomics 12:397–416

Dong Z, Hegarty JM, Zhang J, et al (2017) Validation and characterization of a QTL for adult plant resistance to stripe rust on wheat chromosome arm 6BS (*Yr78*). Theor Appl Genet 130:2127–2137

Dong H, Wang R, Yuan Y, Anderson J, Pumphrey M, Zhang Z, Chen J (2018) Evaluation of the potential for genomic selection to improve spring wheat resistance to fusarium head blight in the pacific northwest. Front Plant Sci 9:1–15

Dreisigacker S, Shewayrga H, Crossa J, Arief VN, DeLacy IH, Singh RP, Dieters MJ, Braun H-J (2012) Genetic structures of the CIMMYT international yield trial targeted to irrigated environments. Mol Breed 29:529–541

Dumalasová V, Bartoš P (2009) Will climatic changes Enhance the risk of *Tilletia indica* in Europe? Plant Prot Sci 45:S38–S40

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

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, Samborski DJ (1979) Adult-plant leaf rust resistance in PI 250413, an introduction of common wheat. Can J Plant Sci 59:329–332

Dyck PL, Samborski DJ, Anderson RG (1966) Inheritance of adult-plant leaf rust resistance derived from the common wheat varieties exchange and frontana. Can J Genet Cytol 8:665–671

Dyck PL, Kerber ER, Aung T (1994) An interchromosomal reciprocal translocation in wheat involving leaf rust resistance gene *Lr34*. Genome 37:556–559

Edae EA, Pumphrey MO, Rouse MN (2018) A genome-wide association study of field and seedling response to individual stem rust pathogen races reveals combinations of race-specific genes in North American spring wheat. Front Plant Sci 9:52

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

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS ONE 6:

Endo TR, Gill BS (1996) The deletion stocks of common wheat. J Hered 87:295–307

Everts KL (1992) Effect of early season powdery mildew on development, survival, and yield contribution of tillers of winter wheat. Phytopathology 82:1273

Faris JD, Zhang Z, Lu H, Lu S, Reddy L, Cloutier S, Fellers JP, Meinhardt SW, Rasmussen JB, Xu SS, Oliver RP, Simons KJ, Friesen TL (2010) A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. Proc Natl Acad Sci 107:13544–13549

Faris JD, Liu Z, Xu SS (2013) Genetics of tan spot resistance in wheat. Theor Appl Genet 126:2197–2217

Feng J, Wang M, See DR, Chao S, Zheng Y, Chen X (2018) Characterization of novel gene *Yr79* and four additional quantitative trait loci for all-stage and high-temperature adult-plant resistance to stripe rust in spring wheat PI 182103. Phytopathology 108:737–747

Feuillet C, Travella S, Stein N, Albar L, Nublat A, Keller B (2003) Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L.) genome. Proc Natl Acad Sci U S A 100:15253–15258

Flint-Garcia SA, Thornsberry JM, Buckler ES (2003) Structure of linkage disequilibrium in plants. Annu Rev Plant Biol 54:357–374

Forrest K, Pujol V, Bulli P, Pumphrey M, Wellings C, Herrera-Foessel S, Huerta-Espino J, Singh R, Lagudah E, Hayden M, Spielmeyer W (2014) Development of a SNP marker assay for the *Lr67* gene of wheat using a genotyping by sequencing approach. Mol Breed 34:2109–2118

Forsyth FR (1956) Interaction of temperature and light on the seedling reaction of McMurachy wheat to race 15B of stem rust. Can J Bot 34:745–749

Friesen TL, Meinhardt SW, Faris JD (2007) The *Stagonospora nodorum*-wheat pathosystem involves multiple proteinaceous host-selective toxins and corresponding host sensitivity genes that interact in an inverse gene-for-gene manner. Plant J 51:681–692

Fu Y-B (2014) Genetic diversity analysis of highly incomplete SNP genotypedata with imputations: An empirical assessment. G3 Genes Genomes Genet 4:891–900

Fu Y-B, Peterson GW (2011) Genetic diversity analysis with 454 pyrosequencing and genomic reduction confirmed the eastern and western division in the cultivated barley gene pool. Plant Genome 4:226–237

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

Fuentes-Davila G, Rajaram S, Singh G (1995) Inheritance of resistance to karnal bunt (*Tilletia indica* Mitra) in bread wheat (*Triticum aestivum* L.). Plant Breed 114:250–252

Gao H, Zhu F, Jiang Y, Wu J, Yan W, Zhang Q, Jacobi A, Cai S (2012) Genetic analysis and molecular mapping of a new powdery mildew resistant gene *Pm46* in common wheat. Theor Appl Genet 125:967–973

Gao L, Babiker EM, Nava IC, Nirmala J, Bedo Z, Lang L, Chao S, Gale S, Jin Y, Anderson JA, Bansal U, Park RF, Rouse MN, Bonman JM, Bariana H (2019) Temperature-sensitive wheat stem rust resistance gene Sr15 is effective against *Puccinia graminis* f. sp. *tritici* race TTKSK. Plant Pathol 68:143–151

Gaudelli NM, Komor AC, Rees HA, Packer MS, Badran AH, Bryson DI, Liu DR (2017) Programmable base editing of T to G C in genomic DNA without DNA cleavage. Nature 551:464–471

Gaut BS, Long AD (2003) The lowdown on linkage disequilibrium. Plant Cell 15:1502–1506

Germán S, Barcellos A, Chaves M, Kohli M, Campos P, de Viedma L (2007) The situation of common wheat rusts in the southern cone of America and perspectives for control. Aust J Agric Res 58:620

Gessese M, Bariana H, Wong D, Hayden M, Bansal U (2019) Molecular mapping of stripe rust resistance gene *Yr81* in a common wheat landrace Aus27430. Plant Dis 103:1166–1171

Gootenberg JS, Abudayyeh OO, Kellner MJ, Joung J, Collins JJ, Zhang F (2018) Multiplexed and portable nucleic acid detection platform with *Cas13, Cas12a*, and *Csm6*. Science 360:439–444

Gouache D, Bensadoun A, Brun F, Pagé C, Makowski D, Wallach D (2013) Modelling climate change impact on *Septoria tritici* blotch (STB) in France: Accounting for climate model and disease model uncertainty. Agric For Meteorol 170:242–252

Goyeau H, Park R, Schaeffer B, Lannou C (2006) Distribution of pathotypes with regard to host cultivars in French wheat leaf rust populations. Phytopathology 96:264–273

Gupta S, Kumari K, Muthamilarasan M, Parida SK, Prasad M (2014) Population structure and association mapping of yield contributing agronomic traits in foxtail millet. Plant Cell Rep 33:881–893

Gupta PK, Chand R, Vasistha NK, Pandey SP, Kumar U, Mishra VK, Joshi AK (2018) Spot blotch disease of wheat: the current status of research on genetics and breeding. Plant Pathol 67:508–531

Gupta V, He X, Kumar N, Fuentes-Davila G, Sharma RK, Dreisigacker S, Juliana P, Ataei N, Singh PK (2019) Genome wide association study of karnal bunt resistance in a wheat germplasm collection from Afghanistan. Int J Mol Sci 20

Haley CS, Knott SA (1992) A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity (Edinb) 69:315–324

Han Y, Korban SS (2010) Strategies for map-based cloning in apple. CRC Crit Rev Plant Sci 29:265–284

He C, Holme J, Anthony J (2014a) SNP genotyping: the KASP assay. Methods Mol Biol 1145:75–86

He J, Zhao X, Laroche A, Lu Z-X, Liu H, Li Z (2014b) Genotyping-by-sequencing (GBS), an ultimate marker-assisted selection (MAS) tool to accelerate plant breeding. Front Plant Sci 5:484

He H, Liu R, Ma P, Du H, Zhang H, Wu Q, Yang L, Gong S, Liu T, Huo N, Gu YQ, Zhu S (2020) Characterization of *Pm68*, a new powdery mildew resistance gene on chromosome 2BS of Greek durum wheat TRI 1796. Theor Appl Genet 1–10

Heffelfinger C, Fragoso CA, Lorieux M (2017) Constructing linkage maps in the genomics era with MapDisto 2.0. Bioinformatics 33:2224–2225

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

Heffner EL, Lorenz AJ, Jannink JL, Sorrells ME (2010) Plant breeding with genomic selection: gain per unit time and cost. Crop Sci 50:1681–1690

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

Herrera-Foessel SA, Singh RP, Lillemo M, Huerta-Espino J, Bhavani S, Singh S, Lan C, Calvo-Salazar V, Lagudah ES (2014) *Lr67/Yr46* confers adult plant resistance to stem rust and powdery mildew in wheat. Theor Appl Genet 127:781–789

Herter CP, Ebmeyer E, Kollers S, Korzun V, Miedaner T (2019) An experimental approach for estimating the genomic selection advantage for fusarium head blight and septoria tritici blotch in winter wheat. Theor Appl Genet 132:2425–2437

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

Hiebert CW, Rouse MN, Nirmala J, Fetch T (2017) Genetic mapping of stem rust resistance to *Puccinia graminis* f. sp. *tritici* race TRTTF in the Canadian wheat cultivar Harvest. Phytopathology 107:192–197

Hoisington D, Bohorova N, Fennell S, Khairallah M, Pellegrineschi A, Ribaut J-M (2002) The application of biotechnology to wheat improvement: new tools to improve wheat productivity. In: Curtis BC, Rajaram S, Gomez Macpherson H (eds) Bread wheat Improv Prod Plant Prod Prot Ser no 30, pp 175–198

Honsdorf N, Becker HC, Ecke W (2010) Association mapping for phenological, morphological, and quality traits in canola quality winter rapeseed (*Brassica napus* L.). Genome 53:899–907

Hovmøller MS, Justesen AF (2007) Rates of evolution of avirulence phenotypes and DNA markers in a northwest European population of *Puccinia striiformis* f. sp. *tritici*. Mol Ecol 16:4637–4647

Hovmøller MS, Yahyaoui AH, Milus EA, Justesen AF (2008) Rapid global spread of two aggressive strains of a wheat rust fungus. Mol Ecol 17:3818–3826

Hovmøller MS, Walter S, Justesen AF (2010) Escalating threat of wheat rusts. Science 329:369

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 (2016) 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

Huang L, Brooks SA, Li W, Fellers JP, Trick HN, Gill BS (2003) Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of bread wheat. Genetics 164:655–664

Huang BE, George AW, Forrest KL, Kilian A, Hayden MJ, Morell MK, Cavanagh CR (2012) A multiparent advanced generation inter-cross population for genetic analysis in wheat. Plant Biotechnol J 10:826–839

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 16:1–15

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

Imelfort M, Duran C, Batley J, Edwards D (2009) Discovering genetic polymorphisms in next-generation sequencing data. Plant Biotechnol J 7:312–317

Inoue Y, Vy TTP, Yoshida K, Asano H, Mitsuoka C, Asuke S, Anh VL, Cumagun CJR, Chuma I, Terauchi R, Kato K, Mitchell T, Valent B, Farman M, Tosa Y (2017) Evolution of the wheat blast fungus through functional losses in a host specificity determinant. Science 357:80–83

Jaccoud D, Peng K, Feinstein D, Kilian A (2001) Diversity arrays: a solid state technology for sequence information independent genotyping. Nucleic Acids Res 29:25

James Cook R (1992) Wheat root health management and environmental concern. Can J Plant Pathol 14:76–85

Jin Y, Singh RP (2006) Resistance in U.S. wheat to recent eastern African isolates of *Puccinia graminis* f. sp. *tritici* with virulence to resistance gene *Sr31*. Plant Dis 90:476–480

Jin Y, Szabo LJ, Pretorius ZA, Singh RP, Ward R, Fetch T (2008) Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis 92:923–926

Johnson JW, Baenziger PS, Yamazaki WT, Smith RT (1979) Effects of powdery mildew on yield and quality of isogenic lines of ‘chancellor’ wheat. Crop Sci 19:349–352

Juliana P, Singh RP, Singh PK, Crossa J, Huerta-Espino J, Lan C, Bhavani S, Rutkoski JE, Poland JA, Bergstrom GC, Sorrells ME (2017a) Genomic and pedigree-based prediction for leaf, stem, and stripe rust resistance in wheat. Theor Appl Genet 130:1415–1430

Juliana P, Singh RP, Singh PK, Crossa J, Rutkoski JE, Poland JA, Bergstrom GC, Sorrells ME (2017b) Comparison of models and whole-genome profiling approaches for genomic-enabled prediction of septoria tritici blotch, *Stagonospora nodorum* blotch, and tan spot resistance in wheat. Plant Genome 10

Juliana P, Poland J, Huerta-Espino J, Shrestha S, Crossa J, Crespo-Herrera L, Toledo FH, Govindan V, Mondal S, Kumar U, Bhavani S, Singh PK, Randhawa MS, He X, Guzman C, Dreisigacker S, Rouse MN, Jin Y, Pérez-Rodríguez P, Montesinos-López OA, Singh D, Mokhlesur Rahman M, Marza F, Singh RP (2019) Improving grain yield, stress resilience and quality of bread wheat using large-scale genomics. Nat Genet 51:1530–1539

Juliana P, He X, Kabir MR, Roy KK, Anwar MB, Marza F, Poland J, Shrestha S, Singh RP, Singh PK (2020) Genome-wide association mapping for wheat blast resistance in CIMMYT’s international screening nurseries evaluated in Bolivia and Bangladesh. Sci Rep 10:15972

Juroszek P, von Tiedemann A (2013) Climate change and potential future risks through wheat diseases: a review. Eur J Plant Pathol 136:21–33

Kalavacharla V, Hossain K, Gu Y, Riera-Lizarazu O, Vales MI, Bhamidimarri S, Gonzalez-Hernandez JL, Maan SS, Kianian SF (2006) High-resolution radiation hybrid map of wheat chromosome 1D. Genetics 173:1089–1099

Kao C-H, Zeng Z-B (1997) General formulas for obtaining the MLEs and the asymptotic variance-covariance matrix in mapping quantitative trait loci when using the EM algorithm. Biometrics 53:653

Kassa MT, You FM, Hiebert CW, Pozniak CJ, Fobert PR, Sharpe AG, Menzies JG, Humphreys DG, Rezac Harrison N, Fellers JP, McCallum BD, McCartney CA (2017) Highly predictive SNP markers for efficient selection of the wheat leaf rust resistance gene *Lr16*. BMC Plant Biol 17:45

Keeble-Gagnère G, Isdale D, Suchecki R law, Kruger A, Lomas K, Carroll D, Li S, Whan A, Hayden M, Tibbits J (2019) Integrating past, present and future wheat research with Pretzel. bioRxiv:517953

Keller M, Keller B, Schachermayr G, Winzeler M, Schmid JE, Stamp P, Messmer MM (1999) Quantitative trait loci for resistance against powdery mildew in a segregating wheat x spelt population. Theor Appl Genet 98:903–912

Khlestkina EK, Salina EA (2006) SNP markers: methods of analysis, ways of development, and comparison on an example of common wheat. Russ J Genet 42:585–594

Kislev ME (1982) Stem rust of wheat 3300 years old found in Israel. Science 216:993–994

Klymiuk V, Yaniv E, Huang L, Raats D, Fatiukha A, Chen S, Feng L, Frenkel Z, Krugman T, Lidzbarsky G, Chang W, Jääskeläinen MJ, Schudoma C, Paulin L, Laine P, Bariana H, Sela H, Saleem K, Sørensen CK, Hovmøller MS, Distelfeld A, Chalhoub B, Dubcovsky J, Korol AB, Schulman AH, Fahima T (2018) Cloning of the wheat *Yr15* resistance gene sheds light on the plant tandem kinase-pseudokinase family. Nat Commun 9:3735

Knott DR (1988) Using polygenic resistance to breed for stem rust resistance in wheat. In: Simmonds NW, Rajaram S (eds) Breeding strategies for resistance to the rusts of wheat. CIMMYT, Mexico, pp 39–47

Kolmer JA (1998) Physiologic specialization of *Puccinia recondita* f. sp. *tritici* in Canada in 1996. Can J Plant Pathol 20:176–181

Kolmer JA (2001) Physiologic specialization of *Puccinia triticina* in Canada in 1998. Plant Dis 85:155–158

Kolmer JA, Acevedo MA (2016) Genetically divergent types of the wheat leaf fungus *Puccinia triticina* in Ethiopia, a center of tetraploid wheat diversity. Phytopathology 106:380–385

Kolmer JA, Anderson JA (2011) First detection in North America of virulence in wheat leaf rust (*Puccinia triticina*) to seedling plants of wheat with *Lr21*. Plant Dis 95:1032–1032

Kolmer JA, Hughes ME (2015) Physiologic specialization of *Puccinia triticina* on wheat in the United States in 2013. Plant Dis 99:1261–1267

Kolmer JA, Hanzalova A, Goyeau H, Bayles R, Morgounov A (2013) Genetic differentiation of the wheat leaf rust fungus *Puccinia triticina* in Europe. Plant Pathol 62:21–31

Kolmer JA, Mirza JI, Imtiaz M, Shah SJA (2017) Genetic differentiation of the wheat leaf rust fungus *Puccinia triticina* in Pakistan and genetic relationship to other worldwide populations. Phytopathology 107:786–790

Kolmer JA, Bernardo A, Bai G, Hayden MJ, Chao S (2018a) Adult plant leaf rust resistance derived from Toropi wheat is conditioned by Lr78 and three minor QTL. Phytopathology 108:246–253

Kolmer JA, Chao S, Brown-Guedira G, Bansal U, Bariana H (2018b) Adult plant leaf rust resistance derived from the soft red winter wheat cultivar 'Caldwell' maps to chromosome 3BS. Crop Sci 58:152–158

Kolmer JA, Su Z, Bernardo A, Bai G, Chao S (2018c) Mapping and characterization of the new adult plant leaf rust resistance gene *Lr77* derived from Santa Fe winter wheat. Theor Appl Genet 131:1553–1560

Konieczny A, Ausubel FM (1993) A procedure for mapping Arabidopsis mutations using co-dominant ecotype-specific PCR-based markers. Plant J 4:403–410

Kover PX, Valdar W, Trakalo J, Scarcelli N, Ehrenreich IM, Purugganan MD, Durrant C, Mott R (2009) A multiparent advanced generation inter-cross to fine-map quantitative traits in *Arabidopsis thaliana*. PLoS Genet 5:

Krattinger S, Wicker T, Keller B (2009a) Map-based cloning of genes in Triticeae (wheat and barley). In: Genetics and genomics of the Triticeae. Springer, New York, pp 337–357

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

Kumar S, Kashyap PL, Singh GP, He X, Gupta V, Bainsla NK, Chawade A, Singh PK (2020) Breeding for wheat blast resistance. In: Wheat Blast. CRC Press, pp 163–174

Kumar S, Singroha G, Singh GP, Sharma P (2021) Karnal bunt of wheat: etiology, breeding and integrated management. Crop Prot 139:

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

Lagudah ES, Krattinger SG, Herrera-Foessel S, Singh RP, Huerta-Espino J, Spielmeyer W, Brown-Guedira G, Selter LL, Keller B (2009) Gene-specific markers for the wheat gene *Lr34/Yr18/Pm38* which confers resistance to multiple fungal pathogens. Theor Appl Genet 119:889–898

Lan C, Hale IL, Herrera-Foessel SA, Basnet BR, Randhawa MS, Huerta-Espino J, Dubcovsky J, Singh RP (2017a) Characterization and mapping of leaf rust and stripe rust resistance loci in hexaploid wheat lines UC1110 and PI610750 under Mexican environments. Front Plant Sci 8:1–11

Lan C, Randhawa MS, Huerta-Espino J, Singh RP (2017b) Genetic analysis of resistance to wheat rusts. In: Methods in molecular biology. Humana Press Inc., pp 137–149

Lander ES, Botstein S (1989) Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185

Lander E, Kruglyak L (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet 11:241–247

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Large EC (1954) Growth stages in cereals illustration of the Feekes scale. Plant Pathol 3:128–129

Levy A, Salas Gonzalez I, Mittelviefhaus M, Clingenpeel S, Herrera Paredes S, Miao J, Wang K, Devescovi G, Stillman K, Monteiro F, Rangel Alvarez B, Lundberg DS, Lu T, Lebeis S, Jin Z, McDonald M, Klein AP, Feltcher ME, Rio TG, Grant SR, Doty SL, Ley RE, Zhao B, Venturi V, Pelletier DA, Vorholt JA, Tringe SG, Woyke T, Dangl JL (2018) Genomic features of bacterial adaptation to plants. Nat Genet 50:138–150

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 1:1–9

Li H, Ye G, Wang J (2007) A modified algorithm for the improvement of composite interval mapping. Genetics 175:361–374

Li Z, Lan C, He Z, Singh RP, Rosewarne GM, Chen X, Xia X (2014) Overview and application of QTL for adult plant resistance to leaf rust and powdery mildew in wheat. Crop Sci 54:1907–1925

Lillemo M, Singh RP, Huerta-Espino J, Chen XM, He ZH, Brown JKM (2007) Leaf rust resistance gene *LR34* is involved in powdery mildew resistance of CIMMYT bread wheat line Saar. In: Wheat production in stressed environments. Springer, Dordrecht, pp 97–102

Lillemo M, Singh RP, William M, Herrera-Foessel S, Huerta-Espino J, Germán S, Campos P, Chaves M, Madriaga R, Xia XC, Liang SS, Liu D, Li ZF, Lagudah ES (2011) Multiple rust resistance and gene additivity in wheat: lessons from multi-location case studies in cultivars Parula and Saar. Borlaug Glob Rust Initiat 2011 Tech Work, 13–16 June, St Paul, Minnesota, USA, oral present, pp 111–120

Litt M, Luty JA (1989) A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am J Hum Genet 44:397–401

Liu K, Muse SV (2005) PowerMaker: an integrated analysis environment for genetic maker analysis. Bioinformatics 21:2128–2129

Liu YG, Shirano Y, Fukaki H, Yanai Y, Tasaka M, Tabata S, Shibata D (1999) Complementation of plant mutants with large genomic DNA fragments by a transformation-competent artificial chromosome vector accelerates positional cloning. Proc Natl Acad Sci USA 96:6535–6540

Liu L, Wang L, Yao J, Zheng Y, Zhao C (2010) Association mapping of six agronomic traits on chromosome 4A of wheat (*Triticum aestivum* L.). Mol Plant Breed 1:1–10

Liu W, Frick M, Huel R, Nykiforuk CL, Wang X, Gaudet D a, Eudes F, Conner RL, Kuzyk A, Chen Q, Kang Z, Laroche A (2014) The stripe rust resistance gene *Yr10* encodes an evolutionary-conserved and unique CC–NBS–LRR sequence in wheat. Mol Plant 7:1740–1755

Liu H, Niu Y, Gonzalez-Portilla PJ, Zhou H, Wang L, Zuo T, Qin C, Tai S, Jansen C, Shen Y, Lin H, Lee M, Ware D, Zhang Z, Lübberstedt T, Pan G (2015) An ultra-high-density map as a community resource for discerning the genetic basis of quantitative traits in maize. BMC Genom 16:1078

Liu J, He Z, Rasheed A, Wen W, Yan J, Zhang P, Wan Y, Zhang Y, Xie C, Xia X (2017) Genome-wide association mapping of black point reaction in common wheat (*Triticum aestivum* L.). BMC Plant Biol 17:220

Liu Y, Salsman E, Fiedler JD, Hegstad JB, Green A, Mergoum M, Zhong S, Li X (2019) Genetic mapping and prediction analysis of FHB resistance in a hard red spring wheat breeding population. Front Plant Sci 10:1–10

Liu Y, Salsman E, Wang R, Galagedara N, Zhang Q, Fiedler JD, Liu Z, Xu S, Faris JD, Li X (2020) Meta-QTL analysis of tan spot resistance in wheat. Theor Appl Genet 133:2363–2375

Loannou PA, Amemiya CT, Garnes J, Kroisel PM, Shizuya H, Chen C, Batzer MA, de Jong PJ (1994) A new bacteriophage P1-derived vector for the propagation of large human DNA fragments. Nat Genet 6:84–89

Long DL, Leonard KJ, Hughes ME (2000) Virulence of *Puccinia triticina* on wheat in the United States from 1996 to 1998. Plant Dis 84:1334–1341

Lopes MS, Dreisigacker S, Peña RJ, Sukumaran S, Reynolds MP (2015) Genetic characterization of the wheat association mapping initiative (WAMI) panel for dissection of complex traits in spring wheat. Theor Appl Genet 128:453–464

Lyon B, Broders K (2017) Impact of climate change and race evolution on the epidemiology and ecology of stripe rust in central and eastern USA and Canada. Can J Plant Pathol 39:385–392

Maccaferri M, Ricci A, Salvi S, Milner SG, Noli E, Martelli PL, Casadio R, Akhunov E, Scalabrin S, Vendramin V, Ammar K, Blanco A, Desiderio F, Distelfeld A, Dubcovsky J, Fahima T, Faris J, Korol A, Massi A, Mastrangelo AM, Morgante M, Pozniak C, N'Diaye A, Xu S, Tuberosa R (2015) A high-density, SNP-based consensus map of tetraploid wheat as a bridge to integrate durum and bread wheat genomics and breeding. Plant Biotechnol J 13:648–663

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, Zhang P, Vautrin S, Šimková H, Bansal U, Luo M-C, Rouse M, Karaoglu H, Periyannan S, Kolmer J, Jin Y, Ayliffe MA, Bariana H, Park RF, McIntosh R, Doležel J, Bergès H, Spielmeyer W, Lagudah ES, Ellis JG, Dodds PN (2015) The wheat *Sr50* gene reveals rich diversity at a cereal disease resistance locus. Nat Plants 1:15186

Manly KF, Cudmore RH, Meer JM (2001) Map Manager QTX, cross-platform software for genetic mapping. Mamm Genome 12:930–932

Marasas CN, Smale M, Singh RP (2003) The economic impact of productivity maintenance research: breeding for leaf rust resistance in modern wheat. Agric Econ 29:253–263

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 diverse resistance spectra to yellow rust. Nat Plants 4:662–668

Markell SG, Milus EA (2008) Emergence of a novel population of *Puccinia striiformis* f. sp. *tritici* in eastern United States. Phytopathology 98:632–639

Marone D, Laidò G, Gadaleta A, Colasuonno P, Ficco DBM, Giancaspro A, Giove S, Panio G, Russo MA, De Vita P, Cattivelli L, Papa R, Blanco A, Mastrangelo AM (2012) A high-density consensus map of A and B wheat genomes. Theor Appl Genet 125:1619–1638

Martínez F, Niks RE, Singh RP, Rubiales D (2001) Characterization of *Lr46*, a gene conferring partial resistance to wheat leaf rust. Hereditas 135:111–114

Mayer KFX, Rogers J, el Dole J, Pozniak C, Eversole K, Feuillet C, Gill B, Friebe B, Lukaszewski AJ, Sourdille P, Endo TR, Kubalakova M, Ihalikova J, Dubska Z, Vrana J, Perkova R, Imkova H, Febrer M, Clissold L, McLay K, Singh K, Chhuneja P, Singh NK, Khurana J, Akhunov E, Choulet F, Alberti A, Barbe V, Wincker P, Kanamori H, Kobayashi F, Itoh T, Matsumoto T, Sakai H, Tanaka T, Wu J, Ogihara Y, Handa H, Maclachlan PR, Sharpe A, Klassen D, Edwards D, Batley J, Olsen O-A, Sandve SR, Lien S, Steuernagel B, Wulff B, Caccamo M, Ayling S, Ramirez-Gonzalez RH, Clavijo BJ, Wright J, Pfeifer M, Spannagl M, Martis MM, Mascher M, Chapman J, Poland JA, Scholz U, Barry K, Waugh R, Rokhsar DS, Muehlbauer GJ, Stein N, Gundlach H, Zytnicki M, Jamilloux V, Quesneville H, Wicker T, Faccioli P, Colaiacovo M, Stanca AM, Budak H, Cattivelli L, Glover N, Pingault L, Paux E, Sharma S, Appels R, Bellgard M, Chapman B, Nussbaumer T, Bader KC, Rimbert H, Wang S, Knox R, Kilian A, Alaux M, Alfama F, Couderc L, Guilhot N, Viseux C, Loaec M, Keller B, Praud S (2014) A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome. Science 345:1251788–1251788

McFadden ES (1930) A successful transfer of emmer characters to vulgare wheat. Agron J 22:1020–1034

McIntosh RA (1998) Breeding wheat for resistance to biotic stresses. Euphytica 100:19–34

McIntosh RA, Wellings C, Park R (1996) Wheat rusts: an atlas of resistance genes. Australas Plant Pathol 25:70–70

McIntosh RA, Dubcovsky J, Rogers WJ, Morris C, Xia XC, Designators L (2017) Catalogue of gene symbols for wheat: 2017 supplement. Page Komugi Wheat Genet. Resour. Database. Kyoto, Japan, updated Jan. 11 p

McMullen MD, Kresovich S, Villeda HS, Bradbury P, Li H, Sun Q, Flint-Garcia S, Thornsberry J, Acharya C, Bottoms C, Brown P, Browne C, Eller M, Guill K, Harjes C, Kroon D, Lepak N, Mitchell SE, Peterson B, Pressoir G, Romero S, Rosas MO, Salvo S, Yates H, Hanson M, Jones E, Smith S, Glaubitz JC, Goodman M, Ware D, Holland JB, Buckler ES (2009) Genetic properties of the maize nested association mapping population. Science 325:737–740

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

Meng L, Li H, Zhang L, Wang J (2015) QTL IciMapping: integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. Crop J 3:269–283

Mert Z, Karakaya A, Düşünceli F, Akan K, Çetin L (2012) Determination of *Puccinia graminis* f. sp. *tritici* races of wheat in Turkey. Turkish J Agric For 36:107–120

Meuwissen THE, Hayes BJ, Goddard ME (2001) Prediction of total genetic value using genome-wide dense marker maps. Genetics 157:1819–1829

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Milus EA, Kristensen K, Hovmøller MS (2009) Evidence for increased aggressiveness in a recent widespread strain of *Puccinia striiformis* f. sp. *tritici* causing stripe rust of wheat. Phytopathology 99:89–94

Milus EA, Lee KD, Brown-Guedira G (2015) Characterization of stripe rust resistance in wheat lines with resistance gene *Yr17* and implications for evaluating resistance and virulence. Phytopathology 105:1123–1130

Ming R, Moore PH, Zee F, Abbey CA, Ma H, Paterson AH (2001) Construction and characterization of a papaya BAC library as a foundation for molecular dissection of a tree-fruit genome. Theor Appl Genet 102:892–899

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

Mohan M, Nair S, Bhagwat A, et al (1997) Genome mapping, molecular markers and marker-assisted selection in crop plants. Mol Breed 3:87–103

Monaco AP, Larin Z (1994) YACs, BACs, PACs and MACs: artificial chromosomes as research tools. Trends Biotechnol 12:280–286

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 RP, Lagudah E (2015) A recently evolved hexose transporter variant confers resistance to multiple pathogens in wheat. Nat Genet 47:1494–1498

Moose SP, Mumm RH (2008) Molecular plant breeding as the foundation for 21st century crop improvement. Plant Physiol 147:969–977

Mourad AMI, Sallam A, Belamkar V, Mahdy E, Bakheit B, Abo El-Wafaa A, Stephen Baenziger P (2018a) Genetic architecture of common bunt resistance in winter wheat using genome-wide association study. BMC Plant Biol 18:280

Mourad AMI, Sallam A, Belamkar V, Wegulo S, Bowden R, Jin Y, Mahdy E, Bakheit B, El-Wafaa AA, Poland J, Baenziger PS (2018b) Genome-wide association study for identification and validation of novel SNP markers for *Sr6* stem rust resistance gene in bread wheat. Front Plant Sci 9:380

Mourad AMI, Alomari DZ, Alqudah AM, Sallam A, Salem KFM (2019a) Recent advances in wheat (*Triticum* spp.) breeding. In: Advances in plant breeding strategies: cereals. Springer International Publishing, pp 559–593

Mourad AMI, Sallam A, Belamkar V, Wegulo S, Bai G, Mahdy E, Bakheit B, Abo El-Wafa A, Jin Y, Baenziger PS (2019b) Molecular marker dissection of stem rust resistance in Nebraska bread wheat germplasm. Sci Rep 9:1–10

Mourad AMI, Belamkar V, Baenziger PS (2020) Molecular genetic analysis of spring wheat core collection using genetic diversity, population structure, and linkage disequilibrium. BMC Genom 21:434

Muqaddasi QH, Zhao Y, Rodemann B, Plieske J, Ganal MW, Röder MS (2019) Genome-wide association mapping and prediction of adult stage *Septoria tritici* blotch infection in european winter wheat via high-density marker arrays. Plant Genome 12:1–13

Neelam K, Brown-Guedira G, Huang L (2013) Development and validation of a breeder-friendly KASPar marker for wheat leaf rust resistance locus *Lr21*. Mol Breed 31:233–237

Nirmala J, Chao S, Olivera P, Babiker EM, Abeyo B, Tadesse Z, Imtiaz M, Talbert L, Blake NK, Akhunov E, Pumphrey MO, Jin Y, Rouse MN (2016) Markers linked to wheat stem rust resistance gene *Sr11* effective to *Puccinia graminis* f. sp. *tritici* race TKTTF. Phytopathology 106:1352–1358

Nsabiyera V, Qureshi N, Bariana HS, Wong D, Forrest KL, Hayden MJ, Bansal UK (2016) Molecular markers for adult plant leaf rust resistance gene *Lr48* in wheat. Mol Breed 36:1–9

Nsabiyera V, Bariana HS, Qureshi N, Wong D, Hayden MJ, Bansal UK (2018) Characterisation and mapping of adult plant stripe rust resistance in wheat accession Aus27284. Theor Appl Genet 131:1459–1467

Nsabiyera V, Baranwal D, Qureshi N, Kay P, Forrest K, Valárik M, Doležel J, Hayden MJ, Bariana HS, Bansal UK (2020) Fine mapping of *Lr49* Using 90 K SNP chip array and flow-sorted chromosome sequencing in wheat. Front Plant Sci 10:1787

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

Oerke EC, Dehne HW (2004) Safeguarding production—losses in major crops and the role of crop protection. Crop Prot 23:275–285

Olivera Firpo PD, Jin Y, Rouse M, Badebo A, Fetch T, Singh RP, Yahyaoui A (2012) Races of *Puccinia graminis* f. sp. *tritici* with combined virulence to *Sr13* and *Sr9e* in a field stem rust screening nursery in Ethiopia. Plant Dis 96:623–628

Olivera Firpo PD, Newcomb M, Szabo LJ, Rouse M, Johnson J, Gale S, Luster DG, Hodson DP, Cox JA, Burgin L, Hort M, Gilligan CA, Patpour M, Justesen AF, Hovmøller MS, Woldeab G, Hailu E, Hundie B, Tadesse K, Pumphrey M, Singh RP, Jin Y (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 PD, Newcomb M, Flath K, Sommerfeldt-Impe N, Szabo LJ, Carter M, Luster DG, 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

Ordoñez ME, Kolmer JA (2007) Virulence phenotypes of a worldwide collection of *Puccinia triticina* from durum wheat. Phytopathology 97:344–351

Ornella L, Sukhwinder-Singh Perez P, Burgueño J, Singh R, Tapia E, Bhavani S, Dreisigacker S, Braun HJ, Mathews K, Crossa J (2012) Genomic prediction of genetic values for resistance to wheat rusts. Plant Genome 5:136–148

Pakeerathan K, Bariana H, Qureshi N, Wong D, Hayden M, Bansal U (2019) Identification of a new source of stripe rust resistance *Yr82* in wheat. Theor Appl Genet 132:3169–3176

Paran I, Michelmore RW (1993) Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor Appl Genet 85:985–993

Pardey PG, Beddow JM, Kriticos DJ, Hurley TM, Park RF, Duveiller E, Sutherst RW, Burdon JJ, Hodson D (2013) Right-sizing stem-rust research. Science 340:147–148

Parida SK, Anand Raj Kumar K, Dalal V, Singh NK, Mohapatra T (2006) Unigene derived microsatellite markers for the cereal genomes. Theor Appl Genet 112:808–817

Park RF (2007) Stem rust of wheat in Australia. Aust J Agric Res 58:558

Parks R, Carbone I, Murphy JP, Marshall D, Cowger C (2008) Virulence structure of the eastern U.S. wheat powdery mildew population. Plant Dis 92:1074–1082

Patpour M, Hovmøller MS, Justesen AF, Newcomb M, Olivera Firpo PD, Jin Y, Szabo LJ, Hodson DP, Shahin AA, Wanyera R, Habarurema I, Wobibi S (2016) Emergence of virulence to *SrTmp* in the Ug99 race group of wheat stem rust, *Puccinia graminis* f. sp. *tritici*, in Africa. Plant Dis 100:522

Patpour M, Justesen AF, Tecle AW, Yazdani M, Yasaie M, Hovmøller MS (2020) First report of race TTRTF of wheat stem rust (*Puccinia graminis* f. sp. *tritici*) in Eritrea. Plant Dis 104:973

Paux E, Sourdille P, Salse J, Saintenac C, Choulet F, Leroy P, Korol A, Michalak M, Kianian S, Spielmeyer W, Lagudah E, Somers D, Kilian A, Alaux M, Vautrin S, Bergès H, Eversole K, Appels R, Safar J, Simkova H, Dolezel J, Bernard M, Feuillet C (2008) A physical map of the 1-gigabase bread wheat chromosome 3B. Science 322:101–104

Peng JH, Bai Y, Haley SD, Lapitan NLV (2009) Microsatellite-based molecular diversity of bread wheat germplasm and association mapping of wheat resistance to the Russian wheat aphid. Genetica 135:95–122

Periyannan S (2018) Sustaining global agriculture through rapid detection and deployment of genetic resistance to deadly crop diseases. New Phytol 219:45–51

Periyannan S, Moore J, Ayliffe M, Bansal U, Wang X, Huang L, Deal K, Luo M, Kong X, Bariana H, Mago R, McIntosh R, Dodds P, Dvorak J, Lagudah E (2013) The gene *Sr33*, an ortholog of barley *Mla* genes, encodes resistance to wheat stem rust race Ug99. Science 341:786–788

Peters JM, Koo BM, Patino R, Heussler GE, Hearne CC, Qu J, Inclan YF, Hawkins JS, Lu CHS, Silvis MR, Harden MM, Osadnik H, Peters JE, Engel JN, Dutton RJ, Grossman AD, Gross CA, Rosenberg OS (2019) Enabling genetic analysis of diverse bacteria with Mobile-CRISPRi. Nat Microbiol 4:244–250

Poland J, Rutkoski J (2016) Advances and challenges in genomic selection for disease resistance. Annu Rev Phytopathol 54:79–98

Poland J, Endelman J, Dawson J, Rutkoski J, Wu S, Manes Y, Dreisigacker S, Crossa J, Sánchez-Villeda H, Sorrells M, Jannink J (2012a) Genomic selection in wheat breeding using genotyping-by-sequencing. Plant Genome 5:103–113

Poland JA, Brown PJ, Sorrells ME, Jannink J-L (2012b) Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. PLoS ONE 7:

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 84:203–203

Pretorius ZA, Pakendorf KW, Marais GF, Prins R, Komen JS (2007) Challenges for sustainable cereal rust control in South Africa. Aust J Agric Res 58:593

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 38:904–909

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Qiu L, Wang H, Li Y, Wang W, Liu Y, Mu J, Geng M, Guo W, Hu Z, Ma J, Sun Q, Xie C (2020) Fine mapping of the wheat leaf rust resistance gene *LrLC10* (*Lr13*) and validation of its co-segregation markers. Front Plant Sci 11:470

Qureshi N, Bariana H, Forrest K, Hayden M, Keller B, Wicker T, Faris J, Salina E, Bansal U (2017a) Fine mapping of the chromosome 5B region carrying closely linked rust resistance genes *Yr47* and *Lr52* in wheat. Theor Appl Genet 130:495–504

Qureshi N, Bariana H, Kolmer JA, Miah H, Bansal U (2017b) Genetic and molecular characterization of leaf rust resistance in two durum wheat landraces. Phytopathology 107:1381–1387

Qureshi N, Bariana H, Kumran VV, Muruga S, Forrest KL, Hayden MJ, Bansal U (2018a) A new leaf rust resistance gene *Lr79*mapped in chromosome 3BL from the durum wheat landrace Aus26582. Theor Appl Genet 131:1091–1098

Qureshi N, Bariana HS, Zhang P, McIntosh R, Bansal UK, Wong D, Hayden MJ, Dubcovsky J, Shankar M (2018b) Genetic relationship of stripe rust resistance genes *Yr34* and *Yr48* in wheat and identification of linked KASP markers. Plant Dis 102:413–420

Qureshi N, Kandiah P, Gessese MK, Nsabiyera V, Wells V, Babu P, Wong D, Hayden M, Bariana H, Bansal U (2018c) Development of co-dominant KASP markers co-segregating with Ug99 effective stem rust resistance gene *Sr26* in wheat. Mol Breed 38:1–9

Ramirez-Gonzalez RH, Segovia V, Bird N, Fenwick P, Holdgate S, Berry S, Jack P, Caccamo M, Uauy C (2015) RNA-Seq bulked segregant analysis enables the identification of high-resolution genetic markers for breeding in hexaploid wheat. Plant Biotechnol J 13:613–624

Randhawa M, Bansal U, Valárik M, Klocová B, Doležel 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 35:1–8

Rao AS, Brakke MK (1969) Relation of soil-borne wheat mosaic virus and its fungal vector, *Polymyxa graminis*. Phytopathology 59:581–587

Rapilly F (1979) Yellow rust epidemiology. Annu Rev Phytopathol 17:59–73

Rasheed A, Wen W, Gao F, Zhai S, Jin H, Liu J, Guo Q, Zhang Y, Dreisigacker S, Xia X, He Z (2016) Development and validation of KASP assays for genes underpinning key economic traits in bread wheat. Theor Appl Genet 129:1843–1860

Reif JC, Gowda M, Maurer HP, Longin CFH, Korzun V, Ebmeyer E, Bothe R, Pietsch C, Würschum T (2011) Association mapping for quality traits in soft winter wheat. Theor Appl Genet 122:961–970

Ribaut JM, Hoisington D (1998) Marker-assisted selection: new tools and strategies. Trends Plant Sci 3:236–239

Ribaut JM, Jiang C, Hoisington D (2002) Simulation experiments on efficiencies of gene introgression by backcrossing. Crop Sci 42:557–565

Ritland K (2005) Multilocus estimation of pairwise relatedness with dominant markers. Mol Ecol 14:3157–3165

Röder MS, Plaschke J, König SU, Börner A, Sorrells ME, Tanksley SD, Ganal MW (1995) Abundance, variability and chromosomal location of microsatellites in wheat. MGG Mol Gen Genet 246:327–333

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Roelfs AP, Singh RP, Saari EE (1992) Rust diseases of wheat: concepts and methods of disease management. In: Hettel GP (ed). CIMMYT, Mexico, pp 1–81

Rosewarne GM, Herrera-Foessel SA, Singh RP, Huerta-Espino J, Lan CX, He ZH (2013) Quantitative trait loci of stripe rust resistance in wheat. Theor Appl Genet 126:2427–2449

Royse DJ, Gregory LV, Ayers JE, Cole H (1980) Powdery mildew of wheat: relation of yield components to disease severity. Can J Plant Pathol 2:131–136

Rutkoski JE, Heffner EL, Sorrells ME (2011) Genomic selection for durable stem rust resistance in wheat. Euphytica 179:161–173

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, Singh RP, Huerta-Espino J, Bhavani S, Poland J, Jannink JL, Sorrells ME (2015) Genetic gain from phenotypic and genomic selection for quantitative resistance to stem rust of wheat. Plant Genome 8:1–10

Ruud AK, Windju S, Belova T, Friesen TL, Lillemo M (2017) Mapping of SnTox3–Snn3 as a major determinant of field susceptibility to septoria nodorum leaf blotch in the SHA3/CBRD × Naxos population. Theor Appl Genet 130:1361–1374

Ruud AK, Dieseth JA, Ficke A, Furuki E, Phan HTT, Oliver RP, Tan K-C, Lillemo M (2019) Genome-wide association mapping of resistance to septoria nodorum leaf blotch in a nordic spring wheat collection. Plant Genome 12:

Šafář J, Bartoš J, Janda J, Bellec A, Kubaláková M, Valárik M, Pateyron S, Weiserová J, Tušková R, Číhalíková J, Vrána J, Šimková H, Faivre-Rampant P, Sourdille P, Caboche M, Bernard M, Doležel J, Chalhoub B (2004) Dissecting large and complex genomes: flow sorting and BAC cloning of individual chromosomes from bread wheat. Plant J 39:960–968

Saintenac C, Zhang W, Salcedo A, Rouse MN, Trick HN, Akhunov E, Dubcovsky J (2013) Identification of wheat gene *Sr35* that confers resistance to Ug99 stem rust race group. Science 341:783–786

Salimath SS, Bhattacharyya MK (1999) Generation of a soybean BAC library, and identification of DNA sequences tightly linked to the *Rps1-k* disease resistance gene. Theor Appl Genet 98:712–720

Salimath SS, De Oliveira AC, Godwin ID, Bennetzen JL (1995) Assessment of genome origins and genetic diversity in the genus *Eleusine* with DNA markers. Genome 38:757–763

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

Saunders DGO, Pretorius ZA, Hovmøller MS (2019) Tackling the re-emergence of wheat stem rust in western Europe. Commun Biol 2:9–11

Savary S, Willocquet L, Pethybridge SJ, et al (2019) The global burden of pathogens and pests on major food crops.Nat Ecol Evol 3:430–439

Schachermayr G, 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

Scheben A, Wolter F, Batley J, Puchta H, Edwards D (2017) Towards CRISPR/Cas crops—bringing together genomics and genome editing. New Phytol 216:682–698

Scherm B, Balmas V, Spanu F, Pani G, Delogu G, Pasquali M, Migheli Q (2013) *Fusarium culmorum*: causal agent of foot and root rot and head blight on wheat. Mol Plant Pathol 14:323–341

Sears ER (1954) The aneuploids of common wheat. E R Sears 572:1–58

Semagn K, Bjørnstad Å, Xu Y (2010) The genetic dissection of quantitative traits in crops. Electron J Biotechnol 13:1–45

Shah L, Rehman S, Ali A, Yahya M, Riaz MW, Si H, Ma C, Lu J (2018) Genes responsible for powdery mildew resistance and improvement in wheat using molecular marker-assisted selection. J Plant Dis Prot 125:145–158

Sharma I (ed) (2012) Disease resistance in wheat. CABI Publishing, Wallingford, pp 1–336

Shaul O (2015) Unique aspects of plant nonsense-mediated mRNA decay. Trends Plant Sci 20:767–779

Shewry PR, Tatham AS (2016) Improving wheat to remove coeliac epitopes but retain functionality. J Cereal Sci 67:12–21

Shi X, Zeng H, Xue Y, Luo M (2011) A pair of new BAC and BIBAC vectors that facilitate BAC/BIBAC library construction and intact large genomic DNA insert exchange. Plant Meth 7:33

Shizuya H, Birren B, Kim UJ, Mancino V, Slepak T, Tachiiri Y, Simon M (1992) Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in *Escherichia coli* using an F-factor-based vector. Proc Natl Acad Sci USA 89:8794–8797

Simmonds NW, Rajaram S (1988) Breeding strategies for resistance to the rusts of wheat. CIMMYT Publ Repos

Singh RP (1992a) Expression of wheat leaf rust resistance gene *Lr34* in seedlings and adult plants. Plant Dis 76:489

Singh RP (1992b) Association between gene *Lr34* for leaf rust resistance and leaf tip necrosis in wheat. Crop Sci 32:874–878

Singh RP, Hodson DP, Jin Y, et al (2006) Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. CAB Rev Perspect Agric Vet Sci Nutr Nat Resour 1

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, Huerta-Espino J, Pfeiffer W, Figueroa-Lopez P (2004a) Occurrence and impact of a new leaf rust race on durum wheat in northwestern Mexico from 2001 to 2003. Plant Dis 88:703–708

Singh RP, Rajaram S, Saini RG, Huerta-Espino J, William M (2004b) Genetics of host-pathogen interaction and breeding for durable resistance. In: Plant breeding. Springer, The Netherlands, pp 145–166

Singh RP, Hodson DP, Jin Y, Huerta-Espino J, Kinyua MG, 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

Singh S, Sharma I, Sehgal SK, Bains NS, Guo Z, Nelson JC, Bowden RL (2007) Molecular mapping of QTLs for Karnal bunt resistance in two recombinant inbred populations of bread wheat. Theor Appl Genet 116:147–154

Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Njau P, Wanyera R, Herrera-Foessel SA, Ward RW (2008a) Will stem rust destroy the world's wheat crop? Adv Agron 98:271–309

Singh RP, Huerta-Espino J, William M (2008b) Breeding for resistance to biotic stresses. Plant Breed Arnel R Hallauer Int Symp 310–322

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 (2015) 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

Singla J, Lüthi L, Wicker T, Bansal U, Krattinger SG, Keller B (2017) Characterization of *Lr75*: a partial, broad-spectrum leaf rust resistance gene in wheat. Theor Appl Genet 130:1–12

Soller M, Brody T, Genizi A (1976) On the power of experimental designs for the detection of linkage between marker loci and quantitative loci in crosses between inbred lines. Theor Appl Genet 47:35–39

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Somers DJ, Banks T, DePauw R, Fox S, Clarke J, Pozniak C, McCartney C (2007) Genome-wide linkage disequilibrium analysis in bread wheat and durum wheat. Genome 50:557–567

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB (2005) Development and mapping of microsatellite (SSR) markers in wheat. Theor Appl Genet 110:550–560

Song J, Yang D, Xu J, Zhu T, Chen YE, Zhang J (2016) RS-1 enhances CRISPR/Cas9- and TALEN-mediated knock-in efficiency. Nat Commun 7:10548

Sorrells ME, Gustafson JP, Somers D, Chao S, Benscher D, Guedira-Brown G, Huttner E, Kilian A, McGuire PE, Ross K, Tanaka J, Wenzl P, Williams K, Qualset CO, Van Deynze A (2011) Reconstruction of the synthetic W7984 × Opata M85 wheat reference population. Genome 54:875–882

Stacklies W, Redestig H, Scholz M, Walther D, Selbig J (2007) pcaMethods—a bioconductor package providing PCA methods for incomplete data. Bioinformatics 23:1164–1167

Stekhoven DJ, Bühlmann P (2012) Missforest-non-parametric missing value imputation for mixed-type data. Bioinformatics 28:112–118

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

Steuernagel B, Witek K, Jones JDG, Wulff BBH (2017) MutRenSeq: a method for rapid cloning of plant disease resistance genes. In: Methods in molecular biology. Humana Press Inc., pp 215–229

Su Z, Jin S, Zhang D, Bai G (2018) Development and validation of diagnostic markers for *Fhb1* region, a major QTL for Fusarium head blight resistance in wheat. Theor Appl Genet 131:2371–2380

Tanksley SD, Ganal MW, Martin GB (1995) Chromosome landing: a paradigm for map-based gene cloning in plants with large genomes. Trends Genet 11:63–68

Taylor J, Butler D (2017) R package ASMap: efficient genetic linkage map construction and diagnosis. J Stat Softw 79:1–29

Tembo B, Mulenga RM, Sichilima S, M'siska KK, Mwale M, Chikoti PC, Singh PK, He X, Pedley KF, Peterson GL, Singh RP, Braun HJ (2020) Detection and characterization of fungus (*Magnaporthe oryzae* pathotype *Triticum*) causing wheat blast disease on rain-fed grown wheat (*Triticum aestivum* L.) in Zambia. PLoS One 15:e0238724

Thind AK, Wicker T, Šimková H, Fossati D, Moullet O, Brabant C, Vrána J, Doležel J, Krattinger SG (2017) Rapid cloning of genes in hexaploid wheat using cultivar-specific long-range chromosome assembly. Nat Biotechnol 35:793–796

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

Torriani SFF, Melichar JPE, Mills C, Pain N, Sierotzki H, Courbot M (2015) *Zymoseptoria tritici*: a major threat to wheat production, integrated approaches to control. Fungal Genet Biol 79:8–12

Toth J, Pandurangan S, Burt A, Mitchell Fetch J, Kumar S (2019) Marker-assisted breeding of hexaploid spring wheat in the canadian prairies. Can J Plant Sci 99:111–127

Turuspekov Y, Ormanbekova D, Rsaliev A, Abugalieva S (2016) Genome-wide association study on stem rust resistance in Kazakh spring barley lines. BMC Plant Biol 16:6

Umehara Y, Inagaki A, Tanoue H, Yasukochi Y, Nagamura Y, Saji S, Otsuki Y, Fujimura T, Kurata N, Minobe Y (1995) Construction and characterization of a rice YAC library for physical mapping. Mol Breed 1:79–89

Upadhyaya HD, Wang YH, Gowda CLL, Sharma S (2013) Association mapping of maturity and plant height using SNP markers with the sorghum mini core collection. Theor Appl Genet 126:2003–2015

Utz HF, Melchinger AE (1996) PLABQTL: a program for composite interval mapping of QTL. J Agric Genomics 2:1–6

Van Ginkel M, Trethowan RM, Ammar K, Wang J, Lillemo M (2002) Guide to bread wheat breeding at CIMMYT. CIMMYT, Mexico, p 59

Van Ooijen JW (2006) JoinMap ® 4 software for the calculation of genetic linkage maps in experimental populations. JoinMap, Kyazma

Vignal A, Milan D, SanCristobal M, Eggen A (2002) A review on SNP and other types of molecular markers and their use in animal genetics. Genet Sel Evol 34:275

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

Visser B, Meyer M, Park RF, Gilligan CA, Burgin LE, Hort MC, Hodson DP, Pretorius ZA (2019) Microsatellite analysis and urediniospore dispersal simulations support the movement of *Puccinia graminis* f. sp. *tritici* from southern Africa to Australia. Phytopathology 109:133–144

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78

Vos P, Hogers R, Bleeker M, Reijans M, Van De Lee T, Hornes M, Friters A, Pot J, Paleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Voss-Fels KP, Cooper M, Hayes BJ (2019) Accelerating crop genetic gains with genomic selection. Theor Appl Genet 132

Wan AM, Chen XM, He ZH (2007) Wheat stripe rust in China. Aust J Agric Res 58:605–619

Wang J-K (2009) Inclusive composite interval mapping of quantitative trait genes. Acta Agron Sin 35:239–245

Wang M, Chen X (2017) Stripe Rust Resistance. In: Stripe rust. Springer, Dordrecht, pp 353–558

Wang S, Basten CJ, Zeng ZB (2007) Windows QTL Cartographer 2.5. http://statgen.ncsu.edu/qtlcart/WQTLCart.htm

Wang Q, Tian F, Pan Y, Buckler ES, Zhang Z (2014a) A SUPER powerful method for genome wide association study. PLoS One 9:

Wang S, Wong D, Forrest K, Allen A, Chao S, Huang BE, Maccaferri M, Salvi S, Milner SG, Cattivelli L, Mastrangelo AM, Whan A, Stephen S, Barker G, Wieseke R, Plieske J, Lillemo M, Mather D, Appels R, Dolferus R, Brown-Guedira G, Korol A, Akhunova AR, Feuillet C, Salse J, Morgante M, Pozniak C, Luo MC, Dvorak J, Morell M, Dubcovsky J, Ganal M, Tuberosa R, Lawley C, Mikoulitch I, Cavanagh C, Edwards KJ, Hayden M, Akhunov E (2014b) Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. Plant Biotechnol J 12:787–796

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

Wang H, Sun S, Ge W, Zhao L, Hou B, Wang K, Lyu Z, Chen L, Xu S, Guo J, Li M, Su P, Li X, Wang G, Bo C, Fang X, Zhuang W, Cheng X, Wu J, Dong L, Chen W, Li W, Xiao G, Zhao J, Hao Y, Xu Y, Gao Y, Liu W, Liu Y, Yin H, Li J, Li X, Zhao Y, Wang X, Ni F, Ma X, Li A, Xu SS, Bai G, Nevo E, Gao C, Ohm H, Kong L (2020) Horizontal gene transfer of Fhb7 from fungus underlies Fusarium head blight resistance in wheat. Science 368

Wanyera R, Kinyua MG, Jin Y, Singh RP (2006) The spread of stem rust caused by *Puccinia graminis* f. sp. *tritici*, with virulence on *Sr31* in wheat in Eastern Africa. Plant Dis 90:113–113

Weeden NF, Hemmatt M, Lawson DM, Lodhi M, Bell RL, Manganaris AG, Reischs BI, Brown SK, Ye GN (1994) Development and application of molecular marker linkage maps in woody fruit crops. Euphytica 77:71–75

Wellings CR (2011) Global status of stripe rust: a review of historical and current threats. Euphytica 179:129–141

Wellings CR, McIntosh RA (1990) *Puccinia striiformis* f.sp. *tritici* in Australasia: pathogenic changes during the first 10 years. Plant Pathol 39:316–325

Wellings CR, Wright DG, Keiper F, Loughman R (2003) First detection of wheat stripe rust in Western Australia: evidence for a foreign incursion. Australas Plant Pathol 32:321–322

William HM, Singh RP, Huerta-Espino J, Rosewarne G (2007) Characterization of genes for durable resistance to leaf rust and yellow rust in CIMMYT spring wheats. In: Buck HT, Nisi JE, Salomón N (eds) Wheat production in stressed environments. Developments in plant breeding. Springer, Dordrecht, pp 65–70

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Wulff BBH, Moscou MJ (2014) Strategies for transferring resistance into wheat: from wide crosses to GM cassettes. Front Plant Sci 5

Xia L, Ma Y, He Y, Jones HD (2012) GM wheat development in China: current status and challenges to commercialization. J Exp Bot 63:1785–1790

Xu K, He X, Dreisigacker S, He Z, Singh PK (2019) Anther extrusion and its association with fusarium head blight in CIMMYT wheat germplasm. Agronomy 10:47

Yao J, Wang L, Liu L, Zhao C, Zheng Y (2009) Association mapping of agronomic traits on chromosome 2A of wheat. Genetica 137:67–75

Yin X, Biswal AK, Dionora J, Perdigon KM, Balahadia CP, Mazumdar S, Chater C, Lin H-C, Coe RA, Kretzschmar T, Gray JE, Quick PW, Bandyopadhyay A (2017) CRISPR-Cas9 and CRISPR-Cpf1 mediated targeting of a stomatal developmental gene *EPFL9* in rice. Plant Cell Rep 36:745–757

Yu J, Pressoir G, Briggs WH, Bi IV, Yamasaki M, Doebley JF, McMullen MD, Gaut BS, Nielsen DM, Holland JB, Kresovich S, Buckler ES (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nat Genet 38:203–208

Yu GT, Cai X, Harris MO, Gu YQ, Luo MC, Xu SS (2009) Saturation and comparative mapping of the genomic region harboring hessian fly resistance gene *H26* in wheat. Theor Appl Genet 118:1589–1599

Yu X, Bai G, Liu S, Luo N, Wang Y, Richmond DS, Pijut PM, Jackson SA, Yu J, Jiang Y (2013) Association of candidate genes with drought tolerance traits in diverse perennial ryegrass accessions. J Exp Bot 64:1537–1551

Yu LX, Barbier H, Rouse MN, Singh S, Singh RP, Bhavani S, Huerta-Espino J, Sorrells ME (2014) A consensus map for Ug99 stem rust resistance loci in wheat. Theor Appl Genet 127:1561–1581

Yu LX, Chao S, Singh RP, Sorrells ME (2017) Identification and validation of single nucleotide polymorphic markers linked to Ug99 stem rust resistance in spring wheat. PLoS One 12

Zeng ZB (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468

Zeng ZB, Kao CH, Basten CJ (1999) Estimating the genetic architecture of quantitative traits. Genet Res 74:279–289

Zhang J, Hao C, Ren Q, Chang X, Liu G, Jing R (2011) Association mapping of dynamic developmental plant height in common wheat. Planta 234:891–902

Zhang W, Chen S, Abate Z, Nirmala J, Rouse MN, Dubcovsky J (2017a) Identification and characterization of *Sr13*, a tetraploid wheat gene that confers resistance to the Ug99 stem rust race group. Proc Natl Acad Sci USA 114:E9483–E9492

Zhang Y, Bai Y, Wu G, Zou S, Chen Y, Gao C, Tang D (2017b) Simultaneous modification of three homoeologs of *TaEDR1* by genome editing enhances powdery mildew resistance in wheat. Plant J 91:714–724

Zhang C, Huang L, Zhang H, Hao Q, Lyu B, Wang M, Epstein L, Liu M, Kou C, Qi J, Chen F, Li M, Gao G, Ni F, Zhang L, Hao M, Wang J, Chen X, Luo MC, Zheng Y, Wu J, Liu D, Fu D

(2019a) An ancestral NB-LRR with duplicated 3′UTRs confers stripe rust resistance in wheat and barley. Nat Commun 10

Zhang J, Zhang P, Hewitt T, Li J, Dundas I, Schnippenkoetter W, Hoxha S, Chen C, Park R, Lagudah E (2019b) A strategy for identifying markers linked with stem rust resistance in wheat harbouring an alien chromosome introgression from a non-sequenced genome. Theor Appl Genet 132:125–135

Zhu Z, Hao Y, Mergoum M, Bai G, Humphreys G, Cloutier S, Xia X, He Z (2019) Breeding wheat for resistance to Fusarium head blight in the Global North: China, USA, and Canada. Crop J 7:730–738

# Chapter 3
# Resistance to Biotic Stress: Theory and Applications in Maize Breeding

**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**

**Abstract** By virtue of its higher genetic diversity, maize has better adaptability to various climatic situations and has high yield potential than other cereals. However, the incidence of pests and diseases at different stages of the crop can reduce the yield drastically. Several strategies have been adopted to manage biotic stresses in maize to maintain the yielding ability. Apart from the chemical method of disease

R. N. Gadag · G. Mukri (✉) · K. Shilpa
Division of Genetics, ICAR-Indian Agricultural Research Institute, PUSA Campus, New Delhi 110012, India
e-mail: ganapati.mukri@icar.gov.in

J. S. Bhat
Regional Research Centre (ICAR-Indian Agricultural Research Institute), Dharwad, Karnataka 580005, India

R. Gogoi
Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, PUSA Campus, New Delhi 110012, India

S. B. Suby · A. K. Das
ICAR-Indian Institute of Maize Research, PAU Campus, Ludhiana 141004, India

S. Yadav
ICAR-National Institute for Plant Biotechnology, PUSA Campus, New Delhi 110012, India

P. Yadava
Division of Plant Physiology, ICAR-Indian Agricultural Research Institute, PUSA Campus, New Delhi 110012, India

M. L. Nithyashree
Division of Agricultural Economics, ICAR-Indian Agricultural Research Institute, PUSA Campus, New Delhi 110012, India

G. K. Naidu
All India Coordinated Research Project on Maize, University of Agricultural Sciences, Dharwad 580005, India

S. K. Yadav
Department of Biophysics, University of Delhi, South Campus, New Delhi 110012, India

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021

C. Kole (ed.), *Genomic Designing for Biotic Stress Resistant Cereal Crops*,
https://doi.org/10.1007/978-3-030-75879-0_3

management, improving the crop for natural resistance has paid much dividend for sustainable maize production. With the advent of high throughput phenotyping method followed by genotyping, targeted trait improvement has become easy. Molecular marker technology—a non-destructive method—enables indirect selection of genotypes without exposing them to epiphytotic condition. This has been found to be efficient over existing traditional methods of screening followed by selection. The information on QTLs, novel genomic resources have provided better understanding of tolerance traits. Although GE technologies have been successful in development of genotypes to combat pathogens in important crops, they are not yet fully exploited for the management of insect pests. The most important limitation has been the lack availability of target genes at present against the insect pests. Genome editing is becoming powerful tool which enables the possibilities of developing resistant gene by targeted gene modification. Though maize is recalcitrant to regeneration, protoplast transient assay made easy the utilization of CRISPR technology in developing disease resistant maize. Institutional support followed by policy intervention makes new technological interventions finding way for improving crops. Social beliefs and ethical issues should be taken care while targeting next generation breeding approaches to develop insect or disease resistant maize.

**Keywords** Biotic stresses · Genetic diversity · Breeding approaches · Molecular mapping · New biotechnological tools · Transgenics · Social issues

## 3.1 Introduction

Maize (*Zea mays* L.) is the most important cereal crop worldwide after wheat and rice with the global production of 1147 Mt. Among the top corn producing countries, United States hold the first position with the yield of 11.86 tha$^{-1}$, followed by European Union, Ukraine, China, Argentina and India. Generally, maize is grown for grain or fodder and silage production. It is having direct economic value on mankind as grain is primarily grown for human consumption, especially in tropics. In Asia, compared to human food, the demand for maize as an animal feed will have more impact on the production scenario. More than doubling of production is expected from the present level of 165 Mt to almost 400 Mt in 2030 (Paliwal et al. 2000). The Food and Agriculture Organisation (FAO) predicts the requirement of an additional 60 Mt of maize grain to meet the demand by 2030. Maize is a versatile crop, having wider adaptability to different climatic situations, from temperate to tropical conditions. Being a C4 crop, maize has the highest yield potentiality compared to other cereals, but due to the damage by insect and pest attack, global maize production is under threat. One of the main deterrents to achieving grain yield in maize is its susceptibility to many pests and diseases (Devi and Thakur 2018).

## 3.2 Description on Different Biotic Stresses

### *3.2.1 Maize Diseases*

Among the maize diseases caused by fungi, bacteria and viruses, fungal diseases like banded leaf and sheath blight (BLSB), turcicum leaf blight (TLB), maydis leaf blight (MLB), post-flowering stalk rots (PFSR) complex, downy mildews (DM), rust, smut, seed rots and seedling blights, leaf spots and blights etc. are of major concern (Saxena et al. 2008). Under favourable conditions, these diseases cause immense losses to both quantity and quality of grain produced. World maize trade in 2019–20 is now forecast to reach nearly 167 Mt, almost unchanged from the previous season despite experiencing annual global yield loss recorded up to 20–41% in maize (FAO 2020).

**Maydis leaf blight** (MLB) disease or southern corn leaf blight (SCLB), caused by *Bipolaris maydis* (Nishik. and Miyake) Shoemaker [*Cochliobolus heterostrophus* (Drech.) Drech.] is one of the impending threats to global maize production. The pathogen *B. maydis* possesses three physiological races viz. race O, race T (Hooker 1972; Ullstrup 1972), and race C (Wei et al. 1988). The race-T is more prevalent in the United States of America (USA). In USA, it resulted in an epidemic during 1970 by the extensive usage of CMS-T cytoplasm based maize lines to develop commercial maize hybrids. The race C is prevalent in China and is pathogenic on maize inbred lines having CMS-C cytoplasm (Wei et al. 1988). On the other hand, the race 'O' is predominantly prevalent in the southern Atlantic coast of the USA, India, Africa, and Western Europe (Balint Kurti et al. 2007), which can infect all types of susceptible maize cultivars, irrespective of the cytoplasm (Smith 1975) and can reduce the grain yield up to 41% (Sharma et al. 2005).

**Banded leaf and sheath blight (BLSB)** disease is caused by a versatile soil borne fungus *Rhizoctonia solani* f. sp. *sasakii* (Kuhn) Exner [teleomorph: *Corticium sasakii*, syn. *Thanatephorus cucumeris* Frank (Donk)] which is not producing any spores. Generally, this pathogen is identified by characteristics of the mycelium and sclerotia. The pathogen is an imperfect fungus (Deutermycetes) belonging to AG 1-1A anastomosis group of *R. solani* isolates (Yang and Li 2012; Hooda et al. 2015).

**Post-flowering stalk rots** (PFSR) are the world's most destructive diseases of corn. Diseases such as Fusarium Stalk Rot (*Fusarium verticillioides* (Sacc) Nirenberg, Syn *F. moniliformae*), Charcoal Rot (*Macrophomina phaseolina* (Tassi) Goid.) and Late Wilt (*Cephalosporium maydis* Samra, Sabet. and Hingorani) are commonly associated with PFSR. Among them, charcoal rot (*M. phaseolina*) is dominant one and occurs as a complex along with *F. verticilloides* in some locations. *M. phaseolina* is an anamorphic ascomycete of the family Botryosphaeriaceae and causes the disease charcoal rot on a broad range of plants in many areas of the world. The lack of a known teleomorph has hindered its proper taxonomy (Crous et al. 2006).

**Turicum leaf blight** (TLB) or northern corn leaf blight (NCLB) is another important disease caused by an Ascomycete *Exserohilum turcicum* (Pass.) Leonard and Suggs [*Setosphaeria turcica* (Luttr.) K. J. Leonard and Suggs, formerly known as *Helminthosporium turcicum*] which belongs to family Pleosporacae (Leonard et al. 1989). In the United States various races of the pathogen exist, of which race 'O' was predominant in the mid-1970s, Race 1 was the most prevalent race in the region by the mid-1990s (Ferguson and Carson 2007). The Indian scenario of the races of *S. turcica* is blurred so far.

**Downy mildews** (DM) are caused by a group of Oomycetes like *Perenosclerospora sorghi* Weston &Uppal (Sorghum downy mildew), *Sclerophthora rayssiae* Kenneth, Koltin & Wahl (Brown stripe downy mildew), *Peronosclerospora sacchari* Miyake and Shaw (Sugarcane downy mildew) and *Pernosclespora heteropogoni* (Rajashan downy mildew). All these genera cause both external and systemic infection. As a result, the severely affected plants do not produce any ear or tassel or in most cases deformed ears are developed that directly affect the grain yield (Kenneth 1970; Bock et al. 2000; Isakeit and Jaster 2005).

**Rusts** in maize are of two types. The common rust is caused by *Puccinia sorghi* Schwein (also known as *Puccinia maydis*). The second one is polysora rust or tropical rust or southern rust caused by *Puccinia polysora* Underw. The physiological races of *P. polysora* were reported long back by Ryland and Storey (1955). Seventeen virulence patterns were identified among the 60 isolates tested (Casela and Ferreira 2002). *Puccinia sorghi* can cause severe damage to susceptible maize varieties and limit production mainly in tropical countries. However, the threat has largely been overcome by resistant varieties. *Puccinia sorghi* is no longer a serious problem on maize although late season plantings are severely affected. Commonly the hosts of *P. sorghi* are maize and *Oxalis* species (wood sorrel). Different spore-producing stages *of P. sorghi* occur on each host, but the sexual stages occur on *Oxalis*.

### *3.2.2 Maize Insects*

About two dozen insect species cause economic damage to maize globally (Ortega and de Leon 1974; Guthrie 1989). The most damaging and difficult to manage among them are the stalk borers. They feed on the foliage in the beginning and later bore into the stalk, where it kills plants or drastically reduce the yield by stalk tunneling which affects xylem and phloem transportation, leading to stunted plant growth. Since maize has high foliage compensation ability, yield reduction is mainly caused by stalk damage. The pests coming under this category are European corn borer [*Ostrinia nubilalis* (Hübner)] in North America, Europe and North Africa, Asian corn borer or Oriental corn borer [*Ostrinia furnacalis* (Guenee)], spotted stemborer [*Chilo partellus* (Swinhoe)], Mediterranean corn borer or pink stem borer [*Sesamia nonagrioides* (Lefebvre)] or pink borer [*Sesamia cretica* (Led)], African maize borer [*Sesamia calamistis* (Hmps)], pink stem borer

[*Sesamia inferens* (Walker)], African maize stalk borer [*Busseola fusca* (Fuller)], African sugarcane borer [*Eldana saccharina* (Walker)], Southwestern corn borer [*Diatraea grandiosella* (Dyar)], American sugarcane borer [*Diatraea saccharalis* (Fabricius)], neotropical corn borer [*Diatraea lineolata* (Walker)].

The only foliage feeder which cause economic loss because of its voraceous feeding habit is fall armyworm [*Spodoptera frugiperda* (J. E. Smith)]; causes direct damage to corn ears too. This pest is currently posing a global challenge since its invasion in Africa in 2016, Asia in 2018 and Australia in 2020. The pests directly causing aesthetic and economic damage to corn ears are corn earworms [*Helicoverpa zea* (Boddie)] and *Helicoverpa armigera* (Hübner), where the former is more damaging and restricted to Americas. The economically damaging corn rootworm complex, [*Diabrotica* spp. viz., the western corn rootworm (*Diabrotica virgifera virgifera* LeConte), the northern corn rootworm (*Diabrotica barberi* Smith and Lawrence) and the southern corn rootworm (*Diabrotica undecimpunctata* howardi Barber)] cause damage to roots, where 15% yield loss per each damaged node is predicted (Tinsley et al. 2013). Corn rootworm species are native to the western hemisphere, however, WCR, the most damaging among all, invaded Europe (Berger 2001).

Corn leaf aphid [*Rhopalosiphum maidis* (Fitch) is the globally distributed sucking pest of maize, which usually attack pre-tasseling stage to grain filling stage and occasionally cause economic damage. Average density of 818 aphids at V10–VT stage corn can cause 28% yield reduction (Al Eryan and El Tabbakh 2004). Plant hoppers *Cicadulina mbila* (Naude) and *Peregrinus maidis* (Ashmead), cause damage primarily by acting as vector of viral diseases like maize streak virus (MSV) and maize stripe virus (MStV) respectively in maize (Roca De Doyle and Autrey 1992).

The main storage pests of maize, which cause loss of quality and quantity of maize grains across the world, are maize weevil [*Sitophilus zeamais* (Motschulsky)], angoumois grain moth, [*Sitotroga cerealella* (Olivier)], the lesser grain weevil [*Sitophilus oryzae* (Linnaeus)] and the larger grain borer [*Prostephanus truncatus* (Horn)]. *P. truncatus* the most damaging among all, is restricted to Americas and Africa. Grain weight losses due to *S. zeamais* and *P. truncatus* can go up to 20 and 35% respectively (Tefera et al. 2016). In addition, several minor and potential pests attack maize around the world causing less frequent economic damage.

Occurrence and severity of insect pests of maize vary by geographical location and vary by season within a geographical area. Since insects are cold-blooded animals, they generally prefer a temperature range of 25–35 °C. They undergo diapause in harsh summer periods, influencing their number of generations produced in a year; so is the severity of damage. For example, two generations of ECB are observed in maize crop of United States, whereas only one generation occurs in central Europe (Bohn et al. 1999).

Similarly, *C. partellus*, the most destructive native pest of maize in India, is more prevalent in *kharif* crop, where the extent of crop loss was about 27–80% (Jalali et al. 2014). Whereas in Nepal, a country with less geographic and climatic

variability, a narrow range of yield reduction (27–30%) was reported (Sharma and Gautam 2010). Severity of infestation and yield losses caused by maize stemborers varies in African continent, where geography, season, cultivars and cultivation practices are the contributory variables. In East Africa, *C. partellus*, *C. orichalcociliellus*, *E. saccharina*, *B. fusca*, and *S. calamistis* are major maize stemborers where, the later three occurs as major pests in West Africa also. In South Africa, *B. fusca* and *C. partellus* are the only major pests (Kfir et al. 2002; Sharma and Gautam 2010).

## 3.3 Stages and Extent of Damage

Among the various biotic factors causing damage to the maize crop, diseases viz., maydis leaf blight (MLB), banded leaf and sheath blight (BLSB), downy mildews (DM), rust, smut, and post flowering stalk rots (PFSR) etc. are most important (Singh and Shahi 2012). Under ideal circumstances, these diseases inflict immense losses both in quantity and quality of grain produced (Yadav et al. 2015). Annually around one percent of the total grain yield is reduced by BLSB alone in India (Sharma et al. 2005). But premature death of plants by diseases can cause drastic reduction in grain yield near to 97% (Sagar and Bhusal 2019). Similarly, MLB causes considerable yield losses even up to 70% (Kumar et al. 2009). Losses due to the downy mildews from India and several SE Asian countries have been accounted as high as 40–60%. In southern India especially Tamil Nadu and Karnataka have been reported downy mildew epidemics at various times. The projected losses resulted by major diseases of maize in India is nearer to 13.2% of which foliar diseases (5%), stalk rots, root rots and ear rots (5%) are accountable for substantial yield reduction. A wide range of crop yield losses caused by maize diseases has been tabulated in (Table 3.1). Similarly, occurrence and severity of insect pests of maize vary by geographical location and season within a geographical area.

Most vulnerable stages of maize to these pests are three leaf stage to flowering stages. However, European corn borer (ECB), the most destructive among all, also damages at reproductive stage where stalk breakage; tassel, ear and kernel damage, and ear/cob drop are common (Chiang and Hodson 1950). ECB had been causing crop losses of about one billion US$ annually in United States alone prior to the introduction of Bt corn hybrids (Hutchison et al. 2010). All hybrids were susceptible to ECB in Europe, where 0.28% and 6.05% grain yield reduction with every one percent damaged plant and one ECB larva per plant respectively was reported (Bohn et al. 1999). The only foliage feeder which cause economic loss because of its voracious feeding habit is fall armyworm [*Spodoptera frugiperda* (J. E. Smith)], which cause direct damage to corn ears too. The pests directly causing aesthetic and economic damage to corn ears are corn earworm [*Helicoverpa zea* (Boddie)], and less frequently by *Helicoverpa armigera*. The sucking pests of maize viz., maize leafhopper [*Cicadulina mbila* (Naude)], corn leaf aphid [*Rhopalosiphum maidis* (Fitch)], cause more indirect damage by acting as vectors of viral diseases in maize.

**Table 3.1** Important maize diseases along with their causal agents and yield losses

| S. No. | Disease | Causal agent | Losses (%) | Reference |
|---|---|---|---|---|
| 1 | Turcicum blight/Northern corn leaf blight | *Helminthosporium turcicum* (*Exerohilum turcicum*) | 20–90 | Razzaq et al. (2019) |
| 2 | Maydis blight/Southern corn leaf blight | *Bipolaris maydis* (*Cochliobolus heterotropus*) | 9.7–11.7 | Manjunatha et al. (2019) |
| 3 | Gray leaf spot | *Cercospora zeae* | 5–30 | Ward et al. (1999) |
| 4 | Curvularia leaf spot | *Cochliobolus lunatus* | 10–60 | Akinbode 2010 |
| 5 | Brown spot | *Physoderma maydis* | 6–20 | Lal and Chakravarti (1976) |
| 6 | Southern corn/Polysora rust | *Puccinia polysora* | 50–100 | Liu et al. (2016) |
| 7 | Common corn rust | *Puccinia sorghi* | 18–49 | Groth et al. (1983) |
| 8 | Eye spot | *Aureobasidium zeae* | 14–44 | Chang et al. (1990) |
| 9 | Head smut | *Sporisorium reilianum* | Up to 30 | Njuguna 2001 |
| 10 | Common smut | *Ustilago zeae* | 40–100 | Pope and McCarter (1992) |
| 11 | Ear rot | *Fusarium verticillioides* | 5–15 | Ako et al. (2003) |
| 12 | Sorghum downy mildew and Rajasthan downy mildew | *Peronosclerospora sorghi* and *P. heteropogoni* | 30 | Singh and Kaur (2018) |
| 13 | Banded leaf and sheath blight | *Rhizoctonia cerealis* or *solani* f. sp. *sasakii* | 10–90 | Sagar and Bhusal (2019) |
| 14 | Various stalk rot | *Macrophomina phaseolina*, *Pythium inflatum* | 30–35 | Costa et al. (2019) |
| 15 | Fusarium stalk rot | *Fusarium verticillioides* | 10 | Archana et al. (2019) |
| 16 | Root rot | *Fusarium graminearum* | 25–30 | Hebbar et al. (1992) |
| 17 | Maize dwarf mosaic | Maize dwarf mosaic virus (MDMV) | 0–90 | Goldberg and Brakke (1987) |
| 18 | Maize rough dwarf | Maize rough dwarf virus (MRDV) | 10–70 | Dovas et al. 2004 |
| 19 | Bacterial stalk rot | *Dickeya zeae* | 85–90 | Kaur et al. (2014) |

*Source* Dey et al. (2015)

A. Damage by *Chilo partellus* B. Damage by Fall armyworm

**Fig. 3.1** Insect damage in maize (Photo courtesy, Suby S. B, IIMR, New Delhi). **a** Damage by *Chilo partellus* **b** damage by Fall armyworm

The storage pests of maize which cause loss of quality and quantity of maize grains across the worlds are, greater rice weevil or maize weevil [*Sitophilus zeamais* (Motschulsky)], angoumois grain moth, [*Sitotroga cerealella* (Olivier)]. In addition to these, other insects also damage maize crop significantly under favourable conditions (described in the previous section) (Figs. 3.1 and 3.2).

### *3.3.1 Disease Management*

The disease management strategy by cultural methods is reported to be effective in the major diseases. In case of BLSB, stripping of the lower leaves can restrict the occurrence and spread of the disease (Sharma and Hembram 1990; Kaur et al. 2020). Management of crop debris, deep tillage, crop rotation with non-host species, decreasing plant density and timely showing can help reduce the incidence of MLB disease (Kaur et al. 2014). Ridge planting and paired row planting methods were successful in minimizing MLB disease severity. The PFSR disease can also be managed by crop rotation with non-cereal crops, deep summer ploughing in April and May, burning of crop residues. In addition, avoidance of the water stress condition at the time of flowering by providing irrigation till grain filling stage significantly reduces PFSR disease occurrence. Various cultural practices such as soil solarization, balanced soil fertility, crop rotation with non-host crop and flooding as well as fallowing can reduce late wilt disease (*Cephalosporium maydis* Samra, Sabet and Hingorani) severity and losses (Degani et al. 2018). However, all these cultural practices will only be successful if all farmers in the vicinity harmonize their activities.

Management of crop diseases using chemicals is the mainstay till date. The wider use of chemical pesticides is due to their more effectiveness, ease of

**Fig. 3.2** Fungal disease of maize (Photo courtesy, Robin Gogoi, IARI, New Delhi). **a** Banded leaf and sheath blight; **b** maydis leaf blight; **c** turcicum leaf blight; **d** charcoal rot (Post flowering stalk rot); **e** smut; **f** common rust

application, availability and stability. Chemical pesticides are generally fast-acting, may damage the crop less than those caused by the diseases. The fungicides have long been recommended are Mancozeb @2.5 g/L against common rust, Polysora rust, MLB and TLB; Propiconozole 25% EC (Tilt) @1 ml/Lagainst rusts; Metalaxyl MZ @2 g/L against downy mildews; Carbendazim, Tebuconazole, Hexaconazole @1 gm or ml/L, Azoxystrobin 18.2% + Difenconazole 11.4% w/w SC against BLSB disease. The pre-flowering stalk rot disease can be minimized using bleaching powder containing 33% chlorine @10 kg/ha as soil drench at pre-flowering in standing crop. Foliar spray with the combination of Carbendazim 12% + Mancozeb 62.7% was reported to be as effective against Fusarium stalk rot disease.

Biocontrol approach is an important measure for plant disease control without posing adverse effect on the environment (Gogoi et al. 2018). Mechanisms such as antibiosis, siderophore production, induced resistance, and competition are the modes of action of the bioagents (Yobo et al. 2004). Several micro-organisms are known to parasitize *Rhizoctonia* species and those are mainly fungi like

*Trichoderma*, *Gliocladium*, and *Laetisaria*, bacteria (*Pseudomonas fluorescence*) and nematodes (*Aphelenchus avenae*) (Singh and Shahi 2012). BLSB disease incidence could be drastically reduced by applying *P. fluorescens* and *T. harzianum* in the field and it improves plant growth as well (Sivakumar et al. 2000; Meena et al. 2003). Combined use of seed and foliar treatment with fluorescent Pseudomonas from maize rhizoplane was most effective against BLSB (Gamliel and Katan 1993) and the result was on par with the systemic fungicide carbendazim. In case of Fusarium stalk rot, seed treatment with *T. harzianum* (4 g/kg seed) along with soil application of castor or neem cake (250 kg/ha), 15 days prior to sowing helps in disease management (Saravanakumar et al. 2017). Application of *Trichoderma* formulations in furrows after mixing with FYM @1 kg/100 kg FYM at least 10 days before its use in the field in moist condition (Hussain et al. 1990) and seed treatment with talc-based powder formulation of *T. viride* (*T. asperellum*) @12 g/kg seed can check the appearance of charcoal rot disease (Shekhar and Kumar 2010). Thus, the ultimate goal of reducing fungicide use in maize production could be achieved by using different bio-origin fungicides in rotations with traditional fungicides. But successful biological control of the diseases requires more knowledge-intensive strategies.

Resistance of the host plant plays a significantly important role in integrated disease management approach. Therefore, identification of resistance genes against the aggressive pathogens and combining them with high grain yield is a priority. Crop diseases, especially the BLSB of maize, can be managed by using different management strategies at some level. It includes cultural practices, chemical management, host resistance and biological control. But the studies revealed that none of the disease managerial measures alone is absolutely effective. Hence, identification of climate resilient components and their combination for integrated disease management (IDM) modules development are expected to provide best management of the diseases like BLSB (Hooda et al. 2015). Use of fungicides and bio-control agents viz., *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* as seed and soil treatment can also restrict the BLSB disease to some extent. Seed treatment with *Trichoderma harzianum* (6 g/kg of seeds) and 2 sprays of 0.25% Mancozeb at 40 and 50 DAS are found effective for the management of turcicum leaf blight disease (Khedekar et al. 2010). Seed treatment with a combination of carbendazim + *T. viride* revealed maximum increase in seed germination (89.4%) followed by reduction in disease severity (83.8%) of Fusarium stalk rot (*F. verticillioides*) in maize (Khokhar et al. 2014). In Nepal, IDM approach was reported to be the most appropriate technology for management of stalk rot complex which is an exclusively soil borne nature (Subedi et al. 2016).

### 3.3.2 Insect Management

The losses caused by the insect pests can be managed by adapting several strategies. Among the various strategies, the use of chemical insecticides is the major one across the globe, but, it has negative impacts viz., ecological damage, environmental pollution, human health hazards and development of resistance in the insect pests. The host plant resistance (HPR) is a most effective alternative and economical approach to control insect pests. Breeding for resistant cultivar is a sustainable approach. In USA, the efforts towards breeding insect resistant maize cultivars has started after the discovery of European corn borer in 1917 (Guthrie 1989).

The success of breeding program to develop resistant cultivars depends on availability of broad germplasm base, knowledge of resistance mechanism, efficient and reliable screening techniques, mode of inheritance, selection of right breeding procedure, etc. In the recent past, new molecular techniques have facilitated plant breeding and brought improvements in cultivars resistance against insect pests (Guthrie 1989). Identification, development and utilization of sources of resistance against different insect pests of maize play important role in designing management strategies (Mihm 1997).

Historically, many cultivars with insect resistance have been developed utilizing conventional breeding methods. In CIMMYT, sub-tropical source populations were developed with multiple borer resistance (MBR population) by following recombination and recurrent selection under artificial infestation with southwestern corn borer (SWCB, *Diatraea grandiosella*), sugarcane borer (SCB, *Diatraea saccharalis*), European corn borer (ECB, *Ostrinia nubilalis*) and fall armyworm (FAW, *Spodoptera frugiperda*) (Mihm 1985). From the different organizations diverse source populations were obtained and used for development of MBR population.

## 3.4 Traditional Breeding Approaches

Traditional breeding comprises all those breeding methods that have been developed since the origin of agriculture and are still commonly used even today. Conventional breeding can be defined as the development or improvement of crop cultivars with the help of natural processes and conservative tools for manipulating plant genome within the natural genetic boundaries of the species (Acquaah 2015), in contrast to molecular plant breeding, which utilizes modern, sophisticated and sometimes radical tools.

In any breeding programme involving incorporation of a new a trait, including disease resistance, breeder has to consider the phenomenon of ‘trait compensation’ by which the gains in other desired characters may suffer (like yield potential) due to addition of a new trait (Badu Apraku and Fakorede 2017). Therefore, breeder has to consider the economic sustainability of incorporation of biotic stress resistance. For this, breeder has to consider the frequency and extent of biotic stress in the

target area and extent of economic damage caused. Breeder may opt for major-gene resistance (qualitative resistance conferred by R-genes) or minor gene resistance (quantitative resistance conferred by QTLs). The major gene resistance has complete expression with high levels of resistance, simple inheritance and is usually race specific. But, this type of resistance may be quickly defeated by co-evolving parasites. However, some cases of durable major-gene resistance have been reported (Badu Apraku and Fakorede 2017). The durable resistance is defined as "the resistance that remains effective when a cultivar is grown widely in environments favouring disease development" (review by Michelmore 2003). The concept of durable resistance has proved a very useful concept in disease resistance breeding. The example of such durable resistance has been seen in Indian inbreds. The maize inbred lines CM104 and CM105 have shown resistance to turcicum leaf blight (TLB) as well as maydis leaf blight (MLB) at 19 diverse locations in India for more than 14 years. Furthermore, these lines registered resistant reaction also in countries like Hawaii, Nigeria and Kenya for TLB, and Cameroon, Mexico, Hawaii and Korea for MLB (Sharma et al. 1993a, b).

Quantitative resistance provides intermediate or partial resistance to the parasite in contrast to qualitative resistance and is thought to be controlled by a set of genes that are distinct from, or showing partial similarity with those involved in qualitative resistance (Wisser et al. 2005; Fu et al. 2009). Quantitative resistance is expected to be more durable as many minor genes with small effects exert lower selection pressure and presents greater hurdles to overcome by the parasite (Parlevliet 2002). Even though a large number of quantitative resistance sources have been reported, especially for disease resistance in plants (Young 1996), there is no clear understanding of genetic basis or the mechanisms of defense involved in quantitative resistance.

Once the decision on the type of resistance to be used in breeding is made, the next step is to identify suitable sources of resistance. The resistance may be found in the primary gene pool of the crop and often within the related species. Sources of resistance have been reported in related taxonomic groups, viz., landraces, commercial cultivars, wild progenitors, related species and genera. Further, breeder has to bear in mind that use of germplasm with common genetic base should be minimized or avoided in disease breeding programmes. Devastating epidemics have been observed, as with the southern leaf blight in USA, when the genetic or cytoplasmic homogeneity was achieved. In general, breeding for biotic stress resistance in maize can be depicted as follows (Fig. 3.3).

Conventional approaches for biotic stress in brief are discussed for diseases and insects separately in the following paragraphs.

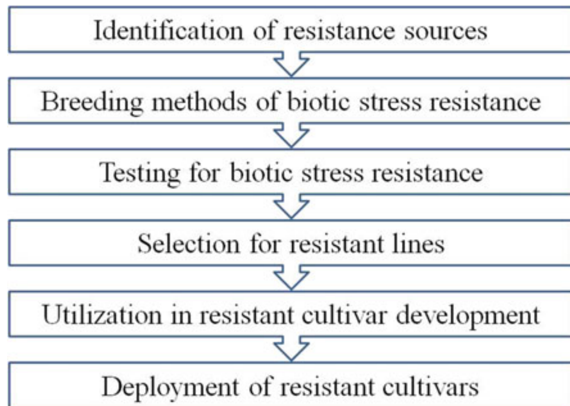



**Fig. 3.3** Flow diagram for breeding for biotic stress resistance in maize

### *3.4.1 Conventional Approaches in Breeding for Disease Resistance*

The systematic efforts on conventional approaches for disease resistance began after Biffen's (1905) demonstration in wheat that disease resistance in plants is under genetic control. This was further strengthened by works of Flor (1946) on flax rust and understanding of the genetics of pathogenicity, and Van der Planck (Plank 1963), who suggested two types of resistance, viz., vertical (qualitative) and horizontal (quantitative) resistance.

Breeding for disease resistance in maize, as in other crops, begins with the screening germplasm to identify resistant sources (donors). Precision phenotyping for disease resistance using disease hot spots or artificial epiphytotic or disease screening hubs is the most important step into identify stable sources of resistance. In the next step, backcross breeding scheme is used to introgress resistance gene from the donor parent into an agronomically superior line or inbred (Fig. 3.3). To achieve this, knowledge on the genetic architecture of disease resistance genes in maize need to be explored to assess the nature of resistance (qualitative or quantitative) in the donor parent (Ali and Yan 2012).

In other major cereal crops like wheat and rice, qualitative disease resistance is extensively used. In contrast, a few major resistance genes (R genes) have been identified and utilized in maize (Ramakrishna et al. 2002), such as *Ht* genes against northern leaf blight (Welz and Geiger 2000) and the *Rp* genes against common rust. This is because, the majority of resistance available against diseases in maize is quantitative disease resistance (QDR). The major reason for the predominance of QDR in maize might be due to its outcrossing nature and hence, it is substantially more genetically diverse than wheat or rice (Buckler et al. 2001). Maize breeders, therefore, have more diversity available to them within adapted germplasm and effective QDR to maize pathogens is available and widely utilized, compared with wheat or rice breeders. This might also be due to the fact that maize is attacked by

fewer commercially important biotrophic pathogens than that of wheat. Furthermore, it is possible to bring together multiple small-effect QTLs to achieve effective levels of QDR in maize through population improvement schemes. Hence, population improvement approaches are more commonly used in maize for improving both agronomic performance and disease resistance. Therefore, it is important to collect and evaluate germplasm continuously to identify new sources of disease-resistant genes, which in turn enables the breeder to incorporate multiple disease resistance into breeding populations before deriving varieties from such populations.

Resistance sources to foliar diseases of maize including maydis leaf blight (MLB, southern corn leaf blight-SCLB), turcicum leaf blight (TLB), northern corn leaf blight-NCLB) (Ayiga Aluba et al. 2015; Bhat et al. 2017; Kurosawa et al. 2018), gray leaf spot (GLS) (Dhami et al. 2015), polysora and common rust, downy mildew (DM), some viral diseases, *Aspergillus* contamination (Hooda et al. 2012; Badu Apraku and Fakorede 2017) have been identified and are incorporated successfully through conventional breeding. In addition, multiple disease resistance (MDR) in maize has been reported (Martins et al. 2019). MDR loci conferring resistance to SCLB, GLS, and NCLB are believed to have relatively small effects individually and the effects may be below the detection threshold to detect them as individual loci (Balint Kurti et al. 2010; Martins et al. 2019). However, there has been a very little progress in resistance against banded leaf and sheath blight, post flowering stalk rots and ear rots (Ali and Yan 2012).

Gene pyramiding and multiline development are not popularly used as incorporation of multiple genes becomes a tedious and lengthy process. These strategies are expected to become much more practical in future. Furthermore, many *R* genes confer resistance against only one or few strains of pathogen and do not provide broad-spectrum resistance as in case QDR. Nonetheless, understanding of the function of R genes at molecular level and of downstream signal transduction pathways might provide strategies to overcome these deficiencies (Balconi et al. 2012).

### *3.4.2 Conventional Approaches in Breeding for Insect Resistance*

Breeding for Insect resistance start with screening of a germplasm for variability in the level of resistance of a genotype to target pest by quantifying the effect of insect on plants and the effect of plants on insect (Mihm 1985). The most essential components for successful screening programme for insect resistance are a broad germplasm base, the established population of a target pest and its mass production. Standardization of the most susceptible stage of plant, the dose of insect for infesting plants, and an accurate phenotyping method are to be established before screening. Once the resistance source is found, a suitable breeding scheme is

followed. Compilation and reviews on mechanisms of resistance, its genetics, sources of resistance and conventional breeding for insect resistance in maize for Americas and Africa have been published (Mihm 1985, 1997; Guthrie 1989; Wiseman and Davis 1990; Mugo et al. 2001; Kumar 2002; Brooks et al. 2007).

Breeding for insect resistance in corn began in 1920s for ECB resistance (Guthrie 1989). International Maize and Wheat Improvement Center (CIMMYT) derived a source population (sub-tropical) with multiple borer resistance (MBR) to combat multiple pests in the area of release of a new cultivar and for increased durability of resistance. MBR was developed from germplasm sources resistant to SWCB, SCB, ECB and FAW, through conventional pedigree breeding using resistant germplasm sources of Mississippi State University, CIMMYT population 47, Antigua populations, Cornell University and University of Missouri. MBR population is characterized by tough and fibrous leaf tissue where the cell wall components are reinforced with phenolic acids, which reduces digestibility and nutritional value of the plants to the pests (Bergvinson et al. 1994). Later, MBR was found to possess good amount of resistance to *C. partellus* and *B. fusca* and served as stemborer resistance source around the world. Its success was attributed to additive variation of the polygenes involved in resistance and the genotypes derived from MBR showing general combining ability as the primary source of variation among $F_1$ for resistance and grain yield (Mugo et al. 2001).

Of this, the landmark populations like Antigua Gpo2 population served the basis of insect resistant lines released around the world. Corn host plant research unit of USDA-ARS extensively worked on this and other resistant sources to derive many insect resistant lines, of which Mp496 (Scott and Davis 1981) was the pioneer, derived from Antigua Gpo2 by direct selection. Subsequently, many superior lines resistant to FAW, SWCB and P. rust such as Mp703 (eight generations of selection by selfing resistant plants of Gpo2 population by Williams and Davis 1980), Mp704 (eight generations of selection by selfing the cross between Mp496 and an S2 population of Republica Dominica Gpo1 by Williams and Davis 1982), Mp701 and Mp702 (selection from bulk populations derived from crosses involving Antigua Gpo. 1 and Antigua Gpo. 2, and Republica Dominica Gpo1 respectively by Scott et al. 1982), Mp705, Mp706, and Mp707 (selfing selections from MpSWCB-4(l) for eight generations by Williams and Davis 1984), Mp708 (in addition to FAW and SWCB this line is resistant to root knot nematode. developed by selfing selections from a cross of Mp704 and Tx601 for eight generations by Williams et al. 1990), Mp713 and Mp714 (Mp713 derived from MBR population and Mp714 from GT-DDSA, a corn earworm resistant population Williams and Davis 2000), and Mp716 (derived from a cross between Mp708 and Mp78:518 by Williams and Davis 2002).

HPR was explored for stored product pests at CIMMYT, where Caribbean germplasm bank accessions like Guadeloupe and Cuba land races served as LGB resistant source (Kumar 2002). Subsequently, “CubaGuard” was derived by recurrent section and selfing under LBG pressure.

Generally, the lines resistant to one pest tended to be resistant to other pests and diseases, indicative of a broad-spectrum resistance. This could be the result of

co-evolution of maize pests with its host plant under different ecologies or man-made evolution by accelerated resistance breeding efforts. For instance, the line Mp496, released in 1981 has good resistance to FAW, and fairly good resistance to ECB, sorghum downy mildew, maize chlorotic dwarf and moderate resistance to maize dwarf mosaic and southern corn rust (Scott and Davis 1981). This suggests that there might be few genetic regions operate in tandem to give broad-spectrum resistance, as observed in the inbred lines, Mp704 and Mp708 with leaf feeding resistance to FAW and SWCB (Brooks et al. 2007).

### *3.4.3 Limitations of Conventional Approaches in Breeding for Biotic Stress Resistance*

Although conventional breeding has achieved tremendous results for many traits and since many years, it also has some serious limitations. First, it takes very long time to achieve desired results. Second, breeding can only be done between two sexually compatible lines. Third, when hybridization is done, many other traits are transferred along with the trait/s of interest—both positive and negative traits resulting in linkage drag. Fourth, the use of distant relative or tertiary gene pool in breeding for resistance poses following problems. (i) failure to get $F_1$ seed between the crop and the donor species, (ii) sterile interspecific or intergeneric hybrid, and (iii) poor recombination between the chromosomes of crop and the donor species (Harlan and De Wet 1971). When distant hybridization is used, the resistance may be realised after the removal of undesirable genes trough many generations of backcrossing.

## 3.5 Genetic Resources of Resistant Genes

The array of genetic resources at our disposal, together with new biotechnology techniques, gives us with a healthy measure of optimism for meeting the world's future food requirements (Hoisington et al. 1999). The genepool of maize consists of two genera, *Zea* and *Tripsacum,* of family Poaceae. These species are housing tremendous genetic diversity that is potentially useful in maize improvement either through hybridization or through special techniques, such as embryo rescue. The genepool classification is based on the ease of genetic exchange through sexual reproduction (Harlan and de Wet 1971). The cultivated species of the genus *Zea* (*Z. mays* ssp. *mays*) represents the primary genepool and all other taxa in the genus *Zea* that are popularly known as "teosintes" form the secondary genepool. All the species in the genus *Tripsacum*, not easily crossable with cultivated maize and require special techniques, are classified as tertiary genepool. The genetic resources with biotic stress resistance have been summarized below Table 3.2.

**Table 3.2** List of germplasm resources of maize with potential to improve biotic stress tolerance

| Sl. No. | Biotic stress | Germplasm | Reference |
|---|---|---|---|
| *Primary genepool* | | | |
| 1 | Foliar diseases | Tuxpeno crema-land race | Kloeppe et al. (1999) |
| 2 | Downy mildew | Suwan-1 (OPV-Thailand) | Dhillon et al. (2002) |
| 3 | Multiple diseases | Prabhat (OPV-India) | Dhillon et al. (2002) |
| 4 | *Sitophilus zeamais* (maize weevil) | Palomero Toluqueno (Popcorn landrace) | Arnason et al. (1993) |
| 5 | *Prostephanus truncates* (Larger grain borer) | Caribbean land races | Kumar (2002) |
| 6 | Northern leaf blight (inbred lines of maize) | DMSC 16-1, Gen1858, HKI PC 4B-1, HKI 141-1, HKI 141-2, CML141 | Hooda et al. (2012) |
| 7 | Southern leaf blight (inbred lines of maize) | DMSC 16-2, V351-1, CM 114, CML 165, CML 167, HKI-139 | |
| 8 | Brown stripe downy mildew (inbred lines of maize) | CUBA 380, DMSC36, HKI-PC-4B-1, DTPYC9-F46-3-1, ESM-11-3, LM 6, LM 12, LM 16, V 355, V 341-1, CM 123, CM 149, CM 500, | |
| 9 | Post flowering stalk rot | WINPOP-1, WINPOP-2, WINPOP-3, WINPOP-21, WINPOP-21, WINPOP-43-1, HKI-2-6-2-4(1-2)-4, HKI 226, HKI 1040-5, CML 451(P2) | |
| 10 | Polysora rust | DMSC 16-1, DMSC 16-2, WINPOP-43-1, WINPOP-43-2, HKI-2-6-2, HKI1040-5, PFSR/51016-1, LM 16, CM 105, HKI 141-1, | |
| 11 | Rajasthan downymildew | LM15, CM114, HKI C 78, DMHOC 4, PFSR- R9, PFSR-S3, PFSR- R10, JCY3-7-1-2-1 | |
| 12 | Curvularia leaf spot | LM11, LM 12, LM 16, V 335, V 341, V 351, CM121, CM 123, CM 144, CM 502, HKI 141, CML384, CML 395 | |
| 13 | Multiple disease resistant (MDR) | LM11, LM 12, LM 16, V 335, V 341, V 351, CM121, CM 123, CM 144, CM 502, HKI 141, HKI 1352-5-8-9, CML384, CML 395 | |
| 14 | Fall army worm | CMS 23, CMS 24, Zapalote Chico, CMS 45, Amarillo Cristalino, WP 1, RR 060, MG 05, Guatemala 786, NõdzobPrê, Puerto Rico 13 | Viana and Guimarães (1997) |
| *Secondary genepool* | | | |
| 1 | *Corn Smut disease* | *Teosinte* | Mammadov et al. (2018) |
| 2 | *H. turcicum* | *Z. diploperennis* | |
| 3 | *H. maydis* | | |

(continued)

**Table 3.2** (continued)

| Sl. No. | Biotic stress | Germplasm | Reference |
|---|---|---|---|
| 4 | Maize chlorotic dwarf virus | *Z. diploperennis* | Findley et al. (1982) |
| 5 | *Fusarium spp.* | *Z. spp. mexicana* | Pásztor and Borsos (1990) |
| 6 | Downey mildew | *Z. spp. mexicana* | Mammadov et al. (2018) |
| 7 | Corn borer | *Z. mays spp. mexicana* | Pásztor and Borsos (1990) |
| 8 | Asiatic corn borer | *Z. mays spp. mexicana,* | Ramirez (1997) |
| 9 | Asiatic corn borer | *Z. mays spp. diploperennis,* | |
| 10 | Asiatic corn borer | *Z. mays spp. perennis* | |
| 11 | Corn rootworm | *T. dactyloides* | Prischmann et al. 2009 |
| 12 | *S. frugiferda* | *Z. diploperennis* | Farias Rivera et al. (2003) |
| 13 | *H.turcicum*, *H.maydis* | *Z. diploperennis* | Wei et al. (2003) |
| 14 | Northern leaf blight | *Teosinte* | Ott (2009) |
| 15 | *Ustilagomaydis* | *Teosinte* | Chavan and Smith (2014) |
| *Tertiary genepool* | | | |
| 1 | *Colletotricum graminicola* | *T. dactyloides* | Bergquist (1979) |
| 2 | *Rust disease* | *T. dactyloides* | Mammadov et al. (2018) |
| 3 | *P. sorghi* (RpTd gene) | *T. dactyloides* | Bergquist (1981) |
| 4 | *Helminthosporimturcicum* | *T. dactyloides* | Bergquist (1979) |
| 5 | *H. maydis* | | |
| 6 | *Erwinia stewartii* | | |
| 7 | *Puccinia sorghi* | | |

### 3.5.1 Utilization of Identified Novel Genes in Maize Improvement

Despite the importance of maize as a major staple crop globally, only a few biotic stress resistance genes have been identified and validated through mutagenesis or transgenic approaches. The resistance genes so far identified and cloned against disease resistance include two qualitative resistance genes, *Rp1-D* and *Hm1*, and four quantitative resistant genes with relatively large effects, *ZmHtn1, ZmWAK, ZmTrx*, and *Rcg1*. Besides, some genes which are strongly implicated in disease resistance and several QTLs against different diseases have been reported. Insect resistance is largely quantitative in maize and few QTLs have been identified. In addition, Cry protein genes have been used to develop maize transgenics resistant against lepidopteran insects. These genes are summarized as follows (Table 3.3).

## 3.6 Diversity Analysis

The genetic diversity analysis in a crop germplasm provides breeders with valuable information to select parents for hybridization and for diverse inbred development (Ertiro et al. 2017). This in turn helps in classifying and describing inbreds into distinct heterotic groups and help in determining the genetic variability in the selected accessions/lines for target traits (Semagn et al. 2012). Several authors have documented the extent of genetic diversity in maize. The genetic diversity analyses in maize germplasm collection have been carried out in maize by both morphological and molecular approaches. Even though diversity analysis using morphological traits has many disadvantages (Botha and Venter 2000), it provides an excellent analysis of variation at phenotypic level coupled with the information on Genotype × Environment interaction. The characterization of accessions through phenotypic descriptors is the first step to classify, describe and assess the potential of available germplasm. Such an exercise will enhance the value of these germplasm in maize breeding (Prasanna and Sharma 2005; Wasala et al. 2013). The inbred lines of tropical and subtropical regions have more alleles and greater gene diversity than temperate inbred lines. Hence, tropical germplasm may be useful in temperate regions as well. It is observed that only 80% alleles present in land races are present in improved inbred lines of maize, implying that substantial additional genetic diversity can be found in landraces. Moreover, compared to the progenitor (teosinte), maize has fewer alleles and hence alleles present in teosinte can provide additional source of genetic diversity for use in maize improvement (Vigouroux et al. 2005). In India, well characterized landraces through SSR marker analysis led to the better understanding of population structure (Prasanna et al. 2010). Molecular marker-based study involving progenitor and wild relatives provided insights into the domestication events in maize (Matsuoka et al. 2002).

**Table 3.3** Gens/QTLs identified conferring resistance to maize diseases

| Biotic stress | Gene/QTL | Method | Mechanism | Reference |
|---|---|---|---|---|
| *Genes implicated in biotic stress resistance in maize* | | | | |
| Aspergillus ear rot | *ZmLOX3* (dQTL) | Implicated by Mutant analysis | Lipoxygenase | Gao et al. (2009) |
| Downey mildew | *GRMZM2G028643, GRMZM2G128315, & GRMZM2G330907* | Candidate gene | LRR (leucine rich repeat) | Kim et al. (2020) |
| Downey mildew | *AC210003.2_FG004* | Candidate gene | Peroxidase (POX) gene | Kim et al. (2020) |
| Northern leaf blight and Stewart's wilt | *pan1* (dQTL) | Implicated by fine-mapping and mutant analysis | Receptor-like kinase | Jamann et al. (2014) |
| Northern leaf blight | *Remorin* (dQTL) | Implicated by fine-mapping and mutant analysis | Remorin_C domain (PFAM 03763) | Jamann et al. (2016) |
| Northern leaf blight, southern leaf blight, and grey leaf spot | *GST* (dQTL) | Implicated by association analysis | Glutathione S-transferase | Wisser et al. (2011) |
| Shoot fly (*Atherigonana spp.*) | *qDH9.1* (and *qEC9.1*) | QTL mapping | NA | Vikal et al. (2020) |
| Fall army worm | *gl15* (*glossy 15*) | Candidate gene | NA | Brooks et al. (2007) |
| Maize leaf feeding insects | *Mir* cysteine proteinase gene family | Candidate gene | NA | Cordero et al. (1994) |
| *Helicoverpazeae* | Maize *Rip3:1* | Ribisomal inactivating protein | Impair susceptible ribosomes | Dowd et al. (2003) |
| European corn borer | QTLs on chromosomes 1, 2, 4, 5, 6, 8 | $F_{2:3}$ mapping population derived from a cross B73Ht (susceptible) × Mo47 (resistant) | Leaf feeding | Jampatong et al. (2002) |
| *Cloned genes of disease resistance in maize* | | | | |
| Maize leaf blight and ear mold | *Hm1* | Transposon tagging | NADPH-dependent HC-toxin reductase | Johal and Briggs 1992 |

(continued)

**Table 3.3** (continued)

| Biotic stress | Gene/QTL | Method | Mechanism | Reference |
|---|---|---|---|---|
| Common rust | *Rp1-D* | Transposon tagging | NB-LRR | Collins et al. 1999 |
| *Exerohilum turcicum* | *Htn1* (dQTL) | Fine-mapping, mutant analysis | Wall-associated receptor-like protein | Hurni et al. 2015 |
| Sugarcane mosaic virus disease | *ZmTrxh* (dQTL) | Transposon tagging | Atypical h-type thioredoxin | Liu et al. 2017 |
| Anthracnose stalk rot | *Rcg1* (dQTL) | Transposon tagging | NB-LRR | Frey2005 |
| Head smut | *ZmWAK*(dQTL) | Sequential fine-mapping, and RNAi | Wall-associated kinase | Zuo et al. (2015) |
| *Transgenes for insect resistance in maize* | | | | |
| Corn rootworm | *Cry34Ab1*, *Cry35Ab1* | Transgenic | Bt toxin-Cry protein | Hellmich and Hellmich 2012 |
| Lepidopteran insects | *cry1A.105*, *cry2Ab*, *CryIF*, *Cry3Bb1*, *mCry3A*, *Vip3A* | Transgenic | Bt toxin-Cry protein | Castagnola and Jurat-Fuentes (2012) |

## 3.7 Glimpse on Classical Mapping in Maize

The morphological marker is a genetic trait detectable by a naked eye and that aids to identify, predict, or characterize the trait linked to it. For instance, the traits such as seed colour, seed shape, flower colour, leaf pigmentation, leaf shape, flower color, pubescence color, awn type and length, fruit shape, stem length, and such other agronomic traits. These markers are easy to identify without any special instrument or modern technique. Use of markers as an assisting tool to select the plants with desired traits had started in breeding long time ago. Since ancient times, various morphological markers have been used to investigate the variation for utilization in plant breeding (Karaköy et al. 2014) and in construction of linkage maps by classical two- and/or three-point tests. Some of these markers are linked with other agronomic traits and thus can be used in indirect selection. Markers of this type have been used in resistance breeding. For instance, the tomato *Tm-2* gene for resistance to tobacco mosaic virus (TMV) is linked to an anthocyaninless seedling marker (Robinson et al. 1970) and a peach mildew resistance gene is linked to the size of foliar glands (Connors 1922).

In maize, insect resistance is significantly correlated with morphological features. For instance, dense waxes on stem and leaf surface against southwestern corn borer (Hedin et al. 1993) and fall armyworm (Yang et al. 1993), low trichome density against corn earworm (Widstrom et al. 1979), silica against European corn borer (Rojanaridpiched et al. 1984), and tight husks against corn earworm (Wiseman et al. 1977). These plant characteristics have been considered while breeding for insect resistance in maize through conventional plant breeding approaches. However main disadvantages of morphological markers are, they are limited in number, influenced by the plant growth stages, various environmental factors (Eagles et al. 2001), and some have deleterious effects, pleiotropy, epistasis, and rare polymorphism.

Traditional method of identification of disease/insect resistance gene is time consuming and affect much by environmental condition prevailed. Hence markers linked to the trait of interest came as an improvement over traditional method of identification and mapping of genes. Before mapping a gene of interest, understanding the inherence of particular trait is at most important. In maize, one recessive major gene, *rhm*1, found to confers resistance to race O of *Cochliobolus heterostrophus* (Zaitlin et al. 1993). Resistance is associated with relatively few changes in gene expression or protein levels (Simmons et al. 2001). Monogenic resistance was reported in case of MLB (Faluyi and Olorede 1984) initially followed by the role of QTL in its expression later. It was established that in the adult plant, *rhm1* confers a level of quantitative resistance (Thompson et al. 1987) and *rhm* was mapped to the short arm of chromosome 6 with two restriction fragment length polymorphism (RFLP) marker loci (UMC85 and p144). The gene *Hm2* and *Hm1A* confer adult plant resistance to *C. carbonum* race (Balint Kurti et al. 2007, 2008). MLB resistance QTL are found in the same bin in populations derived from two or more different crosses (McMullen and Simcox 1995; Wisser et al. 2006).

Carson et al. (2004) identified a total of 11 QTLs governing resistance against MLB. Another six significant QTLs (LOD > 3.1) were identified for resistance to MLB which were located on the chromosome 1, 2, 3, 6, 7 and 8 (Balint Kurti and Carson 2006). Seven potential QTLs, and the two strongest among them being located on chromosome 3 (bin 3.04) and 9 (bin 9.04), were reported by recently, Kump et al. (2011) identified 32 QTLs using nested association mapping population. As pointed out earlier, disease resistance in maize is mostly quantitative in nature. It can be noted that many dQTLs (disease QTLS) and only few R genes (qualitative resistance) have been reported in maize. Wisser et al. (2006) compiled the information from 50 publications on mapping of disease resistance pertaining to 11 different diseases in maize. In all, these papers reported the locations of 437 dQTLs, 17 R-genes, and 25 R gene analogs. The analysis of the distribution of resistance loci indicated that the dQTLs are distributed over all 10 chromosomes and covered 89% of the genetic map. Further, it indicated the presence of clusters of dQTLs for multiple diseases. There is an evidence for the association of dQTL with maturity related QTL. On the dQTL consensus map, each maize chromosome had co-localizing dQTL for at least two different diseases. Also, MDR was found to be associated with many common chromosomal segments. These distinct dQTL distributions for the different diseases imply that certain breeding schemes may be more suitable for some diseases (Wisser et al. 2006).

### 3.7.1 Map-Based Cloning of Genes for Resistance

Northern corn leaf blight (NCLB) is one of the most devastating foliar diseases caused by the fungus *Exserohilum turcicum* (teleomorph *Setosphaeria turcica*) and result in huge economic loss in maize. *Htn1* locus has been reported to confer quantitative and partial resistance against NCLB (Gevers HO 1975) and mapped at the locus to a 23.1-cM interval of chromosome 8. Inclusion of additional marker within the interval narrowed down the interval to a 4.7-cM with the flanking markers MA0003 (SNP) and bnlg1782. This distance represented 1.3-Mb on physical map which was sequenced in resistant parent RP4Htn1 using a BAC library. Further using sequence-based approaches narrowed down *Htn1* between newly designed SNP markers MA0024 and MA0013 representing a 131.7-kb distance carrying three putative candidate genes *ZmWAK-RLK1*, *ZmWAK-RLK2* and *ZmWAK-RLP1*. Later, Jamann et al. (2016) fine mapped the maize *remorin* (*ZmREM6.3*) locus and demonstrated its role in conferring quantitative resistance against NCLB.

Resistance to BLSB has been reported to be governed by multiple genes, and till now genes with major effect has not been reported. Further, maize varieties with complete resistance are not available. Hence, unravelling the genetic mechanisms and mining resistance genes can be a boon for BLSB resistance breeding. Li et al. (2019) performed GWAS for BLSB using 542,438 SNPs (MAF $\geq$ 0.05) in the association panel of 318 maize inbred lines consisted of 133 tropical or subtropical,

78 temperate and 71 of mixed origin. Wide phenotypic variation for lesion length was observed with average lesion length 0.8–14.13 cm in the panel. GWAS analysis using the general linear model (GLM) could identify 28 SNPs ($P < 1 \times 10^{-5}$) corresponding to nine loci and distributed on four (1, 4, 7 and 8) linkage group. Out of 28 SNPs, the most significant SNP chr4.S_180199219 ($P < 1.84 \times 10^{-6}$) at chromosome 4 was present in second exon of the gene *GRMZM2G109140*. The gene was designated as *ZmFBL41* as the predicted F-box protein (41 kDa) shares 79% sequence similarity with rice *OsFBX61*. Resequencing of *ZmFBL41* and comparative analysis of susceptible (28) and resistant (23) lines identified four SNPs in the second exon in strong LD along with the lead SNP 2867 ($r^2 > 0.8$). These five SNPs could be assigned to two haplotypes, viz., resistant (haplotype 1) and susceptible (haplotype 2). However, these haplotypes did not affect the *ZmFBL41* expression level. To confirm the role of *ZmFBL41* in BLSB resistance, disease incidence and expression level of zmfbl41 carrying Mutator insertion in the 5′ UTR was compared with inbred line W22 which showed 28% reduced expression as well as disease index in *zmfbl41*. Further, transgenic rice cultivar Zhonghua 11 overexpressing the susceptible $ZmFBL41^{B73}$ allele developed longer lesions. Hence, *ZmFBL41*$^{B73}$ was found to be a negative regulator of BLSB resistance and degrade a target protein, cinnamyl alcohol dehydrogenase (ZmCAD).

## 3.8 Association Studies in Maize

Importance of discovering durable pest and disease resistance necessitates additional genetic mapping of diseases tolerant genes. Genome wide association mapping identifies regions of the genome associated with different biotic stresses and gives clue for directional selection to accelerated crop improvement. Majority of the biotic stress resistance in maize are governed by many genes and its inheritance is quantitative in nature. In order to analyse quantitative characteristics, association mapping utilizes ancestral recombination and natural genetic variation within a population and is based on the linkage disequilibrium principle (Geiringer 1944; Lewontin and Kojima 1960). The non-random co-segregation of alleles into two loci is one of the functional concepts of linkage disequilibrium.For association mapping research design, this observation is important as it can be used to calculate the marker density desired for scanning relatively undiscovered regions of the genome as well as the maximum resolution that can be obtained in the target population for genotype-phenotype associations (Ersoz et al. 2009).

The first association study at genome wide scale was reported in maize, in 2018, in which 8590 loci, in 553 elite maize inbred lines were used. Large scale Genome wide analysis provides new opportunity to understand the genetic architecture of complex quantitative traits such as biotic stress tolerance. More than 40 QTLs map for phenologic traits and kernel related traits in maize which are indirectly responsible for stress tolerance (Li et al. 2013). There exist successful and practical examples of association mapping in maize which give a new avenue for

identification and/or introgression of rare alleles into elite maize germplasm via a molecular marker assisted breeding. In a wide range of African agro-ecologies, Genome wide association mapping (GWAS) was used in maize inbred and double haploid lines to map several complex traits including disease and insect resistance, for example, resistance to maize chlorotic mottle virus and response to the Mediterranean corn borer (MCB) (Awata et al. 2019) (Table 3.4).

In the past few decades understanding of disease tolerance has been improved by the inclusion of GWAS techniques in the identification of marker trait association and trait specific identification of genotypes. However, relatively small portion of phenotypic variation for a trait can be explained in any given GWAS. So further, genomic studies to uncover this missing part can be explore in future.

**Table 3.4** Some of the biotic stress tolerant traits dissected via a GWAS in maize are given below

| Traits category | Phenotype | Population | Sample size | Number of markers | Reference |
|---|---|---|---|---|---|
| Stress resistance | Disease resistance | IAP | 1487 | 8.2 K | Van Inghelandt et al. (2012) |
| | | IAP | 527 | 557 K | Chen et al. (2015) |
| | | IAP | 1687 | 201 K | Zila et al. (2014) |
| | | IAP | 999 | 56 K | Ding et al. (2015) |
| | | IAP | 890 | 56 K | Mahuku et al. (2016) |
| | | IAP | 818 | 43.4 K | Chen et al. (2016) |
| | | IAP | 274 | 426 K | Mammadov et al. (2015) |
| | | IAP | 287 | 461 K | Tang et al. (2015), Warburton et al. (2015) |
| | | IAP | 280 | 459 K | Gowda et al. (2015) |
| | | IAP | 267 | 47 K | Zila et al. (2013) |
| | | IAP | 346 | 60 K | Farfan et al. (2015) |
| | | IAP | 267 | 287 K | Horn et al. (2014) |
| | | USNAM | 4892 | 1.6 M | Poland et al. (2011), Kump et al. (2011) |
| | Insect resistance | IAP | 302 | 246 K | Samayoa et al. (2015) |
| | Hyper sensitive response | IAP | 231 | 47 K | Olukolu et al. (2013) |
| | | USNAM | 3381 | 26.5 M | Olukolu et al. (2014) |

*Source* Xiao et al. (2017)

## 3.9 Genomics-Aided Breeding for Traits Conferring Resistance

### *3.9.1 Structural and Functional Genomic Resources*

*Mutant Libraries*

Mutants are one of the most important functional genomics resources in plants and transposon tagging is the widely used approach for gene cloning in maize. Transposon tagging has been used to clone many important genes in maize including the well-known domestication gene (*tb1*) (Doebley and Wang 1997). Maize genes have been tagged using active Mu in different research programmes including Uniform Mu (McCarty et al. 2005) which is widely used by the maize researchers and have uniform Mu-insertion for 30% of maize genes. Some of the other programmes are Maize Targeted Mutagenesis database, Trait Utility System for Corn, Mu array, RescueMu, Photosynthetic Mutant Screen (Brutnell 2002). Maize mutant libraries have also been constructed through targeting induced local lesions in genomes (TILLING) (Till et al. 2004; Lu et al. 2018) and much higher number (80%) of genes have been reported to cover using this approach (Lu et al. 2018).

*High Resolution Mapping Populations*

Number of high-resolution mapping populations have been developed by maize researchers and are freely available for genetic mapping (https://maizegdb.org/stock_catalog). Intermated B73-Mo17 (IBM) is one of such intermated RIL (IRIL) population which was derived through initial intermating among $F_2$ (B73 × Mo17) individuals for four generations and thereafter selfing through single-seed descent (SSD) method. The additional four generation of recombination supported higher (2.7-fold) recombination fraction and longer (3.86-fold) map length (Lee et al. 2002). The another most important available resource is nested association mapping (NAM) population generated by crossing 25 founder lines with the common parent (B73) (Yu et al. 2008). This population has the advantage of both linkage and association mapping (McMullen et al. 2009) and captured approximately three recombination event per gene including total ~136,000 recombination events. These populations have been used to dissect the genetic basis of different traits including trait like disease resistance to southern leaf blight caused by *Cochliobolus heterostrophus* (Balint Kurti et al. 2007; Kump et al. 2011). Further, the "Goodman" maize panel representing the diversity of public breeding programs consists of 302 inbred lines have been characterized using high throughput sequencing and used to dissect the genetic basis of different disease resistance traits including resistance to ear rot resistance (Zila et al. 2013), aflatoxin (Farfan et al. 2015), *Fusarium verticillioides* infection (Stagnati et al. 2019) etc. MaizeGo panel (http://www.maizego.org/Resources.html) consisting of 540 maize lines is another association panel representing the largest AMP panel ever assembled for maize

(Yang et al. 2011), which has also been used to explore disease resistance traits including other traits (Ding et al. 2015; Li et al. 2019).

### 3.9.2 Details of Genome Sequencing

Schnable et al. (2009) released the first reference genome (B73 RefGen_v1) of maize based on the sequencing of bacterial artificial chromosomes (BAC) and phasmids. Subsequently, the reference genome has been improved (B73 RefGen_v4) using single-molecule real-time (SMRT) sequencing and high-resolution optical mapping with rapid increase (52-fold) in contig length than previous version with notable progresses in intergenic spaces and centromeres assembly. Comparison of inbred lines with B73 reference genome revealed millions of SNPs and InDels along with many presence/absence variation (PAV), structural variations (SVs), copy-number variation expression presence/absence variation (ePAV) etc. (Springer et al. 2009; Lai et al. 2010; Fu et al. 2013; Hirsch et al. 2014; Jin et al. 2016; Bukowski et al. 2018; Sun et al. 2018). However, identification and mapping of new SNPs has been limited by the use of single reference genome only, which restrict the use of genome data, detection of SVs, and exploration of genetic diversity in real sense. Since 2016, multiple genomes, viz., PH207 (Hirsch et al. 2016), mexicana (Yang et al. 2017a), Mo17 (Yang et al. 2017a, b; Sun et al. 2018), W22 (Springer et al. 2018), HZS (Li et al. 2019), and SK (Yang et al. 2019) have been sequenced, which can be used as representative genomes. Moreover, B73 Ref_V4, Mo17 and SK genome assemblies are of much high quality which can be advantageous for genome annotation, identification of promoters and TEs (Yang et al. 2019).

## 3.10 Genetic Engineering for Biotic Stress Resistance in Maize

### 3.10.1 Disease Resistance

Over expression of *Mcchit1* gene in maize significantly reduced frequency and size of lesions compared to the control plants after 5 days inoculation of *Exserohilum turcicum* (Zhu et al. 2011). Transgenic maize expressing an enhanced green fluorescent protein fused to a ZEN-degrading enzyme (zhd101) was evaluated against *F. graminearum* infection. When the seeds were artificially contaminated by immersion in a ZEN solution for 48 h at 28 °C, the total amount of the mycotoxin in the transgenic seeds was consistently reduced to less than 1/10 of that in the wild type (Igawa et al. 2007). Overexpression of *ZmRACK1* in maize enhanced the expression levels of the pathogenesis-related protein genes, *PR-1* and *PR-5* by 2.5–

3 folds, and production of reactive oxygen species production and reduced the symptoms caused by *Exserohilum turcicum* (Wang et al. 2014a, b).Transgenic maize developed by constitutively expressing the Totivirus antifungal protein KP4 exhibited the robust resistance to *U. maydis* and expressed high levels of KP4 without any apparent negative impact on plant development (Allen et al. 2011). Transgenic maize developed by expressing the sorghum *y1* gene encoding a MYB transcription factor yellow seed1 (y1), an orthologue of the maize gene pericarp color1 (p1). LC-MS profiling of fungus-challenged transgenic maize leaves exhibited the increase in luteolinidin and flavonoids content in leaves which facilitated resistance to *Colletotrichum graminicola* infection (Ibraheem et al. 2015). Heterologous expression (under control of the constitutive CaMV 35S promoter) of a *Lablab purpureus* L. α-amylase inhibitor-like protein (*AILP*) in maize was performed and tested against *A. flavus*. Fungal growth has been observed to reduce from 35 to 72% in transgenic maize kernels which, in turn, facilitated into a 62–88% reduction in aflatoxin content (Rajasekaran et al, 2019). Expression of siRNAs (targeting *amy1, aflR* and *aflM* genes) in maize has been reported to provide excellent protection against *A. flavus* (Gilbert et al. 2018; Masanga et al. 2015 and Raruang et al. 2020). Up to 72% reduction in growth of *A. flavus* has been reported in maize expressing Tachyplesin1-derived synthetic peptide AGM182 (Rajasekaran et al. 2018).

An hpRNA targeting P1 protein (protease) gene of *Maize dwarf mosaic virus* (MDMV) was transformed in maize and the transgenic lines were showing excellent protection against MDMV disease (Zhang et al. 2010). Transgenic maize expressing *Maize dwarf mosaic virus* strain B (MDMV-B) coat protein provided resistance to inoculations with MDMV-A or MDMV-B and to mixed inoculations of MDMV and maize chlorotic mottle virus (Murry et al. 1993).To overcome the low efficiency of agronomic protection from maize dwarf mosaic disease, susceptible maize inbred line was transformed with *Agrobacterium* harbouring hpRNA expression vectors containing inverted-repeat sequences of different lengths targeting coat protein (*cp*) gene of MDMV. The MDMV resistance mediated by RNA interference was observed to be relative to the length of the inverted-repeat sequence, the copy number of T-DNA integration and the repeatability of integration sites. A longer hpRNA expression construct shows more efficiency than a shorter one (Zhang et al. 2011). Transgenic maize expressing mutated *Maize streak virus* replication-associated protein provided a higher survival rates than non-transgenic control plants after MSV inoculation. Similar results exhibited by transgenic hybrid developed by crossing $T_2$ Hi-II with the widely grown, commercial, highly MSV-susceptible, white maize genotype WM3 (Shepherd et al. 2007). Transgenic maize plants expressing dsRNAof *Sugarcane mosaic virus* (SCMV)-*NIb* gene provided 60–85% resistance to SCMV inoculums in field. For silencing of *Rice black-streaked dwarf virus* (RBSDV) coding gene with gene silencing suppressor, amiRNA were constructed and transformed in maize inbred lines Z31. The disease resistance of transgenic homozygous maize with the anti-rough dwarf virus amiRNA has been enhanced as compared to wild type.

### *3.10.2 Insect Resistance*

Crystal toxin protein encoding genes i.e. *Cry1Ab, Cry1Ah, mCry3A,* etc. derived from bacterium *Bacillus thuringiensis* have been cloned downstream to CMV35S or maize ubiquitin promoter and transformed in maize individually or in combinations through micro projectile bombardment or *Agrobacterium* mediated gene transfer. Foreign genes integration into maize genome and their stability was confirmed through PCR and Southern blot analysis. A synthetic gene encoding a truncated version of the Cry1Ab protein was introduced into immature embryos of an elite line of maize. Hybrid plants obtained through crossing of transgenic elite inbred lines with commercial inbred lines were showing excellent resistance against corn borer infestation (Koziel et al. 1993). The gene *Cry1Ab* also deployed commercially for control of pyralid stem borers of maize (Baumgarte and Tebbe 2005).

The *cry1Ah* gene from *B. thuringiensis* isolate BT8 was cloned in two plant expression vectors. In the first construct, intron of maize *ubiqutin1* gene was inserted between the maize Ubiquitin promoter and *cry1Ah* gene (pUUOAH) and the second construct contained Ubiquitin promoter and *cry1Ah* gene without intron (pUOAH). Both the constructs were introduced into maize and stable transgenic plants were obtained. The ELISA results of $T_1$ and $T_2$ generation plants exhibited that the expression of Cry1Ah protein in the construct containing the *ubi1* intron (pUUOAH) was 20% higher than that of the intronless construct (pUOAH). Bioassay results showed that the transgenic maize harbouring *cry1Ah* with *ubi1* intron had high resistance to the Asian corn borers than that of the harbouring intronless construct. MIR604 transgenic corn, expressing them Cry3A protein were evaluated for survivorship of western corn rootworm, *Diabrotica virgifera virgifera* LeConte, larvae and compared with the isoline corn at three Missouri sites during 2005 and 2006. The mortality of *D. v. virgifera* due to the mCry3A protein was recorded an average of 94.88% across all seasons. The emergence of beetles was delayed 5.5 days by 50% (Hibbard et al. 2010). Transgenic crops producing insecticidal toxins from the bacterium *B. thuringiensis* are widely planted to manage agricultural insect pests. However, widespread adoption of Bt crops has led to the evolution of Bt resistance among insects. The western corn rootworm, *Diabrotica v. virgifera*, is among the most serious pests of maize in the mid-western United States and is currently managing with Bt maize. While the genes such as *Cry3Bb1*, and the closely related *mCry3A* and *eCry3.1Ab* conferring resistance against western corn rootworm are widely distributed within the Midwest, fewer cases of *Cry34/35Ab1* resistance have been observed and planting of *Cry34/35Ab1* maize is one of the methods used to manage Cry3-resistant rootworm. It has been found that fields with high levels of root injury in *Cry34/35Ab1* maize by western corn rootworm were associated with Cry34/35Ab1-resistant western corn rootworm (Gassmann et al. 2020).

## 3.11 Bioinformatics as a Tool for Studying Biotic Stress Tolerance in Maize

As whole genome information is rapidly becoming available for various pests and pathogens afflicting maize crop, it has opened up a new avenue for designing rational management strategies against these biotic stresses. The most successful biotic stress resistance deployment in maize during last two decades has been that of commercialization and widespread adoption of herbicide tolerance and insect resistance transgenic traits in maize hybrids. The GM Approval Database developed by International Service for the Acquisition of Agri-biotech Applications (ISAAA) provides the most comprehensive and updated information on approved transgenic events for managing biotic stresses. So far 108 herbicide tolerant events have been approved for cultivation; while 117 events have been approved for insect resistance. The initial sequencing of maize genome (Schnable et al. 2009) and subsequent deluge in sequencing data for various maize inbred lines provided a new and powerful tool for resistance breeding. Over last several years, extensive germplasm screening work had been conducted to identify natural genetic variation in maize germplasm for resistance against various biotic stresses. A number of unique resistant lines have been reported. But, deployment of these resistance sources in elite maize hybrids becomes difficult in absence of information on genomic regions controlling those resistance phenotypes. To address this challenge and hasten the mapping work of biotic stress tolerant genes, a number of bioinformatics resources have been developed. Some of the bioinformatics resources relevant for biotic stress research in maize are listed in Table 3.5. Extensive genomic, transcriptomic, proteomic and metabolomic data of maize with respect to inoculation/infection/infestation with various maize insect pests, pathogens etc. are available in general bioinformatics resources, like National Centre for Biotechnology Information (NCBI) portal. NCBI also hosts similar data for various maize insect pests, pathogens species per se.

## 3.12 Rationale of Genome Designing, Limitations and Prospect of Genomic Designing

The advent of genomics assisted breeding and genome manipulation techniques promises a real revolution in plant breeding, biotechnology and genetic engineering. The use of molecular markers and genomic tools has accelerated the process of plant breeding. The emergence of genome and gene editing tools aid in targeted editing of the genomes and allows the investigations into fundamental basis of biological systems and help achieve the goals of higher productivity and quality of crops coupled with biotic and abiotic stress resistance/tolerance (Kamburova et al. 2017; Tyagi et al. 2020). In contrast to conventional plant breeding methods, these enable greater precision with lesser population size to achieve targeted results

**Table 3.5** Bioinformatics resources relevant for biotic stress research in maize

| S. No. | Name of resource | Main features | Primary developer/ host of database | Reference/URL |
|---|---|---|---|---|
| 1 | Bacterial Pesticidal Protein Resource Center | Comprehensive information on Bt/ non-Bt pesticidal proteins for academics, regulators, and research and development personnel | University of Sussex, Cardiff University, and University of Florida | https://www.bpprc.org/ |
| 2 | BtToxin_Digger | A comprehensive and high-throughput pipeline for mining toxin protein genes from *Bacillus thuringiensis* | Huazhong Agricultural University | Liu et al. (2020) |
| 3 | CryProcessor | Open source tool to carry out massive screening for novel 3d-Cry toxins and obtain sequences of specific domains for further comprehensive in silico experiments in constructing artificial toxins | All-Russia Research Institute for Agricultural Microbiology | Shikov et al. (2020) |
| 4 | CryGetter | A tool to automate retrieval and analysis of Cry protein data | Instituto Federal de EducaçãoCiência e Tecnologia de São Paulo | Buzatto et al. (2016) |
| 5 | Insects in Indian Agro-ecosystems database | A pictorial database of maize insect-pests in India | ICAR-National Bureau of Agricultural Insect Resources | https://www.nbair.res.in/Databases/insectpests/pestsearch.php?cropname=Maize |
| 6 | USDA Ag Data Commons | Data from: Datasets for transcriptomic analyses of maize leaves in response to Asian corn borer feeding and/or jasmonic acid and other genomic data | United States Department of Agriculture | Zhang et al. (2016) |
| 7 | International Herbicide-Resistant Weed Database | Global and constantly updated database of herbicide tolerant | Global Herbicide Resistance Action Committee and | http://www.weedscience.org/Home.aspx |

(continued)

Table 3.5 (continued)

| S. No. | Name of resource | Main features | Primary developer/ host of database | Reference/URL |
|---|---|---|---|---|
| | | weeds in maize and other crops | CropLife International | |
| 8 | Maize Genetics and Genomics Database (MaizeGDB) | Genome browser; Genome and gene annotation browser; Nested Association Mapping (NAM) founder lines (25) genome browser; qTeller: a *comparative* RNA-seq expression platform; Metabolic pathways; etc. | United States Department of Agriculture-Agricultural Research Service (USDA-ARS) | Portwood et al. (2019) https://www.maizegdb.org/ |
| 9 | MaizeMine | Gene, Gene expression, Proteins, Homology, Functions, Variations, etc. | University of Missouri | Elsik et al. (2018) http://maizemine.rnet.missouri.edu:8080/maizemine/begin.do |

quickly. Mutagenesis can provide variations, but such as undirected mutagenesis may result in unwanted off-target effects. With the genomic and genome editing tools, it is possible to introduce mutations at specific target loci of interest, which can be analysed and tested for resistance to stresses. Additionally, it also allows the introduction of transgenes at a defined chromosomal location. These technologies are powerful, versatile and will greatly facilitate efficient expression and avoid negative side effects caused, usually by integration of transgene into a different gene. These new tools are expected to facilitate breeding of stress-resistant transgenic or transgene free crops in relatively short time, (Borel 2017). The genome editing (GE) with specialized nucleases will aid in introducing targeted and accurate deletions, insertions, and replacement at site-specific genomic locations. Examples of the use of specialized nucleases include, Zinc Finger Nucleases, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), Oligonucleotide-directed mutagenesis, RNA-dependent DNA methylation, and precision breeding for crop plant improvement (Doudna and Charpentier 2014; Gray and Brady 2016).

The GE tools have been successfully used to control diseases caused by fungi, bacteria, and viruses. In general, CRISPR/cas technique has been used in two ways to control pathogens by editing the genes required for infection process; (i) modifying pathogen genes (ii) modifying plant host genes. GE has been successful in controlling the powdery mildew by editing the host susceptibility factor (mildew-resistance locus-*MLO*) in wheat and tomato (Wang et al. 2014a, b;

Nekrasov et al. 2017), developing resistance against rice blast (*Magnaporthe oryzae*) and bacterial blight (*Xanthomonas oryzae* pv. *oryzae*), and bacterial speck of tomato (Ortigosa et al. 2019). Furthermore, GE has potential in controlling RNA and DNA viruses of plants (Ali et al. 2015). The GE by CRISPR/cas is expected to play important role in development of resistant genotypes in relatively short time (Kamburova et al. 2017). For maize, which is recalcitrant to regeneration, protoplast transient assay is becoming an efficient tool for testing CRISPR target before starting the transformation of embryos or scutellum derived calli by Agrobacterium or particle bombardment (Gao et al. 2010). The only report on development of GE turcicum leaf blight resistant maize implied the potential of GE in maize for development of disease resistant genotypes as well. The above information does not imply that genome-editing technology is the substitute for conventional or GM or molecular breeding techniques; most probably they have to coexist. However, genome editing would apparently deliver certain benefits better, quickly and with high precision (Lassoued et al. 2019).

Although GE technologies have been successful in development of genotypes to combat pathogens in important crops, they are not yet fully exploited for the management of insect pests. The most important limitation has been the lack of availability of target genes at present against the insect pests. Once such genes are available, targeted mutagenesis of host plants through GE will be able to manage their respective pests (Tyagi et al. 2020).

Although the GE system looks straightforward, it too has limitations. It is difficult to practice gene insertion and in vitro regeneration in recalcitrant crops. There is a need for optimization and development of protocols for plant genome editing such as plant compatible set of vector systems, efficient plant transformation protocols and delivery systems, efficient screening of transformation events, which can be streamlined to enable rapid product development (Schenke and Cai 2020). GE requires implementation of proper bioinformatics specific pipelines, setting up workflows and transformation efficiency. Moreover, mutating plant genes may intervene with the normal cellular and development functions and may affect crop performance. In addition, GE for improved disease resistance depends on the availability of genome sequence information of both plant host and pathogen. At present, information on genes involved in host/pathogen interactions is limited. Also, targeting individual pathogen genes may not be efficient, due to the emergence of new strains with altered virulence and host ranges. QTL analysis for Mediterranean corn borer resistance revealed low percentage of phenotypic variance, which makes marker assisted selection for improving resistance less possible. Pleiotropism or linkage between genes would also imbalance resistance and agronomic traits (Ordas et al. 2009). Polygenic nature of maize resistance to *Busseolafusca* and *Chilopartellus*, which involves additive, dominance, and epistatic effects and its low to moderate heritability makes breeding for HPR difficult in maize (Murenga et al. 2018). Thus, clearly quantitative nature of maize resistance to insect pests which involves polygenes, often with low heritability that vary in spatial and temporal expression makes conventional breeding a challenging task. Since the genome diverts its energy for expressing many resistance traits at the cost

of its yield, the negative relationship between insect resistance and yield is the expected normal consequence. Thus, achieving desirable level of genetic gains is nearly an impossible task. Thus, the application of genomic tools for improving insect resistance in maize is not attempted at a practical level. There is also concern regarding the biosafety and regulatory issues on products developed through GE technologies (Khatabi et al. 2019). Hence, forthcoming regulatory protocols will play role in deciding the mode of testing and commercialization of Genome Edited crop varieties.

## 3.13 Social, Political and Regulatory Issues

In contrast to traditional plant breeding, new biotechnological tools have both pros and cons in crop improvement. Acceptance of the tools and products obtained by new biotechnological tools are debatable. Always there is a counterargument for utilization of NBT in agriculture. Though NBT has scientific potential, they have been, and are being considered as a fundamentally controversial invention in some countries. Any technology will be successful only after its wide acceptance by consumers, regulators, and non-governmental organizations (NGOs) (Hall and Martin 2005). The acceptance of innovation depends on its extent of socio-political legitimacy, where political influences and cultural aspects matter (Aldrich and Fiol 1994).

To develop insect and pest resistant maize genotypes, genetic engineering played major role in recent years. The genetic engineering in maize has provided economic advantages to some marginal farmers/adopters in the early years. Sustained gains will typically be expected in those situations in which farmers are economically able with the institutional support, such as access to credit, extension services, affordable inputs, and markets.

Institutional factor favours economic benefits to small-scale farmers. Yield can be enhanced and stabilized by improving germplasm, environmental conditions, management practices, and socioeconomic and physical infrastructure for which investments in GE crop R&D may be just one potential strategy to solve agricultural-production and food-security problems. Decision of policy-makers determines much and the ways in which resources are distributed among the different categories of farmers to improve production depends on agricultural policies. Though scientist says genetically engineered crops are economically viable option, but because of credit constraints and the money and time spent on redundant insecticide applications especially by small scale farmers made them apparently non-viable at least in some cases. These outcomes indicated an initial lack of familiarity with genetic-engineering technology and strongly suggested the need for extension services for small-scale farmers, especially during initial deployment (Hamburger 2018).

Precision plant breeding plays an important role in accelerated crop improvement. Genome editing enabled next generation biotechnological tools made

breeding/improving crops with site-specific genetic modification a reality. Mutation/change in the DNA sequence leading to the novel genetic architecture is a natural phenomenon that takes several years, but CRISPR technology based base editing techniques can lead to novel beneficial alterations in plants in quick time. However, controversial debate whether at all and how to regulate genome edited plants has essentially led to the formation of two contrasting schools of thought. Possibility of generation of off targets that would lead to abnormal changes in the ecology/plant system needs attention and gained importance as a matter of discussion (Lassoued et al. 2018). There is differential opinion across different countries. New Policy under the single umbrella is required to facilitate the utilization of novel, fast track breeding systems. Institution support for scientific community as well as farming community will make proper utilization of novel ideas, which support targeted breeding to achieve expected goals in plant breeding (Sprink et al. 2020).

## 3.14 Future Perspective

Maize is a crop of future of the world; having highest yield potential and providing raw material for many agro-based industries. It is having higher adaptability to various agro-climatic conditions than any other cereal crop. However, insect pests and diseases are affecting maize crop. Integration of different breeding methods along with biotechnological tools is must to develop sustainable resistance breeding mechanism against biotic stresses. Application of New Breeding Tools enables breeding against disease and pest in crop in general and maize in particular. Genomic resources developed in maize play important role in identification of novel genes for pest and disease resistance and understanding on their tolerance mechanism. Sequencing and re-sequencing approaches made genomic assisted maize improvement possible. Utilization of next generation tools and techniques surely finds answer to emerging biotic threats to maize in years to come. Although genome editing is one of the potential novel technologies, recalcitrant nature of maize to transformation and/or availability of little information on maize transformation protocols are responsible for slower pace in its successful utilization in maize. Hence, research efforts on these aspects and related to transgenics followed by application of CRISPR technology may provide answer to biotic stress tolerance in maize.

## References

Acquaah G (2015) Conventional plant breeding principles and techniques. In: Acquaah G (ed) Advances in plant breeding strategies: breeding, biotechnology and molecular tools. Springer, Cham, Switzerland, pp 115–158

Akinbode OA (2010) Evaluation of antifungal efficacy of some plant extracts on *Curvularia lunata*, the causal organism of maize leaf spot. African J Environ Sci Tech 4(11):797–800

Ako M, Schulthess F, Gumedzoe MY, Cardwell, KF (2003) The effect of Fusarium verticillioides on oviposition behaviour and bionomics of lepidopteran and coleopteran pests attacking the stem and cobs of maize in West Africa Entomologia Experimentalis et Applicata 106(3):201–210

Al Eryan MA, El Tabbakh SS (2004) Forecasting yield of corn, Zea mays infested with corn leaf aphid, Rhopalosiphum maidis. J Appl Entomo 128(4):312–315

Aldrich HE, Fiol CM (1994) Fools rush in? The institutional context of industry creation. Acad Manag Rev 19:645–670

Ali F, Yan J (2012) Disease resistance in maize and the role of molecular breeding in defending against global threat. J Integrat Plant Bio 54(3):134–151

Ali Z, Abulfaraj A, Idris A, Ali S, Tashkandi M et al (2015) CRISPR/Cas9-mediated viral interference in plants. Genome Bio 16(1):238–242

Allen A, Islamovic E, Kaur J, Gold S, Shah D et al (2011) Transgenic maize plants expressing the Totivirus antifungal protein, KP4, are highly resistant to corn smut. Plant Biotech J 9(8):857–864

Archana R, Lohithaswa HC, Pavan R (2019) Genetic analysis of Fusarium stalk rot resistance in maize (*Zea mays* L.). J Pharmacog Phytochem 19:58–61

Arnason JT, Baum B, Gale J, Lambert JD, Bergvinson D et al (1993) Variation in resistance of Mexican landraces of maize to maize weevil *Sitophilus zeamais*, in relation to taxonomic and biochemical parameters. Euphytica 74(3):227–236

Awata LAO, Ifie BE, Tongoona P, Danquah E, Jumbo MB et al (2019) Maize lethal necrosis and the molecular basis of variability in concentrations of the causal viruses in co-infected maize plant. J Genet Mol Virol 9(1):1–19

Ayiga Aluba J, Edema R, Tusiime G, Asea G, Gibson P (2015) Response to two cycles of S1 recurrent selection for turcicum leaf blight in an open pollinated maize variety population. Adv Appl Sci Res 6(12):4–12

Badu Apraku B, Fakorede MAB (2017) Breeding for disease resistance in maize. Advances in genetic enhancement of early and extra-early maize for Sub-Saharan Africa. Springer, Cham, Switzerland, pp 379–410

Balconi C, Stevanato P, Motto M, Biancardi E (2012) Breeding for biotic stress resistance/ tolerance in plants. In: Balconi C (eds) Crop production for agril improve. Springer, Dordrecht, pp 57–114

Balint Kurti PJ, Carson ML (2006) Analysis of quantitative trait loci for resistance to southern leaf blight in juvenile maize. Phytopathology 96(3):221–225

Balint Kurti PJ, Zwonitzer JC, Wisser RJ, Carson ML, Oropeza Rosas MA et al (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

Balint Kurti GG, Zou S, Brown A (2008) Optimal control theory for manipulating molecular processes. Adv Chem Phy 138:43–94

Balint Kurti PJ, Yang J, Van Esbroeck G, Jung J, Smith ME (2010) Use of a maize advanced intercross line for mapping of QTL for northern leaf blight resistance and multiple disease resistance. Crop Sci 50(2):458–466

Baumgarte S, Tebbe CC (2005) Field studies on the environmental fate of the Cry1Ab Bt-toxin produced by transgenic maize (MON810) and its effect on bacterial communities in the maize rhizosphere. Mol Ecol 14(8):2539–2551

Berger HK (2001) The western corn rootworm (*Diabrotica virgifera virgifera*): a new maize pest threatening Europe. EPPO Bull 31(3):411–414

Bergquist RR (1979) Selection for disease resistance in a maize breeding programme. II. Introgression of an alien genome from Tripsacum dactyloides conditioning resistance in Zea mays. In: Proceedings of the tenth meeting of the Maize and Sorghum Section of *Eucarpia*, 17–19 Sept 1979, Varna Bulgaria, pp 200–206

Bergquist RR (1981) Transfer from Tripsacum dactyloides to corn of a major gene locus conditioning resistance to Puccinia sorghi. Phytopathology 71(5):518–520

Bergvinson DJ, Arnason JT, Mihm JA, Jewell DC (1994) Phytochemical basis for multiple borer resistance in maize. In: Mihm JA (ed) Insect resistant maize: recent advances and utilization; Proceedings of an International Symposium Held at the International Maize and Wheat Improvement Center (CIMMYT), 27 Nov–3 Dec 1994 CIMMYT27, pp 82–90

Bhat JS, Mukri G, Patil BS (2017) Turcicum leaf blight resistance in maize: field screening of new inbreeds and hybrids. Int J Adv Res Sci Eng 6(9):141–149

Biffen RH (1905) Mendel's laws of inheritance and wheat breeding. J Agril Sci 1(1):4–48

Bock CH, Jeger MJ, Mughogho LK, Cardwell KF, Mtisi E et al (2000) Variability of Peronosclerospora sorghi isolates from different geographic locations and hosts in Africa. Mycol Res 104(1):61–68

Bohn M, Kreps RC, Klein D, Melchinger AE (1999) Damage and grain yield losses caused by European corn borer (Lepidoptera: Pyralidae) in early maturing European maize hybrids. J Econ Entomol 92(3):723–731

Borel B (2017) When the pesticides run out. Nature 543:302–304

Botha AM, Venter E (2000) Molecular marker technology linked to pest and pathogen resistance in wheat breeding. South African J Sci 96(5):233–240

Brooks TD, Shaun Bushman B, Paul Williams W, McMullen MD, Buckley PM (2007) Genetic basis of resistance to fall armyworm (Lepidoptera: Noctuidae) and southwestern corn borer (Lepidoptera: Crambidae) leaf-feeding damage in maize. J of Econ Entomol 100(4):1470–1475

Brutnell TP (2002) Transposon tagging in maize. Funct Integrat Genom 2(1–2):4–12

Buckler ES, Thornsberry JM, Kresovich S (2001) Molecular diversity, structure and domestication of grasses. Genet Res 77(3):213–218

Bukowski R, Guo X, Lu Y, Zou C, He B et al (2018) Construction of the third-generation Zea mays haplotype map. Gigascience 7(4):1–12

Buzatto D, de Castro França S, Zingaretti SM (2016) CryGetter: a tool to automate retrieval and analysis of Cry protein data. BMC Bioinfo 17(1):1–14

Carson ML, Stuber CW, Senior ML (2004) Identification and mapping of quantitative trait loci conditioning resistance to southern leaf blight of maize caused by *Cochliobolus heterostrophus* race O. Phytopathology 94(8):862–867

Casela CR, Ferreira AS (2002) Variability in isolates of Puccinia polysora in Brazil. Fitopatologia Brasileira 27(4):414–416

Castagnola AS, Jurat Fuentes JL (2012) Bt crops: past and future. In: Castagnola AS (ed) Bacillus thuringiensis biotechnology. Springer, Dordrecht, pp 283–304

Chang MS, Hudon M, Devaux A (1990) Inheritance of resistance to Kabatielle eyespot of maize. Phytoprotection 71(3):107–112

Chavan S, Smith SM (2014) A rapid and efficient method for assessing pathogenicity of Ustilago maydis on maize and teosinte lines. J Visual Exp 83:

Chen G, Wang X, Hao J, Yan J, Ding J (2015) Genome-wide association implicates candidate genes conferring resistance to maize rough dwarf disease in maize. PLoS ONE 10(11):

Chen J, Shrestha R, Ding J, Zheng H, Mu C et al (2016) Genome-wide association study and QTL mapping reveal genomic loci associated with Fusarium ear rot resistance in tropical maize germplasm. Genes, Genomes, Genet 6(12):3803–3815

Chiang HC, Hodson AC (1950) Stalk breakage caused by European corn borer and its effect on the harvesting of field corn. J Econ Entomol 43(4):415–422

Collins N, Drake J, Ayliffe M, Sun Q, Ellis J et al (1999) Molecular characterization of the maize Rp1-D rust resistance haplotype and its mutants. Plant Cell 11(7):1365–1376

Connors CH (1922) Inheritance of foliar glands of the peach. Proc Am Soc Hort Sci 18:20–26

Cordero MJ, Raventós D, San Segundo B (1994) Expression of a maize proteinase inhibitor gene is induced in response to wounding and fungal infection: systemic wound-response of a monocot gene. Plant J 6(2):141–150

Costa RV, Simon J, Cota LV, da Silva DD, de Almeida REM et al (2019) Perdas na produtividade de milho safrinha em razão de podridões da base do colmo. Pesquisa Agropecuária Brasileira 54(X):283–290

Crous PW, Liebenberg MM, Braun U, Groenewald JZ (2006) Re-evaluating the taxonomic status of Phaeoisariopsis griseola, the causal agent of angular leaf spot of bean. Studies Mycol 55:163–173

Degani O, Dor S, Movshowitz D, Fraidman E, Rabinovitz O (2018) Effective chemical protection against the maize late wilt causal agent, Harpophora maydis, in the field. PLoS ONE 13(12):

Devi B, Thakur BR (2018) Integrated management of banded leaf and sheath blight of maize caused by Rhizoctonia solani f. sp. sasakii. Proc Natl Acad Sci, India Section B: Bio Sci 88 (2):769–777

Dey U, Harlapur SI, Dhutraj DN, Suryawanshi AP, Bhattacharjee R (2015) Integrated disease management strategy of common rust of maize incited by Puccinia sorghi Schw. African J Microbio Res 9(20):1345–1351

Dhami NB, Kim SK, Paudel A, Shrestha J, Rijal TR (2015) A review on threat of gray leaf spot disease of maize in Asia. J Maize Res Dev 1(1):71–85

Dhillon BS, Vasal SK, Prasanna BM (2002) In: VL Chopra and Prakash S (eds) Evolution and adaptation of cereal crops. Oxford & IBH, New Delhi, pp 99–133

Ding J, Ali F, Chen G, Li H, Mahuku G et al (2015) Genome-wide association mapping reveals novel sources of resistance to northern corn leaf blight in maize. BMC Plant Bio 15(1):206

Doebley J, Wang RL (1997) Genetics and the evolution of plant form: an example from maize. In: Doebley J (ed) Cold spring harbor symposia on quantitative biology. Cold Spring Harbor Labor Press 62:361–367

Doudna JA, Charpentier E (2014) The new frontier of genome engineering with CRISPR-Cas9. Science 346:6213

Dovas CI, Eythymiou K, Katis NI (2004) First report of maize rough dwarf virus (MRDV) on maize crops in Greece. Plant Pathol 53(2):238

Dowd PF, Zuo WN, Gillikin JW, Johnson ET, Boston RS (2003) Enhanced resistance to *Helicoverpa zea* in tobacco expressing an activated form of maize ribosome-inactivating protein. J Agril Food Chem 51(12):3568–3574

Eagles HA, Bariana HS, Ogbonnaya FC, Rebetzke GJ, Hollamby GJ et al (2001) Implementation of markers in Australian wheat breeding. Australian J Agril Res 52(12):1349–1356

Elsik CG, Tayal A, Unni DR, Nguyen HN, Gardiner J et al (2018) MaizeMine: a data mining warehouse for MaizeGDB. In: Plant and animal genome XXVI conference, Jan 13–17

Ersoz ES, Yu J, Buckler ES (2009) Applications of linkage disequilibrium and association mapping in maize. Molecular genetic approaches to maize improvement. Springer, Berlin, Heidelberg, pp 173–195

Ertiro BT, Semagn K, Das B, Olsen M, Labuschagne M et al (2017) Genetic variation and population structure of maize inbred lines adapted to the mid-altitude sub-humid maize agro-ecology of Ethiopia using single nucleotide polymorphic (SNP) markers. BMC Genomics 18(1):1–11

Faluyi JO, Olorode O (1984) Inheritance of resistance to *Helminthosporium maydis* blight in maize (Zea mays L.). Theor Appl Genet 67(4):341–344

FAO (2020) http://www.fao.org/3/ca8032en/ca8032en.pdf

Farfan IDB, Gerald N, Murray SC, Isakeit T, Huang PC et al (2015) Genome wide association study for drought, aflatoxin resistance, and important agronomic traits of maize hybrids in the sub-tropics. PLoS ONE 10(2):

Farias Rivera LA, Hernandez Mendoza JL, Molina Ochoa J, Pescador Rubio A (2003) Effect of leaf extracts of teosinte, Zea diploperennis L., and a Mexican maize variety, criollo 'uruapeño', on the growth and survival of the fall armyworm (Lepidoptera: Noctuidae). Florida Entomol 86 (3):239–243

Ferguson LM, Carson ML (2007) Temporal variation in Setosphaeria turcica between 1974 and 1994 and origin of races 1, 23, and 23 N in the United States. Phytopathology 97(11):1501–1511

Findley WR, Nault LR, Styer WE, Gordon DT (1982) Inheritance of maize chlorotic dwarf virus resistance in maize × Zeadiploperennis backcrosses. Maize News Lett 56:165–166

Flor HH (1946) Genetics of pathogenicity in *Melampsora lini*. J Agric Res 73:335–357

Frey TJ (2005) Fine-mapping, cloning, verification, and fitness evaluation of a QTL, Rcg1, which confers resistance to Colletotrichum graminicola in maize. PhD dissertation, University of Delaware

Fu D, Uauy C, Distelfeld A, Blechl A, Epstein L et al (2009) A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. Science 323(5919):1357–1360

Fu J, Cheng Y, Linghu J, Yang X, Kang L et al (2013) RNA sequencing reveals the complex regulatory network in the maize kernel. Nature Commun 4(1):1–12

Gamliel A, Katan J (1993) Suppression of major and minor pathogens by fluorescent pseudomonads. Phytopathology 83:68–75

Gao X, Brodhagen M, Isakeit T, Brown SH, Göbel C et al (2009) Inactivation of the lipoxygenase ZmLOX3 increases susceptibility of maize to Aspergillus spp. Mol Plant Microbe Interact 22 (2):222–231

Gao M, Kawabe M, Tsukamoto T, Hanada H, Tao R (2010) Somatic embryogenesis and Agrobacterium-mediated transformation of Japanese apricot (Prunus mume) using immature cotyledons. Scientia Horti 124(3):360–367

Gassmann AJ, Shrestha RB, Kropf AL, St Clair CR, Brenizer BD (2020) Field-evolved resistance by western corn rootworm to Cry34/35Ab1 and other Bacillus thuringiensis traits in transgenic maize. Pest Manage Sci 76(1):268–276

Geiringer H (1944) On the probability theory of linkage in Mendelian heredity. Ann Math Stat 15 (1):25–57

Gilbert MK, Majumdar R, Rajasekaran K, Chen ZY, Wei Q et al (2018) RNA interference-based silencing of the alpha-amylase (amy1) gene in Aspergillus flavus decreases fungal growth and aflatoxin production in maize kernels. Planta 247(6):1465–1473

Gogoi R, Bisht S, Mathuria RC, Venkatesh I (2018) Biological control of maize diseases. In: Singh D, Chakraborty BN, Pandey RN, Sharma P (eds) *Biological* control of crop diseases: recent advances & perspectives, Part 2: Cereals, pulses, oilseeds and other crops. Indian Phytopathological Society. Today & Tomorrow's Printers and Publishers, New Delhi, pp 479–499

Goldberg KB, Brakke MK (1987) Concentration of maize chlorotic mottle virus increased in mixed infections with maize dwarf mosaic virus, strain B. Phytopathology 77(2):162–167

Gowda M, Das B, Makumbi D, Babu R, Semagn K et al (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

Gray SB, Brady SM (2016) Plant developmental responses to climate change. Dev Bio 419(1):64–77

Groth JV, Zeyen RJ, Davis DW, Christ BJ (1983) Yield and quality losses caused by common rust (Puccinia sorghi Schw.) in sweet corn (Zea mays) hybrids. Crop Protect 2(1):105–111

Guthrie WD (1989) Breeding for insect resistance in maize. Plant Breed Rev 6:209–243

Hall JK, Martin MJ (2005) Disruptive technologies, stakeholders and the innovation value-added chain: a framework for evaluating radical technology development. R&D Manag 35(3):273–284

Hamburger DJ (2018) Normative criteria and their inclusion in a regulatory framework for new plant varieties derived from genome editing. Front Bioeng Biotechnol 6:176

Harlan JR, de Wet JM (1971) Toward a rational classification of cultivated plants. Taxon 20 (4):509–517

Hebbar KP, Davey AG, Merrin J, Dart PJ (1992) Rhizobacteria of maize antagonistic to *Fusarium moniliforme*, a soil-borne fungal pathogen: colonization of rhizosphere and roots. Soil Bio Biochem 24(10):989–997

Hedin PA, Davis FM, Williams WP (1993) 2-hydroxy-4, 7-dimethoxy-1, 4-benzoxazin-3-one (N-O-Me-DIMBOA), a possible toxic factor in corn to the southwestern corn borer. J chem Eco 19(3):531–542

Hellmich RL, Hellmich KA (2012) Use and impact of Bt maize. Nature Edu Knowl 3(10):4
Hibbard BE, Clark TL, Ellersieck MR, Meihls LN, El Khishen AA et al (2010) Mortality of western corn rootworm larvae on MIR604 transgenic maize roots: field survivorship has no significant impact on survivorship of F1 progeny on MIR604. J Econ Entomol 103(6):2187–2196
Hirsch CN, Foerster JM, Johnson JM, Sekhon RS, Muttoni G et al (2014) Insights into the maize pan-genome and pan-transcriptome. Plant Cell 26(1):121–135
Hirsch CN, Hirsch CD, Brohammer AB, Bowman MJ, Soifer I et al (2016) Draft assembly of elite inbred line PH207 provides insights into genomic and transcriptome diversity in maize. Plant Cell 28(11):2700–2714
Hoisington D, Khairallah M, Reeves T, Ribaut JM, Skovmand B et al (1999) Plant genetic resources: what can they contribute toward increased crop productivity? Proc Nat Acad Sci 96 (11):5937–5943
Hooda KS, Sekhar JC, Chikkappa G, Kumar S, Pandurange KT et al (2012) Identifying sources of multiple disease resistance in maize. Maize J 1:82–84
Hooda R, Bhardwaj NK, Singh P (2015) Screening and identification of ligninolytic bacteria for the treatment of pulp and paper mill effluent. Water Air Soil Pollut 226(9):1–11
Hooker AL (1972) Southern leaf blight of corn—present status and future prospects. J Environ Qual 1(3):244–249
Horn F, Habekuß A, Stich B (2014) Genes involved in barley yellow dwarf virus resistance of maize. Theor Appl Genet 127(12):2575–2584
Hurni S, Scheuermann D, Krattinger SG, Kessel B, Wicker T et al (2015) The maize disease resistance gene Htn1 against northern corn leaf blight encodes a wall-associated receptor-like kinase. Proc Nat Acad Sci 112(28):8780–8785
Hussain S, Ghaffar A, Aslam M (1990) Biological control of Macrophomina phaseolina charcoal rot of sunflower and mung bean. J Phytopathol 130(2):157–160
Hutchison WD, Burkness EC, Mitchell PD, Moon RD, Leslie TW et al (2010) Areawide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. Science 330(6001):222–225
Ibraheem F, Gaffoor I, Tan Q, Shyu CR, Chopra S (2015) A sorghum MYB transcription factor induces 3-deoxyanthocyanidins and enhances resistance against leaf blights in maize. Molecules 20(2):2388–2404
Igawa T, Takahashi Ando N, Ochiai N, Ohsato S, Shimizu T et al (2007) Reduced contamination by the Fusarium mycotoxin zearalenone in maize kernels through genetic modification with a detoxification gene. Appl Env Microbio 73(5):1622–1629
Isakeit T, Jaster J (2005) Texas has a new pathotype of Peronosclerospora sorghi, the cause of sorghum downy mildew. Plant Dis 89(5):529–529
Jalali SK, Yadavalli L, Ojha R, Kumar P, Sulaikhabeevi SB et al (2014) Baseline sensitivity of maize borers in India to the *Bacillus thuringiensis* insecticidal proteins Cry1A.105 and Cry2Ab2. Pest Manage Sci 71(8):1082–90
Jamann TM, Poland JA, Kolkman JM, Smith LG, Nelson RJ (2014) Unraveling genomic complexity at a quantitative disease resistance locus in maize. Genetics 198(1):333–344
Jamann TM, Luo X, Morales L, Kolkman JM, Chung CL et al (2016) A remorin gene is implicated in quantitative disease resistance in maize. Theor Appl Genet 129(3):591–602
Jampatong C, McMullen MD, Barry BD, Darrah LL, Byrne PF et al (2002) Quantitative trait loci for first-and second-generation European corn borer resistance derived from the maize inbred Mo47. Crop Sci 42(2):584–593
Jin M, Liu H, He C, Fu J, Xiao Y et al (2016) Maize pan-transcriptome provides novel insights into genome complexity and quantitative trait variation. Sci Rep 6:18936
Johal GS, Briggs SP (1992) Reductase activity encoded by the HM1 disease resistance gene in maize. Science 258(5084):985–987
Kamburova VS, Nikitina EV, Shermatov SE, Buriev ZT, Kumpatla SP et al (2017) Genome editing in plants: an overview of tools and applications. Int J Agron

Karaköy T, Baloch FS, Toklu F, Özkan H (2014) Variation for selected morphological and quality-related traits among 178 faba bean landraces collected from Turkey. Plant Genetic Res 12(1):5–11

Kaur H, Singh S, Grewal MS (2014) Effect of nitrogen levels on foliar and stalk rot diseases of Kharif maize. J Res 51(2):142–145

Kaur H, Kumar S, Hooda KS, Gogoi R, Bagaria P et al (2020) Leaf stripping: an alternative strategy to manage banded leaf and sheath blight of maize. Indian Phytopathol 73:203–211

Kenneth R (1970) Downy mildews of Gramineae in Israel. Indian Phytopathol 23:371–377

Kfir R, Overholt WA, Khan ZR, Polaszek A (2002) Biology and management of economically important lepidopteran cereal stem borers in Africa. Ann Rev Entomol 47(1):701–731

Khatabi B, Gharechahi J, Ghaffari MR, Liu D, Haynes PA et al (2019) Plant-microbe symbiosis: what has proteomics taught us? Proteomics 19(16):1800105

Khedekar SA, Harlapur SI, Shripad K, Benagi VI, Deshpande VK (2010) Integrated management of turcicum leaf blight of maize caused by Exserohilum turcicum (Pass.) Leonard and Suggs. Karnataka J Agril Sci 23(2):372–373

Khokhar MK, Sharma SS, Gupta R (2014) Integrated management of post flowering stalk rot of maize caused by Fusarium verticillioides. Indian Phytopathol 67(3):228–233

Kim HC, Kim KH, Song K, Kim JY, Lee BM (2020) Identification and validation of candidate genes conferring resistance to downy mildew in maize (Zea mays L.). Genes 11(2):1–20

Kloeppe JW, Rodriguez Kabana R, Zehnder AW, Murphy JF, Sikora E et al (1999) Plant root-bacterial interactions in biological control of soilborne diseases and potential extension to systemic and foliar diseases. Australasian Plant Pathol 28(1):21–26

Koziel MG, Beland GL, BowmanC Carozzi NB, Crenshaw R et al (1993) Field performance of elite transgenic maize plants expressing an insecticidal protein derived from Bacillus thuringiensis. Biotechnology 11(2):194–200

Kumar H (2002) Resistance in maize to the larger grain borer, Prostephanus truncatus (Horn) (*Coleoptera: Bostrichidae)*. J Stored Prod Res 38(3):267–280

Kumar S, Rani A, Jha MM (2009) Evaluation of plant extracts for management of maydis leaf blight of maize. Ann Plant Protect Sci 17(1):130–132

Kump KL, Bradbury PJ, Wisser RJ, Buckler ES, Belcher AR et al (2011) Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. Nat Genet 43(2):163–168

Kurosawa RDNF, Vivas M, Amaral ATD, Ribeiro RM, Miranda SB et al (2018) Popcorn germplasm resistance to fungal diseases caused by *Exserohilum turcicum* and *Bipolaris maydis*. Bragantia 77(1):36–47

Lai J, Li R, Xu X, Jin W, Xu M et al (2010) Genome-wide patterns of genetic variation among elite maize inbred lines. Nat Genet 42(11):1027–1030

Lal BB, Chakravarti BP (1976) Assessment of loss due to brown spot of maize caused by Physoderma maydis. Indian Phytopathol 29(4):449–450

Lassoued R, Smyth SJ, Phillips PW, Hesseln H (2018) Regulatory uncertainty around new breeding techniques. Front Plant Sci 9:1291

Lassoued R, Macall DM, Hesseln H, Phillips PW, Smyth SJ (2019) Benefits of genome-edited crops: expert opinion. Transgen Res 28(2):247–256

Lee M, Sharopova N, Beavis WD, Grant D, Katt M et al (2002) Expanding the genetic map of maize with the intermated B73 × Mo17 (IBM) population. Plant Mol Bio 48(5–6):453–461

Leonard KJ, Levy Y, Smith DR (1989) Proposed nomenclature for pathogenic races of Exserohilum turcicum on corn. Plant Dis 73(9):776–777

Lewontin RC, Kojima KI (1960) The evolutionary dynamics of complex polymorphisms. Evolution:458–472

Li H, Peng Z, Yang X, Wang W, Fu J et al (2013) Genome-wide association study dissects the genetic architecture of oil biosynthesis in maize kernels. Nat Genet 45(1):43–50

Li N, Lin B, Wang H, Li X, Yang F et al (2019) Natural variation in Zm FBL41 confers banded leaf and sheath blight resistance in maize. Nat Genet 51(10):1540–1548

Liu J, Jiang Y, Zeng J, Ji G, Liu L et al (2016) Analysis of the main occurrence characteristics and causes of the southern corn rust in China in 2015. China Plant Prot 36:44–47
Liu Q, Liu H, Gong Y, Tao Y, Jiang L et al (2017) An atypical thioredoxin imparts early resistance to Sugarcane mosaic virus in maize. Mol Plant 10(3):483–497
Liu H, Zheng J, Yu Y, Ye W, Peng D et al (2020) BtToxin_Digger: a comprehensive and high-throughput pipeline for mining toxin protein genes from Bacillus thuringiensis. https://doi.org/10.1101/2020.05.26.114520
Lu X, Liu J, Ren W, Yang Q, Chai Z et al (2018) Gene-indexed mutations in maize. Mol Plant 11 (3):496–504
Ott O (2009) The search for novel resistance alleles: screening teosinte-maize introgression lines for resistance to northern leaf blight. PhD Thesis, CornellUniversity, NY, USA
Mahuku G, Chen J, Shrestha R, Narro LA, Guerrero KVO et al (2016) Combined linkage and association mapping identifies a major QTL (qRtsc8-1), conferring tar spot complex resistance in maize. Theor Appl Genet 129(6):1217–1229
Mammadov J, Sun X, Gao Y, Ochsenfeld C, Bakker E et al (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
Mammadov J, Buyyarapu R, Guttikonda SK, Parliament K, Abdurakhmonov IY et al (2018) Wild relatives of maize, rice, cotton, and soybean: treasure troves for tolerance to biotic and abiotic stresses. Front Plant Sci 9:886–893
Manjunatha C, Gogoi R, Singh B, Jeevan B, Rai SN et al (2019) Phenotypic and physiological characterization of maize inbred lines resistant and susceptible to maydis leaf blight. Indian Phytopathol 72(2):217–224
Martins LB, Rucker E, Thomason W, Wisser RJ, Holland JB et al (2019) Validation and characterization of maize multiple disease resistance QTL. Genes Genomes Genet 9(9):2905–2912
Masanga JO, Matheka JM, Omer RA, Ommeh SC, Monda EO et al (2015) Downregulation of transcription factor *aflR* in *Aspergillus flavus* confers reduction to aflatoxin accumulation in transgenic maize with alteration of host plant architecture. Plant Cell Rep 34(8):1379–1387
Matsuoka Y, Mitchell SE, Kresovich S, Goodman M, Doebley J (2002) Microsatellites in Zea–variability, patterns of mutations, and use for evolutionary studies. Theor Appl Genet 104(2–3):436–450
McCarty DR, Mark Settles A, Suzuki M, Tan BC, Latshaw S et al (2005) Steady-state transposon mutagenesis in inbred maize. Plant J 44(1):52–61
McMullen MD, Simcox KD (1995) Genomic organization of disease and insect resistance genes in maize. Mol Plant Microbe Interact 8:811–815
McMullen MD, Kresovich S, Villeda HS, Bradbury P, Li H et al (2009) Genetic properties of the maize nested association mapping population. Science 325(5941):737–740
Meena RL, Rathore RS, Mathur K (2003) Efficacy of biocontrol agents against Rhizoctonia solani f. sp. sasakii causing banded leaf and sheath blight of maize. J Mycol Plant Pathol 33(2):310–312
Michelmore RW (2003) The impact zone: genomics and breeding for durable disease resistance. Curr Opin Plant Bio 6(4):397–404
Mihm JA (1985) Breeding for host plant resistance to maize stem-borers. Int J Trop Insect Sci 6 (3):369–377
Mihm JA (1997) Insect resistant maize: recent advances and utilization. In: Proceedings of an international symposium held at the international maize and wheat improvement center (CIMMYT), 27 Nov-3 Dec CIMMYT
Mugo SN, Bergvinson D, Hoisington D (2001) Options in developing stemborer-resistant maize: CIMMYT's approaches and experiences. Int J Trop Insect Sci 21(4):409–415
Murenga M, Derera JD, Mugo S, Tongoona P (2018) A review of genetic analysis and response to selection for resistance to Busseola fusca and Chilo partellus, stem borers in tropical maize germplasm: a Kenyan perspective. Maydica 61(1):1–11

Murry LE, Elliott LG, Capitant SA, West JA, Hanson KK et al (1993) Transgenic corn plants expressing MDMV strain B coat protein are resistant to mixed infections of maize dwarf mosaic virus and maize chlorotic mottle virus. Biotechnology 11(12):1559–1564

Nekrasov V, Wang C, Win J, Lanz C, Weigel D et al (2017) Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion. Sci Rep 7(1):1–6

Njuguna JGM (2001) Combating head smut of maize caused by Sporisorium reilianum through resistance breeding. In: Seventh Eastern and Southern Africa regional Maize conference, pp 110–112

Olukolu BA, Negeri A, Dhawan R, Venkata BP, Sharma P et al (2013) A connected set of genes associated with programmed cell death implicated in controlling the hypersensitive response in maize. Genetics 193(2):609–620

Olukolu BA, Wang GF, Vontimitta V, Venkata BP, Marla S et al (2014) A genome-wide association study of the maize hypersensitive defense response identifies genes that cluster in related pathways. PLoS Genet 10(8):

Ordas B, Malvar RA, Santiago R, Sandoya G, Romay MC et al (2009) Mapping of QTL for resistance to the Mediterranean corn borer attack using the intermated B73 × Mo17 (IBM) population of maize. Theor Appl Genet 119(8):1451–1459

Ortega A, de Leon C (1974) Maize insects and diseases. In: Proceedings World-wide maize improvement in the 70's and the role for CIMMYT, El Batan, Mexico, pp 7–1

Ortigosa A, Gimenez Ibanez S, Leonhardt N, Solano R (2019) Design of a bacterial speck resistant tomato by CRISPR/Cas9-mediated editing of Sl JAZ 2. Plant Biotech J 17(3):665–673

Paliwal RL, Granados G, Lafitte HR, Violic AD, Marathée J P (2000) In: Paliwal RL, Granados G, Lafitte HR, Violic AD, Marathée JP (eds) Tropical maize: improvement and production. FAO, Raom Italy, pp 363–374

Parlevliet JE (2002) Durability of resistance against fungal, bacterial and viral pathogens; present situation. Euphytica 124(2):147–156

Pásztor K, Borsos O (1990) Inheritance and chemical composition in inbred maize (Zea mays L.) × teosinte (Zea mays subsp. mexicana Schräder/Iltis) hybrids (Preliminary communication). Növénytermelés 39(3):193–213

Plank JE (1963) Plant diseases-epidemics and control. Academic Press, 111 Fifth Avenue New York, p 10003

Poland JA, Bradbury PJ, Buckler ES, Nelson RJ (2011) Genome-wide nested association mapping of quantitative resistance to northern leaf blight in maize. Proc Nat Acad Sci 108(17):6893–6898

Pope DD, McCarter SM (1992) Evaluation of inoculation methods for inducing common smut on corn ears. Phytopathology 82(9):950–955

Portwood JL, Woodhouse MR, Cannon EK, Gardiner JM, Harper LC et al (2019) MaizeGDB 2018: the maize multi-genome genetics and genomics database. Nucleic Acids Res 47(D1): D1146–D1154

Prasanna BM, Sharma L (2005) The landraces of maize (Zea mays L.): diversity and utility. Indian J Plant Genet Resour 18(2):155–168

Prasanna BM, Pixley K, Warburton ML, Xie CX (2010) Molecular marker-assisted breeding options for maize improvement in Asia. Mol Breed 26(2):339–356

Prischmann DA, Dashiell KE, Schneider DJ, Eubanks MW (2009) Evaluating Tripsacum-introgressed maize germplasm after infestation with western corn rootworms (Coleoptera: Chrysomelidae). J Appl Entomol 133(1):10–20

Rajasekaran K, Sayler RJ, Sickler CM, Majumdar R, Jaynes JM et al (2018) Control of Aspergillus flavus growth and aflatoxin production in transgenic maize kernels expressing a tachyplesin-derived synthetic peptide, AGM182. Plant Sci 270:150–156

Rajasekaran K, Sayler RJ, Majumdar R, Sickler CM, Cary JW (2019) Inhibition of *Aspergillus flavus* growth and aflatoxin production in transgenic maize expressing the α-amylase Inhibitor from Lablab purpureus L. J Visual Exper 15(144):

Ramakrishna W, Emberton J, Ogden M, SanMiguel P, Bennetzen JL (2002) Structural analysis of the maize Rp1 complex reveals numerous sites and unexpected mechanisms of local rearrangement. Plant Cell 14(12):3213–3223
Ramirez DA (1997) Gene introgression in maize (Zea mays ssp mays L.). Philippine J Crop Sci 22 (2):51–63
Raruang Y, Omolehin O, Hu D, Wei Q, Han ZQ et al (2020) Host induced gene silencing targeting *Aspergillus flavus aflM* reduced aflatoxin contamination in transgenic maize under field conditions. Front Microbio 11:754
Razzaq T, Khan MF, Awan SI (2019) Study of northern corn leaf blight (NCLB) on maize (Zea mays L.) genotypes and its effect on yield. Sarhad J Agric 35(4):1166–1174
Robinson RW, Provvidenti R, Schroeder WT (1970) A marker gene for tobacco mosaic resistance. Rep Tomato Genet Coop 20:55–56
Roca De Doyle MM, Autrey LJC (1992) Assessment of yield losses as a result of co-infection by maize streak virus and maize stripe virus in Mauritius. Annals Appl Bio 120(3):443–450
Rojanaridpiched C, Gracen VE, Everett HL, Coors JF, Pugh BF et al (1984) Multiple factor resistance in maize to European corn borer. Maydica (Italy) 29(3):305–315
Ryland AK, Storey HH (1955) Physiological races of Puccinia polysora Underw. Nature 176 (4483):655–656
Sagar GC, Bhusal K (2019) Banded leaf and sheath bight (BLSB) of maize, its introduction, losses and management. Plant Physiol 1(2):106
Samayoa LF, Malvar RA, Olukolu BA, Holland JB, Butrón A (2015) Genome-wide association study reveals a set of genes associated with resistance to the Mediterranean corn borer (Sesamia nonagrioides L.) in a maize diversity panel. BMC Plant Bio 15(1):35
Saravanakumar K, Li Y, Yu C, Wang QQ, Wang M et al (2017) Effect of Trichoderma harzianum on maize rhizosphere microbiome and biocontrol of Fusarium Stalk rot. Sci Rep 7(1):1–13
Saxena G, Verma PC, Rahman LU, Banerjee S, Shukla RS et al (2008) Selection of leaf blight-resistant Pelargonium graveolens plants regenerated from callus resistant to a culture filtrate of Alternaria alternata. Crop Protect 27(3–5):558–565
Schenke D, Cai D (2020) Applications of CRISPR/Cas to improve crop disease resistance–beyond inactivation of susceptibility factors. Iscience 23:1–10
Schnable PS, Ware D, Fulton RS, Stein JC, Wei F et al (2009) The B73 maize genome: complexity, diversity, and dynamics. Science 326(5956):1112–1115
Scott GE, Davis FM (1981) Registration of Mp496 Inbred of Maize1 (Reg. No. PL 56). Crop Sci 21(2):353
Scott GE, Davis FM, Williams WP (1982) Registration of Mp701 and Mp702 germplasm lines of maize. Crop Sci 22:1270
Semagn K, Magorokosho C, Vivek BS, Makumbi D, Beyene Y et al (2012) Molecular characterization of diverse CIMMYT maize inbred lines from eastern and southern Africa using single nucleotide polymorphic markers. BMC Genom 13(1):1–11
Sharma PN, Gautam P (2010) Assessment of yield loss in maize due to attack by the maize borer, Chilo partellus (Swinhoe). Nepal J Sci Tech 11:25–30
Sharma RC, Hembram D (1990) Leaf stripping: a new method to control banded leaf and sheath blight of maize. Curr Sci 59(15):745–746
Sharma RC, De Leon C, Payak MM (1993a) Diseases of maize in South and South-East Asia: problems and progress. Crop Protect 12(6):414–422
Sharma RC, Payak MM, Mukherjee BK (1993b) Durable resistance in maize—a case study from India. In: Durability of disease resistance. Springer, Berlin, Heidelberg, pp 227–229
Sharma S, Duveiller E, Basnet R, Karki CB, Sharma RC (2005) Effect of potash fertilization on Helminthosporium leaf blight severity in wheat, and associated increases in grain yield and kernel weight. Field Crops Res 93(2–3):142–150
Shekhar M, Kumar S (2010) Potential biocontrol agents for the management of Macrophomina phaseolina, incitant of charcoal rot in maize. Arch Phytopathol Plant Protect 43(4):379–383

Shepherd DN, Mangwende T, Martin DP, Bezuidenhout M, Thomson JA et al (2007) Inhibition of maize streak virus (MSV) replication by transient and transgenic expression of MSV replication-associated protein mutants. J Gen Virol 88(1):325–336
Shikov AE, Malovichko YV, Skitchenko RK, Nizhnikov AA, Antonets KS (2020) No more tears: mining sequencing data for novel bt cry toxins with cryprocessor. Toxins 12(3):204
Simmons CR, Grant S, Altier DJ, Dowd PF, Crasta O et al (2001) Maize rhm1 resistance to Bipolaris maydis is associated with few differences in pathogenesis-related proteins and global mRNA profiles. Mol Plant-Microbe Interact 14(8):947–954
Singh RS, Kaur N (2018) Biochemical and molecular characterization of a new pullulan producer Rhodosporidium paludigenum PUPY-06. J Appl Biol Biotech 6(1):28–37
Singh A, Shahi JP (2012) Banded leaf and sheath blight: an emerging disease of maize (Zea mays L). Maydica 57(3):215–219
Sivakumar G, Sharma RC, Rai SN (2000) Biocontrol of banded leaf and sheath blight of maize by peat based Pseudomonas fluorescens formulation. Indian Phytopathol 53(2):190–192
Smith DR (1975) Expression of monogenic chlorotic-lesion resistance to Helminthosporium maydis in corn
Springer NM, Ying K, Fu Y, Ji T, Yeh CT et al (2009) Maize inbreds exhibit high levels of copy number variation (CNV) and presence/absence variation (PAV) in genome content. PLoS Genet 5(11):
Springer NM, Anderson SN, Andorf CM, Ahern KR, Bai F et al (2018) The maize W22 genome provides a foundation for functional genomics and transposon biology. Nat Genet 50(9):1282–1288
Sprink T, Wilhelm RA, Spök A, Robienski J, Schleissing S et al (2020) Plant genome editing-policies and governance. Front Media SA 11:284
Stagnati L, Lanubile A, Samayoa LF, Bragalanti M, Giorni P et al (2019) A genome wide association study reveals markers and genes associated with resistance to Fusarium verticillioides infection of seedlings in a maize diversity panel. Genes Genomes Genet 9 (2):571–579
Subedi S, Subedi H, Neupane S (2016) Status of maize stalk rot complex in western belts of Nepal and its integrated management. J Maize Res Dev 2(1):30–42
Sun S, Zhou Y, Chen J, Shi J, Zhao H et al (2018) Extensive intraspecific gene order and gene structural variations between Mo17 and other maize genomes. Nat Genet 50(9):1289–1295
Tang JD, Perkins A, Williams WP, Warburton ML (2015) Using genome-wide associations to identify metabolic pathways involved in maize aflatoxin accumulation resistance. BMC Genom 16(1):673
Tefera T, Mugo S, Beyene Y (2016) Developing and deploying insect resistant maize varieties to reduce pre-and post-harvest food losses in Africa. Food Security 8(1):211–220
Thompson CJ, Movva NR, Tizard R, Crameri R, Davies JE et al (1987) Characterization of the herbicide-resistance gene bar from Streptomyces hygroscopicus. EMBO J 6(9):2519–2523
Till BJ, Reynolds SH, Weil C, Springer N, Burtner C et al (2004) Discovery of induced point mutations in maize genes by TILLING. BMC Plant Bio 4(1):12
Tinsley NA, Estes RE, Gray ME (2013) Validation of a nested error component model to estimate damage caused by corn rootworm larvae. J Appl Entomol 137(3):161–169
Tyagi S, Kesiraju K, Saakre M, Rathinam M, Raman V et al (2020) Genome editing for resistance to insect pests: an emerging tool for crop improvement. ACS Omega 5(33):20674–20683
Ullstrup AJ (1972) The impacts of the southern corn leaf blight epidemics of 1970–1971. Ann Rev Phytopathol 10(1):37–50
Van Inghelandt D, Melchinger AE, Martinant JP, Stich B (2012) Genome-wide association mapping of flowering time and northern corn leaf blight (*Setosphaeria turcica*) resistance in a vast commercial maize germplasm set. BMC Plant Bio 12(1):56
Viana PA, Guimarães PEO (1997) Maize resistance to the lesser cornstalk borer and fall armyworm in Brazil. In *Embrapa Milho e Sorgo-Artigo em anais de congresso (ALICE)*. In: International symposium on insect resistant in maize: recent advances and utilization, 1994, Mexico, Proceedings. CIMMYT, Mexico, pp 112–116

Vigouroux Y, Mitchell S, Matsuoka Y, Hamblin M, Kresovich S et al (2005) An analysis of genetic diversity across the maize genome using microsatellites. Genetics 169(3):1617–1630

Vikal Y, Kaur A, Jindal J, Kaur K, Pathak D et al (2020) Identification of genomic regions associated with shoot fly resistance in maize and their syntenic relationships in the sorghum genome. PLoS ONE 15(6):

Wang B, Yu J, Zhu D, Chang Y, Zhao Q (2014a) Maize ZmRACK1 is involved in the plant response to fungal phytopathogens. Int J Mol Sci 15(6):9343–9359

Wang Y, Cheng X, Shan Q, Zhang Y, Liu J et al (2014b) Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat Biotech 32(9):947–951

Warburton ML, Tang JD, Windham GL, Hawkins LK, Murray SC et al (2015) Genome-wide association mapping of Aspergillus flavus and aflatoxin accumulation resistance in maize. Crop Sci 55(5):1857–1867

Ward JM, Stromberg EL, Nowell DC, Nutter FW Jr (1999) Gray leaf spot: a disease of global importance in maize production. Plant Dis 83(10):884–895

Wasala SK, Guleria SK, Sekhar JC, Mahajan V, Kalyani Srinivasan et al (2013) Analysis of yield performance and genotype × environment effects on selected maize (Zea mays) landrace accessions of India. Indian J Agril Sci 83(3):287–293

Wei JK, Liu KM, Chen JP, Luo PC, Stadelmann OYL (1988) Pathological and physiological identification of race C of Bipolaris maydis in China. Phytopathology 78(5):550–554

Wei WH, Zhao WP, Song YC, Liu LH, Guo LQ et al (2003) Genomic in situ hybridization analysis for identification of introgressed segments in alloplasmic lines from Zea mays × Zea diploperennis. Hereditas 138(1):21–26

Welz HG, Geiger HH (2000) Genes for resistance to northern corn leaf blight in diverse maize populations. Plant Breed 119(1):1–14

Widstrom NW, McMillian WW, Wiseman BR (1979) Ovipositional preference of the corn earworm and the development of trichomes on two exotic corn selections. Env Entomol 8 (5):833–839

Williams WP, Davis FM (1980) Registration of Mp703 germplasm line of maize. Crop Sci 20:418

Williams WP, Davis FM (1982) Registration of Mp704 germplasm line of maize. Crop Sci 22:1269

Williams WP, Davis FM (1984) Registration of Mp705, Mp706, and Mp707 germplasm lines of maize. Crop Sci 24(6):1217

Williams WP, Davis FM (2000) Registration of maize germplasms Mp713 and Mp714. Crop Sci 40(2):584

Williams WP, Davis FM (2002) Registration of maize germplasm line Mp716. Crop Sci 42 (2):671–672

Williams WP, Davis FM, Windham GL (1990) Registration of maize germplasm line Mp708. Crop Sci 30:757

Wiseman BR, Davis FM (1990) Plant resistance to insects attacking corn and grain sorghum. Florida Entomol 73(3):446–458

Wiseman BR, Widstrom NW, McMillian WW (1977) Ear characteristics and mechanisms of resistance among selected corns to corn earworm. Florida Entomol:97–103

Wisser RJ, Sun Q, Hulbert SH, Kresovich S, Nelson RJ (2005) Identification and characterization of regions of the rice genome associated with broad-spectrum, quantitative disease resistance. Genetics 169(4):2277–2293

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

Wisser RJ, Kolkman JM, Patzoldt ME, Holland JB, Yu J et al (2011) Multivariate analysis of maize disease resistances suggests a pleiotropic genetic basis and implicates a GST gene. Proc Nat Acad Sci 108(18):7339–7344

Xiao Y, Liu H, Wu L, Warburton M, Yan J (2017) Genome-wide association studies in maize: praise and stargaze. Mol Plant 10(3):359–374

Yadav OP, Hossain F, Karjagi CG, Kumar B, Zaidi PH et al (2015) Genetic improvement of maize in India: retrospect and prospects. Agril Res 4(4):325–338
Yang G, Li C (2012) General description of Rhizoctonia species complex. In: Plant pathology. Intech Open, p 362
Yang G, Wiseman BR, Isenhour DJ, Espelie KE (1993) Chemical and ultrastructural analysis of corn cuticular lipids and their effect on feeding by fall armyworm larvae. J Chem Ecol 19 (9):2055–2074
Yang T, Stoopen G, Yalpani N, Vervoort J, de Vos R et al (2011) Metabolic engineering of geranic acid in maize to achieve fungal resistance is compromised by novel glycosylation patterns. Metabol Engin 13(4):414–425
Yang N, Xu XW, Wang RR, Peng WL, Cai L et al (2017a) Contributions of Zea mays subspecies mexicana haplotypes to modern maize. Nat Commun 8(1):1–10
Yang Q, Balint Kurti P, Xu M (2017b) Quantitative disease resistance: dissection and adoption in maize. Mol Plant 10(3):402–413
Yang N, Liu J, Gao Q, Gui S, Chen L et al (2019) Genome assembly of a tropical maize inbred line provides insights into structural variation and crop improvement. Nat Genet 51(6):1052–1059
Yobo KS, Laing MD, Hunter CH, Morris MJ (2004) Biological control of Rhizoctonia solani by two Trichoderma species isolated from South African composted soil. South African J Plant Soil 21(3):139–144
Young ND (1996) QTL mapping and quantitative disease resistance in plants. Ann Phytopathol 34 (1):479–501
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
Zaitlin D, DeMars S, Ma Y (1993) Linkage of rhm, a recessive gene for resistance to southern corn leaf blight, to RFLP marker loci in maize (Zea mays) seedlings. Genome 36(3):555–564
Zhang ZY, Fu FL, Gou L, Wang HG, Li WC (2010) RNA interference-based transgenic maize resistant to maize dwarf mosaic virus. J Plant Bio 53(4):297–305
Zhang J, Zhao Y, Gan D (2011) Construction of marker-free siRNA complex expression vector against MDMV and MRDV. J Anhui Agril Uni 38(2):232–237
Zhang Y, Huang Q, Pennerman KK, Yu J, Liu Z et al (2016) Datasets for transcriptomic analyses of maize leaves in response to Asian corn borer feeding and/or jasmonic acid. Data Brief 7:1010–1014
Zhu Y, Zhao F, Zhao D (2011) Regeneration and transformation of a maize elite inbred line via immature embryo culture and enhanced tolerance to a fungal pathogen *Exserohilum turcicum* with a balsam pear class I chitinase gene. African J Agril Res 6(7):1923–1930
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. Genes Genomes Genet 3(11):2095–2104
Zila CT, Ogut F, Romay MC, Gardner CA, Buckler ES et al (2014) Genome-wide association study of Fusarium ear rot disease in the USA maize inbred line collection. BMC Plant Bio 14 (1):372
Zuo W, Chao Q, Zhang N, Ye J, Tan G et al (2015) A maize wall-associated kinase confers quantitative resistance to head smut. Nat Genet 47(2):151–157

# Chapter 4
# Molecular Strategies for Managing Disease Resistance in Barley

**Rekha Malik, Pawan Kumar, RPS Verma, Sonia Sheoran, Dinesh Kumar, Lokendra Kumar, Sanjaya Gyawali, and G. P. Singh**

**Abstract** Barley is one of the oldest cultivated crops in world that has been cultivated across diverse ecological regions since centuries. This varied agro-climatic cultivation of barley makes it vulnerable to various biotic stresses like fungal, viral, bacterial diseases and insect pest infestations. In order to reduce yield losses due to biotic stresses, breeding for disease resistance became most important in barley improvement. Disease resistance breeding has been successfully implemented in barley with release of disease resistant and insect-pest tolerant varieties using conventional breeding methods in past. With the advancement of molecular technologies like, QTL mapping, genome wide association mapping, gene editing, genomic selection and embryo rescue, identification of resistant gene from

R. Malik (✉) · R. Verma · S. Sheoran · D. Kumar · L. Kumar · G. P. Singh
ICAR-Indian Institute of Wheat and Barley Research (IIWBR), Karnal 132001, Haryana, India
e-mail: rekha.malik@icar.gov.in

R. Verma
e-mail: pibarley.iiwbr@icar.gov.in

S. Sheoran
e-mail: Sonia.Sheoran@icar.gov.in

D. Kumar
e-mail: Dinesh.Kumar3@icar.gov.in

L. Kumar
e-mail: Lokendra.Kumar@icar.gov.in

G. P. Singh
e-mail: director.iiwbr@icar.gov.in

P. Kumar
ICAR-Central Soil Salinity Research Institute (CSSRI), Karnal 132001, Haryana, India

S. Gyawali
Washington State University Mount Vernon NWREC, 16650 State Route 536, Mount Vernon, WA 98273, USA

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021

C. Kole (ed.), *Genomic Designing for Biotic Stress Resistant Cereal Crops*,
https://doi.org/10.1007/978-3-030-75879-0_4

wild-type/secondary gene pool and their incorporation in cultivated barley has become an achievable target in recent years. More than hundred major genes and QTLs have been identified against wide range of pathogens and insect-pests. Recently available high-throughput genotyping platforms have promoted development of high-density genetic maps that will further support marker assisted selection with refined disease breeding strategies in barley. This chapter reviews the biotic stresses, germplasm variability and traditional disease breeding in past and the status and scope of present day molecular marker and next generation technologies for developing biotic stress resistance in barley.

**Keywords** Barley · Biotic stresses · Disease resistance genes · Molecular approaches

## 4.1 Introduction

Barley is one of the ancient crops domesticated around 8000 BC that is cultivated and used across the world. In terms of total production, barley ranks fourth in the world among cereals after wheat, maize, and rice. It is grown by nearly 100 countries on about 50 million hectares (ha) with around 145 m tones production (FAOSTAT 2020). Amongst different continents, Europe is the largest in terms of barley area (49.8%) and production (61.1%) followed by Asia and Africa. In terms of productivity also Europe is highest with 3.4 t/ha amongst all continents to closely followed by America (3.3 t/ha). The largest barley-producing countries in the world are Russian Federation, Germany, France, Canada, Spain, and Turkey, while in terms of area cultivated Russian Federation, Ukraine, Australia, Spain, Turkey, and Canada are major countries (FAOSTAT 2020). In terms of yield, European countries Ireland, Germany, France, UK, Denmark, Austria, and Sweden are having more than 5.0 t/ha, while Argentina, Canada, USA, China, and Brazil are countries outside Europe with more than 3.5 t/ha yield levels, which is well above the world average of 2.77 t/ha. The major barley importing countries include Saudi Arabia followed by China, Iran, Japan, Algeria, Jordan, Libya, Morocco, and Tunisia (FAOSTAT 2020). Barley is the most widely grown cereal in the world that is well adapted to different global climates ranging from temperate to arctic and subarctic regions due to its genetic evolution and wider genetic diversity (Kendal 2016). Barley is often considered the only possible rainfed cereal crop under low input and stressful environments, like drought, heat, and cold. This adaptability to extreme and marginal conditions has led to widespread cultivation of this cereal throughout the world (Bothmer et al. 1995). The range of barley cultivation is from the tropics to high latitudes (>600 N) in Iceland and Scandinavia as well as in high altitudes up to 4500 m above sea level in the Himalayas (Bothmer et al. 2003; Ceccarelli et al. 2008). Historically, owing to its rich dietary fiber and readily available energy, barley was utilized by the Roman gladiators, who were also called “hordearii” (Curry 2008). The wide adaptability of barley is supported by the availability of

early spring varieties suitable for long cold weather-short summer, winter varieties for temperate weather and varieties with drought tolerance for dry hot regions (Van Oosterom and Acevedo 1992). Barley has a versatile end-use purpose and caters to different economic sectors such as animal feed, food, alcoholic beverage, medicines, and restricted use in biofuels production (Baik and Ullrich 2008). Globally, around 55–60% of barley production is used for feed, 30–40% for malt, 2–3% for food, and 5% for seed (Ullrich 2010). In dry regions of West Asia and North Africa, it is mainly used for grazing by sheep and goats, which in turn comes to human food chain (Ceccarelli et al. 2008). Some amount of barley is also utilized for producing alcohol for the renewable energy source. In addition to brewing and distillation, barley malt is prominently used in healthy food drinks and medicines as a diuretic, an excellent source of dietary fiber and functional food ingredient (Zhou 2009). Barley is categorized as hulled and hulless type due to morphological appearance of its grain (Ullrich 2010). In hulled barley, the lemma and palea are fused to the pericarp while in hulless the chaff is easily separated from grain. Hulless barley is mainly used for food consumption and beverage production and hulled is grown for feed and fodder purposes (Ullrich 2010). The cultivated barley is a diploid species with 2n = 14 chromosomes and large genome size (>5.1 gigabases) consisting of highly repetitive sequences, almost 12 times the size of the rice genome (Bennett and Smith 1976, IBGSC 2012). It is self-pollinating and can either be cross-pollinated or self-incompatible with some wild species like *Hordeum bulbosum*.

It is grown across wider agroclimatic regions and is commonly affected by various diseases and insect pests. These biological factors have a drastic effect during the germination, development, ripening, and subsequently in the storage on the quantity and quality of barley crop and produce (Kiesling 1985). There are four major groups of pathogens—bacteria, fungi, viruses, and nematodes affecting the yield and quality of barley grains causing widespread substantial economic losses to producers and industries (Kerr et al. 2019). Most of the diseases and insects affecting barley crops are effectively contained using approaches like integrated insect pest management, developing resistant varieties, and new molecular tools for studying and identifying new resistant genes and technologies.

## 4.2 Description of Different Biotic Stresses

Barley is one of the most widely adopted crops and cultivated from tropical to subarctic regions of the world. Major barley growing areas of the world are from European Union (France, Germany, UK, and Demark) to Russia, Australia, and North America to China and Middle East countries (Iran, Iran, Kazakhstan). With this much wider area of cultivation, numerous biotic stresses (diseases and pests) affect crop production at a severe to moderate rate. More than 100 pathotypes/races/biotypes have been identified in barley for fungal, bacterial, viral, and nematodes diseases as listed in Tables 4.1. The fungal diseases can be managed by cultural

practices like crop rotation, removal of weeds, and alternate host from the field and border of the field. In the initial stage of the disease uprooting and burning of infected plants is effective. Cultural methods of control are effective to manage the disease only in the initial stage. If a disease crosses the economic threshold level (ETL) in a crop then chemical methods should be used to eradicate the disease from the field. Integrated disease management (IDM) and integrated pest management (IPM) are the most common practices by farmers to control the economic loss of barley yield. All these integrated practices are labor, time, and money consuming which increase the cost of crop production. The intensive spray of fungicides or pesticides for diseases and insects had reported residual effects on humans and cattle feed, degradation of soil health, and loss of biodiversity in the agricultural area. Monotonous cropping and frequent spray of chemicals have developed resistance in the pathogens and insects (Castro et al. 2012). Conventional breeding has been used successfully to develop resistant varieties for different diseases using primary and secondary gene pools (Pickering et al. 1995, 2000, 2004). Primary introduction, pedigree method, and backcross breeding are the most common methods used for the development of disease-resistant varieties through conventional methods of breeding. CI 4196, Zhedar 2, Svanhals, Imperia, Chevron, and NDB112 are some of the best line varieties identified by conventional breeding and have been used in disease-resistant varieties development programs throughout the world for decades (Steffenson et al. 1996; Bai and Shaner 2004). But evolution in the pathogens and identification of new biotypes or races of pathogens with time, continuous use of the same genotypes/parents in breeding programs made the crop susceptible to diseases. For long-term and stable resistance to new races of pathogens identification of new resistance genes is mandatory and needs time. Exploring the secondary and tertiary gene pools for resistance could be a good source of resistance for biotic stresses. The secondary gene pool has also been identified as a resistance source for many diseases (Pickering et al. 1995).

## 4.3 Genetic Resources of Resistance Genes

The genetic variation available in a population of a crop is the first and most important requirement for a breeding program of any specific goal (yield improvement, quality improvement, abiotic and biotic stress tolerance, or resistance development). Variation present not only in cultivars or varieties but also in close wild relative species can be used for resistance development in plants depending upon the liability and feasibility of successful crossing and fertile progenies of hybrids. Harlan and Jan (1971) proposed the concept of gene pool beyond its original definition (based on the origin of the population) and defined the gene pool for plants depending upon the difficulty and compatibility of crossing over between the species or wild relatives. So, according to Harlan and De Wet concept barley species are divided into three major gene pools:

**Table 4.1** List of diseases reported in barley with their causal organism effected plant part and symptoms (modified from Paulitz and Steffenson 2011 and Gangwar et al. 2018)

| Sensitive plant part/ stage | Disease | Causal organism | Symptoms |
|---|---|---|---|
| Ear head | Ergot | *Claviceps purpurea* | Flowers oozing sticky substance; Dirty ear-head appearance; Diseased kernels turn to black mass of fungal mycelia |
| | False loose smut | *Ustilago avenae* (*U. nigra*) | Olive brown spore masses on the head with reduced bracts |
| | Loose smut | *Ustilago tritici* (*U. nuda*) | Early emergence of heads; Dark green or black masses in place of kernels |
| | Scab (Fusarium Head Blight, FHB) | *Fusarium graminearum* | Initial bleaching on some of the florets in the spike. Under favorable conditions, premature blight of whole spike may occur. As the disease progress head discoloration occur. Kernels become shriveled, white, and chalky |
| | Covered smut | *Ustilago hordei* | Stunted growth; late emergence of heads; Kernels replaced with grey fungal masses |
| Leaves and awns | Leaf (brown) rust | *Puccinia hordei* | Small orange-brown circular spore masses on the upper surface of leaves |
| Leaves, tiller and ear head | Downy mildew (Crazy top) | *Sclerophthora rayssiae* | Dwarfed and/or deformed plants; Flag leaves yellow; Leathery leaves; Heads distorted; No seed formation |
| Leaves and tillers | Powdery mildew | *Blumeria graminis f. sp. hordei* | Initially the lower leaf surface shows white, cottony patches of fungal growth with chlorotic spots on the upper surface of these patches. As disease progress, cottony patches become dull gray- brown in color due to development of fruiting bodies (cleistothecia) |
| Leaves and glumes | Basal glume rot | *Pseudomonas syringae pv. atrofaciens* | Brown discoloration at base of the glume; Dark line where glume attaches to spike; Water-soaked spots on leaves; Yellow and necrotic spots on leaves |

(continued)

**Table 4.1** (continued)

| Sensitive plant part/ stage | Disease | Causal organism | Symptoms |
|---|---|---|---|
| | Black chaff and bacterial streak | *Xanthomonas translucens pv. translucens* | Dark red-brown transparent water-soaked lesions on leaves with the browning of glumes |
| | Spot blotch/ leaf blight | *Bipolaris sorokiniana (Drechslera sorokiniana), Cochliobolus sativus (Teleomorph)* | Dark brown round spots that join to make irregular patches with yellowing around the net |
| Leaves | Barley mosaic | Barley mosaic virus (BMV) | Irregular chlorotic streaks or necrotic patches on leaves with upward rolling of leaf margins and yellow discoloration |
| | Barley stripe mosaic | Barley stripe mosaic virus (BSMV) | White mottling with mild stripes; Mosaic on leaves, and lethal necrosis on stunted plants |
| | Barley yellow dwarf | Barley yellow dwarf virus (BYDV) | Stunted growth of plants; Yellow green blotches at leaf tip, leaf margin or leaf blade; Leaves turning bright yellow, red or purple |
| | Barley yellow streak mosaic | *Barley yellow streak mosaic virus (BYSMV)* | Pale yellow streaks and stripes parallel to the mid-rib leading to mosaic pattern and stunting plant growth |
| | Bacterial stripe | *Pseudomonas syringae pv. striafaciens* | Small, water-soaked coalescing lesions with expanded narrow, yellowish margins |
| | Scald | *Rhynchosporium secalis* | Appearance of dark, pale or bluish gray lesions on leaves. As the disease progress, these spots enlarge into oval lesions with bluish gray centers and dark brown margins |
| | Net Type Net Blotch (NTNB) | *Pyrenophora teres f. teres* | Dark green water-soaked spots; Narrow brown blotches with netted appearance, surrounding tissue yellow; Stripes running the length of leaf |
| Seedling, leaves | Stripe disease | *Drechslera (Pyrenophora) graminea* | Small yellow spots on seedling leaves; Yellow to tan stripes along leaf blade before heading; red margins on stripes; Death of diseased tissue; Stunted plants |
| Stem, leaves, | | *Pyrenophora teres f. maculata* | Chocolate brown like-patterns on leaves, leaf sheaths, and |

(continued)

**Table 4.1** (continued)

| Sensitive plant part/ stage | Disease | Causal organism | Symptoms |
|---|---|---|---|
| glumes and seeds | Spot Type Net Blotch (STNB) | | glumes with yellowing around the net |
| Leaf and stem | Anthracnose | *Colletotrichum cereale* | Dark, yellow water-soaked lesions on stems and leaves |
| Collar stem, roots, lower leaves | Common root rot and seedling blight | *Cochliobolus sativus (Bipolaris sorokiniana)* | Brown lesions on leaves near soil extending to stem; resembles drought; Death of lower leaves; Rotting roots |
| Roots | Molya disease | *Heterodera avenae, Heterodera filipjevi* | Knots in roots, stunting, early senescence, and uneven appearance of infected plants |

4.3.1 Primary gene pool: includes all cultivars/varieties/genotypes, landraces of cultivated barley of species *Hordeum vulgare* ssp. *vulgare* and wild relative *H. vulgare* ssp. spontaneous (K. Koch.). All varieties or cultivars of the primary gene pool can be crossed to each other easily to get a fertile progeny.

4.3.2 Secondary gene pool: includes single species *Hordeum bulbosum* L., responsible for sharing basic *Hordeum* genome. *Hordeum bulbosum* L. is a valuable genetic resource for the improvement of barley crop by introgression of biotic stress resistance from it (Pickering et al. 1995, 2000, 2004). Genotypes of this species can be crossed with primary gene pool species but with some difficulties like less fertile progeny, deletion of few or more chromosomes, etc. It has been reported that crossing *H. bulbosum* with *H. vulgare* results in the development of haploid plants by the elimination of chromosomes form H. bulbosum (Pickering et al. 1995).

4.3.3 Tertiary gene pool: The tertiary gene pool of barley is very outsized and comprises all other remaining wild species (Von Bothmer et al. 2003). The tertiary gene pool for barley involved more than 30 species of barley (*H. glaucum*, *H. marinum*, and *H. murinum*, etc.). Crossing between these species and cultivated barley is very difficult. To obtain the seed from the cross the tertiary gene pool with the primary gene pool special techniques like embryo rescue, protoplast fusion, doubling of the chromosome, etc. are required as some tertiary gene pool have tetraploid and hexaploid species too. Because of all these difficulties, tertiary gene pool has been used very less in barley crop improvement and no successful cross has been reported to date.

## 4.4 Genetic Variability and Traditional Breeding for Disease Resistance

As one of the oldest crops and the fourth major cereal crop in the world breeding work in barley also started very early. Use of barley as human food in areas where other cereal crops were not grown and industrial use for bear production made it a crop of interest for researchers too. In early 1900s, plant breeding started for barley as pure line selections (Ramage 1987) in Europe and USA from cultivated barley which were mixture of landraces. The varieties "Atlas", "O.A.C.21", "Chevallier", "Svahals", "Hannchen" were developed from the landraces and introduction and have dominated the crop cultivated area for long periods (Ramage 1987). Pedigree and bulk methods have also been used in barley for the development of the barley varieties for specific desired traits like malting and yield improvement, these two methods are still popular among the conventional breeders for variety development (Swanston 1997). Even for biotic stresses like rust a few of the earliest studies in cereal crops were conducted in a barley crop. Backcross breeding is a widely used breeding method for resistance transfer in crops and had been used in barley too for the transfer of resistant gene to high yielding but susceptible cultivars. Earlier, crossing for the resistant varieties development were made using resistant resource/cultivar available in primary gene pool i.e. cultivated barley (*H. vulgare*). Steptoe x Morex doubled haploid barley mapping population was the first product of the North American Barley Genome Mapping Project. Before the development of mapping population in barley, Tsuchiya and Singh mapped the barley chromosomes by telotrisomic analysis in (1982). Graner et al. constructed the restriction fragment length polymorphism (RFLP) map for barley map 1991.

Phenotypic diversity of barley germplasm had been studied to large extent since 1970s. It is estimated that more than 4,00,000 accessions of barley have been conserved worldwide in different gene banks. ICARDA barley germplasm has been assessed for diversity assessment in various studies up to different extents. Tolbert et al. (1979) analyzed the 1700 accessions collected from different parts of the world. While, Kumar et al. (2018a) studied the agro-morphological diversity of 310 barley accessions in Indian conditions introduced from ICARDA. USDA-ARS National Small Grains Collection (NSGC) is one of the world's largest barley germplasm collections. Its core collection is a subset having ~10% of the entire collection and is being assessed for various agronomic traits, resistance to diseases and pests (Kumar et al. 2020). Agro-morphological diversity of barley germplasm has been studied in several studies but the set of germplasm was even smaller than 200 genotypes (Jain et al. 2014; Yadav et al. 2015; Kumar et al. 2017; Banjarey et al. 2017; Yadav et al. 2018).

With the advancement in biotechnology and availability of molecular markers, PCRs, and next-generation sequencing (NGS), genotyping of large germplasm has become feasible and a large number of studies have been performed for genetic diversity of available germplasm region wise or location wise for quality traits, biotic and abiotic stresses (Sallam et al. 2018; Verma et al. 2020). In the last 2–3

decades various markers have been used in barley for genotyping of germplasm for various traits viz. inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) (Fernandez et al. 2002; Giancarla et al. 2012), simple sequence repeat (SSR, Hamza et al. 2004). A total of 953 cultivated barley accessions collected from different parts of the world were analyzed for agronomic and genetic diversity by Malysheva-Otto et al. (2006). Milner et al. (2019) analyzed the genetic diversity of 21,405 barley accessions by collecting DNA samples from different gene banks of the world using single nucleotide polymorphism (SNP)s. NGS has made it possible to sequence the whole genome of barley of about haploid genome of size ~5.3 Gb in seven chromosomes (International Barley Genome Sequencing Consortium). Various studies have been reported for biotic stress resistance using genetic variability of barley germplasm. About 40,000 barley accessions which include breeding lines, cultivars, landraces, and genetic stocks from more than 100 countries were screened for stripe rust reaction on barley at the mature plants and some promising resistance sources were identified (Brown et al. 2001). Several genes have shown resistance to stripe rust even at the seedling stage of barley (Chen and Line 1999; Brown et al. 2001). Chen and Line (2003) identified 26 different genes conferring resistance to stripe rust in 18 barley lines. Unlike other cereals, most of the stripe rust resistance genes in barley have recessive gene action (Chen and Line 1999, 2003). For Fusarium head blight (FHB) CI 4196, Zhedar 2, Svanhals, and Imperia (Bai and Shaner 2004) were identified as the resistant source and among these CI 4196 has been considered as the best source of FHB resistance. Surprisingly, all the reported resistant cultivars for FHB are two-rowed barley except Chevron (an old Switzerland cultivar) which is the best FHB resistant source among six-rowed and good for malting, it has been used in many breeding programmes across the world. In USA, large germplasm of six-rowed barley (about 8200 accessions from across the world) were screened for FHB resistance in the field, and only 13 showed resistance similar to that of Chevron (Steffenson 2003; Bai and Shaner 2004). In Japan and China, over 10,000 barley accessions from different countries have been screened for FHB resistance, but only several dozen accessions had a low level of FHB (Zhou et al. 1991; Steffenson 2003). Results of all these studies showed that two-rowed barley had better resistance as compare to six-rowed barley and among the two-rowed barley hulled barley reported higher resistance to FHB (Steffenson 2003; Legge et al. 2004).

The stem rust resistance genes Rpg1, Rpg4, and Rpg5 in barley have been cloned and molecular studies have been advanced to understand the basis of their resistance (Kleinhofs et al. 2009). Several of the early studies for rust resistance in cereal crops were initiated with barley leaf rust and slow-rusting cv. Vada (Parlevliet 1976). Many different alleles for powdery mildew resistance were identified in both cultivated (Jørgensen 1994) and wild barley (Dreiseitl and Dinoor 2004). The effectiveness of these alleles used in resistant varieties has been prolonged in the cultivated fields due to the appearance of a new type of virulence.

For spot blotch resistance several sources have been identified in both cultivated and wild barley (Arabi 2005; Bilgic et al. 2006), but the one that has proved widely effective resistance for many years in commercial six-rowed malting cultivars is line

NDB112 (Steffenson et al. 1996). The durable resistance contributed by NDB112 is controlled chiefly by a major effect quantitative trait locus (QTL) on chromosome 1H (Steffenson et al. 1996). Verma et al. (2011) studied the regulation of corn leaf aphid resistance in barley and reported monogenic or oligogenic regulation of trait. Genetic diversity for reaction to RLS has been reported in both winter and spring barley cultivars (Pinnschmidt and Hovmøller 2004; Leistrumaite and Liatukas 2006; Manninger et al. 2008; Pinnschmidt and Jørgensen 2009). Resistant cultivars are being recommended for growers in RLS-prone areas. Additionally, research is being advanced to breed cultivars with higher levels of resistance to RLS. Four barley yellow dwarf virus genetic studies have identified several genes for resistance, but the one used most commonly inbreeding is Ryd2 or Yd2 because of its uniformly effective in all genetic backgrounds and effectiveness against various BYDV strains.

## 4.5 Association Mapping Studies

For the development of resistance for any biotic stress the first important key is to understand the molecular mechanisms of concerned the disease or insect pest. Gene for gene hypothesis proposed by Flor (1971) opened up a wide area of research and opportunity for researchers in the development of resistant varieties for plant diseases. The Flor hypothesis depicted that there is virulent or avirulent gene in plant which triggered the result of action of pathogen on plant whether the plant will be susceptible or resistant to the disease. The finding of these resistant alleles has helped the conventional plant breeding for resistant varieties' development for barley against fungal, viral, and bacterial diseases. Molecular markers had been used in barley crop also for resistant genes' identification in several studies since the mapping populations were developed in barley. Linkage disequilibrium (LD) is defined as the nonrandom association of alleles at different loci (Slatkin 2008). Apart from the major genes, a number of unexploited minor alleles could be identified using LD based association mapping. A number of resistant alleles have been identified using molecular markers in various cereal crops (Gyawali et al. 2018; Shrestha et al. 2019; Tessmann et al. 2019; Singh et al. 2020).

QTL mapping is a "classical approach" and will continue to be the main tool for gene tagging in crops The major drawback of QTL linkage mapping is that it is deficient in fine mapping, as only a few available meiotic events are used in the mapping (Jannink and Walsh 2002) and its very expensive (Stich et al. 2006). Association mapping is based on nonrandom association of alleles at different loci among phenotype and marker [i.e. Linkage disequilibrium (LD)] overcome the drawback of bi-parental QTL mapping. Association mapping uses natural populations for mapping purposes, thus it provide higher mapping resolution due to a number of recombination events that occurred over a long development history of population. LD can be caused by many underlying factors i.e. including mutation, genetic drift, founder effects, selection, and inbreeding level plants. LD decay is

faster outcrossing crops compared to inbreeding plants. Apart from higher resolution of mapping and multiple allele evaluation, association study has advantages over bi-parental traditional QTL-mapping as it same precious time of plant scientist and cost-effective as the development of bi-parental population is a long, tedious and costly approach (Hansen et al. 2001; Kraakman et al. 2006).

The genome-wide association study (GWAS) approach is being used successfully to identify and position stem rust resistance genes in both wild and cultivated barley germplasm (Steffenson et al. 2007). Tsai et al. (2020) used 1317 advanced breeding lines of spring barley for genome-wide association mapping and identified two markers on chromosome 4H and one marker in an unknown region significantly associated with ramularia leaf spot disease caused by the fungus Ramularia collocygni, and these three markers were also identified in powdery mildew resistance by using multivariate GWAS. Gyawali et al. (2018) used 336 genotypes of barley and identified about 10 QTLs for spot blotch resistance at the seedling and adult plant stage. Kraakman et al. (2006) used 148 cultivars of spring barley for GWAS and identified about five QTLs for barley yellow dwarf virus resistance. Adhikari et al. (2020) reported association mapping of 3490 elite barley breeding lines and identified 12 QTLs for resistance/susceptibility for net form of net blotch.

## 4.6 Molecular Mapping of Resistance Genes and QTLs

Barley crop in field can be damaged by fungal, bacterial and viral pathogens and insect-pests. Resistance against biotic stresses in barley is regulated by both mono- and polygenic traits. Developing disease resistant varieties has remained the major goal in barley breeding to avoid yield losses and provide environment sustainable production (Kiesling 1985). Chemical applications as insecticides and pesticides have supported in minimizing yield loss due to biotic stresses but they affected cost of cultivation as well as posed health hazards. In general, traditional breeding methods are used to develop disease resistant genotypes with high yield and superior grain quality to minimize yield losses. Breeding for developing biotic stress resistance has remained successful and many disease resistant high yielding varieties has been developed by breeders. Still this classical approach of developing disease resistance was tedious and time consuming with short and narrow resistance against pathogens (Ali et al. 2019). Meanwhile, development of molecular markers led exponential use of DNA based technologies in crop improvement including barley. The first molecular marker system reported was restriction fragment length polymorphism (RFLP, Botstein et al. 1980) and first RFLP based genetic map in barley was reported by Graner et al. (1991). Over the time, PCR based molecular markers became dominant in evaluation of different traits at DNA level with the availability of SSR based high density maps in barley (Varshney et al. 2007). This application enabled easy application of molecular marker technology in identification of genes/QTLs and their use in marker assisted selection (MAS) and marker assisted breeding (MAB) for disease resistance in barley (Hudcovicova et al. 2008;

Harwood 2016; Sayed and Baum 2018; Yu et al. 2018; Kis et al. 2019; Wang et al. 2019). In gene mapping approach, bi-parental population developed from contrasting parents i.e. one carrying resistance for specific pathogenic virulence and other with complete susceptibility, was developed. This population is then mapped to identify the co-segregation of resistance with respect to specific locus of a gene (Drader and Kleinhofs 2010; Hamwieh et al. 2018). The first report of resistance gene mapping was given by Graner et al. (2000) for *Rph7* gene conferring resistance for leaf rust. This was followed by identification and localization of many resistance genes in the last two decades for most of prevailing diseases in barley (Table 4.2). These new genes/loci identified in landraces, old cultivars, wild types can be mapped and targeted for introgression in elite cultivars for improved resistance against pathogens using molecular breeding approaches.

Apart from major resistance genes, minor genes/QTLs are also considered promising to develop durable resistance per se. In earlier time, epidemiological and biometric studies were used to identify minor resistance genes in crops including barley. With the evolution of marker systems and user friendly statistical analysis in the last two decades, identification of QTLs has resulted in hundreds of reports on molecular linkage mapping for both biotic and abiotic stress resistance (Graner et al. 2000; Varshney et al. 2004). In addition, high throughput technologies further provided better insight on germplasm variability for new genes through genome-wide association studies. QTL mapping in plants mainly involved biparental population developed mostly from two parents, resistant and susceptible, for desirable trait. Fixed progeny lines (F6-F8) are then phenotyped in field conditions under natural hot-spots or epiphytotic conditions to generate disease data. This information along with genotypic data of fixed population is then analyzed statistically facilitating the dissection of quantitative resistance into individual Mendelian loci. For the first time in barley, Heun (1992) reported QTLs on 5H and 7H chromosomes for powdery mildew (*Blumeria graminis*). Since then QTL mapping has become the most studied area of barley research for disease resistance to identify QTLs conferring durable adult plant resistance for most of the diseases in barley (Toojinda et al. 2000; Li et al. 2006; Castro et al 2012; Grewal et al. 2012; Hickey et al. 2012; Jain et al. 2013; Esvelt Klos et al. 2016; Romero et al. 2018). Important QTLs identified and reported in different genotypic backgrounds over the last two decades are in barley listed in Table 4.3.

## 4.7 Marker-Assisted Strategies for Transferring Genes/ QTLs Against Diseases

The availability of mapped major genes and QTLs for disease resistance in barley facilitated their utilization through marker assisted selection (MAS), backcross breeding (MAB) and gene pyramiding for developing disease resistance in barley (Hudcovicova et al. 2008; Sayed and Baum 2018; Singh et al. 2019). MAS involves

**Table 4.2** List of resistance genes mapped and localized in barley for barley diseases

| Disease/Pathogen | Gene | Chromosome location | Marker type/linked marker | Reference |
|---|---|---|---|---|
| *Leaf rust Puccinia hordei* | *Rph27* | 4H | DArT-Seq | Rothwell et al. (2020) |
| | *Rph26* | 1H | CM_1194 | Yu et al. (2018) |
| | *Rph24* | 6H | 3,999,875, 3,265,068, 3,272,559, and 3,272,930 | Ziems et al. (2017) |
| | *Rph23* | 7H | bPb-8660 and bPb-9601; Ebmac0603 | Singh et al. (2015) |
| | *Rph22* | 2H | H35_26334 & H35_45139 | Johnston et al. (2013) |
| | *Rph21* | 4H | GBM1044 & GBM1220 | Sandhu et al. (2012) |
| | *Rph16* | 2H | GBR 1185 | Perovic et al. (2004) |
| | *Rph13* | 3H | *Hv*KASP_*Rph13*plus | Jost et al. (2020) |
| | *Rph7* | 3H | TC2863-12.4 and ABG70 | Mammadov et al. (2007) |
| | *Rph5* | 3H | | |
| | *Rph6* | 3H | MWG2021 & BCD 907 | Zhong et al (2003) |
| | *Rph3* | 7H | EBmac755 | Park et al (2003) |
| | *Rph*$_{\mathrm{MBR1012}}$ | 1H | GMS021 & GBS546 | Konig et al. (2012) |
| | *RphC* | 5H | DART4872 and DART7508 | Dracatos et al. (2014) |
| Stripe rust *Puccinia striiformis* | *Rdg2a* | 7H | MWG2018 | Arru et al. (2003) Tacconi et al. (2001) |
| *Puccinia graminis* Stem rust | *Rpg1* | 7H | ABG704-MWG036B | Kilian et al. (1994) |
| | *Rpg4* | 5H | ABG391 | Kilian et al. (1997) |
| *Erysiphe graminis* f. sp. *hordei* (powdery mildew) | | 6H | DArT markers (4,793,171, 3,258,880 3,264,002& 3,432,488) | Piechota et al. (2020) |
| | *Mla* | 7H | GBM1126 &GBM1060 | Soldanova et al. (2013) |

(continued)

**Table 4.2** (continued)

| Disease/Pathogen | Gene | Chromosome location | Marker type/linked marker | Reference |
|---|---|---|---|---|
| | | | GBMS192 &GBM1060 | |
| Leaf scald *Rhynchosporium secalis* | *Rrs1* | 3H | 11_0010 and 11_0823 | Hofmann et al. (2013) |
| Yellow mosaic virus *Polymyxa graminis* | *Rym17* | 3H | ABG070 | Kai et al. (2012) |
| | *Rym 18* | 4H | Bmag0490 | Kai et al. (2012) |
| | *Rym13* | 4H | HVM67& GBM1015 | Humbroich et al. (2010) |
| *Loose smut Ustilago nuda* | *Un8* | | Un8 SNP4; 0498L15 F8/R8 | Zang et al. (2015) |
| *Wheat stripe rust* | *Rps6* | 7H | FPC 320 | Dawson et al. (2016) |
| Spot blotch *Cochliobolus sativus* | *Scs6* | 1H | Bc183711 and Bc13291 | Leng et al. (2018) |
| Spot blotch *Bipolaris sorokiniana* | *Rbs7* | 6H | M13.06 and M13.37 | Wang et al. (2019) |

indirect selection of phenotype carrying desirable trait at allele level using gene specific or closely linked markers. Ordon et al. (1995) were the first group to report marker assisted introgression of *ym*4 gene from Frankainto Igri background for barley yellow mosaic disease. This was followed by many studies on resistance genes introgression for viral diseases (BaMMV/BaYMV and BYDY) in elite barley background using MAS (Schiemann and Backers 2000; Ovesna et al. 2000; Jefferies et al. 2003). Grewal et al. (2008b) reported transfer of loose smut resistance gene *Run*8 and covered smut resistance gene *Ruhq* in barley line CDC McGwire using the SCAR marker Un8-700R for loose smut and combination of RAPD (OPO6780) and STS (Hor2) markers, respectively. Similarly, SCAR marker E-ACT/M-CAA-170a was used to introgress the resistance gene *Rsp*2 for Septoria speckled leaf blotch (SSLB) in recurrent lines M110 and M96-46 (Zhong et al. 2006). Resistance for Scald (*Rhynchosporim commune*) was developed in barley elite lines Arta and Tadmor by marker-assisted transfer of *Rrs*1 gene using SSR markers Bmac209, Bmac67, EBmac871, Bmag6 and HVS3 (Sayed and Baum 2018). Richardson et al. (2006) reported introgression of three QTLs using closely linked SSR markers viz. 1H (GMS021, Bmac203 & Bmac399), 4H (EBmac679, EBmac788 & HvMLO3) and 5H (Bmag337 and GBM1039) providing resistance against *Puccinia striiformis* f.sp. *hordei* in barley cultivar Baronesse.

**Table 4.3** List of QTLs identified for major diseases in barley

| Disease | Pathogen | Linked/flanking markers | QTL name/position | References |
|---|---|---|---|---|
| Leaf rust | *Puccinia hordei* | Hv0963-Bpb-8580 | *QTL 5H* | Li et al. (2013) |
| | | Bmag173-Bmag009 | *QTL (6H)* | Castro et al. (2012) |
| | | GBMS137 and Bmag13 | *Qrph2.1; Qrph3.1* | Li et al. (2006) |
| Stripe rust | *Puccinia striiformis* | | *Qpsh*.316A.2Hb<br>*Qpsh*.316A.7H | Klos et al. 2020 |
| | | SCRI_RS_196285est (G), | *QPsh.FW6-6H.2* | Belcher er al. (2018) |
| | | SCRI_RS_188827, SCRI_ RS_157611, and SCRI_RS_10818 | *Qpsh*4Hb | Esvelt Klos et al. (2016) |
| | | *act8-BMAC213* | *QTL 5(1H)* | Toojinda et al. (2000) |
| Stem rust | *Puccinia graminis* | S2H_26426904<br>S4H_18920429<br>S3H_582311657<br>S7H_534521877 | *Rpg*-qtl-HH-Hie-2H<br>*Rpg*-qtl-HH-Hie-4H<br>*Rpg*-qtl-HH-Hip-3H<br>*Rpg*-qtl-HH-Hie-7H | Case et al. 2018 |
| *Fusarium*Head Blight (FHB) | *Fusarium* species | BOPA2_12_31203 and BOPA1_2251-643 | QTL on 7H chromosome | Huang et al. (2018) |
| | | bPb5755 and bPb1181 | *Qrgz-2H-14* | Yu et al. (2010) |
| | | FXLRRfor_XLRRrev119 – STS_FEgtaMacg677 | *QTL (2H)* | Hori et al. (2006) |
| *Fusarium* Crown Rot | *Fusarium* species | K01150 and WMS6 | *Qcrs.cpi-4H* | Chen et al. (2013a) |

(continued)

**Table 4.3** (continued)

| Disease | Pathogen | Linked/flanking markers | QTL name/position | References |
|---|---|---|---|---|
| (FCR) | | bPb-6065 and bPb-8619 bPb-7278 and bPb-0619 | *Qcrs.cpi-1H Qcrs.cpi-3H* | Chen et al. (2013b) |
| | | bPb-4747 and bpb-6765 | *Qcrs.cpi-3H* | Li et al. (2009) |
| Powdery mildew | *Blumeria graminis* | SC_C5-SNP46 and SC_C2- SNP54 DC_C5-SNP52 and DC_C2- SNP57 | Rbgnq1 and Rbgnq2 | Romero et al. (2018) |
| | | SNP4H_1 (A), SNP4H_1 (G), | QPM.PPP-4H (mlo) | Bengtsson et al. (2017) |
| | | bPb-0837 | *QTL (5HS)* | Hickey et al. (2012) |
| | | bPb-8179 and bPb-7769 bPb-5638 | *QPm.TxFr.5H* and *QPm. TxFr.7HQPm.YeFr.1H* | Li and Zhou (2011) |
| | | GBM1126 and HvM004 EBmac0755 | *QTL (7HS)* | Silvar et al. (2010) |
| | | | *Rbgq1, Rbgq2,* nd *Rbgq3* | Shtaya et al. (2006) |
| Net blotch (NB) | *Pyrenophora teres* | SCRI_RS_140499 and SCRI_RS_8410 | AL_QRptt5-2 | Wonneberger et al. (2017) |
| | | i_SCRI_RS_186193, i_BK_12, i_SCRI_RS_186193, i_SCRI_RS_200895 | *Rpt5, QRpts2Sa, QNFNBAPR.AI/S-7Ha* | Vatter et al. (2017) |
| | | HVM62b bPb–9604 and bPb–6127 | *QRpt6 QRcss1* and *QRcs3* | Grewal et al. (2012) |
| | | Bmag0807-Bmag0496 Bmac0209–Bmag0841 | *QTL (6H) QTL (3H)* | Gupta et al. (2010) |
| | | HVM74 and Bmag496 HVM03 and Bmac181 | *QRpt6 QRpts4* | |

(continued)

**Table 4.3** (continued)

| Disease | Pathogen | Linked/flanking markers | QTL name/position | References |
|---|---|---|---|---|
| | | | | Grewal et al. (2008a) |
| | | *Xksua3b* and *Xwg719d Xcdo786-Xabc156a* | *Rpt QTL QTL (2H)* | Ma et al. (2004) |
| Net type net blotch<br>Spot type net blotch | *Pyrenophora teres f. teres*<br>*P. teres f. maculate* | HVM0060 and Bmag0173 Bmag0496 HVHOTR0001 | *QTL (3HS)* and *QTL (6HS) QTL (6HS) QTL (2HL)* | Cakir et al. (2011) |
| *Septoria* speckled leaf blotch (SSLB)<br>Net form net blotch | *Septoria passerine*<br>*Pyrenophora teres f. teres* | Bmag500<br>Ebmac787 and Ebmac874 | *Rsp4 QTL(6H)* | St. Pierre et al. (2010) |
| Leaf stripe | *Pyrenophora graminea* | *Pcr 1* | *QTL (2H)* | Arru et al. (2003) |
| Nonparasitic leaf spots (NPLS) | – | *EBmac0635* | *QNpls.lfl-4H* | Behn et al. (2004) |
| Spot blotch | *Cochliobolus sativus* | 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*<br>*Rcs-qtl-2H- SCRI_RS_233272*<br>*Rcs-qtl-4H- SCRI_RS_168399*<br>*Rcs-qtl-5H- SCRI_RS_138933* | Haas et al. (2016) |
| | *Puccinia hordei* | EBmac684-Bmac093 | QTL (2H, 3H, 6H, 7H) | |

(continued)

**Table 4.3** (continued)

| Disease | Pathogen | Linked/flanking markers | QTL name/position | References |
|---|---|---|---|---|
| Spot blotch and Leaf rust | *Cochliobolus sativus* | Bmag606-Bmag013<br>Bmac316-Bmag500<br>Bmag173-Bmag009<br>Ris44-Bmac156<br>Bmag213-Bmag770, EBmac684-Bmac093<br>Bmag136-Bmag603<br>Bmac316-Bmag500<br>Bmac156-Bmag135 | QTL (1H, 2H, 3H, 6H, 7H) | Castro et al. (2012) |
| *Cereal yellow dwarf virus* (CYDV) | *Poleovirus* | 12_30872<br>11_20247 | *Qcyd.MaBu-1 Qcyd. MaBu-2* | Del Blanco et al. (2014) |
| Barley leaf blotch/scald | *Rhynchosporium secalis* | 11_11098 and 11_10169 | *QTL (7H)* | Looseley et al. (2012) |
| | | bPb-7356 and Bmag0006 | *QSc.YeFr.3H* | Li and Zhou (2011) |
| | | GBM1281 and GemS13 | *QTL (2HS)* | Wagner et al. (2008) |
| | | | *Rrsq1*, *Rrsq2*, *Rrsq3*, and *Rrsq4* | Shtaya et al. (2006) |
| Yellow mosaic virus | *Polymyxa graminis* | *MWG2134* | *QTL (2H)* | Miyazaki et al. (2001) |
| Barley yellow dwarf virus-PAV | | 3262224S3, 3258686S5, 4789985D, 4789803D, 3263957S5, 5249736S7, 3257158S7 | *Ryd2*, | Hu et al. (2019) |

The shelf-life of varieties carrying single major gene against particular pathogen is short due to evolution and development of new pathogen race over time to adapt already available resistance genes in these varieties. Nelson (1978) suggested gene pyramiding aiming at horizontal resistance system to increase resistance spectrum (Nelson 1978). In barley also, gene pyramiding was attempted for major and minor gene (QTLs) pyramiding especially for stripe rusts and viral diseases (Castro et al. 2003; Werner et al. 2005; Richardson et al. 2006). Pyramids of stripe rust resistance QTLs were developed to study the level of stripe rust resistance in relation to combined QTLs and lesser disease susceptibility was observed in lines carrying more number of loci on 1H, 4H and 5H chromosomes (Castro et al. 2003). Werner et al. (2005) reported gene pyramiding of resistance genes (*rym*4, *rym*5, *rym*9 and *rym*11) against barley yellow mosaic virus complex. Although ample number of genes/QTLs have been mapped and reported in barley using molecular tools still the reports of marker assisted transfer of these is few over the last two decades. The reason behind lesser reports of MAS and MABC in barley might be because resistance conferred by major gene (mongenic) are easy to handle without intervention of molecular technology and most of the economic viable QTLs are not manageable at molecular level due to too many minor genes (QTLs) involvement.

## 4.8 Transgenic Approaches for Developing Disease Resistance

Transgenesis is another technology established, validated and utilized to transfer new candidate gene of interest in crop plants. Flor (1971) reported gene for gene theory stating that outcome of plant pathogen interaction is mainly regulated by resistance locus (R) present in plant and avirulence locus (*avr*) of pathogen. If both are present then plant shows resistance towards that particular pathogen. This information led to transfer of genes for disease resistance within species, across species and across genera (Dong and Ronald 2019). In barley, Ritala et al. (1994) were the first group to report development of fertile barley from particle bombardment of immature embryo. Since then numerous studies were reported using biolistic transformation method for successful transfer of desirable genes in barley as shown in Table 4.4.

These efforts resulted in well-established *Agrobacterium* mediated transformation system in barley using androgenic pollens and immature embryo for introducing transgene (Kumlehn et al. 2006; Hensel et al. 2008). The cultivar Golden Promise was proved to be most promising genotype for genetic transformation studies in barley especially for *Agrobacterim* mediated approach as summarized in Table 4.5. Other cultivars amenable for developing transgenics are Igri, Harrington, Clipper, Sloop, Galena and Tafeno (Goedeke et al. 2007). As a result a number of genes have been transferred in barley for developing resistance against diseases like

*Rpg*1 (Horvarth et al. 2003), wheat *Lr*34 (Risk et al. 2013), *Sr22* (Hatta et al. 2020, and *Lr*67*res* (Milne et al. 2019).

After successful transformation and integration of a new gene in host genotype different methods are used for regeneration of transgenic plant. At present genetic transformation method is not difficult to implement successfully but the in-depth knowledge of cellular regulation and predictable transient expression of transformed gene is much required for successful generation of transgenic plants.

## 4.9 Genomic-Aided Breeding for Resistance in Traits

### *4.9.1 Barley Genome Sequencing*

Barley is a diploid plant species with seven chromosomes consisting a huge genome of size nearly 5.1 gigabases (Gb). Of the total 5100 Mbp genome more than 80% is repetitive DNA. To decode the barley genome the International Barely Genome Sequencing Consortium (IBGSC) was established in the year 2006 (Schulte et al. 2009). Two heuristic approaches have been used to decode the barely genome viz. (1) BAC-by-BAC approach and (2) shot-gun whole genome sequencing. Till date ca. 550 K BAC (bacterial artificial chromosome) clones have been fingerprinted and assembled to contig. In further efforts a robust consensus physical map will be developed by combining contigs of ca. 350 K sequenced BAC clones and SNP based genetic map. On the other hand, IBSC has successfully developed a physical map of 4.98 Gbp (98% of total genome) of which 3.90 Gbp is anchored to high resolution genetic map. The structurally and functionally annotated barley genome is available on a public domain database called EnsmblePlants, (https://plants.ensembl.org/Hordeum_vulgare/Info/Index). This framework supports structural and functional information on more than 26,159 high-confidence barley genes which includes BLAST facility, homology details with other decoded plant species, transcript details, information on functional proteins and transcriptome data. Additionally, efforts have been made to high-resolution genome assembly at chromosome scale. For this purpose, BAC clones with minimum tiling path were sequenced by Illumina short read sequencing using population sequencing methodology. Followed to this a BAC-based super scaffold was constructed by combining high-resolution genetic map and a highly contiguous optical map. This super scaffold was then re-ordered and re-oriented with chromosome conformation capture sequencing (Hi–C) to develop a final chromosome-scale assembly representing 4.97 Gbp of 5.10 Gbp barley genome and 39,734 high-confidence genes. Both, chloroplast and mitochondrial genomes of barley have also been decoded (Middleton et al. 2014; Hisano et al. 2016). Deep sequencing of the transcriptome (RNA-seq) from the cultivar Morex and FL-cDNAs from the cultivar Haruna Nijo helped to annotate the reference genome of the cultivar Morex. A de novo RNA-seq-based genotyping procedure for barley strains used in breeding programs

**Table 4.4** Transgenic studies reported in barley using biolistic mediated transformation and GUS assay

| Disease/Pathogen | Gene | Compound | Promoter/vector | Genotype Used | References |
|---|---|---|---|---|---|
| Powdery mildew *Blumeria graminis* f. sp. *tritici* | *LEMK1* | LRR-RLK | NA | Maythorpe | Rajaraman et al. (2016) |
| | *CslD2* | Cellulose synthase like D2 | NA | Maythorpe | Douchkov et al. (2016) |
| | *BEC* | Blumeria effector | pTA30 | Golden Promise | Pliego et al. (2013) |
| | *BI-1* | Bax inhibitor 1 | pIPKTA30N | Golden Promise | Eichmann et al. (2010) |
| | *Avra10; GTF1* | Effector gene | pIPKTA30N, pIPKb007_BgGTF1 | Golden Promise | Nowara et al. (2010) |
| | *BI-1* | Bax inhibitor 1 | NA | Golden Promise | Babaeizad et al. (2009) |
| | *GLP4 and TaGLP4* | Superoxide dismutase | pGY1 | Golden Promise | Christensen et al. (2004) |
| *FHB* *Fusarium graminearum* | *FsTri101* | 3-O-acetyltransferase | pUBR1 | Conlon | Manoharan et al. (2006) |
| *Botrytis cinerea* | *Vst1* | | Stilbene synthase | Igri | Leckband and Lörz (1998) |

**Table 4.5** Transgenic studies with *Agrobacterium* mediated transformation approach in barley

| Disease/Pathogen | Gene | Compound | Selection | Promoter/Vector | Reference |
|---|---|---|---|---|---|
| Leaf rust *Puccinia hordei* | *Sr22* | Stem rust resistance gene | Hygromycin | pVec8 | Hatta et al. (2018) |
| | *Lr67res* | Lr67 hexose transporter variant | Hygromycin | pVec8 | Milne et al. (2019) |
| Stem rust *P. graminis* f.sp. *tritici* | *Lr34res* | ABC transporter | Hygromycin | P6u and pWBVec8 | Risk et al. (2013) |
| | *Rpg1* | Receptor-like protein | PCR | pNRG040 | Horvath et al. (2003) |
| FHB *Fusarium graminearum* | *ICS* | Isochorismate synthase | Glufosinate ammonium | PC186 | Hao et al. (2018) |
| Powdery Mildew *Blumeria graminis f. sp. Hordei* | *ADH-1* | Alcohol dehydrogenase 1 | Hygromycin | pIPKb007 | Käsbauer et al. (2018) |
| | *CslD2* | Cellulose synthase- like D2 | Hygromycin | pIPKb009 | Douchkov et al. (2016) |
| | *LEMK1* | LRR-malectin domain-containing transmembrane RLK | Hygromycin | pIPKb009 | Rajaraman et al. (2016) |
| | *Mtk* | Metchnikowin | GFP | pGY1-GFP expression vector | Rahnamaeian and Vilcinskas (2012) |
| | *BI-1* | BAX inhibitor-1 | Hygromycin | pIPKTA30N-BI-1 | Eichmann et al. (2010) |
| | *BI-1* | BAX inhibitor-1 | Hygromycin | pLH6000 | Babaeizad et al. (2009) |
| | *Mtk* | Metchnikowin | PCR | pLH6000 | Rahnamaeian et al. (2009) |
| | *racb-G15V* | RAC/ROP family G protein | Hygromycin | pSB181 | Schultheiss et al. (2005) |
| *Pyrenophora teres f. teres* Net blotch | *CSD1* | Superoxide dismutase | Hygromycin | pSTARGATE | Lightfoot et al. (2017) |
| Wheat Dwarf virus | *RepA* | Replication- associated gene | Hygromycin | pIPKb002 | Cejnar et al. (2018) |
| | amiRNAs | Artificial microRNAs | Hygromycin | pCUbiVirusBuster171 | Kis et al. (2016) |

*Barley cultivar Golden Promise is used for transformation

has been implemented. Using 150 samples from 108 strains, de novo RNA-seq-based genotyping detected 181,567 SNPs and 45,135 indels, located in 28,939 transcribed regions distributed throughout the Morex genome (Mascher et al. 2017). Automated gene annotation of the barley reference sequence assembly was based on four datasets providing independent gene evidence information. This included (1) RNA sequencing (RNA-seq) data; (2) reference protein predictions from barley, rice, *B. distachyon* and *S. bicolor*; (3) published barley full-length complementary DNA (fl-cDNA) sequences; and (4) newly generated barley PacBio Iso-Seq data. This identified 83,105 putative gene loci including protein-coding genes, noncoding RNAs, pseudogenes and transcribed transposons. Recently, Liu et al. (2020) reported a high-quality draft assembly of wild barley accession (AWCS276; henceforth named as WB1), which consists of 4.28 Gb genome and 36 395 high-confidence protein-coding genes. It is inferred that the WB1 genome contains more genes involved in resistance and tolerance to biotic and abiotic stresses by comparing with the genome of the cultivated genotype Morex.

EnsemblPlants provides easy access to the most updated barley genome assembly, including chromosome sequences, genes, transcripts, and predicted proteins. Additional annotation can be done with a number database available in public domain using variety of bioinformatic techniques. GSDS server helps in studying gene structure with interactive graphical outputs. A variety of sequence statistics and multiple sequence alignment can be obtained from MEGA tool for evolutionary and phylogenetic studies. ProtParm from ExPasy provides physical and structural information with on proteins using amino acid sequences. Web-based serves like ARGOT and AgriGo provide information on gene ontology in very user-friendly manners. Protein domains can be studied using CDD search whereas, structural information of these domains can be retrieved from MEME suites. The KEGG web server further allows identifying enzymatic roles of functional protein in various metabolic pathways in plants. STICH and STRING servers finally help to identify chemical-protein and protein-protein interactions from the amino acid sequences.

## 4.10 Structural and Functional Genomics Resources

Multiple genomic resources (Table 4.6) for barley have been developed to empower genomic assisted barley improvement programs. These genomic resources are available in public domain and are useful for structural and functional annotation of putative genes of agronomic importance. These resources mainly include large array of expressed sequence tags (ESTs), SSR markers, cDNA library, BAC cloning library, gene expression database, SNP database, proteome, transcriptome database, complete genome sequence assembly and high-density linkage maps.

**Table 4.6** Barley genomic and functional resources available on public domain

| Name | Use | URL |
|---|---|---|
| Barley DB | Seed collection, cDNA sequence | http://www.shigen.nig.ac.jp/barley/ |
| barleyGenes | RNA-seq data | https://ics.hutton.ac.uk/barleyGenes/ |
| bex-db | cDNA, gene expression | https://barleyflc.dna.affrc.go.jp/bexdb/ |
| EnsemblPlants | Browser, BLAST | http://plants.ensembl.org/Hordeum_vulgare |
| GrainGenes | Markers, maps, mutants, *etc*. | http://www.graingenes.org |
| HarvEST | cDNA sequence | http://harvest.ucr.edu/ |
| IPK (IBSC) barley BLAST server | BLAST | https://webblast.ipkgatersleben.de/barley_ibsc/ |
| PLEXdb | Gene expression analysis | http://www.plantgdb.org/prj/PLEXdb/ |
| STITCH | Chemical protein interaction | http://stitch.embl.de/ |
| STRING` | Protein protein interaction database | https://string-db.org/ |
| eFP expression browser | Visual assessment of expression data | http://bar.utoronto.ca/efpbarley/cgi-bin/efpWeb.cgi |
| BarleyNet | Functional gene network | http://www.inetbio.org/barleynet |
| Phytozome v13 | Plant comparative genomics resource | https://phytozome.jgi.doe.gov/pz/portal.html |

## 4.11 Genome Editing

CRISPR/Cas9 based genome editing system offers many avenues to scientists to that can efficiently produce mutations in desired genes. Lawrenson et al. (2015) exploited the wheat promoter of the *TaU6* snRNA gene for *Sp*Cas9-mediated gene-editing of barley *HvPM19*, which encodes an ABA-inducible plasma membrane protein. Holme et al. (2020) edited *HvPAPhy*, a barley phytase gene using a similar construct. Kapusi et al. (2017) used the *Sp*Cas9 system to disrupt a barley *Endo-N-acetyl-*β*-D-glucosaminidase* (*ENGase*) gene by employing the rice *OsU6* promoter to drive the sgRNA, reaching a *Sp*Cas9-induced mutation frequency of 78%. In barley MORC1 (Microrchidia proteins) was further analyzed by Kumar et al. (2018b) using a highly efficient RNA-guided Cas9 gene-editing system. Kis et al. (2019) created a highly efficient resistance against wheat dwarf virus (inhibit an economically important, phloem-limited, insect transmitted virus) in barley by employing CRISPER/Cas9 system. Recently Garcia-Gimenez et al. (2020) performed tar*geted mutation of barley* using CRISPR to generate mutations in members of the gene superfamily responsible for making (1,3;1,4)-β-D-Glucan led

to specific differences in grain quality, composition and content of this compound. in case of lacking natural resistance resources, the CRISPR/Cas9 system can be utilized to establish extremely efficient resistance in monocotyledonary plants to combat an economically important, insect vector-transmitted, destructive DNA virus. However, the selection of potent sgRNAs and ensuring their proper expression are prerequisites of the optimal result. However, transformation and genome-editing experiments may suffer from some limitations resulting from the low transformation potential of some accessions. Hisano and Sato (2016) identified loci controlling transformation amenability in the regions of chromosomes 2H and 3H in an $F_2$ population derived from a cross between the cultivars Golden Promise and Haruna Nijo. Introducing these genomic regions in target haplotypes may increase their transformation efficiency and genome-editing capabilities.

Advancements in molecular biology and barely genomics have enabled plants scientists to improve their efficiency in genetic characterization of barely germplasm and identification of agronomically important genes. In recent years, availability of high throughput genotyping techniques such as genotyping-by-sequencing, SNP arrays and KASP markers have not only allowed barley breeders to identify genetic diversity at single nucleotide level but also the effect of single nucleotide polymorphisms on various phenotypes. With the help of these techniques, breeders are now able to identify population structure and to develop core collections. Precision in gene discovery has also improved due to availability these markers. Availability of reference genome and several other genomic resources allows a scientist to functionally annotate the significant genomic regions using various web-based tools before going on the sophisticated wet lab methods. Furthermore, survey of literature reveals that since the availability of reference barley genome and high throughput genotyping techniques number of studies in reference to characterization of germplasm, gene identification, and high-density QTL mapping have increased by nearly 70% however, most of these studies are aimed to genomewide association mapping for grain quality traits and various stress tolerance in barley.

## 4.12 Future Perspectives

Barley as crop has gained importance due to its commercial and versatile end use in distillation, brewing, food and feed sector. As an agriculture crop, barley has got attention of researchers because of its wide cultivation from temperate to tropical regions. The traditional breeding approach of hybridization and selection was majorly used in the last century to enhance biotic stress resistance in barley. Later development and implementation of molecular markers system motivated researchers to map various major genes and QTLs for disease resistance against major pathogens. With the onset of high throughput techniques and information generated in International Barley Genome Sequencing Consortium, research in barley is gaining momentum in important research areas like yield, disease

resistance and quality. Support of genomic assisted technologies like GWAS, genome editing, CRISPER, gene cloning and numerous genotypic platforms like SNP genotyping, microrray, DArT etc. and well established doubled haplod method have led to generate precise information of host (R) and pathogen genes (*Avr*) to understand their interaction and regulation at cellular level. In future also, a number of untapped gees/QTLs will be identified and introgressed in the elite barley cultivars as well as resistance genes will be cloned and transferred. The near future is promising with the availability of powerful game changing tools like DH and CRISPER mediated gene editing which will definitely going to impact barley research in developing robust resistance against the major pathogens.

## References


Adhikari A, Steffenson BJ, Smith KP, Smith M, Dill-Macky R (2020) Identification of quantitative trait loci for net form net blotch resistance in contemporary barley breeding germplasm from the USA using genome-wide association mapping. Theor Appl Genet 133(3):1019–1037

Ali MA, Shahzadi M, Zahoor A, Dababat AA, Toktay H, Bakhsh A, Nawaz MA, Li H (2019) Resistance to cereal cyst nematodes in wheat and barley: an emphasis on classical and modern approaches. Intl J Mol Sci 20(2):432–449

Arabi MIE (2005) Inheritance of partial resistance to spot blotch in barley. Plant Breed 124 (6):605–607

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

Babaeizad V, Imani J, Kogel KH, 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(3):455–463

Bai G, Shaner G (2004) Management and resistance in wheat and barley to Fusarium head blight. Ann Rev Phytopathol 42:135–161

Baik BK, Ullrich SE (2008) Barley for food: characteristics, improvement, and renewed interest. J Cereal Sci 48(2):233–242

Banjarey P, Kumar P, Verma S, Tikle AN, Malik R, Sarker A, Verma RPS (2017) Comparative analysis of agro-morphological and molecular variations in huskless barley (*Hordeum vulgare* L) under Central Agro-Climatic Zone of India. Intl J Curr Microbiol Appl Sci 6(12):2821–2829

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 (7):1229–1235

Belcher AR, Cuesta-Marcos A, Smith KP, Mundt CC, Chen X, Hayes PM (2018) TCAP FAC-WIN6 Elite Barley GWAS Panel QTL I Barley stripe rust resistance QTL in facultative and winter six-rowed malt barley breeding programs identified via GWAS. Crop Sci 58 (1):103–119

Bengtsson T, Ahman I, Manninen O, Reitan L, Christerson T, Due Jensen J, Krusell L, Jahoor A, Orabi J (2017) A novel QTL for powdery mildew resistance in nordic spring barley (*Hordeum vulgare* L ssp *vulgare*) revealed by genome-wide association study. Front Plant Sci 8(1954):1–11

Bennett MD, Smith JB (1976) Nuclear DNA amounts in angiosperms. Philos Trans R Soc Lond B Biol Sci 274:227–274

Bilgic H, Steffenson BJ, Hayes PM (2006) Molecular mapping of loci conferring resistance to different pathotypes of the spot blotch pathogen in barley. Phytopathology 96(7):699–708

Bothmer RV, Jacobsen N, Baden C, Jorgensen RB, Linde Laursen I (1995) An ecogeographical study of the genus hordeum, 2nd edn. Systematic and ecogeographical studies on crop genepools, 7. IBPGR, Rome, Italy, 129, CABI

Bothmer RV, Sato K, Kamatsuda T, Yasuda S, Fischbeck G (2003) The domestication of cultivated barley. In: Von Bothmer R, van Hintum T, Knüpffer H, Sato K (eds) Diversity in Barley (*Hordeum vulgare*), pp 9–27. Elsevier Press, Amsterdam, Netherlands

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Amer J Hum Genet 32(3):314–331

Brown WM Jr, Hill JP, Velasco VR (2001) Barley yellow rust in North America. Annu Rev Phytopathol 39(1):367–384

Cakir M, Gupta S, Li C, Hayden M, Mather DE, Ablett GA, Platz GJ, Broughton S, Chalmers KJ, Loughman R, Jones MG (2011) Genetic mapping and QTL analysis of disease resistance traits in the barley population Baudin × AC Metcalfe. Crop Pasture Sci 62(2):152–161

Case AJ, Bhavani S, Macharia G, Pretorius Z, Coetzee V, Kloppers F, Tyagi P, Brown-Guedira G, Steffenson BJ (2018) Mapping adult plant stem rust resistance in barley accessions Hietpas-5 and GAW-79. Theor Appl Genet 131(10):2245–2266

Castro AJ, Capettini FLAVIO, Corey AE, Filichkina T, Hayes PM, Kleinhofs ANDRIS, Kudrna D, Richardson K, Sandoval-Islas S, Rossi C, Vivar H (2003) Mapping and pyramiding of qualitative and quantitative resistance to stripe rust in barley. Theor Appl Genet 107(5):922–930

Castro AJ, Gamba F, German S, Gonzalez S, Hayes PM, Pereyra S, Perez C (2012) Quantitative trait locus analysis of spot blotch and leaf rust resistance in the BCD47 × Baronesse barley mapping population. Plant Breed 131(2):258–266

Ceccarelli S, Grando S, Capettini F, Baum M (2008) Barley breeding for sustainable production. In: Kang MS, Priyadarshan PM (eds) Breeding major food staples. Blackwell Publishing, Iowa, USA, pp 193–225

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 Agri 17(11):2492–2500

Chen X, Line RF (1999) Recessive genes for resistance to *Puccinia striiformis* f sp *hordei* in barley. Phytopathology 89(3):226–232

Chen X, Line RF (2003) Identification of genes for resistance to *Puccinia striiformis* f sp *hordei* in 18 barley genotypes. Euphytica 129(1):127–146

Chen G, Liu Y, Ma J, Zheng Z, Wei Y, McIntyre CL, Zheng YL, Liu C (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(3):

Chen GD, Liu YX, Wei YM, McIntyre CL, Zhou MX, Zheng YL, Liu CJ (2013b) Major QTL for Fusarium crown rot resistance in a barley landrace. Theor Appl Genet 126(10):2511–2520

Christensen AB, Thordal-Christensen H, Zimmermann G, Gjetting T, Lyngkjær MF, Dudler R, Schweizer P (2004) The germin-like protein GLP4 exhibits superoxide dismutase activity and is an important component of quantitative resistance in wheat and barley. Mol Plant-Microbe Interact 17(1):109–117

Curry A (2008) The gladiator diet: how to eat, exercise, and die a violent death. Archaeology 61 (6):28–30

Dawson AM, Ferguson JN, Gardiner M, Green P, Hubbard A, Moscou MJ (2016) Isolation and fine mapping of *Rps*6: an intermediate host resistance gene in barley to wheat stripe rust. Theor Appl Genet 129(4):831–843

Del Blanco IA, Hegarty J, Gallagher LW, Fal BW, Brown-Guedira G, Pellerin E, Dubcovsky J (2014) Mapping of QTL for tolerance to Cereal Yellow Dwarf Virus in two-rowed spring barley. Crop Sci 54(4):1468–1475

Dong OX, Ronald PC (2019) Genetic engineering for disease resistance in plants: recent progress and future perspectives. Plant Physiol 01224

Douchkov D, Lueck S, Hensel G, Kumlehn J, Rajaraman J, Johrde A, Doblin MS, Beahan CT, Kopischke M, Fuchs R, Lipka V (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(2):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 *Rph*9/*Rph*12 locus on chromosome 5HL in barley. BMC Plant Biol 14(1):1598–1599

Drader T, Kleinhofs A (2010) A synteny map and disease resistance gene comparison between barley and the model monocot *Brachypodium distachyon*. Genome 53(5):406–417

Dreiseitl A and Dinoor A (2004) Phenotypic diversity of barley powdery mildew resistance sources. Genet Res Crop Evol 51(3):251–257

Eichmann R, Bischof M, Weis C, Shaw J, Lacomme C, Schweizer P, Duchkov D, Hensel G, Kumlehn J, Hückelhoven R (2010) BAX INHIBITOR-1 is required for full susceptibility of barley to powdery mildew. Mol. Plant Microbe In 23(9):1217–1227

Esvelt Klos K, Gordon T, Bregitzer P, Hayes P, Chen XM, Del Blanco IA, Fisk S, Bonman JM (2016) Barley stripe rust resistance QTL: development and validation of SNP markers for resistance to *Puccinia striiformis* f sp *hordei*. Phytopathology 106(11):1344–1351

FAOSTAT (2020) Food and Agriculture Organization of the United Nations Statistics Division portal. http://faostat3.fao.org/. Accessed on Jan 2021

Fernandez M, Figueiras A, Benito C (2002) The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. Theor Appl Genet 104(5):845–851

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

Gangwar OP, Bhardwaj SC, Singh GP, Prasad P, Kumar S (2018) Barley disease and their management: an Indian perspective. Wheat Barley Res 10(3):138–150

Garcia-Gimenez G, Barakate A, Smith P, Stephens J, Khor SF, Doblin MS, Hao P, Bacic A, Fincher GB, Burton RA, Waugh R (2020) Targeted mutation of barley (1, 3; 1, 4)-β-glucan synthases reveals complex relationships between the storage and cell wall polysaccharide content. Plant J 104:1009–1022

Giancarla V, Emilian MADOŞĂ, Radu S, Sorin C, Sorina P, Cerasela P (2012) The use of RAPD and ISSR markers for genetic diversity among some barley cultivars. Roman Biotechnol Lett 17(4):7493–7503

Goedeke S, Hensel G, Kapusi E, Gahrtz M, Kumlehn J (2007) Transgenic barley in fundamental research and biotechnology. Transgen Plant J 1(1):104–117

Graner A, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G, Herrmann RG (1991) Construction of an RFLP map of barley. Theor Appl Genet 83(2):250–256

Graner A, Streng S, Drescher A, Jin Y, Borovkova I, Steffenson BJ (2000) Molecular mapping of the leaf rust resistance gene *Rph*7 in barley. Plant Breed 119(5):389–392

Grewal TS, Rossnagel BG, Scoles GJ (2008a) Validation of molecular markers for covered smut resistance and marker-assisted introgression of loose and covered smut resistance into hulless barley. Mol Breed 21(1):37–48

Grewal TS, Rossnagel BG, Pozniak CJ, Scoles GJ (2008b) Mapping quantitative trait loci associated with barley net blotch resistance. Theor Appl Genet 116(4):529–539

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(1):267–279

Gupta S, Li CD, Loughman R, Cakir M, Platz G, Westcott S, Bradley J, Broughton S, Lance R (2010) Quantitative trait loci and epistatic interactions in barley conferring resistance to net type net blotch (*Pyrenophora teres* f teres) isolates. Plant Breed 129(4):362–368

Gyawali S, Chao S, Vaish SS, Singh SP, Rehman S, Vishwakarma SR, Verma RPS (2018) Genome wide association studies (GWAS) of spot blotch resistance at the seedling and the adult plant stages in a collection of spring barley. Mol Breed 38(5):62–75

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(10):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 Protec 36:50–56

Hamza S, Hamida WB, Rebaï A, Harrabi M (2004) SSR-based genetic diversity assessment among Tunisian winter barley and relationship with morphological traits. Euphytica 135 (1):107–118

Hansen M, Kraft T, Ganestam S, Saell T, Nilsson NO (2001) Linkage disequilibrium mapping of the bolting gene in sea beet using AFLP markers. Genet Resour 77(1):61–66

Hao Q, Wang W, Han X, Wu J, Lyu B, Chen F, Caplan A, Li C, Wu J, Wang W, Xu Q (2018) Isochorismate-based salicylic acid biosynthesis confers basal resistance to Fusarium graminearum in barley. Mol Plant Patho 19(8):1995–2010

Harlan Jack R, de Wet Jan MJ (1971) Toward a rational classification of cultivated plants. Taxon 20(4):509–517

Harwood W (2016) Barley as a cereal model for biotechnology applications, pp 80–87. Biotechnology of Major Cereals CABI, Wallingford

Hatta MAM, Arora S, Ghosh S, Matny O, Smedley MA, Yu G, Chakraborty S, Bhatt D, Xia X, Steuernagel B, Richardson T, Mago R, Lagudah ES, Patron NJ, Ayliffe M, Rouse MN, Harwood WA, Periyannan S, Steffenson BJ, Wulff BBH (2020) The wheat Sr22, Sr33, Sr35 and Sr45 genes confer resistance against stem rust in barley. Plant Biotechnol J 1–12

Hatta MAM, Johnson R, Matny O, Smedley MA, Yu G, Chakraborty S, Bhatt D, Xia X, Arora S, Steuernagel B, Richardson T (2018) The wheat Sr22 Sr33Sr35 and Sr45 genes confer resistance against stem rust in barley. bioRxiv : https://doi.org/10.1101/374637

Hensel G, Valkov V, Middlefell-Williams J, Kumlehn J (2008) Efficient generation of transgenic barley: the way forward to modulate plant–microbe interactions. J Plant Physiol 165(1):71–82

Heun M (1992) Mapping quantitative powdery mildew resistance of barley using a restriction fragment length polymorphism map. Genome 35(6):1019–1025

Hickey LT, Lawson W, Platz GJ, Fowler RA, Arief V, Dieters M, Germán S, Fletcher S, Park RF, Singh D, Pereyra S (2012) Mapping quantitative trait loci for partial resistance to powdery mildew in an Australian barley population. Crop Sci 52(3):1021–1032

Hisano H, Sato K (2016) Genomic regions responsible for amenability to *Agrobacterium*—mediated transformation in barley. Sci rep 6(1):1–11

Hisano H, Tsujimura M, Yoshida H, Terachi T, Sato K (2016) Mitochondrial genome sequences from wild and cultivated barley (*Hordeum vulgare*). BMC Genom 17(1):1–12

Horvath H, Rostoks N, Brueggeman R, Steffenson B, Von Wettstein D and Kleinhofs A (2003) Genetically engineered stem rust resistance in barley using the Rpg1gene. Proceed Nat Acad Sci 100(1):364–369

Hofmann K, Silvar C, Casas AM, Herz M, Büttner B, Gracia MP, Contreras-Moreira B, Wallwork H, Igartua E, Schweizer G (2013) Fine mapping of the Rrs1 resistance locus against scald in two large populations derived from Spanish barley landraces. Theor Appl Genet 126 (12):3091–3102

Holme IB, Madsen CK, Wendt T, Brinch-Pedersen H (2020) Horizontal stacking of PAPhy_a Cisgenes in barley is a potent strategy for increasing mature grain phytase activity. Front Plant Sci 11:

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(1):25–30

Horvath H, Rostoks N, Brueggeman R, Steffenson B, Von Wettstein D, Kleinhofs A (2003) Genetically engineered stem rust resistance in barley using the *Rpg*1 gene. Proc Natl Acad Sci USA 100(1):364–369

Hu H, Choudhury S, Shabala S, Gupta S, Zhou M (2019) Genomic regions on chromosome 5H containing a novel QTL conferring barley yellow dwarf virus-PAV (BYDV-PAV) tolerance in barley. Sci Rep 9(1):1–10

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

Hudcovicova M, Sudyova V, Slikova S, Gregova E, Kraic J, Ordon F, Mihalik D, Horevaj V, Sramkova Z (2008) Marker-assisted selection for the development of improved barley and wheat lines. Acta Agron Hung 56(4):385–392

Humbroich K, Jaiser H, Schiemann A, Devaux P, Jacobi A, Cselenyi L, Habekuss A, Friedt W, Ordon F (2010) Mapping of resistance against Barley mild mosaic virus-Teik (BaMMV)–an rym5 resistance breaking strain of BaMMV–in the Taiwanese barley (Hordeum vulgare) cultivar 'Taihoku A'. Plant Breed 129(3):346–348

IBGSC (2012) International barley genome sequencing consortium. a physical, genetic and functional sequence assembly of the barley genome. Nature 491(7426):711–716

Jain N, Malik R, Pande V, Kumar R, Verma RPS (2013) Identification of genomic regions associated with leaf blight resistance in barley. Intl J Adv Biotechnol Res 4(4):553–559

Jain N, Malik R, Selvakumar R, Kumar R, Pande V, Verma RPS (2014) Screening of barley germplasm for leaf blight (*Bipolaris sorokiniana*) resistance. Indian J Agric Res 48(1):67–71

Jannink JL, Walsh B (2002) Association mapping in plant populations. Quantitative genetics, genomics and plant breeding, pp 59–68

Jefferies SP, King BJ, Barr AR, Warner P, Logue SJ, Langridge P (2003) Marker-assisted backcross introgression of the Yd2 gene conferring resistance to barley yellow dwarf virus in barley. Plant Breed 122(1):52–56

Jørgensen JH and Wolfe M (1994) Genetics of powdery mildew resistance in barley. Crit Rev Plant Sci 13(1):97–119

Johnston PA, Niks RE, Meiyalaghan V, Blanchet E, Pickering R (2013) *Rph*22: mapping of a novel leaf rust resistance gene introgressed from the non-host *Hordeum bulbosum* L into cultivated barley (*Hordeum vulgare* L). Theor Appl Genet 126(6):1613–1625

Jost M, Singh D, Lagudah E, Park RF, Dracatos P (2020) Fine mapping of leaf rust resistance gene *Rph*13 from wild barley. Theor Appl Genet 1–9

Kai H, Takata K, Tsukazaki M, Furusho M, Baba T (2012) Molecular mapping of *Rym*17, a dominant and rym18 a recessive barley yellow mosaic virus (BaYMV) resistance genes derived from *Hordeum vulgare* L. Theor Appl Genet 3:577–583

Kapusi E, Corcuera-Gómez M, Melnik S, Stoger E (2017) Heritable genomic fragment deletions and small indels in the putative ENGase gene induced by CRISPR/Cas9 in barley. Front Plant Sci 8:540

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

Kendal E (2016) GGE biplot analysis of multi-environment yield trials in barley (*Hordeum vulgare* L) cultivars. Ekin J Crop Breed Genet 2(1):90–99

Kerr ED, Phung TK, Caboche CH, Fox GP, Platz GJ, Schulz BL (2019) The intrinsic and regulated proteomes of barley seeds in response to fungal infection. Anal Biochem 580:30–35

Kiesling RL (1985) The diseases of barley. Barley 26:269–312

Kilian A, Steffenson BJ, Saghai Maroof MA, Kleinhofs A (1994) RFLP markers linked to the durable stem rust resistance gene Rpg1 in barley. Mol Plant Microbe Interact 7(2):298–301

Kilian A, Chen J, Han F, Steffenson B, Kleinhofs A (1997) Towards map-based cloning of the barley stem rust resistance genes Rpg1 and rpg4 using rice as an intergenomic cloning vehicle. Takuji Sasaki, Graham Moore: Oryza: From Molecule to Plant. Springer, Dordrecht, Netherlands, pp 187–195

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(3):427–437

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 17 (6):1004–1006

Kleinhofs A, Brueggeman R, Nirmala J, Zhang L, Mirlohi A, Druka A, Rostoks N, Steffenson BJ (2009) Barley stem rust resistance genes: structure and function. The Plant Genome 2(2):109–120

Klos K, Hayes P, Del Blanco IA, Chen X, Filichkin T, Helgerson L, Fisk S, Bregitzer P (2020) Quantitative trait loci for field resistance to barley stripe rust derived from malting line 95SR316A. Crop Sci 60(4):1844–1853

König J, Kopahnke D, Steffenson BJ, Przulj N, Romeis T, Röder MS, Ordon F, Perovic D (2012) Genetic mapping of a leaf rust resistance gene in the former Yugoslavian barley landrace MBR1012. Mol Breed 30(3):1253–1264

Kraakman ATW, Martinez F, Mussiraliev B, Van Eeuwijk FA, Niks RE (2006) Linkage disequilibrium mapping of morphological, resistance, and other agronomically relevant traits in modern spring barley cultivars. Mol Breed 17(1):41–58

Kumar P, Tikle AN, Verma RPS, Rekha M (2017) Diversity assessment of hulled barley (*Hordeum vulgare* L) accessions by agro-morphological traits and SSR markers. Res J Biotechnol 12:11

Kumar N, Galli M, Ordon J, Stuttmann J, Kogel KH, Imani J (2018a) Further analysis of barley MORC 1 using a highly efficient RNA-guided Cas9 gene-editing system. Plant Biotechnol J 16 (11):1892–1903

Kumar P, Pratap S, Verma RP, Tikle AN, Malik R (2018b) Diversity assessment of hulled barley (*Hordeum vulgare* L) accessions of ICARDA in Indian condition using cluster analysis. Indian J Agric Res 52(4):429–433

Kumar P, Banjarey P, Malik R, Tikle AN, Verma RPS (2020) Population structure and diversity assessment of barley (*Hordeum vulgare* L) introduction from ICARDA. J Genet 99(1):1–9

Kumlehn J, Serazetdinova L, Hensel G, Becker D, Loerz H (2006) Genetic transformation of barley (*Hordeum vulgare* L) via infection of androgenetic pollen cultures with *Agrobacterium tumefaciens*. Plant Biotechnol J 4(2):251–261

Lawrenson T, Shorinola O, Stacey N, Li C, Østergaard L, Patron N, Uauy C, Harwood W (2015) Induction of targeted, heritable mutations in barley and *Brassica Oleracea* using RNA-guided *Cas9* nuclease. Genome Biol 16(1):

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(8):1004–1012

Legge WG, Therrien MC, Tucker JR, Banik M, Tekauz A, Somers D, Savard ME, Rossnagel BG, Lefol E, Voth D, Zatorski T (2004) Progress in breeding for resistance to Fusarium head blight in barley. Can J Plant Pathol 26(4):436–442

Leistrumaite A, Liatukas Z (2006) Resistance of spring barley cultivars to the new disease Ramularia leaf spot, caused by *Ramularia collo-cygni*. Agron Res 4:251–255

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(7):1531–1539

Li HB, Zhou MX (2011) Quantitative trait loci controlling barley powdery mildew and scald resistances in two different barley doubled haploid populations. Mol Breed 27(4):479–490

Li JZ, Huang XQ, Heinrichs F, Ganal MW, Röder MS (2006) Analysis of QTLs for yield components, agronomic traits, and disease resistance in an advanced backcross population of spring barley. Genome 49(5):454–466

Li HB, Zhou MX, Liu CJ (2009) A major QTL conferring crown rot resistance in barley and its association with plant height. Theor Appl Genet 118(5):903–910

Li C, Gupta S, Zhang XQ, Westcott S, Yang J, Park R, Platz G, Loughman R, Lance R (2013) A major QTL controlling adult plant resistance for barley leaf rust. In: Zhang G, Li C, Liu X (eda) Barley Sciences, pp 285–300. Springer, Dordrecht, Netherlands

Lightfoot DJ, Mcgrann GR, Able AJ (2017) The role of a cytosolic superoxide dismutase in barley–pathogen interactions. Mol Plant Pathol 18(3):323–335

Liu M, Li Y, Ma Y, Zhao Q, Stiller J, Feng Q, Tian Q, Liu D, Han B, Liu C (2020) The draft genome of a wild barley genotype reveals its enrichment in genes related to biotic and abiotic stresses compared to cultivated barley. Plant Biotechnol J 18(2):443–456

Looseley ME, Newton AC, Atkins SD, Fitt BD, Fraaije BA, Thomas WTB, Keith R, Macaulay M, Lynott J, Harrap D (2012) Genetic basis of control of *Rhynchosporium secalis* infection and symptom expression in barley. Euphytica 184(1):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(3):291–296

Malysheva-Otto LV, Ganal MW, Röder MS (2006) Analysis of molecular diversity, population structure and linkage disequilibrium in a worldwide survey of cultivated barley germplasm (*Hordeum vulgare* L). BMC Genet 7(1):6–20

Mammadov JA, Brooks WS, Griffey CA, Saghai Maroof MA (2007) Validating molecular markers for barley leaf rust resistance genes *Rph*5 and *Rph*7. Plant Breed 126(5):458–463

Manninger K, Mátrai T, Muranyi I (2008) Survey of winter barley fields for leaf spot diseases: epidemic spread of Ramularia leaf spot in Hungary in 2008, pp 123–124

Manoharan M, Dahleen LS, Hohn TM, Neate SM, Yu XH, Alexander NJ, McCormick SP, Bregitzer P, Schwarz PB, Horsley RD (2006) Expression of 3-OH trichothecene acetyltransferase in barley (*Hordeum vulgare* L) and effects on deoxynivalenol. Plant Sci 171(6):699–706

Mascher M, Gundlach H, Himmelbach A, Beier S, Twardziok SO, Wicker T, Radchuk V, Dockter C, Hedley PE, Russell J, Bayer M (2017) A chromosome conformation capture ordered sequence of the barley genome. Nature 544(7651):427–433

Middleton CP, Senerchia N, Stein N, Akhunov ED, Keller B, Wicker T, Kilian B (2014) Sequencing of chloroplast genomes from wheat, barley, rye and their relatives provides a detailed insight into the evolution of the *Triticeae* tribe. PLoS ONE 9(3):

Milne RJ, Dibley KE, Schnippenkoetter W, Mascher M, Lui AC, Wang L, Lo C, Ashton AR, Ryan PR, Lagudah ES (2019) The wheat *Lr67* gene from the sugar transport protein 13 family confers multipathogen resistance in barley. Plant Physiol 179(4):1285–1297

Milner SG, Jost M, Taketa S, Mazón ER, Himmelbach A, Oppermann M, Weise S, Knüpffer H, Basterrechea M, König P, Schüler D (2019) Genebank genomics highlights the diversity of a global barley collection. Nat Genet 51(2):319–326

Miyazaki C, Osanai E, Saeki K, Ito K, Konishi T, Sato K, Saito A (2001) Mapping of quantitative trait loci conferring resistance to barley yellow mosaic virus in a Chinese barley landrace Mokusekko 3. Breed Sci 51(3):171–177

Nelson RR (1978) Genetics of horizontal resistance to plant diseases. Annu Rev Phytopathol 16 (1):359–378

Nowara D, Gay A, Lacomme C, Shaw J, Ridout C, Douchkov D, Hensel G, Kumlehn J, Schweizer P (2010) HIGS: host-induced gene silencing in the obligate biotrophic fungal pathogen *Blumeria graminis*. Plant Cell 22(9):3130–3141

Ordon F, Bauer E, Graner A (1995) Marker-based selection for the ym4 BaMMV-resistance gene in barley using RAPDs. Agronomie 15:481–485

Ovesna J, Vacke J, Kucera L, Chrpová J, Novakova I, Jahoor A, Š Ip V (2000) Genetic analysis of resistance in barley to barley yellow dwarf virus. Plant Breed 119(6):481–486

Park RF, Poulsen D, Barr AR, Cakir M, Moody DB, Raman H, Read BJ (2003) Mapping genes for resistance to *Puccinia hordei* in barley. Aust J Agric Res 54(12):1323–1333

Parlevliet JE (1976) Partial resistance of barley to leaf rust, *Puccinia hordei* III The inheritance of the host plant effect on latent period in four cultivars. Euphytica 25(1):241–248

Paulitz TC, Steffenson BJ (2011) Biotic stress in barley: disease problems and solutions. In: Steven EU (ed) Barley production, improvement, and uses, pp 307–354. WileyBlackwell, New Jersey, USA

Perovic D, Stein N, Zhang H, Drescher A, Prasad M, Kota R, Kopahnke D, Graner A (2004) An integrated approach for comparative mapping in rice and barley with special reference to the *Rph*16 resistance locus. Funct Integr Genom 4(2):74–83

Pickering RA, Hill AM, Michel M, Timmerman-Vaughan GM (1995) The transfer of a powdery mildew resistance gene from *Hordeum bulbosum* L to barley (*H vulgare* L) chromosome 2 (2I). Theor Appl Genet 91(8):1288–1292

Pickering RA, Malyshev S, Künzel G, Johnston PA, Korzun V, Menke M, Schubert I (2000) Locating introgressions of *Hordeum bulbosum* chromatin within the *H. vulgare* genome. Theor Appl Genet 100(1):27–31

Pickering R, Niks RE, Johnston PA, Butler RC (2004) Importance of the secondary genepool in barley genetics and breeding II Disease resistance, agronomic performance and quality. Czech J Genet Plant Breed 40(3):79–85

Piechota U, Słowacki P, Czembor PC (2020) Identification of a novel recessive gene for resistance to powdery mildew (*Blumeria graminis* f sp *hordei*) in barley (*Hordeum vulgare*). Plant Breed 139(4):730–742

Pinnschmidt HO, Hovmøller MS (2004) Resistance against net blotch, scald and Ramularia of barley. DJF Rapp 98:61–71

Pinnschmidt HO, Jørgensen LN (2009) Yield effects of Ramularia leaf spot on spring barley. Asp Appl Biol 92:57–66

Pliego C, Nowara D, Bonciani G, Gheorghe DM, Xu R, Surana P, Whigham E, Nettleton D, Bogdanove AJ, Wise RP, Schweizer P (2013) Host-induced gene silencing in barley powdery mildew reveals a class of ribonuclease-like effectors. Mol Plant-Microbe Interact 26(6):633–642

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(1):115–124

Rahnamaeian M, Langen G, Imani J, Khalifa W, Altincicek B, Von Wettstein D, Kogel KH, Vilcinskas A (2009) Insect peptide metchnikowin confers on barley a selective capacity for resistance to fungal ascomycetes pathogens. J Exp Bot 60(14):4105–4114

Rajaraman J, Douchkov D, Hensel G, Stefanato FL, Gordon A, Ereful N, Caldararu OF, Petrescu AJ, Kumlehn J, Boyd LA, Schweizer P (2016) An LRR/malectin receptor-like kinase mediates resistance to non-adapted and adapted powdery mildew fungi in barley and wheat. Front Plant Sci 7:1836–1849

Ramage RT (1987) A history of barley breeding methods. Plant Breed Rev 5:95–138

Richardson KL, Vales MI, Kling JG, Mundt CC, Hayes PM (2006) Pyramiding and dissecting disease resistance QTL to barley stripe rust. Theor Appl Genet 113(3):485–495

Risk JM, Selter LL, Chauhan H, Krattinger SG, Kumlehn J, Hensel G, Viccars LA, Richardson TM, Buesing G, Troller A, Lagudah ES (2013) The wheat *Lr34* gene provides resistance against multiple fungal pathogens in barley. Plant Biotechnol J 11(7):847–854

Ritala A, Aspegren K, Kurtén U, Salmenkallio-Marttila M, Mannonen L, Hannus R, Kauppinen V, Teeri TH, Enari TM (1994) Fertile transgenic barley by particle bombardment of immature embryos. Plant Mol Biol 24(2):317–325

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(5):1031–1045

Rothwell CT, Singh D, Dracatos PM, Park RF (2020) Inheritance and characterization of *Rph27*: a third race-specific resistance gene in the barley cultivar quinn. Phytopathology 110(5):1067–1073

Sallam A, Amro A, Ammar EA, Dawood MF, Kumamaru T, Baenziger PS (2018) Genetic diversity and genetic variation in morpho-physiological traits to improve heat tolerance in Spring barley. Mol Biol Rep 45(6):2441–2453

Sandhu KS, Forrest KL, Kong S, Bansal UK, Singh D, Hayden MJ, Park RF (2012) Inheritance and molecular mapping of a gene conferring seedling resistance against *Puccinia hordei* in the barley cultivar Ricardo. Theor Appl Genet 125(7):1403–1411

Sayed H, Baum M (2018) Marker-assisted selection for scald (*Rhynchosporium commune* L) resistance gene(s) in barley breeding for dry areas. J Plant Protec Res 335–344

Schiemann A, Backes G (2000) The use of molecular markers in practical barley breeding In 8th International Barley Genetics Symposium, Adelaide University, pp 42–44

Schulte D, Close TJ, Graner A, Langridge P, Matsumoto T, Muehlbauer G, Sato K, Schulman AH, Waugh R, Wise RP, Stein N (2009) The international barley sequencing consortium at the threshold of efficient access to the barley genome. Plant Physiol 149(1):142–147

Schultheiss H, Hensel G, Imani J, Broeders S, Sonnewald U, Kogel KH, Kumlehn J, Hückelhoven R (2005) Ectopic expression of constitutively activated RACB in barley enhances susceptibility to powdery mildew and abiotic stress. Plant Physiol 139(1):353–362

Shrestha V, Awale M, Karn A (2019) Genome Wide Association Study (GWAS) on disease resistance in Maize. Disease resistance in crop plants. Springer, Cham, pp 113–130

Shtaya MJY, Marcel TC, Sillero JC, Niks RE, Rubiales D (2006) Identification of QTLs for powdery mildew and scald resistance in barley. Euphytica 151(3):421–429

Silvar C, Dhif H, Igartua E, Kopahnke D, Gracia MP, Lasa JM, Ordon F, Casas AM (2010) Identification of quantitative trait loci for resistance to powdery mildew in a Spanish barley landrace. Mol Breed 25(4):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(1):62–69

Singh B, Mehta S, Aggarwal SK, Tiwari M, Bhuyan SI, Bhatia S, Islam MA (2019) Barley, disease resistance, and molecular breeding approaches. In: Wani SH (ed) Disease resistance in crop plants, pp 261–299. Springer, Cham, Switzerland

Singh S, Sehgal D, Kumar S, Arif MAR, Vikram P, Sansaloni CP, Fuentes-Dávila G, Ortiz C (2020) GWAS revealed a novel resistance locus on chromosome 4D for the quarantine disease Karnal bunt in diverse wheat pre-breeding germplasm. Sci Rep 10(1):1–11

Slatkin M (2008) Linkage disequilibrium—understanding the evolutionary past and mapping the medical future. Nat Rev Genet 9(6):477–485

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(3):95–102

St Pierre S, Gustus C, Steffenson B, Dill-Macky R, Smith KP (2010) Mapping net form net blotch and Septoria speckled leaf blotch resistance loci in barley. Phytopathology 100(1):80–84

Steffenson BJ (2003) Fusarium head blight of barley: impact, epidemics, management, and strategies for identifying and utilizing genetic resistance. Fusarium head blight of wheat and barley, pp 241–95

Steffenson BJ, Hayes PM, Kleinhofs A (1996) Genetics of seedling and adult plant resistance to net blotch (*Pyrenophora teres* f *teres*) and spot blotch (*Cochliobolus sativus*) in barley. Theor Appl Genet 92(5):552–558

Steffenson BJ, Olivera P, Roy JK, Jin Y, Smith KP, Muehlbauer GJ (2007) A walk on the wild side: mining wild wheat and barley collections for rust resistance genes. Aust J Agric Res 58 (6):532–544

Stich B, Maurer HP, Melchinger AE, Frisch M, Heckenberger M, van der Voort JR, Peleman J, Sørensen AP, Reif JC (2006) Comparison of linkage disequilibrium in elite European maize inbred lines using AFLP and SSR markers. Mol Breed 17(3):217–226

Swanston JS (1997) Waxy starch barley genotypes with reduced β-glucan contents. Cereal Chem 74(4):452–455

Tacconi G, Cattivelli L, Faccini N, Pecchioni N, Stanca AM, Vale G (2001) Identification and mapping of a new leaf stripe resistance gene in barley (*Hordeum vulgare* L). Theor Appl Genet 102(8):1286–1291

Tessmann EW, Dong Y, Van Sanford DA (2019) GWAS for Fusarium head blight traits in a soft red winter wheat mapping panel. Crop Sci 59(5):1823–1837

Tolbert DM, Qualset CO, Jain SK, Craddock JC (1979) A diversity analysis of a world collection of barley. Crop Sci 19(6):789–794

Toojinda T, Broers LH, Chen XM, Hayes PM, Kleinhofs A, Korte J, Kudrna D, Leung H, Line RF, Powell W, Ramsay L (2000) Mapping quantitative and qualitative disease resistance genes in a doubled haploid population of barley (*Hordeum vulgare*). Theor Appl Geneti 101 (4):580–589

Tsai HY, Janss LL, Andersen JR, Orabi J, Jensen JD, Jahoor A, Jensen J (2020) Genomic prediction and GWAS of yield, quality and disease-related traits in spring barley and winter wheat. Sci Rep 10(1):1–15

Tsuchiya T, Singh RJ (1982) Chromosome mapping in barley by means of telotrisomic analysis. Theor Appl Genet 61(3):201–208

Ullrich SE (2010) Significance, adaptation, production, and trade of barley. In: Ullrich SE (ed) Barley: production, improvement and uses. Wiley-Blackwell, Oxford, UK, pp 3–13

Van Oosterom EJ, Acevedo E (1992) Adaptation of barley (*Hordeum vulgare* L) to harsh Mediterranean environments. Euphytica 62(1):1–14

Varshney RK, Marcel TC, Ramsay L, Russell J, Röder MS, Stein N, Waugh R, Langridge P, Niks RE, Graner A (2007) A high density barley microsatellite consensus map with 775 SSR loci. Theor Appl Genet 114(6):1091–1103

Varshney RK, Prasad M, Graner A (2004) Molecular marker maps of barley: a resource for intra- and interspecific genomics. In: Lörz H, Wenzel G (eds) Biotechnology in agriculture and forestry, vol 55. Mol Markers Sys. Springer, Heidelberg: 229–243

Vatter T, Maurer A, Kopahnke D, Perovic D, Ordon F, Pillen K (2017) A nested association mapping population identifies multiple small effect QTL conferring resistance against net blotch (*Pyrenophora teres* f *teres*) in wild barley. PLoS ONE 12(10):

Verma R, Malik R, Kumar R, Singh S (2011) Genetics of corn leaf aphid (*Rhopalosiphum maidis*) resistance in barley. Cereal Res Commun 39(1):130–136

Verma S, Yashveer S, Rehman S, Gyawali S, Kumar Y, Chao S, Sarker A, Verma RPS (2020) Genetic and Agro-morphological diversity in global barley (*Hordeum vulgare* L) collection at ICARDA. Genet Resour Crop Evol 1–16

Von Bothmer R, van Hintum T, Knüpffer H, Sato K (eds) 2003. Diversity in Barley (*Hordeum vulgare*), pp 9–28. Elsevier, Netherlands

Wagner C, Schweizer G, Krämer M, Dehmer-Badani AG, 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(1):113

Wang R, Leng Y, Zhao M, Zhong S (2019) 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(1):41–51

Werner K, Friedt W, Ordon F (2005) Strategies for pyramiding resistance genes against the barley yellow mosaic virus complex (BaMMV, BaYMV, BaYMV-2). Mol Breed 16(1):45–55

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(4):

Yadav SK, Singh AK, Malik R (2015) Genetic diversity analysis based on morphological traits and microsatellite markers in barley (*Hordeum vulgare*). Indian J Agri Sci 85(10):37–44

Yadav RK, Gautam S, Palikhey E, Joshi BK, Ghimire KH, Gurung R, Adhikari AR, Pudasaini N, Dhakal R (2018) Agro-morphological diversity of Nepalese naked barley landraces. Agri Food Secur 7(1):86

Yu GT, Franckowiak JD, Neate SM, Zhang B, Horsley RD (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(2):111–118

Yu X, Kong HY, Meiyalaghan V, Casonato S, Chng S, Jones EE, Butler RC, Pickering R, Johnston PA (2018) Genetic mapping of a barley leaf rust resistance gene *Rph26* introgressed from *Hordeum bulbosum*. Theor Appl Genet 131(12):2567–2580

Zang W, Eckstein PE, Colin M, Voth D, Himmelbach A, Beier S, Stein N, Scoles GJ, Beattie AD (2015) Fine mapping and identification of a candidate gene for the barley Un8 true loose smut resistance gene. Theoret Appl Genet 128(7):1343–1357

Zhong S, Effertz RJ, Jin Y, Franckowiak JD, Steffenson BJ (2003) Molecular mapping of the leaf rust resistance gene *Rph6* in barley and its linkage relationships with *Rph5* and *Rph7*. Phytopathology 93(5):604–609

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(9):993–999

Zhou MX (2009) Barley production and consumption. In: Zhangm G, Li C (eds) Genetics and Improvement of barley malt quality, pp 1–17. Springer, Berlin, Heidelberg, Germany

Zhou X, Chao M, Liang X (1991) Screening and testing of barley varieties for scab resistance. Acta Phytopathol Sin 18:261–265

Ziems LA, Hickey LT, Platz GJ, Franckowiak JD, Dracatos PM, Singh D, Park RF (2017) Characterization of *Rph24*: a gene conferring adult plant resistance to *Puccinia hordei* in barley. Phytopathilogy 107(7):834–841

# Chapter 5
# Genomic Designing for Biotic Stress Resistance in Sorghum

**B. Fakrudin, T. N. Lakshmidevamma, 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**

**Abstract** Sorghum (*Sorghum bicolor* (L.) Moench) is an immensely valuable staple cereal crop across semi-arid tropical regions of the world and is regarded as a nutritionally potential crop compared to other cereals with high fibre content, minerals and slow digestibility. A multitude of bacterial, fungal and viral pathogens and several insect pests cause significant losses in yield and quality of sorghum. Management of these biotic stresses using chemicals is quite expensive and environmentally not sustainable. Developing host-plant resistance and use of resistant cultivars has great promise in this direction. Repository of genetic resources and the wild gene pool in sorghum that harbor many biotic resistant genes serve as a rich source to develop resistant cultivars. Crossing programs involving several resistance sources resulted in many resistant varieties, hybrids and parents. The genetic barriers between wild and cultivated sorghum species are still challenging to transfer resistant genes. However, with the recent advances in genomic tools, next generation sequencing/re-sequencing technologies, genetic engineering, more genomic data is being utilized in the sorghum breeding programs. These advanced molecular tools have helped to unravel the genetic architecture and provide a deeper understanding of the marker-trait associations. A maximum number of individuals in the mapping population coupled with large-scale genotyping with markers like SNPs would capture more recombination events, leading to high resolution of QTL

B. Fakrudin (✉) · T. N. Lakshmidevamma · J. Ugalat · R. Gunnaiah · S. P. Gautham Suresh · K. A. Apoorva · M. Doddamani · S. Kadam · K. Rashmi · M. N. Mamathashree · A. Hadimani · M. Faizan · G. Prakash · A. Gowda
Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences Campus, Bengaluru 560065, India
e-mail: bfakrudin@uhsbagalkot.edu.in

J. Khan
Center for Cellular and Molecular Platforms, National Center for Biological Sciences, Bengaluru 560065, India

K. Omkar Babu
Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021

C. Kole (ed.), *Genomic Designing for Biotic Stress Resistant Cereal Crops*,
https://doi.org/10.1007/978-3-030-75879-0_5

mapping. Marker-assisted selection can now be efficiently used for pyramiding multiple resistance genes/QTLs which can accelerate the varietal development process, resulting in durable resistance with great impact on the sorghum yield stability and sustainability. This chapter provides an overview of biotic stresses in sorghum, their impact, various breeding strategies and genomics assisted tools in prospecting sorghum biotic stress resistance besides highlighting the recent concepts and future perspectives for genomic designing.

**Keywords** Sorghum · Biotic stresses · Nutritionally potential · Host-plant resistance · GWAS · QTL

## 5.1 Introduction

Sorghum (*Sorghum bicolor* (L.) Moench), the fifth major cereal crop of the world with a small genome size of 750 Mbp, is a native of Africa and possesses enormous genetic potential as a food crop, especially in disadvantaged marginal situations of the world human habitats (Paterson et al. 2009; Zhang et al. 2017b). It is known to be grown since five thousand years and as a crop of the semi-arid regions, it is being utilized as food and forage for the cattle. Much recently this crop has sustainably prospected for biofuel manufacturing (Calvino and Messing 2012). The food security necessitates a deeper understanding of the food production dynamics in the environmentally challenging situations where sorghum is being cultivated (Mundia et al. 2019). The factors that significantly limit sorghum production comprise adverse weather, poor soil fertility and most importantly the pests and diseases. Insect pests and diseases significantly affect crop yields, grain quality, marketability and its utilization as food and fodder (Savary et al. 2012) which in turn influence the food supply, if measures are not taken (Reynolds et al. 2016). The yield loss in sorghum due to biotic stresses covering all stages is anywhere between 30 and 100 % (Singh and Bandyopadhyay 2000). Global sorghum production at about 57.50 million tons during 2019–20 is expected to increase by 3.94% to meet the demand (Miller magazine 2020). Developing sorghum cultivars resistant to various biotic stresses is critical to sustaining its production to meet the present food, fodder and industrial requirements. Deployment of resistant cultivars to overcome biotic stresses can augment the solution for low productivity of cultivars. Knowledge of the genetic resources/gene pool of sorghum as the source of genes for stresses is crucial to develop biotic resistant cultivars (Kumar et al. 2014). Owing to the faster evolution and development of counter-resistance genes in pathogens and insect-pests to evade the host resistance, it is highly challenging to develop durable, long-lasting resistance through conventional approaches (Witcombe and Hash 2000). An integrated approach involving plant breeding and genomics tools could increase the efficiency and precision of resistance breeding, this has to happen on sustainable pace across different tropical and sub-tropical situations (Perez-de-Castro et al. 2012). The availability of mapping populations, molecular

markers and genetic maps in sorghum over the last two decades offered ample opportunities for the identification of genomic regions/quantitative trait loci (QTLs) associated with traits of interest, especially those associated with biotic stresses. The modern molecular tools can achieve introgression of several resistant genes/ QTLs into a single cultivar through molecular breeding strategies. Marker assisted selection (MAS) has enhanced the selection efficiency in some of the important traits related to resistance against insect pests and pathogens (Dormatey et al. 2020).

Functional genomics tools coupled with diverse genetic backgrounds having broad resistance can be used to provide critical insights into the biotic stress responses (Cuevas et al. 2019). Indeed, the relevance of the sorghum functional genomics is increased with the advent of next-generation sequencing techniques (Paterson et al. 2009). Functional genomics along with the developments in genome sequencing, QTL mapping, RNA-sequencing, and bioinformatics allow a deeper understanding of the genetics of resistance both in plant and pathogen as well as insect pests (Andersen et al. 2018). The RNA-interference (RNAi) in imparting resistance to biotic stresses is becoming practically relevant in sorghum and other millets due to affordability of the technology (Banerjee et al. 2017; Majumdar et al. 2017; Zhang et al. 2017a). Transcription factors (TF), the proteins crucial in governing transcriptional regulation during the entire crop period, can function as switches to control the expression of genes involved in mediating biotic stress resistance. Characterization of transcription factor genes critically involved in plant stress responses and their introgression is vital for enhanced biotic stress resistance in sorghum.

The biotic stress management systems depend on various control approaches such as genetic, physical, chemical, cultural and biological, among others. However, the use of stress-resistant varieties can lead to enhanced ecological fitness, reduced pesticide usage and sustainable production system, resulting in increased yields and grain quality/end-use traits (Singh et al. 2004a). A multidisciplinary and multipronged approach with synergistic integration of morphological and molecular approaches i.e., plant breeding in combination with genomics tools would accelerate and maximize the efficiency of breeding programs for developing biotic stress resistance in sorghum (Dormatey et al. 2020).

## 5.2 Different Biotic Stresses

Sorghum is affected by various biotic factors including multiple insect-pests, diseases and parasitic weeds that cause significant economic losses.

### *5.2.1 Major Diseases of Sorghum*

Major diseases of sorghum are grain mold, anthracnose (*Colletotrichum sublineolum*), ergot (*Claviceps sorghi* and *C. africana*), downy mildew (*Peronosclerospora sorghi*), sorghum rust (*Puccinia purpurea*), charcoal rot/stalk rot (*Macrophomina phaseolina*), bacterial leaf spot (*Pseudomonas syringae* pv. *syringae*), leaf blight (*Helminthosporium turcicum*) and head smut (*Sporisorium reilianum*). *Striga* spp., a parasitic weed is one of the most devastating constraints that can cause a cent per cent yield loss under severe infestation (Esele 1995).

#### 5.2.1.1 Grain Mold

Grain mold is the most devastating disease of sorghum with global distribution. More severe in the *kharif* season, especially, in white-grain sorghum, which is being grown widely in Asia and Africa for food. Grain mold is less severe on colored grain sorghum being grown widely in United States of America (USA), Argentina, Australia and Mexico for feed purpose. It is a complex disease caused by more than 40 pathogenic and opportunistic fungi from several genera, including the most common species *Fusarium thapsinum* (Klittich et al. 1997; Cuevas et al. 2019). *Fusarium* spp., *Alternaria* spp., *Curvularia* spp. and *Colletotrichum* spp. were the most principal fungi reported in grain mold (Williams and Rao 1981; Navi et al. 2005). The disease develops with the infection and colonization of spikelet followed by grain colonization leading to the deterioration of seed (Bandyopadhyay et al. 2000). Fungi secrete enzymes that degrade starch in endosperm and germ tissues (Hodges et al. 2000). A devastating effect of grain mold on grain yield, quality, market value and eventually on the grain-based products with an annual loss of US$ 50–80 million was estimated in India (Das 2019).

#### 5.2.1.2 Anthracnose

Anthracnose caused by *Colletotrichum sublineolum* Henn. is one among the most economically damaging sorghum diseases that affect leaves, stems and grain, with significant grain yield reduction over 50–86% (Cota et al. 2017). An estimated grain yield loss of more than 50% was observed in susceptible sorghum cultivars under severe anthracnose epiphytotic in Georgia (Harris et al. 1964). The disease gets aggravated under warm and humid conditions (Tsedaley et al. 2016). Symptoms include the development of small spherical spots on leaves and leaf midribs with red, orange, purple or tan colored wide margins having straw-colored centers. In case of disease severity, the spots increase in number and conjoin covering the entire leaf surface and stem leading to premature plant death. In the centers of the spots, small black fruiting bodies (acervuli) develop (Tesso et al. 2012). A direct

negative impact on grain yield can be envisaged as the infection in later stages of development, is seen on the rachis, panicle branches and seeds.

#### 5.2.1.3 Ergot or Sugary Disease

Ergot is caused by the fungi of the genus *Claviceps*, i.e., *Claviceps sorghi or Sphacelia sorghi*. Over 40 species of *Claviceps* have been reported, major include *C. sorghicola*, *C. africana* and *C. purpurea* (Pazoutova and Frederickson 2005). The infection starts with the sclerotium production within the floret and only the ovaries are infected. Ergot infects unfertilized ovaries individually within a panicle. Male sterile lines are highly susceptible. Severe infection is seen under high rainfall, high humidity, cloudy weather during anthesis and in cool night temperature conditions. The two noticeable signs of the disease are droplets of honeydew oozing from infected florets and the growth of fungal sclerotia. The hard textured sclerotia developed from sphacelia protrude few millimeters outside the glumes. The major threat from ergot is the infection and contamination of the harvested grains by toxic alkaloids present in the sclerotia (Wegulo and Carlson 2011). Major types of ergot alkaloids produced are clavine alkaloids, D-lysergic acid and its derivatives, and ergopeptines which cause a group of symptoms called "ergotism" (Hulvova et al. 2013).

#### 5.2.1.4 Downy Mildew

Downy mildew of sorghum is caused by *Peronosclerospora sorghi.* The disease is manifested as downy whitish growth on the lower leaf surface followed by whitish streaks on both the leaf surfaces. Tissues alongside the white streaks slit later resulting in leaf shredding. Primary infection of the disease is through oospores present in the soil, mycelium in seeds and secondary infection is through air-borne sporangia. Crop rotation with other crops viz., pulses and oilseeds and removal of the infected plants can prevent the secondary spread of the disease (Tesso et al. 2012).

#### 5.2.1.5 Rust

Sorghum rust caused by *Puccinia purpurea*, is of significance as it predisposes sorghum to other diseases such as stalk rot. The disease is manifested as small reddish-brown specks on the lower leaf surface and pustules (uredospori) on both the leaf surfaces as purplish spots which later rupture to release a reddish powdery mass of uredospores. Primary infection is through long cycled rust and secondary infection is by wind-born uridospores. Infection can be minimized through the destruction of the alternate host, *Oxalis corniculata* and spray of mancozeb (Hooker 1985).

#### 5.2.1.6 Charcoal Rot or Stalk Rot

Charcoal rot caused by a soil-borne fungus, *Macrophomina phaseolina*, is a major fungal disease of sorghum worldwide with great destructive potential causing substantial losses in economic yield levels ranging between 14.2 and 46.6% (Mughogho and Pande 1983). After infection, the infected stalk will split open and consequently results in longitudinal shredding of the pith tissue into fibers and disintegration. The stem breaks down to the ground resulting in premature stem lodging that negatively affects the grain and fodder quality. The disease is primarily infected from the soil, weed hosts and can be aggravated by rain or irrigated water (Ghosh et al. 2018).

#### 5.2.1.7 Bacterial Leaf Spot

Bacterial leaf spot is caused by *Pseudomonas syringae* pv. *syringae*. Symptoms include initial water-soaked lesion on the lower leaves which grow and mature, and become elliptical to circular developing red or brown margins. As lesions dry, the centers become light-colored. Most commonly found in the spring season since it is dispersed by rain and wind and becomes insignificant in summer seasons (Te-Beest et al. 2004).

#### 5.2.1.8 Leaf Blight

Causal organism of this disease is *Helminthosporium turcicum* (Syn. *Exserhilum turcicum*). Yield losses can approach 50%. Symptoms of the disease are manifested as small reddish or tan spots that can enlarge to long elliptical reddish-purple or tan lesions of 12 mm wide and 2.5–15 cm long. Sporulation of the fungus on lesions often gives them a dark grey or olive appearance on the surface. The fungus can survive on grasses, on residue and seeds (Lu et al. 2018).

### *5.2.2 Insect Pests of Sorghum*

More than 150 species of insect pests infest sorghum. The key pests are shoot-fly (*Atherigona soccata*), sorghum gall midge (*Stenodiplosis sorghicola*), stem borer (*Chilo partellus*), aphids (*Rhopalosiphum maidis* and *Melanaphis sacchari*), jowar ear head bug (*Calocoris angustatus*), shoot bug (*Peregrinus maidis*), red-headed hairy caterpillar (*Amsacta albistriga*) and ear head caterpillar (*Helicoverpa armigera*) causing significant damage to the crop (Sharma et al. 2006).

#### 5.2.2.1 Shoot-Fly

The shoot-fly (*Atherigona soccata* Rondani) is one of the prominent threats for sorghum production in Asia, Africa and American continent (Sharma et al. 2003). Sorghum shoot-fly, the most devastating pest, alone is responsible for 5% loss out of 12% total insect losses. Grain yield reduction of about 50% (Jotwani et al. 1979) and a still more devastating damage with loss up to 90% was reported (Jotwani et al. 1970). High susceptibility is seen in the early stages of crop growth (5–25 days), especially in the late sown crop during rainy season, whereas, the early-sown crop is more affected during the post-rainy season (Mohammed et al. 2016). Female shoot-fly lays eggs singly on the surface of the leaf, parallel to the mid-rib. The larvae cut the growing point of the apical shoot resulting in a dead heart symptom. Larvae feed on the decaying tissue which may lead to seedling mortality and the crop gets damaged within 1–4 weeks after seedling emergence, present a rosette appearance and fail to produce any grain (Mohammed et al. 2016). Pesticides are being used to control shoot infestation in sorghum crop (Sharma et al. 2007).

#### 5.2.2.2 Sorghum Gall Midge

Sorghum gall midge (*Stenodiplosis sorghicola* Coquillett) is an ubiquitous damaging pest of grain sorghum all over the globe (Young and Teetes 1977). A crop loss of 10–15% was reported and in severity, this can cause cent per cent damage to developing kernels in all the sorghum growing areas (Sharma and Teetes 1995). Eggs hatch and feed on the ovaries resulting in chaffy grains. Certain varieties may be particularly susceptible to egg lay in pre-flowering spikelets (Franzmann and Vaschina 1989). Sorghum midge can be managed by uniform regional planting, such that, all sorghum varieties flower within 7–14 days or through insecticide spray at anthesis. Genetic resistance mechanism that increases non-preference of florets for sorghum midge is eco-friendly and a durable approach.

#### 5.2.2.3 Sorghum Stem Borer

Sorghum stem borer (*Chilo partellus* Swinehoe) infestation begins from over a month after sowing. The larvae feed on the surface of leaf sheath and leaf whorls, bore into the midrib and the shoot which later feed on the internal tissues causing extensive tunnelling and results in ‘dead heart’ formation and consequent killing of young plants. Larvae also infest ear heads and cause tunneling leading to chaffy ear heads and poor grain development. Chemical control includes soil application of phorate or carbofuran at the time of sowing. Many parasitoids viz., green lacewing, ladybird beetle, spider, fire ant, reduviid bug, robber fly, black drongo, big-eyed bug, earwig, ground beetle, pentatomid bug, praying mantis, *Dicyphus hesperus* etc. are effective against stem borer. Greenleaf desmodium can also be used as a control strategy against stem borers in sorghum (Khan et al. 2006).

#### 5.2.2.4 Aphid

Both sorghum aphid (*Rhopalosiphum maidis* Fitch) and sugarcane aphid (*Melanaphis sacchari*) are common in several sorghum growing countries (Singh et al. 2004a). The severity of aphid damage is up to 77% reduction in grain yields (Van Rensburg and Hamburg 1975). Aphids usually attack newly emerged leaves, wherein, the adult and nymphs cause the damage throughout the growing period by piercing and sucking sorghum juice which eventually slows down plant growth leading to plant death. Sorghum is reported to be a preferred host for sugarcane aphid (Bowling et al. 2016). Aphids produce honeydew in plenty on which sooty molds grow, which further hinder grain harvesting and grain quality (Wang et al. 2013). Leaf extracts from neem seed or dursban found to be effective in controlling aphids (Diarisso et al. 2005). Insecticides viz., dimethoate 30 EC and imidacloprid formulations are reported to be most effective in reducing the aphid population.

#### 5.2.2.5 Jowar Ear Head Bug

Jowar ear head bug (*Calocoris angustatus*) is a vigorous, small yellowish-green bug that infests the crop from ear head emergence to dough stage and causes about 54–89% reduction in grain yield levels. During ear head formation, ear head bugs are usually seen covering over the ear heads. Both nymphs as well as adults suck the milky juice from ear heads or developing grains, as a consequence the grains shrink and turn black color leading to chaffy or crinkled grains. Older grains show distinct feeding punctures that reduce grain quality (Sharma 1985).

#### 5.2.2.6 Shoot Bug

Shoot bug (*Peregrinus maidis*) is a sporadic pest of sorghum that can cause heavy damage under favorable conditions. Nymphs and adults suck the sap from young leaves and leaf sheath resulting in unhealthy plants, with reduced plant vigor and yellowing. Under severe infestation, the leaves wither from top-down and later turn reddish finally leading to plant death. The infestation generally leads to twisting of leaves and seldom of panicles emergence—collectively results in over 41% yield toll in India (Subbarayudu 2002). Deep summer ploughing, collection and destruction of larvae, crop rotation with non-host crops, timely sowing, destruction of alternate host plants, field sanitation, rogueing, early uprooting and burning of infested plants can reduce the incidence of pest.

#### 5.2.2.7 Red Headed Hairy Caterpillar

Red headed hairy caterpillar (*Amsacta albistriga, A. moorei*), a polyphagous pest is highly injurious to young sorghum seedlings. Caterpillars are voracious feeders

which feed on leaves by scrapping the under surface of tender leaflets besides flowers and main stem in later stages independently, they spread across fields, which lead to severe crop damage and yield loss (Nagarajan et al. 1957). Use of light traps and digging trenches around the infested field and dusting with insecticide can reduce the pest infestation.

#### 5.2.2.8 Ear Head Caterpillar

The adult ear head caterpillar (*Helicoverpa armigera*) is a medium-sized moth. Caterpillars feed till grain hardening stage and are covered in the inner branches of the ear. Compact panicles are more prone to heavy damage and damaged ears could be easily spotted in the field by their chalky appearance (Bora et al. 1994).

## 5.3 Genetic Resources of Resistance Genes

The sorghum germplasm resources can stand rounds of intense selection to meet diverse requirements of plant breeding due to its rich genetic diversity that stems from five basic races - *Bicolor, Guinea, Caudatum, Kafir*, *Durra* and 10 intermediate races that include -*Guinea-bicolor, Durra-bicolor, Caudatum-bicolor, Guinea-caudatum, Kafir-bicolor, Guinea-kafir, Guinea-durra, Durra-caudatum, Kafir-caudatum, Kafir-durra* (Harlan and de Wet 1972; Venkateswaran et al. 2019). The rich genetic diversity in the gene pool 1 (GP-1) and gene pool 2 (GP-2) and their cross-compatibility with *Sorghum bicolor* have led to the development of successful hybrids. Most significantly, *S. bicolor* subspp. *verticilliflorum* and *S. propinquum* have contributed for yield per se and *S. halepense* has contributed genes for crop duration (Dweikat 2005; Aruna and Cheruku 2019). Wild species, harbouring genes, which are resistant against striga are *arundinaceum, virgatum* and *verticilliflorum* (Cox et al. 1984; Bramel-Cox and Cox 1988). Other potentially useful traits in sorghum's GP-1 and GP-2 include *S. bicolor* subspp. *drummondii* for allelopathic properties and resistance to ergot and nematodes, and *S. halepense* conferring resistance to multiple pests (Dweikat 2005; Baerson et al. 2008). Further, the gene pool 3 (GP-3) of sorghum has enormous potential in gaining grain yield advantage through introgression of specific genes and it is envisaged that the diversity in GP-3 would be of particular use in breeding sorghum for climate change eventualities and dreaded insect pests of sorghum (Venkateswaran 2003; Kamala et al. 2009) (Fig. 1).

The concept of core collection facilitates thorough characterization of accessions for various traits of interest and thereby maximizing the use of the germplasm. The core collection consists of a subset of accessions from the entire collection, capturing most of the species diversity. Further, Upadhyaya and Ortiz (2001) postulated the concept of mini core collection with 10% core collection accessions. Repeated evaluation of the reference collection, core collection and mini-core

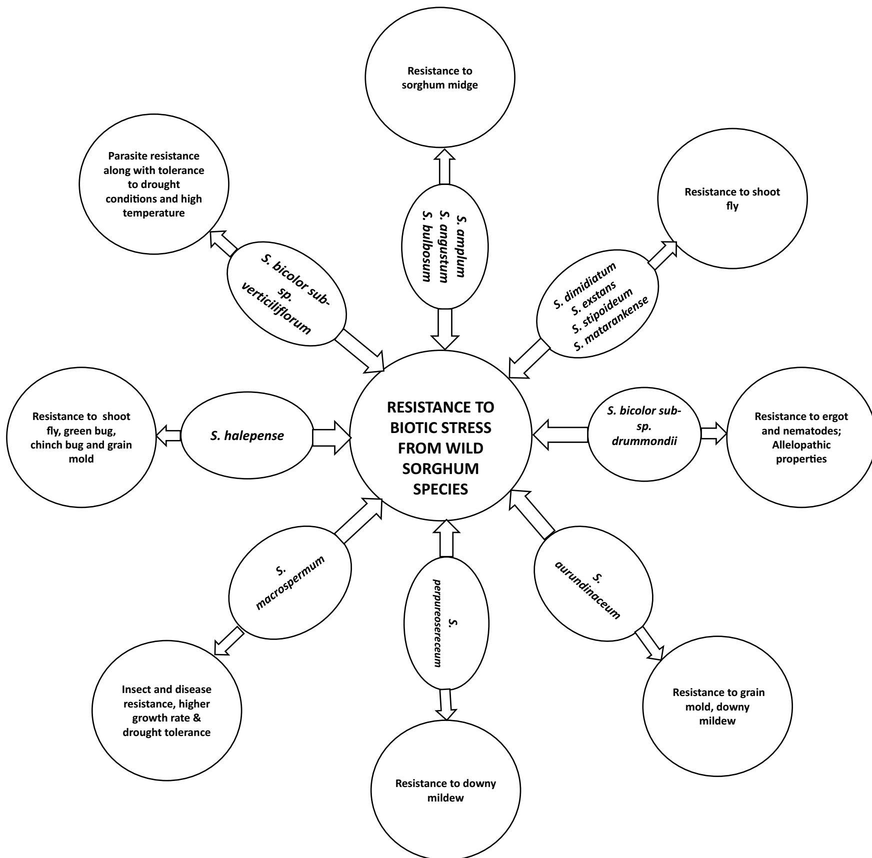



**Fig. 5.1 Resistance to biotic stress from wild sorghum species**. The genus *Sorghum* consists of 25 diverse species, among which few wild species confer resistance to major pests and diseases of sorghum. *S. bicolor subsp. verticiliflorum* gives high yield along with tolerance to drought conditions and high temperatures (Bramel-Cox and Cox 1998; Rich et al. 2004). *S. bicolor subsp. drummandii* has allelopathic properties to ergot disease and nematodes (Mojtahedi et al. 1993; Tsukiboshi et al. 1998). The species *S. amplum, S. angustum, and S. bulbosum* are a source of resistance sorghum midge (Sharma and Franzman 2001). *S. dimidiatum, S. extans, S. stipoideum*, and *S. matarankense* are a source of resistance to major pest of sorghum, shoot fly (Nwanze et al. 1995; Kamala et al. 2009). *S. perpureosereceum* confers resistance to downey mildew (Sharma 2010). *S. halepnse* is resistant to shoot fly, green bug and chinch bug (Nwanze et al. 1995; Dweikat 2005). *S. aurundinaceum* is resistant to and grain mold and downey mildew (Mohan et al. 2008). *S. macrospermum* has resistance to many pests and diseases along with higher growth rate and significant aboveground Dhurrin content under drought conditions (Kuhlman et al. 2008; Cowan et al. 2020)

collections of sorghum germplasm resulted in the identification of accessions useful in breeding for various biotic stresses. These include, ***Grain mold***: White-grained four guinea race accessions and a set of 50 sorghum mini core accessions have been reported to be resistant to grain mold (Sharma et al. 2010). ***Anthracnose***: a set of 13 accessions including IS10302, IS19153, IS20956 and IS24218, recorded minimal mean disease scores (Sharma et al. 2012). ***Leaf blight***: IS2906, IS18417, IS18425, IS18758, IS19667 and IS19669 (Reddy et al. 2004); besides a set of 27 accessions of mini core recorded resistance with a mean disease score of 2 (Sharma et al. 2012); ***Rust***: IS3413, IS13896, IS18417, IS21454 and IS29016; IS473, IS23521, IS23684, IS24503, IS26737 and IS33023 with mean disease severity of 3.8% (Reddy et al. 2004; Sharma et al. 2012); ***Downy mildew***: IS3547, IS20450, IS23992, IS27697, IS28449, IS28747, IS30400 and IS31714, (Reddy et al. 2004; Sharma et al. 2012); ***Potyvirus spp***.: IS7679 and IS20740 (Seifers et al. 2012); Further, accessions such as IS2058, IS18758, IS3547, IS14332, IS17141, IS2333, IS14387, IS3413, IS14390, IS21454 have shown multiple disease resistance (Reddy et al. 2008; Sharma et al. 2010, 2012).

## 5.4 Traditional Breeding for Disease and Pest Resistance

Insect pests and diseases cause considerable loss in grain and fodder yield levels besides the quality of produce. Resistant cultivars that are genetically superior form the cheapest method for minimizing the yield toll. Breeding for host plant genetic resistance is a continuous process in terms of searching for source accessions that are resistant to ever-evolving new races of the pathogens (Singh and Bandyopadhyay 2000; Mohammed et al. 2016). Classical breeding methods such as introduction, selection, backcrossing, pedigree method, recurrent selection schemes continue to play vital roles in evolving resistant cultivars. All through the history of the plant breeding, the crop wild relatives have been the sources of resistance genes (Hariprasanna and Rakshit 2016). However, a multidisciplinary approach to utilize these crop wild relatives is critical in achieving success (Kamala et al. 2016; Ananda et al. 2020). Sorghum germplasm with rich and diverse crop wild relatives offers an excellent opportunity to improve the deficient agronomically superior cultivars/hybrids. In sorghum, wild relatives such as chaeto, hetero, stipo, and parasorghum have been potential sources of biotic stress tolerant genes (Kamala et al. 2002).

### 5.4.1 Traditional Breeding Research for Diseases Resistance

#### 5.4.1.1 Grain Mold

Grain mold being a disease complex, its development mechanisms are fairly described (Waniska et al. 2001). Both major and minor genes with additive and epistatic effects coupled with significant genotype X environment (GXE) interaction have been reported (Stenhouse et al. 1997; Rodriguez-Herrera et al. 2000). Limited success in resistant cultivar development to this disease could be attributed to complex genetics, mechanisms governing resistance and high influence of the environment (Audilakshmi et al. 2011). The conventional breeding prospected 'grain hardness' to improve grain mold resistance in white-grained sorghum used for human consumption in Asian and African counties (Das et al. 2020) and this approach has been successful in developing cultivars with high yield and resistance to grain mold through an expanded systematic screening and selection in segregating progenies of specifically planned crosses (Reddy et al. 2000). The resistant sorghums belonging to the guinea race with open panicles, large glume coverage along with grain hardness need to be involved in the crosses (Reddy et al. 2000). Germplasm from Sudan and Ethiopia which possessed desirable quality with white grains as well as less susceptibility to grain mold under natural conditions was utilized in the development of variety CSV 4, which further served as restorer parent for several hybrids viz., CSH 5, CSH 6 and CSH 9 in India (Ashok Kumar et al. 2011b). Accessions such as E 35-1, CS 3541, SC 108-3, SC 108-4-8 and SC 120 continue to be parents of choice in widening the genetic base of grain mold resistance in sorghum improvement programs of India and Africa (Reddy et al. 2000; Ashok Kumar et al. 2011b).

Pedigree breeding followed by multi location testing led to the identification of many advanced breeding lines which were used to develop high yielding grain mold resistant varieties and hybrids, such as SEPON 77, M 90038 and SEPON 82 × S 34. Many grain mold resistant lines with dwarf and earliness sorghum segments, grain and glumes traits from guinea along with semi-compact heads were developed through pedigree breeding (Stenhouse et al. 1997). A grain mold resistant population was developed with white-grained, color-grained lines coupled with higher grain yielding ability into genetic male-sterility (ms3) background. Repeated half-sib family selections and cycles of random mating resulted in pinning down of several superior lines with resistance, which eventually contributed to the release of grain mold resistant varieties and hybrids in India (Ashok Kumar et al. 2011a). Germplasm sources with wide adaptability and high grain quality along with the grain mold resistance are available. Resistant lines viz., ICSB392, ICSB403, ICSB383, IS13817, IS8614, IS10646, IS25060, IS21599 and IS23585 have been used extensively in the breeding programmes (Reddy et al. 2005).

#### 5.4.1.2 Ergot

Ergot resistant trials, with susceptible A-lines and R-lines, concerning to incidence and severity of the disease revealed Tx2737 as a popular R-line. A male-fertile accession from Ethiopia, IS8525 with high levels of resistance was considered as a potential source for host-plant resistance strategies. Five CMS-lines were crossed with five pollinator lines without fertility restorer genes, wherein, the pollinator lines on an average had low (7–10%) and the CMS female lines had very high (62–82%) ergot severities (Reed et al. 2002). Further, pollen traits such as genetic architecture, pollen quantity and pollen viability have significant correlations with the ergot resistance. The genetic correlations studied among different traits have also have pointed at possibilities of common genetic factors controlling these traits (Parh et al. 2008). Three male-sterile lines in sorghum exhibited noteworthy differences in ovary colonization rates after inoculation (Komolong et al. 2003). Further, ergot severity with high heritability behaved quantitatively and the possible effect of floral traits need to be understood, as the resistance donor was having a short, narrow stigma, least or no stigma.

#### 5.4.1.3 Charcoal Rot or Stalk Rot

Stalk rot resistance in sorghum is associated with a delay in leaf and plant death. Different genetic control mechanisms within SC599-11E for non-senescence and charcoal rot resistance envisaged that these two forms of resistance are not different pointers of the same trait (Tenkouano et al. 1993). The component traits—internode number was associated with two QTLs on linkage group B, the length of infection associated QTL on linkage group D and two QTLs associated with per cent lodging on linkage group I (Reddy et al. 2008; Patil 2011). Stalk rot-resistant sorghum genotypes were unaffected by the pathogen-mediated yield retardation (Bandara et al. 2019). High-temperature stress decreased chlorophyll and *F*v/*F*m. Genotypes PI533946, IS26749, IS23992, RTx7000, and SC35 had the maximum *F*v/*F*m and the genotypes IS19262, SC35, PI576380 and IS27912 had resistance to both pathogens (Perumal et al. 2020).

#### 5.4.1.4 Downy Mildew

Concerted efforts to search and characterize the resistant sources for downy mildew by using the dual approach of sandwich inoculation technique and green-house screening revealed high-level resistance to the disease in a set of six accessions viz., IS28747, IS27697, IS31714, IS28449, IS23992 and IS30400 out of 242 germplasm accessions of sorghum mini-core collection from diverse geographies (Sharma et al. 2010; Rashid et al. 2018).

#### 5.4.1.5 Rust

Eight loci with a significant effect on rust resistance with a total phenotypic variation explained (PVE), varied from 6.8 to 42.6% (Tao et al. 1998). Of the 12 sorghum varieties screened, a local cultivar 'Tetron' was reported to be highly resistant with zero yield loss compared to 40% yield reduction in 97 MW 6129 (NVT11 4). Impact of this disease on seed germination was also observed (Abera and Alemayehu 2012). A set of 13 advanced breeding lines generated from a cross between UPCA-S1 and Numbu revealed significant G × E interaction on leaf rust disease severity.

#### 5.4.1.6 Multiple Disease Resistance

Field studies have revealed that an effective screening strategy can identify resistance sources to multiple pathogens in sorghum germplasm as vast genetic diversity to individual component traits exist across its species and subspecies (Prom et al. 2012). The development of several diseases simultaneously on a susceptible sorghum grown as a mixed stand with either maize or resistant sorghum found to be a good strategy (Ngugi et al. 2001). Over 242 sorghum mini-core collection evaluated to identify resistant ones for anthracnose and leaf-blight diseases resulted in 13 accessions resistant to anthracnose and 27 to leaf blight (Kimball et al. 2019). These accessions with resistance to multiple diseases would be potential sources for sorghum disease resistance breeding programs (Upadhyaya et al. 2013a). Parental genotypes such as 234112, Bt-623, 226057 and 210903 with positive genetic combining ability (GCA) effects -Bt-623 × Gemedi, 210903 × 234112, 210903 × 71708, 74222 × 234112, 74222 × 226057, 234112 × 71708, 226057 × 214852 and 226057 × 214852 with positive specific combining ability (SCA) effects for grain yield and the desirable families: 174222 × 234112, Gemedi × 71708, Bt-623 × 234112, Bt-623 × Gemedi, 226057 × 71708, Chemeda × 71708, and Gemedi × 71708 with negative SCA effect and low anthracnose severity were forwarded as promising populations for resistance breeding (Mengistu et al. 2019).

### *5.4.2 Traditional Breeding Research for Resistance to Insect Pests*

#### 5.4.2.1 Shoot-Fly

Shoot-fly resistance is a complex trait, that depends on the interplay of many component traits of plant, insect and environment. Developing genetically superior resistant cultivars offers a sustainable pest management system with enhanced grain

quality (Sharma et al. 2005; Mohammed et al. 2016). Resistance is manifested in the form of non-preference for oviposition (Dhillon et al. 2006). Systematic screening and further evaluation of sorghum germplasm led to identification of resistant accessions (Sharma et al. 2014a). Use of these resistant accessions in crossing programs led to the development of several shoot-fly resistant (SFR) varieties and hybrids (Kumar et al. 2014). Shoot-fly resistant superior advanced breeding lines such as IS2122, IS18551, IS2146, IS1054, IS2312, SFCR151, ICSV705 and SFCR125 were derived from germplasm (Riyazaddin et al. 2015). Rigorous breeding efforts have evolved cultivars that are significantly tolerant to shoot-fly incidence. Germplasm after infestation by the shoot-fly recovered in varying proportions. The accessions, CSV 22 and RSV 1093 revealed high grain yield potential in addition to shoot-fly resistance, while Phule Yashoda, RSV 1235, IS 2312, and ICSV 574 were high yielding with moderate resistance (Sharma et al. 2015). Similarly, another set with 10 parents, 45 $F_1$'s along with their reciprocals screened for shoot-fly resistance and inferred that the genotypes ICSV 700, ICSV 25019 were useful (Mohammed et al. 2016).

The morphological traits allied with an expression of resistance/susceptibility to shoot-fly exhibited significant GCA effects. The interlard-fishmeal technique used to increase shoot-fly abundance at seedling stage of susceptible cultivars such as Swarna was effective and successful (Chamarthi et al. 2011). In a successful effort, a trait-based pedigree breeding approach was used to develop *kharif* and *rabi* sorghum grain types in both agronomically superior genotypes and genotypes with specific traits of importance. New sources of resistance such as IS923, IS5072, IS1057, IS1071, IS4664, IS1082, IS4663, IS1096, IS2394, IS5636, IS5470 and IS18369 have been mined to infuse and breed for shoot-fly resistance in sorghums (Kumar et al. 2014). A comparative study indicated that upregulation of total soluble sugar, total phenol, prussic acid and chlorophyll play a dominant role to impart resistance in the susceptible sorghum genotypes (Kumari et al. 2020; Salama et al. 2020).

#### 5.4.2.2 Sorghum Gall Midge

Sorghum gall midge was first reported in 1953 in 'Nunaba' varieties from West Africa (Bowden and Neve 1953). Efficient management requires combining several strategies that suppress midge damage and abundance in the field. Resistance to this pest has been attributed to the traits long glumes and non-anthesis. Field tests have suggested that 'Nunaba' varieties were resistant under choice conditions in the presence of an alternative host, but susceptible in the absence of a more favorable host (Passlow 1965). Spikelet flowering time and morphology have a direct influence on the per se resistance. Genotypes that displayed resistance under no-choice conditions in glasshouse and field trials were reported to deploy an antixenosis resistance mechanism (Franzmann 1988). The mechanism of resistance to midge has been recognized as reduced egg-lay (Franzmann 1988; Sharma and Vidyasagar 1994). Midge resistant sorghum hybrids gave higher yields and greater

returns than susceptible hybrids under the same insecticidal spray regime (Teetes et al. 1986). There is little clear evidence on the exact chemical or physical components that affect the antibiosis mechanism of resistance in sorghum. Association between tannins and midge resistance is also noted (Santos and Carmo 1974; Sharma 1985, 1993). The hybrid breeding approach has been successful to achieve resistance (Boozaya-Angoon et al. 1984).

#### 5.4.2.3 Sorghum Stem Borer

Larva of stem borer crawls and feeds on tender leaves that become folded, causing typical "shot hole" symptom. Sorghum genotype IS18573 displayed antibiosis to stem borer in terms of reduced survival and development (Kumar et al. 2006). Induced resistance in sorghum genotypes against stem borer infestation included elevated expression of peroxidase (POD), polyphenol oxidase (PPO), hydrogen peroxide ($H_2O_2$) and malondialdehyde (MDA) (Hussain et al. 2014).

#### 5.4.2.4 Aphid

Aphid has emerged as a potential threat to sorghum. Resistance to this pest was assessed by testing 23 genotypes for tolerance to antixenosis and antibiosis and was compared with the known resistant cultivar 'TX 2783' and the susceptible 'KS 585'. The entries viz., AG1201, AG1301, W844-E, and DKS 37-07 expressed all three forms of resistance to aphids, while H13073 exhibited antibiosis and tolerance (Paudyal et al. 2019). Screening of a diverse set of sorghum genotypes resulted in the identification of several lines with moderate levels of resistance to aphid damage.

### *5.4.3 Traditional Breeding for Resistance to Striga*

Witchweed (*Striga* spp.) infestation is an obstacle to sorghum cultivation and it inflicts both grain and biomass production. Over 20–100% yield reduction has been recorded in Asian and African countries (Ejeta and Gressel 2007; Parker 2009). Co-evolution of sorghum with striga in Africa might have resulted in inherent resistance, which is of scope as a breeding strategy (Shayanowako et al. 2018). Complex interactions between host, parasite and the physical environment have restricted the exploitation of resistance to striga (Ejeta and Gressel 2007). Several novel techniques have been employed to identify unique resistance sources to striga followed by introgression of these genes into selected cultivars with multiple resistance mechanisms. Several high yielding cultivars with striga resistance have been deployed in many African countries (Ejeta and Gressel 2007; Mbuvi et al. 2017). Over 25 sorghum accessions screened for *Striga hermonthica* were shown

resistance along with yield in Nigeria, identified SRN39, Sepon82, Danyana and SAMSORG40 as the top four resistant accessions to *S. hermonthica* (Afolayan et al. 2020). Seeds of acetolactate synthase herbicide-tolerant mutant recorded fewest *Striga* attachments (Tuinstra et al. 2009). Introgression of major quantitative trait loci (QTLs) governing resistance to striga or major genes/transcription factor genes involved are identified, and genetic engineering approach is an effective way forward for their effective transfer and integration (Gressel 2010). Such resistance is difficult to overcome by the parasitic weeds and could be easily backcrossed into local varieties (Wang et al. 2009).

### *5.4.4 Morphological and Biochemical Markers in Disease and Insect Pest Resistance*

Effective screening techniques and availability of biochemical, morphological or DNA markers associated or genetically linked to a specific disease or pest resistance, or at least to the specific component traits, would go a long way in sorghum breeding. Morphological and biochemical markers play a crucial role in the diagnosis and management of various biotic stresses in sorghum. Several phenolic compounds viz., allelochemicals like p-coumarates, p-hydroxybenzoates, flavanols and phytoalexins (3-deoxyanthocyanidins) have a key role in biotic stress resistance (Weir et al. 2004). Higher contents of proanthocyanidins, flavan-4-ols and 3-deoxyanthocyanidins (3-DAs) are involved in host plant resistance. 3-DAs are considered as better markers for resistance to biotic stresses as they associate with resistance to all biotic stresses (Dicko et al. 2005). The peroxidases (POXs) play a vital role against biotic and abiotic stress resistance by forming physical barriers through the synthesis of cell-wall polymers (lignin and suberin) (Cui et al. 1996).

#### 5.4.4.1 Diseases

Antifungal proteins viz., chitinases, glucanases, sormatin and ribosome-inhibiting proteins play a significant role in imparting resistance to grain mold (Rodríguez-Herrera et al. 1999; Bueso et al. 2000). Further, traits like kernel hardness and pericarp color were found to be associated with grain mold resistance (Menkir et al. 1996). Traits such as kernel hardness with red pericarp, tan plant color kernels, high tannins along with a pigmented testa, as well as the pericarp intensifier (*I*) gene can enhance grain mold resistance (Esele 1995; Waniska et al. 2001). Involvement of peroxidases in fungal resistance was also inferred (Luthra et al. 1988).

#### 5.4.4.2 Insect Pests

Insect/pest infestation in sorghum produces prussic acid or hydrocyanide (HCN), a toxic chemical which shows fatal response to herbivores. Two enzymes viz., glycosidase and hydroxynitrile lyases produce HCN from cyanogenic glycosides after mechanical stress or insect feeding (Freeman and Beattie 2008). Salicylic acid, jasmonic acid and abscisic acid, auxin and gibberellic acid are envisaged as defense responses to greenbug feeding (Park et al. 2006). Increased secondary metabolites also take part in shoot-fly resistance mechanism (Sharma et al. 2007). Biotic stress resistance in sorghum shoot-fly infestation is attributed in the form of tolerance, antixenosis (non-preference) and antibiosis which make them repellent to insects for feeding, shelter, egg laying and survival (Chamarthi et al. 2011; Mohammed et al. 2016). Biochemical factors like p-hydroxy benzaldehyde, luteolin, cinnamic acid and apigenin were connected with expression of shoot-fly resistance (Chamarthi et al. 2012). Further, polyphenol oxidase and peroxidase activities were upregulated in resistant genotypes which might have helped plants to tolerate infestation by shoot-fly (Padmaja et al. 2014). The pivotal role of total chlorophyll content, peroxidase and polyphenol activity in imparting resistance to shoot-fly was also noted (Singh et al. 2004b).

Shoot-fly resistance is associated with leaf glossiness and trichomes besides pigmentation and epicuticular wax (Dhillon et al. 2006; Kumar et al. 2014; Kiranmayee et al. 2015). The associated traits include reduced dead hearts incidence, improved leaf glossiness and reduced oviposition incidence (Anandan et al. 2009). Flies are reflected by the glossiness of leaves, larval movement on the surface of leaves is inhibited by dense trichomes, in turn acting as a physical barrier between the leaf and fly to prevent egg deposition (antixenosis). High seedling vigor leads to rapid growth of seedlings which hinder the larvae movement to the central leaf whorl thereby, reducing the frequency of dead hearts (Satish et al. 2009). Glumes of spikelets of gall midge resistant varieties are more tightly closed than those in susceptible varieties. A correlation between tannins and midge resistance was also noted (Santos and Carmo 1974; Sharma 1985, 1993). Spikelet morphology might be associated with antixenosis resistance mechanism (Henzell et al. 1994). A positive association between midge resistance and small glume size has been observed in sorghum (Jadhav and Jadhav 1978).

## 5.5 A Brief Account of Molecular Mapping of Resistance Genes and QTLs

Identification and mapping of major QTLs associated with disease resistance in sorghum pave way for the transfer of QTLs to varieties or parental lines to be used in breeding. Fine mapping of the QTLs would be revamped through the establishment of highly saturated genetic maps, restriction-site associated DNA

sequencing (RAD-seq) and single nucleotide polymorphisms (SNPs) (Zou et al. 2012). Six collinear independent component maps of sorghum were created and integrated into a single resource through the amalgamation of the component maps (Mace et al. 2009). A set of five genetic linkage maps based on RFLP markers were integrated to 10 linkage groups (Xu et al. 1994; Subudhi and Nguyen 2000). Thereafter, various research groups saturated the linkage maps with amplified fragment length polymorphism (AFLP) markers (Ramu et al. 2009). Over 323 RFLPs and 143 simple sequence repeats (SSRs) were utilized for the construction of high-density linkage map (Bhattramakki et al. 2000; Bowers et al. 2003). Later, AFLPs, SSRs and RFLPs markers constituting 2926 markers were further used to saturate the former map (Menz et al. 2002). EST-SSRs and candidate genes-based SSR markers have been used in constructing linkage maps (Ramu et al. 2009; Reddy et al. 2012; Zou et al. 2012).

## *5.5.1 Mapping Populations*

Different research groups across the globe have developed specific bi-parental mapping populations that have paved the way for the identification and mapping of QTLs associated with resistance to several pests and diseases in sorghum. In general cross between two, sometimes more parents are involved in obtaining mapping populations. The parents selected and the mating design for the development of the mapping populations largely depend on the purpose of the study. $F_2$, $F_2$-derived $F_3$ ($F_2$:$F_3$), recombinant inbred lines (RILs), backcross inbred lines (BILs), near-isogenic lines (NILs), doubled haploids (DHs), multi-parent advanced generation intercross (MAGIC), chromosomal segment substitution lines (CSSLs), nested association mapping (NAM) population etc. are the several types of mapping populations being used for QTL mapping (Singh and Singh 2015). For biotic stress resistance BILs, RILs and populations derived from multi-parental lines such as MAGIC and NAMs are more specifically used (Arrones et al. 2020). Identification and mapping of important QTLs for pest and disease resistance in sorghum has enhanced with the use of genomic tools and mapping populations.

## *5.5.2 QTLs Mapped Using Different Mapping Populations*

### 5.5.2.1 QTLs Associated with Diseases

The SNP markers linked to rust resistance have been identified and studied in detail (Upadhyaya et al. 2013b). The GWAS approach elucidated over 64 significant QTLs for rust including the earlier reported ones (Tao et al. 1998; Mohan et al. 2010; Upadhyaya et al. 2013b). Besides, a major QTL of the genome SC414-12E on chromosome 5 explained 20–39% of PVE in four environments (Kimball et al.

2019). Anthracnose resistance gene was identified by a closely linked RAPD marker *OPJ* $01_{1437}$ through bulk segregant analysis of derived RILs. QTLs for anthracnose on chromosome 9 found to be consistent across all the environments tested. The genomic regions reported earlier by Tao et al. (1998) and Klein et al. (2001) for rust and grain mold resistance, were found relevant to ergot disease resistance. Further, the presence of additive and non-additive gene actions for charcoal rot resistance has been noticed (Rao et al. 1993). Three traits, number of internodes crossed by the rot, crop lodging and length of infection conferred genetic basis to charcoal rot (Reddy et al. 2008). Haussmann et al. (2004) revealed five QTLs related to striga resistance in two RIL and were common across two mapping populations.

#### 5.5.2.2 QTLs Associated with Insect Pests

Four major QTLs on chromosome 9 from PI 607900 resistant to greenbug biotype I were identified. Two major QTLs—*sbi09ii* and *sbi09iii* described up to 39.8 and 34.7 per cent variability for greenbug infestation (Punnuri et al. 2013). Three QTLs located on LG-A, LG-G and LG-J, respectively, explained 8.8%, 15% and 33.9%, phenotypic variation for gall midge resistance (Tao et al. 2003). Some shoot fly resistance (SFR) sources identified after evaluating the marker traits in sorghum germplasm have been exploited in breeding programs (Chamarthi et al. 2011; Kumar et al. 2014). Using crosses 296B (susceptible) × IS18551 (resistant) (Satish et al. 2012b) and cross 27B (susceptible) × IS2122 (resistant), SFR QTLs were mapped (Aruna et al. 2011). Four SFR QTLs were introgressed and the progenies harboring different combinations of major QTLs showed resistance which was evidenced by the fewer number of shoot flies (Abinaya et al. 2019). A set of 19 putative QTLs associated with resistance to shoot-fly including *qDH9.1* (dead heart) and *qEC9.1* (oviposition) explaining 15.03 and 18.89% phenotypic variance have been reported (Vikal et al. 2020). The genes producing allelochemicals, receptor kinases, and ubiquitin-proteasome degradation in the pathways as well as the candidate genes, like *cysteine protease* and cytochrome P450 were identified within the predicted QTL regions (Vikal et al. 2020).

## 5.6 Marker-Assisted Breeding for Biotic Stress Management in Sorghum

With the availability of large-scale sorghum genomic resources and use of DNA markers, breeding for desired agronomic traits and biotic stress resistance is becoming increasingly relevant. Affordable high-throughput genotyping coupled with throughput genome sequencing is rendering the use of molecular markers in germplasm diversity assessment, QTL mapping facilitating MAS. Resistance to

major pests and diseases is governed by multiple genes, which are seldom amenable to achieve an appreciable increase in resistance due to their strong influence by the environment (Tao et al. 2003; Mohammed et al. 2016). The molecular breeding approaches have been deployed in many crop species including sorghum to achieve delivery of results quickly with much greater precision (Kiranmayee et al. 2015).

### *5.6.1 Germplasm Characterization*

Genetic resources as classified based on morphological characteristics, divided *Sorghum bicolour* into five major races: *bicolor*, *guinea*, *caudatum*, *kafir*, and *durra* and over ten possible hybrid groups (Harlan and de Wet 1972; Harlan and Stemler 1976; De Wet 1978). There are several 'wild' species and sub-species within *S. bicolor* and races within each subspecies (Snowden 1955). Sorghum has over 3475 accessions with over 242 mini core collection that include all five races and representation of geographic regions (Prasada Rao and Ramanatha Rao 1995; Upadhyaya et al. 2009; Dahlberg et al. 2012). Association mapping in mini-core collection for grain mold resistance using 14,739 SNP markers led to the identification of two linked marker to rust resistance (Upadhyaya et al. 2013b). Over 3367 accessions involving cultivated and wild relatives were genotyped using 41 SSR markers in which 78.3% of the SSR alleles were detected with a mean of 14.9 alleles per marker, comparable to the original allelic richness (Billot et al. 2013). Further, in an another attempt, a genome-wide association analysis using 268,289 SNPs, two loci linked to low seed deterioration and seedling emergence rate was identified (Cuevas et al. 2019).

### *5.6.2 Marker-Assisted Gene Introgression*

Both the efficiency and precision of crop breeding can be achieved with the use of DNA markers. Molecular mapping of major QTLs for disease and pest resistance has facilitated the transfer of QTLs to the agronomically superior varieties in the shortest possible time using MAS strategies (Dormatey et al. 2020). Marker-assisted gene pyramiding (MAGP) can pyramid disease/pest resistance genes into single cultivar (Sanchez et al. 2000). Marker-assisted recurrent selection (MARS) is a strategy to accumulate favorable alleles i.e., multiple QTLs controlling resistance through genotypic selection and inter-crossing in repeated cycles of selection resulting in enhanced efficiency of recurrent selection and accelerated breeding (Ribaut et al. 2010; Dormatey et al. 2020). The process of stacking of genes/QTLs into a single elite cultivar background can now be efficiently performed using backcrossing or pedigree approaches with molecular markers thus eliminating the elaborate and costly process other ways (Kole 2006). Pyramiding of multiple genes/QTLs can lead to improved resistance (Werner et al. 2005).

Molecular breeding tools enable tracking the introgression of several *R*-genes from various sources into a single cultivar (Witcombe and Hash 2000). Three breeding strategies viz., stepwise transfer, simultaneous/synchronized, and convergent backcrossing are being employed for marker-assisted gene pyramiding (MAGP). MAS is needed to select pyramided resistance genes in the segregating progeny generation (Werner et al. 2005).

As many as five putative SFR QTLs for the component traits from IS18551 were introgressed through marker assisted back-cross breeding (MABCB) (Mehtre 2006; Jyothi et al. 2010). SFR QTLs were introgressed into 296B backgrounds and introgression lines (ILs) from 296B × IS18551 and BTx623 × IS18551 (Deshpande et al. 2010; Jyothi et al. 2010; Satish et al. 2012a) were field evaluated for the traits (Reddy et al. 2012). A total 136 $BC_3$ and 30 $BC_4$ plant progenies from crosses $BC_2$ X AKSV 13 R and BC3 X AKSV 13 R, respectively, were screened for the recovery of donor alleles in the elite background (Wagh et al. 2016). In addition, three QTLs associated with shoot-fly resistance were also introgressed into an elite cultivar ICSB 29004 and Parbhani Moti, all the derived introgression lines had higher shoot-fly resistance levels (Gorthy et al. 2017).

A gene associated with leaf blight resistance from G-118 was introgressed into the susceptible cultivar HC-136, using linked DNA marker (Mittal and Boora 2005). RILs with both resistant and susceptible reaction were screened individually with marker *Xtxp 309,* which produced amplification in 23 of the 26 resistant RILs, but no amplification in 25 susceptible RILs. This indicated the potential application of this marker in MAS for gene introgression (Mittal and Boora 2005). Further, eight putative QTLs were detected for resistance to sorghum downy mildew in a set of 50 inbred lines derived from the cross CML153 (susceptible) X CML226 (resistant) using 128 SSRs and 191 SNPs, introgression effort developed 33 resistant lines (Nagabhushan 2014). In a separate effort of using DNA markers for striga resistance, markers spanning through the QTLs conferring resistance to striga parasite were identified and the same markers were used in introgression to make headway towards developing resistant lines (Haussmann et al. 2004; Satish et al. 2012a; Mohamed et al. 2014; Yohannes et al. 2015). The QTLs of striga resistance in N13 were transferred to a farmer-preferred sorghum variety through MABCB using flanking SSR markers (Yohannes et al. 2016; Afolayan et al. 2019).

### *5.6.3 Limitations and Prospects of MAS and MABCB*

MAS and MABCB approaches, even though adopted to breed resistance against biotic stresses, the complex quantitative traits have recorded marginal success as the QTLs for such traits partially explain the phenotype. The impact and application of MAS in plant breeding are still below the hypothetical possibilities. This could be attributed majorly due to the difficulty in identifying major QTLs with an adequate stable effect across environments and genetic backgrounds. The limited number of polymorphic markers in the breeding material and diverse mapping populations is

difficult to compare, assessment of QTL x environment interaction effect pose a complicate interpretation (Collard and Mackill 2008; Ribaut et al. 2010; Delannay et al. 2012). Further, genomic selection is a hope to solve this problem, where unmapped QTLs of small individual effects selected together by the plant breeders (Tuberosa 2012; Sakiyama et al. 2014). High throughput genotyping, phenotyping and more automatic ways would enhance the use of MAS in plant breeding (Gorthy et al. 2017).

## 5.7 Brief on Genetic Engineering for Resistance Traits

Conventional plant breeding methods used to develop cultivars resistant to multiple pests and diseases in sorghum are inadequate when the desirable genes are limited in the gene pool of cross-compatible species or when such genes restricted linkage-drag (Crews and Cattani 2018). Genetic transformation and genome editing enable incorporation of beneficial genes across genera into sorghum with limited genetic diversity in the desired traits (Liu et al. 2014). Insect pests with wide host range, evolving races of pathogens and low level of resistance in the cultivated sorghum germplasm have made molecular plant breeding approaches as highly desirable (Madhusudhana 2015). Insecticidal crystal proteins (CRY) from *Bacillus thuringiensis* (*Bt*) are very effective against the lepidopterans and dipterans. *Bt* and other genes including protease inhibitors, enzymes, secondary plant metabolites and plant lectins are being evaluated to reduce losses due to insect pests (Sharma et al. 2004; Visarada and Kishore 2007). Progress in sorghum transformation has been hindered by the challenges associated with recalcitrance to genetic transformation (Jeoung et al. 2002; Girijashankar et al. 2007). Different gene transfer methods are being used in sorghum so far, which include, Agrobacterium-mediated indirect gene transfer; electroporation and particle bombardment (Ahmed et al. 2018). *Agrobacterium*-mediated transformation is simple and precise in the integration of the transgene. However, monocotyledons such as sorghum are less responsive to agrobacterium infection. *PR* genes for *fusarium stalk rot* disease resistance were introduced into sorghum genotypes through *Agrobacterium*-mediated gene transfer. Despite the basic research, sorghum is still one of the most recalcitrant crops to transformation and regeneration (Raghuwanshi and Birch 2010).

### *5.7.1 Transgenic Resistance to Fungal Diseases*

Sorghum is highly vulnerable to multiple fungal diseases causing decreased grain quality and yield loss. Genes encoding fungal cell wall hydrolyzing enzymes such as glucanases, chitinases and chitosanases are potential transgene candidates for developing fungal disease resistance in sorghum (Muthukrishnan et al. 2001). Chitinases and chitosanases degrade the components of fungal cell walls i.e., chitin

and chitosan and lyse the fungi. The first fungal resistance gene -*rice chitinase* (*G11*) was introduced into the sorghum inbred 'Tx430' along with *bar* gene and a plasmid DNA into the calli of immature zygotic embryos (Zhu et al. 1998). Thaumatin-like proteins (TLPs) are one more class of pathogenesis-related (PR) proteins that have shown antifungal activity and have been used as transgenes for enhancing fungal resistance (Mahdavi et al. 2012). Two *PR* genes viz., *rice chitinase* (*G11*) and *tlp* (Thaumatin-like protein) were introduced into three different sorghum inbred lines (Jeoung et al. 2002). Chitinase gene, *OschiII* fused with CaMV 35S promoter prospected for stalk rot resistance (Muthukrishnan et al. 2001). Transformation of sorghum with *tlp* gene along with green fluorescent protein (*gfp*) under the maize *ubi1* promoter exhibited enhanced resistance to fungal diseases. Expression of *gfp* was highly correlated with the expression of *tlp*, which was further confirmed by western blot analysis (Gao et al. 2005a). *In planta* and *ex planta* anthracnose infection assays revealed transgenic line KOSA-1 to be more resistant to anthracnose in comparison to its non-transgenic wild type AT412 (Ayoo 2008; Anami et al. 2016).

### 5.7.2 Transgenic Resistance to Insect Pests

Insecticidal crystal proteins are potential candidates for insect resistance in many crop plants (Roh et al. 2007; Jain et al. 2016). Many *Bt* toxin genes have been transferred into sorghum to attain resistance against insect pests. Stem borer is an important pest in sorghum. Sorghum genotype BT × 623 was transformed with the *cry1Ac* gene under the control of a wound-inducible promoter from the maize protease inhibitor gene (*mpiC1*) via particle bombardment of shoot apices. Transgenic lines showed up to 60% reduction in leaf damage, 40% larval mortality and 36% weight loss in the survived larvae of stem borer. However, *Bt* protein accumulation under the inducible promoter was very low at 1–8 ng/g of fresh tissue, which led to partial resistance (Girijashankar et al. 2005). Sorghum varieties 115, ICS21B and 5–27 were transformed with the *cry1Ab* gene and the transgenic lines showed high resistance levels to pink rice borer (Liu et al. 2015). Sweet sorghum varieties 'BABUSH' and 'MN-3025' transformed with *cry1Ah* using *Agrobacterium*-mediated transformation have shown high insect-resistance to Asian corn borer (*Ostrinia furnacalis*) (Zhao et al. 2011). Enhanced accumulation of the *Bt* protein in leaves by 30 to 50-fold (35–500 ng/g fresh leaf) by expression of *cry1Aa* and *cry1B* genes under the influence of maize *ubiquitin-1* promoter was reported during the susceptible plant growth period. Leaf consumption by the stem borer in the transgenic sorghum leaves was significantly lower (20–30%) compared to their feeding on non-transgenic lines (77–80%). Transgenic lines also showed a significant reduction in the leaf damage (55–78%) over their non-transgenic controls. Where, the larval mortality was appreciably high (60–90%) in transgenic lines as compared to (14–24%) non-transgenic control (Visarada et al. 2014). Higher expression of *Bt* protein is crucial for achieving superior insect control, which may

be achieved by placing the *Bt* genes under suitable promoters such as maize ubiquitin.

Transgenic glyphosate-resistant crops overexpressing 5-enolpyruvylshikimate-3-phosphate synthase (*cp4 epsps*) gene accelerated widespread use of glyphosate (Duke and Powles 2008) in controlling recalcitrant weeds such as Johnsongrass. RNAi is one of the most successful strategy in target trait improvement apart from its role in identifying gene function by silencing different pathogens/pests as well as plant genes (Stach and Good 2011; Banerjee et al. 2017; Majumdar et al. 2017; Zhang et al. 2017a). Virus-induced gene silencing (VIGS) efficiency in sorghum was significantly enhanced with an antisense strand of a gene in *Brome mosaic virus* (BMV) (Singh et al. 2018) (Table 5.1).

## 5.8 Brief Account on Bioinformatics as a Tool for Biotic Stress Resistance Breeding

Advances in disciplines that are contributing to the generation of genomic resources and data analytics have made a significant impact for structural and functional genomics of sorghum in the past decade. The next-generation sequencing platforms have greatly facilitated advanced assessment of sorghum genome, variety of transcriptome profiling attempts with deep insights into the structural organization of the genome; gene prediction; gene annotation and response of genes in variable biotic and abiotic conditions. The developed bioinformatics tools assist in filtering of data sets of various types, the correct interpretation of specific outcomes of *in silico* analytics of the data and its targeted views to elucidate the candidate gene sets that have potential applications in the breeding of sorghum for various biotic stress resistances (López de Maturana et al. 2019). The use of publicly available genomic data sets has helped the researchers to annotate key genes for their target traits in sorghum. Since the genome of sorghum is sequenced and high throughput datasets are publicly available, the bioinformatics pipelines can effectively identify putative candidate genes for various biotic stress responses. These potential candidate genes would be useful to develop the markers for the genotyping of breeding populations for the identification of a superior lines (de Oliveira et al. 2018). Databases in sorghum include; **SorGSD:** Web-portal with a comprehensive database of genomic variation across all types of cultivated and wild sorghums (Luo et al. 2016) and **SorghumFDB** covering transcription factors, regulators, protein kinases, ubiquitin, monolignol biosynthesis-related enzymes, carbohydrate-active enzymes, cytochrome P450, organelle-genes and R-genes. It acts as a genome browser for comprehensive coverage of gene annotations, miRNA information, gene loci conversions, orthologues in model plants like arabidopsis, maize and rice (Tian et al. 2016).

Through genomics, identified genomic regions could be incorporated to impart resistance to sorghum midge (Yazawa et al. 2013). Transcriptomic analysis of Cv.

**Table 5.1** Transgenes conferring insect and disease resistance in sorghum

| Transgene | Source | Target organism | Transformation method | Reference |
|---|---|---|---|---|
| *Insect resistance* | | | | |
| *CryIAc* | *Bacillus thuringiensis* | Stem borer | Biolistic | Girijashankar et al. (2005) |
| *cry1Ab* | *Bacillus thuringiensis* | Pink rice borer | Agrobacterium | Liu et al. (2015) |
| *cry1Ah* | *Bacillus thuringiensis* | Asian corn borer | Agrobacterium | Zhao et al. (2011) |
| *cry1Aa* and *cry1B* | *Bacillus thuringiensis* | Stem borer | Biolistic and Agrobacterium | Visarada et al. (2014) |
| *Disease resistance* | | | | |
| chitinase (*G11*) | *Oryza sativa* | Fungi | Biolistic | Zhu et al. (1998) |
| chitinase (*chiII*) | *Oryza sativa* | *Fusarium thapsinum* | Biolistic | Muthukrishnan et al. (2001) |
| chitinase | *Oryza sativa* | Fungi | Agrobacterium | Arulselvi et al. (2010) |
| chitinase (*HarChit*) | *Trichoderma harzianum* | *Colletotrichum sublineolum* | Biolistic | Ayoo (2008) |
| chitosanase (*HarCho*) | *Trichoderma harzianum* | *Colletotrichum sublineolum* | Biolistic | Ayoo (2008) |
| Thaumatin-like protein | – | Fungi | Agrobacterium | Gao et al. (2005b) |

SIL-05 and *Bipolaris sorghicola* led to the identification of genes in host-pathogen interaction (Mizuno et al. 2012). The genes encoding hyphae related proteins and enzymes involved in plant cell wall degradation elucidated from pathogen transcriptome data sets besides genes encoding WRKY, receptors of LLR domain and class III peroxidase are relevant for functional genomics analysis in sorghum (Yazawa et al. 2013). Molecular interpretation of charcoal rot defense mechanism was unraveled by expression profiling of genes in resistant and susceptible cultivars (Sharma et al. 2014b). A major QTL on linkage group 5 in the cross of BTx623/SC748-5 for anthracnose resistance analyzed by sequencing genomic DNA of SC748-5 and compared to BTx623 genome sequence (Burrell et al. 2015; Poloni and Schirawski 2016). Transcriptional changes and network analysis were decoded in a resistant and a susceptible genotype of sorghum to sugarcane aphids. A suite of abundantly expressed genes were recovered across genotypes and nucleotide-binding-site -leucine-rich repeat (NBS-LRR) and disease resistance genes were recognized (Kiani and Szczepaniec 2018; Tetreault et al. 2019). Correlation-based network analyses vis-a-vis metabolic pathway analysis revealed that multi-component defense response characterized by a functional defense-related molecular cues are involved in pathogen invasion (Tugizimana et al. 2019). Metabolomics of white sorghum-isolated *Burkholderia andropogonis* interaction revealed the alterations in the levels of phytohormones that marked the onset of defense in sorghum (Mareya et al. 2019, 2020).

## 5.9 Recent Concepts and Strategies Developed

### *5.9.1 Genome Editing*

A multitude of pathogens and insect pests comprising viruses, bacteria, fungi, insects and even parasitic plants affect sorghum globally with significant yield losses which in turn influence the food supply (Mushtaq et al. 2019; Yin and Qiu 2019). The strategy to control various diseases and pests involve widespread use of hazardous pesticides, which can be directly or indirectly deleterious to nature (Tyagi et al. 2020). Developing disease and insect pest resistant crops through various breeding approaches are sustainable and ecofriendly. In addition to the conventional transgenic approach, recent genome editing for biotic stress has greater potential in breeding programs. The biotic stress resistance being complex in nature is governed by several genes each with small effect. Some of these key genes could be potentially edited to create new alleles that can produce a larger desirable effect, thus re-orienting the process of breeding. More recently, genome editing technologies have emerged and evolved to enable rapid and precise manipulation of specific DNA sequences for developing biotic stress-resistant germplasm (Shi et al. 2017; Gao 2018; Yin et al. 2018). Genome editing involves engineered nucleases containing a non-specific nuclease domain fused with a

sequence-specific DNA binding domain, which can cleave the targeted gene precisely that can be repaired through specific genetics approaches. Zinc finger nucleases (ZFN), the first-generation editing technology are the chimeric proteins that consist of Fok1 cleavage domain and non-specific DNA cleavage domain (Fiaz et al. 2019; Ansari et al. 2020). Transcription activator-like effectors nucleases (TALENs) discovered in *Xanthomonas* consists of the amino acid repeats in the central DNA binding domain that recognizes one nucleotide in the target sequence. The repeat variable di-residue (RVD) which is located at 12 and 13 positions determines the specificity of TALEN. Once $TALEN_S$ are translocated to the nucleus, they bind to the target DNA strand in an opposite orientation. The FokI gets dimerized and cleaves at the spacer region resulting in double-strand breaks (DSB) in the target region (Jaganathan et al. 2018; Li et al. 2018). CRISPR/Cas System: clustered regularly interspaced short palindromic repeats (CRISPR), is a prokaryotic system observed for the first time in *Escherichia coli* that contains short repeated sequences separated by spacers with unique sequences (Ishino et al. 1987; Rath et al. 2015). A large recognition (REC) lobe determines the Cas9-specific function, whereas the small nuclease (NUC) incorporates two nuclease domains, RuvC and HNH, and a protospacer adjacent motif (PAM)-interacting domain (PI). The Cas9/single guide RNA (Cas9-sgRNA) complex probes a DNA sequence for rigorous protospacer adjacent motif (PAMs) using the Watson–Crick pairing principle (Song et al. 2016).

#### 5.9.1.1 Genome Editing in Sorghum

The type II CRISPR/Cas, Cas9-sgRNA system was employed in sorghum as well as in arabidopsis and tobacco. *Agrobacterium tumefaciens* mediated method for green fluorescent protein-coding gene transfer was used and mutagenic effects of the Cas9/sgRNA system in immature sorghum embryos were observed (Jiang et al. 2013). CRISPR/Cas9 system has been investigated by targeted editing of cinnamyl alcohol dehydrogenase (CAD) and phytoene desaturase. Genotype TX430 was edited successfully with effective biolistic bombardment (Li et al. 2018). CRISPR/Cas9 approach was applied to edit the *chlorophyll*-a *oxidase* (CAO) gene in sorghum protoplasts (Meng et al. 2020). Fourteen protoplasts showed precise editing in target gene region which could be a possible model for precise editing study in sorghum for improvement concerning agronomically important traits. Although, till date, there is no study reported concerning the use of genome editing for biotic stress resistance in sorghum, the use of the conventional transgenic approach for improving resistance is reported by a number of studies. Transformation of sorghum with *rice chitinase* for resistance against stalk rot, expression of the *cry1Ac* gene against sorghum spotted stem borer, *chitinase & chitosanase* genes against anthracnose, expression of Bt *cry1Ah* gene (Zhu et al. 1998; Muthukrishnan et al. 2001; Girijashankar et al. 2005; Akosambo-Ayoo et al. 2011; Liu and Godwin 2012) envisages that the model transgenic studies in sorghum will pave the path for precise breeding and may facilitate the development of product germplasm governing biotic resistance through the editing of same genes.

### *5.9.2 Nanotechnology*

Nanotechnology is currently being explored for agricultural applications, including, finding solutions for yield loss due to insect pests and diseases (Balaure et al. 2017; Sinha et al. 2017). Current pest management relies on the use of chemicals with all their side effects and environmental concern (Ghormade et al. 2011; Worrall et al. 2018). The nanoparticles, besides other ways, aid enhanced solubility of pesticides, increased shelf life and these protect plants from target pest (Hayles et al. 2017). Nanoparticles of Ag, Cu and Zn could be utilized as a potential method for suppressing diseases in crop plants (Elmer and White 2018; Malandrakis et al. 2019; Vanti et al. 2019). The application of nanotechnology with the use of emerged nanomaterials may heighten the sustainable productivity through effective insect pest and disease management (Giannousi et al. 2013; Imada et al. 2016).

## 5.10 Conclusion and Future Perspectives

Sorghum being an important cereal crop for low endowed and climatically challenging situations of the world, to achieve the sustainability for food and fodder in such regions, the development of biotic stress-resistant cultivars coupled with drought stress tolerance is crucial. As such, yield stability and grain quality are severely affected by various biotic stresses including insect pests, diseases and parasitic weeds, which hamper this crop at all stages including storage. Biotic stresses pose daunting challenges to the realization of its yield potential, and development of resistant cultivars through host plant resistance is most opted, as a great deal of germplasm diversity exists and serves as a source of resistant genes. However, on practical scale, attempts to increase the production of sorghum with the introduction of new high yielding varieties and hybrids have been largely unsuccessful because of their susceptibility to various biotic stresses (Kishore 2001; Kiranmayee et al. 2015). The limited number of resistant accessions and their overall phenotype may constrain the development of new varieties. The wide host range for many of the insect pests and low level of resistance in the cultivated germplasm necessities the use of wild relatives and new parental lines having the potential genes for various biotic stress resistance to mitigate the negative effect. Advent of molecular tools has great scope in accelerating the process of breeding, and in turn resulting the enhanced resistance in the form of horizontal as well as vertical resistance. Besides, the conventional process of breeding is highly labour-intensive and time-consuming (Sharma et al. 2005) and the resistance being highly complex, it is essential to deploy molecular markers linked to QTLs or any gene and these QTLs/genes are to be introgressed to increase the efficiency of conventional breeding (Kumar et al. 2014; Kiranmayee et al. 2015).

Fine mapping of the mapped QTL regions and significant maker trait associations through GWAS is needed. Further validation of QTLs provides great promise

for employing MAS in sorghum improvement. Genes responsible for resistance such as leaf blade glossiness and trichome density and other associated genomic regions need to be cloned and their introgression and expression level studies should be made to enhance the resistance related genetic architecture. Combinatorial approaches with conventional plant resistance along with novel genes such as *Bt* gene for increased resistance is highly desirable. An integrated synergistic system involving plant breeding and genomics research using advanced molecular tools such as high-throughput sequencing and large-scale genotyping technologies followed with MAS is a way forward to improve sorghum biotic stress resistance. Advances in new genomic tools such as genome sequencing, DNA microarrays, RNA-sequencing, real-time PCR, protein expression profiling, metabolomics strategies and bioinformatics allow more in-depth knowledge about the genetics of host defense and host-plant resistance mechanisms to biotic stresses (Kumar et al. 2014). In addition, sequencing of sorghum whole genome and its availability on publically available data sets of genomic resources of various types is expected to accelerate for rapid trait discovery and introgression (McCormick et al. 2018).

Transcription factors are candidates of choice to alter the agronomically relevant traits and to boost the resistance to biotic stresses and several transcription factors families, such as WRKY, NAC, MYB, DREB, and bZIP, in response to biotic stresses have been identified and characterized in sorghum. As such transcription factor responses to biotic stresses are highly complex with larger effects and complex cross-talk between different signal transduction pathways (Baillo et al. 2019). The findings from previous reports indicate the potential application of TF genes to enhance stress resistance in important crops, however, extensive studies for understanding the mechanisms of these TFs are required. Studies involving combinatorial approaches of TFs and small RNAs are expected to unravel the pathways and key genes for biotic stress resistance, such genes will be of key in utilizing the upcoming opportunities such as genome editing and genomics assisted breeding. The availability of complete genome sequences in sorghum and breakthroughs in sequencing technology have facilitated the identification and characterization of TFs (McCormick et al. 2018).

In future, it is crucial to pyramid multiple genes to achieve multiple resistant varieties through MABCB. So far, no QTL has been found to regulate multiple pest/disease resistance in sorghum (Romana et al. 2018). Hence, future research efforts should focus on identifying genetic loci responsible for multiple disease resistance, new sources of resistance, characterization of resistance genes, and dissecting the network of resistance gene regulation (Dormatey et al. 2020). Genomic selection has great promise in exploiting unmapped QTLs of small individual effects at the whole plant level which could be deployed in plant breeding endeavors, this approach expected to be relevant in sorghum as it has great genetic diversity (Yano and Tuberosa 2009). The newly developed genomic approaches would rapidly accelerate applications to many different research areas ranging from marker discovery; genetic diversity; and linkage/association mapping

to the genomic selection, physical mapping, gene discovery and genomic-assisted breeding to improve biotic stress resistance in sorghum.

## References

Abera M, Alemayehu G (2012) Evaluation of improved and local/landrace/sorghum varieties for covered kernel smut. Arch Phytopathol Plant Protect 45:717–723

Abinaya M, Kumaravadivel N, Varanavasiappan S, Kavithamani D (2019) Screening the genotypes of sorghum [*Sorghum bicolor* (L.) Moench] $BC_1F_3$ generation of the cross CO (S) 28 × IS18551 for shoot-fly [*Atherigona soccata* (Rond.)] resistance. Elect J Plant Breed 10:1133–1139

Afolayan G, Aladele S, Deshpande S, Oduoye O, Nwosu D, Michael C, Blay E, Danquah E (2019) Marker-assisted foreground selection for identification of striga resistant backcross lines in *Sorghum bicolor*. Covenant J Physic Life Sci 7:29–36

Afolayan G, Aladele S, Deshpande S, Kolawole A, Nwosu D, Michael C, Blay E, Danquah E (2020) Genetic variation for *Striga hermonthica* resistance and yield among sorghum accessions in Nigeria. J Agric Sci 12:192–202

Ahmed RI, Ding A, Xie M, Kong Y (2018) Progress in optimization of Agrobacterium-mediated transformation in sorghum (*Sorghum bicolor*). Intl J Mol Sci 19:2983

Akosambo-Ayoo L, Bader M, Loerz H, Becker D (2011) Transgenic sorghum (*Sorghum bicolor* L. Moench) developed by transformation with chitinase and chitosanase genes from Trichoderma harzianum expresses tolerance to anthracnose. Afr J Biotechnol 10:3659–3670

Anami SE, Zhang Y-M, Zhang L-X, Zhu L, Jing H-C (2016) Sorghum genetic transformation: current status and future target traits. Biotechnol Major Cereals, pp 48

Ananda GK, Myrans H, Norton SL, Gleadow R, Furtado A, Henry RJ (2020) Wild sorghum as a promising resource for crop improvement. Front Plant Sci 11:1108

Anandan A, Huliraj H, Veerabadhiran P (2009) Analysis of resistance mechanism to *Atherigona soccata* in crosses of sorghum. Plant Breed 128:443–450

Andersen EJ, Ali S, Byamukama E, Yen Y, Nepal MP (2018) Disease resistance mechanisms in plants. Genes 9:339

Ansari WA, Chandanshive SU, Bhatt V, Nadaf AB, Vats S, Katara JL, Sonah H, Deshmukh R (2020) Genome editing in cereals: approaches, applications and challenges. Intl J Mol Sci 21:4040

Arrones A, Vilanova S, Plazas M, Mangino G, Pascual L, Díez MJ, Prohens J, Gramazio P (2020) The dawn of the age of multi-parent MAGIC populations in plant breeding: novel powerful next-generation resources for genetic analysis and selection of recombinant elite material. Biology 9:229

Arulselvi I, Michael P, Umamaheswari S, Krishanaveni S (2010) Agrobacterium mediated transformation of *Sorghum bicolor* for disease resistance. Intl J Pharma Bio Sci 1:272–281

Aruna C, Cheruku D (2019) Genetic improvement of grain sorghum. In: Aruna C, Visarada KBRS, Bhat BV, Tonapi VA (eds) Breeding sorghum for diverse end uses, pp 157–173. Woodhead Publishing, Cambridge, United Kingdom

Aruna C, Bhagwat V, Madhusudhana R, Sharma V, Hussain T, Ghorade R, Khandalkar H, Audilakshmi S, Seetharama N (2011) Identification and validation of genomic regions that affect shoot-fly resistance in sorghum [*Sorghum bicolor* (L.) Moench]. Theor Appl Genet 122:1617–1630

Ashok Kumar A, Reddy BV, Ramaiah B, Sharma R (2011a) Heterosis in white-grained grain mold resistant sorghum hybrids. J SAT Agric Res 9:6

Ashok Kumar A, Reddy BVS, Sharma HC, Hash CT, Srinivasa Rao P, Ramaiah B, Reddy PS (2011b) Recent advances in sorghum genetic enhancement research at ICRISAT. Amer J Plant Sci 2:589–600

Audilakshmi S, Das I, Ghorade R, Mane P, Kamatar M, Narayana Y, Seetharama N (2011) Genetic improvement of sorghum for grain mould resistance: I. Performance of sorghum recombinant inbred lines for grain mould reactions across environments. Crop Protect 30:753–758

Ayoo LMK (2008) Genetic transformation of Kenyan sorghum (*Sorghum bicolor* (L.) Moench) with anti-fungal genes and response to *Colletotrichum sublineolum* infection. Staats-und Universitätsbibliothek Hamburg Carl von Ossietzky

Baerson SR, Dayan FE, Rimando AM, Nanayakkara ND, Liu C-J, Schröder J, Fishbein M, Pan Z, Kagan IA, Pratt LH (2008) A functional genomics investigation of allelochemical biosynthesis in *Sorghum bicolor* root hairs. J Biol Chem 283:3231–3247

Baillo EH, Kimotho RN, Zhang Z, Xu P (2019) Transcription factors associated with abiotic and biotic stress tolerance and their potential for crops improvement. Genes 10:771

Balaure PC, Gudovan D, Gudovan I (2017) Nanopesticides: a new paradigm in crop protection. In: New pesticides and soil sensors, Academic Press. Elsevier, pp 129–192

Bandara AY, Weerasooriya DK, Tesso TT, Little CR (2019) Stalk rot resistant sorghum genotypes are resilient to pathogen-mediated photosystem II quantum yield retardation. Crop Protect 124: p104852

Bandyopadhyay R, Butler DR, Chandrashekar A, Reddy RK, Navi SS (2000) Biology, epidemiology, and management of sorghum grain mold. Technical and institutional options for sorghum grain mold management: proceedings of an international consultation. ICRISAT, Pattancheru, Andhra Pradesh, India, pp 18–19

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

Bhattramakki D, Dong J, Chhabra AK, Hart GE (2000) An integrated SSR and RFLP linkage map of *Sorghum bicolor* (L.) Moench. Genome 43:988–1002

Billot C, Ramu P, Bouchet S, Chantereau J, Deu M, Gardes L, Noyer J-L, Rami J-F, Rivallan R, Li Y (2013) Massive sorghum collection genotyped with SSR markers to enhance use of global genetic resources. PLoS ONE 8:59714

Boozaya-Angoon D, Starks K, Weibel D, Teetes G (1984) Inheritance of resistance in sorghum, *Sorghum bicolor*, to the sorghum midge, *Contarinia sorghicola* (Diptera: Cecidomyiidae). Environ Entomol 13:1531–1534

Bora D, Saharia D, Hussain S (1994) Biology of yellow stem borer, *Scirpophagain certulas* (Wlk.). Crop Res Hissar 8:366–370

Bowden J, Neve R (1953) Sorghum midge and resistant varieties in the Gold Coast. Nature 172:551

Bowers JE, Abbey C, Anderson S, Chang C, Draye X, Hoppe AH, Jessup R, Lemke C, Lennington J, Li Z (2003) A high-density genetic recombination map of sequence-tagged sites for sorghum, as a framework for comparative structural and evolutionary genomics of tropical grains and grasses. Genetics 165:367–386

Bowling RD, Brewer MJ, Kerns DL, Gordy J, Seiter N, Elliott NE, Buntin GD, Way M, Royer T, Biles S (2016) Sugarcane aphid (Hemiptera: Aphididae): a new pest on sorghum in North America. J Integr Pest Manag 7(1):1–13

Bramel-Cox PJ, Cox TS (1988) Use of wild sorghums in sorghum improvement. In: Wilkinson D (ed) Proc 43rd Annu Corn & Sorghum Industry Res Conf Amer Seed Trade Assoc, Washington D.C, pp 13–26

Bueso FJ, Waniska RD, Rooney WL, Bejosano FP (2000) Activity of antifungal proteins against mold in sorghum caryopses in the field. J Agric Food Chem 48:810–816

Burrell AM, Sharma A, Patil NY, Collins SD, Anderson WF, Rooney WL, Klein PE (2015) Sequencing of an anthracnose-resistant sorghum genotype and mapping of a major QTL reveal strong candidate genes for anthracnose resistance. Crop Sci 55:790–799

Calvino M, Messing J (2012) Sweet sorghum as a model system for bioenergy crops. Curr Opin Biotechnol 23:323–329

Chamarthi S, Sharma H, Sahrawat K, Narasu L, Dhillon M (2011) Physico-chemical mechanisms of resistance to shoot-fly, *Atherigona soccata* in sorghum, *Sorghum bicolor*. J Appl Entomol 135:446–455

Chamarthi SK, Vijay PM, Sharma HC, Narasu LM (2012) Constitutive and inducible resistance to *Atherigona soccata* (Diptera: Muscidae) in *Sorghum bicolor*. J Econ Entomol 105:1069–1076

Collard BC, Mackill DJ (2008) Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. Phil Trans Roy Soc B: Biol Sci 363:557–572

Cota LV, Souza AGC, Costa RV, Silva DD, Lanza FE, Aguiar FM, Figueiredo JEF (2017) Quantification of yield losses caused by leaf anthracnose on sorghum in Brazil. J Phytopathol 165:479–485

Cox T, House L, Frey KJ (1984) Potential of wild germplasm for increasing yield of grain sorghum. Euphytica 33:673–684

Crews TE, Cattani DJ (2018) Strategies, advances, and challenges in breeding perennial grain crops. Sustainability 10:1–7

Cuevas HE, Prom LK, Cruet-Burgos CM (2019) Genome-wide association mapping of anthracnose (*Colletotrichum sublineolum*) resistance in NPGS Ethiopian sorghum germplasm. G3: Genes Genomes Genet 9:2879–2885

Cui Y, Magill J, Frederiksen R, Magill C (1996) Chalcone synthase and phenylalanine ammonia-lyase mRNA levels following exposure of sorghum seedlings to three fungal pathogens. Physiol Mol Plant Pathol 49:187–199

Dahlberg J, Berenji J, Sikora V, Latković D (2012) Assessing sorghum [*Sorghum bicolor* (L) Moench] germplasm for new traits: food, fuels and unique uses. Maydica 56:85–92

Das IK (2019) Advances in sorghum disease resistance. Breeding sorghum for diverse end uses. Woodhead Publishing, Elsevier, pp 313–324

Das IK, Aruna C, Tonapi V (2020) Sorghum grain mold. ICAR-Indian Institute of Millets Research, Hyderabad, India

De Oliveira AA, Pastina MM, da Costa Parrella RA, Noda RW, Simeone MLF, Schaffert RE, de Magalhães JV, Damasceno CMB, Margarido GRA (2018) Genomic prediction applied to high-biomass sorghum for bioenergy production. Mol Breed 38:49

De Wet J (1978) Special paper: systematics and evolution of sorghum sect. Sorghum (Gramineae). Amer J Bot 65:477–484

Delannay X, McLaren G, Ribaut J-M (2012) Fostering molecular breeding in developing countries. Mol Breed 29:857–873

Deshpande S, Jyothi T, Folkertsma R, Sharma H, Bhasker Raj A, Yousuf A, Hash C (2010) Marker-assisted backcrossing of shoot-fly resistance in sorghum [*Sorghum bicolor* (L.) Moench]. In: Abstracts, national symposium on genomics and crop improvement: relevance and reservations, pp 25–27

Dhillon M, Sharma H, Reddy B, Singh R, Naresh J (2006) Inheritance of resistance to sorghum shoot-fly, *Atherigona soccata*. Crop Sci 46:1377–1383

Diarisso NY, Diourté M, Pendleton B (2005) Use of local plants to control sorghum insect pests in the field. Intl Sorghum Millets News 146:60–62

Dicko MH, Gruppen H, Barro C, Traoré AS, van Berkel WJ, Voragen AG (2005) Impact of phenolic compounds and related enzymes in sorghum varieties for resistance and susceptibility to biotic and abiotic stresses. J Chem Ecol 31:2671–2688

Dormatey R, Sun C, Ali K, Coulter JA, Bi Z, Bai J (2020) Gene pyramiding for sustainable crop improvement against biotic and abiotic stresses. Agronomy 10:1255

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

Dweikat I (2005) A diploid, interspecific, fertile hybrid from cultivated sorghum, *Sorghum bicolor*, and the common Johnson grass weed *Sorghum halepense*. Mol Breed 16:93–101

Ejeta G, Gressel J (2007) The Striga scourge in Africa: a growing pandemic. Integrating new technologies for Striga control—towards ending the Witch-Hunt'. World Scientific, Singapore, pp 3–16

Elmer W, White JC (2018) The future of nanotechnology in plant pathology. Annu Rev Phytopathol 56:111–133

Esele J (1995) Foliar and head diseases of sorghum. Afr Crop Sci J 3:185–189

Fiaz S, Ahmad S, Noor MA, Wang X, Younas A, Riaz A, Riaz A, Ali F (2019) Applications of the CRISPR/Cas9 system for rice grain quality improvement: perspectives and opportunities. Intl J Mol Sci 20:888

Franzmann B (1988) Components of resistance to sorghum midge in grain sorghum. In: McWhirter KS, Downes RW, Read BJ (eds) Proceedings of 9th Australian plant breeding conference, agriculture research institute. Private Mail Bag, Wagga-Wagga, New South Wales, Australia, pp 277–278

Franzmann B, Vaschina B (1989) Sorghum midge oviposition in preflowering and flowering panicles. Agric Sci 119–129

Freeman BC, Beattie GA (2008) An overview of plant defenses against pathogens and herbivores. Plant Health Instructor. https://doi.org/10.1094/phi-i-2008-0226-01

Gao C (2018) The future of CRISPR technologies in agriculture. Nat Rev Mol Cell Biol 19:275–276

Gao Z, Jayaraj J, Muthukrishnan S, Claflin L, Liang G (2005a) Efficient genetic transformation of sorghum using a visual screening marker. Genome 48:321–333

Gao Z, Xie X, Ling Y, Muthukrishnan S, Liang GH (2005b) Agrobacterium tumefaciens-mediated sorghum transformation using a mannose selection system. Plant Biotechnol J 3:591–599

Ghormade V, Deshpande MV, Paknikar KM (2011) Perspectives for nano-biotechnology enabled protection and nutrition of plants. Biotechnol Adv 29:792–803

Ghosh T, Biswas M, Guin C, Roy P (2018) A review on characterization, therapeutic approaches and pathogenesis of *Macrophomina phaseolina*. Plant Cell Biotechnol Mol Biol 19:72–84

Giannousi K, Avramidis I, Dendrinou-Samara C (2013) Synthesis, characterization and evaluation of copper-based nanoparticles as agrochemicals against *Phytophthora infestans*. RSC Adv 3:21743–21752

Girijashankar V, Sharma HC, Sharma KK, Swathisree V, Prasad LS, Bhat B, Royer M, San Secundo B, Narasu ML, Altosaar I (2005) Development of transgenic sorghum for insect resistance against the spotted stem borer (*Chilo partellus*). Plant Cell Rep 24:513–522

Girijashankar V, Sharma K, Balakrishna P, Seetharama N (2007) Direct somatic embryogenesis and organogenesis pathway of plant regeneration can seldom occur simultaneously within the same explant of sorghum. J SAT Agric Res 3:1–3

Gorthy S, Narasu L, Gaddameedi A, Sharma HC, Kotla A, Deshpande SP, Are AK (2017) Introgression of shoot-fly (*Atherigona soccata* L. Moench) resistance QTLs into elite post-rainy season sorghum varieties using marker-assisted backcrossing (MABC). Front Plant Sci 8:1494

Gressel J (2010) Needs for and environmental risks from transgenic crops in the developing world. New Biotechnol 27:522–527

Hariprasanna K, Rakshit S (2016) Economic importance of sorghum. In: Rakshit S, Wang Y-H (eds) The sorghum genome. Springer, Cham, Switzerland, pp 1–25

Harlan JR, de Wet JM (1972) A simplified classification of cultivated sorghum 1. Crop Sci 12:172–176

Harlan JR, Stemler A (1976) The races of sorghum in Africa. Origins African Plant Domestication 465:478

Harris HB, Johnson BJ, Dobson JW, Luttrell ES (1964) Evaluation of anthracnose on grain sorghum 1. Crop Sci 4:460–462

Haussmann B, Hess D, Omanya G, Folkertsma R, Reddy B, Kayentao M, Welz H, Geiger H (2004) Genomic regions influencing resistance to the parasitic weed *Striga hermonthica* in two recombinant inbred populations of sorghum. Theor Appl Genet 109:1005–1016

Hayles J, Johnson L, Worthley C, Losic D (2017) Nanopesticides: a review of current research and perspectives. New pesticides and soil sensors. Elsevier, pp 193–225

Henzell RG, Franzmann BA, Brengman RL (1994) Sorghum midge resistance research in Australia. Intl Sorghum Millets Newsl 35:41–47

Hodges R, Hall A, Jayaraj K, Jaiswal P, Potdar N, Yogand B (2000) Quality changes in farm-stored sorghum grain grown in the wet or dry season in southern India-a technical and social study. ACIAR proceedings, pp 333–344

Hooker AL (1985) Corn and sorghum rusts. In: Roelfs AP, Bushnell WR (eds) The Cereal Rusts. Vol. II. Diseases, Distribution, Epidemiology, and Control. Academic Press, Orlando, FL, pp 207–236

Hulvova H, Galuszka P, Frébortová J, Frébort I (2013) Parasitic fungus *Claviceps* as a source for biotechnological production of ergot alkaloids. Biotechnol Adv 31:79–89

Hussain B, War AR, Sharma HC (2014) Jasmonic and salicylic acid-induced resistance in sorghum against the stem borer *Chilo partellus*. Phytoparasitica 42:99–108

Imada K, Sakai S, Kajihara H, Tanaka S, Ito S (2016) Magnesium oxide nanoparticles induce systemic resistance in tomato against bacterial wilt disease. Plant Pathol 65:551–560

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 Bacteriol 169:5429–5433

Jadhav R, Jadhav L (1978) Studies on preliminary screening of some sorghum hybrid and varieties against earhead midge (*Contarinia sorghicola* Coq.). J Maharashtra Agric Univ 3:187–188

Jaganathan D, Ramasamy K, Sellamuthu G, Jayabalan S, Venkataraman G (2018) CRISPR for crop improvement: an update review. Front Plant Sci 9:985

Jain D, Saharan V, Pareek S (2016) Current status of *Bacillus thuringiensis*: insecticidal crystal proteins and transgenic crops. In: Al-Khayri JM, Jain M, Johnson DV (eds) Advances in plant breeding strategies: agronomic, abiotic and biotic stress traits. Springer International Publishing, New York, pp 657–698

Jeoung J, Krishnaveni S, Muthukrishnan S, Trick H, Liang G (2002) Optimization of sorghum transformation parameters using genes for green fluorescent protein and β-glucuronidase as visual markers. Hereditas 137:20–28

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:e188–e188

Jotwani M, Marwaha K, Srivastava K, Young W (1970) Seasonal incidence of shoot-fly *(Atherigona varia soccata* Rond) in jowar hybrids at Delhi. Indian J Entomol 32:7–15

Jotwani MG, Knndu GG, Kishore P, Srivastava KP, Sukhani TR, Singh SP (1979) Evaluation of some high yielding sorghum derivatives for resistance to stem borer, *Chilo partellus* (Swinhoe). Indian J Entomol 41:1–4

Jyothi T, Fölkertsma R, Sharma H, Bhasker Raj A, Anwar Y, Hash C (2010) P-128: marker-assisted transfer of shoot-fly resistance in *Sorghum bicolor*. In: National symposium on genomics and crop improvement: relevance and reservations institute of biotechnology. Acharya NG Ranga Agricultural University, Rajendranagar, Hyderabad, India, pp 25–27

Kamala V, Singh S, Bramel P, Rao DM (2002) Sources of resistance to downy mildew in wild and weedy sorghums. Crop Sci 42:1357–1360

Kamala V, Sharma H, Manohar Rao D, Varaprasad K, Bramel P (2009) Wild relatives of sorghum as sources of resistance to sorghum shoot-fly, *Atherigona soccata*. Plant Breed 128:137–142

Kamala V, Sivaraj N, Pandravada S, Rameash K, Sharma H, Sarath Babu B (2016) Potential of wild relatives in sorghum improvement through molecular approaches. In: Conference on national priorities in plant health management February 4–5, 2016, Tirupati, Andhra Pradesh, India, pp 124–125

Khan ZR, Midega CA, Hassanali A, Pickett JA, Wadhams LJ, Wanjoya A (2006) Management of witchweed, *Striga hermonthica*, and stem borers in sorghum, *Sorghum bicolor*, through intercropping with green leaf desmodium, *Desmodium intortum*. Intl J of Pest Manag 52:297–302

Kiani M, Szczepaniec A (2018) Effects of sugarcane aphid herbivory on transcriptional responses of resistant and susceptible sorghum. BMC Genom 19:1–18

Kimball J, Cui Y, Chen D, Brown P, Rooney WL, Stacey G, Balint-Kurti PJ (2019) Identification of QTL for target leaf spot resistance in *Sorghum bicolor* and investigation of relationships between disease resistance and variation in the MAMP response. Sci Rep 9:1–9

Kiranmayee K, Hash CT, Deshpande SP, Varaprasad K, Kishor P (2015) Biotechnological approaches to evolve sorghum (*Sorghum bicolor* (L.) Moench) for drought stress tolerance and shoot-fly resistance. Curr Trends Biotechnol Pharm 9:281–292

Kishore P (2001) Resistance to shoot-fly, *Atherigona soccata* Rondani and stem borer, *Chilo partellus* (Swinhoe) in new germplasm of sorghum. J Entomol Res 25:273–282

Klein R, Rodriguez-Herrera R, Schlueter J, Klein P, Yu Z, Rooney W (2001) Identification of genomic regions that affect grain-mould incidence and other traits of agronomic importance in sorghum. Theor Appl Genet 102:307–319

Klittich CJ, Leslie JF, Nelson PE, Marasas WF (1997) *Fusarium thapsinum* (*Gibberella thapsina*): a new species in section Liseola from sorghum. Mycologia 89:643–652

Kole C (2006) Genome mapping and molecular breeding in plants. Vol on Cereals and Millets. Springer, Berlin, New York

Komolong B, Chakraborty S, Ryley M, Yates D (2003) Ovary colonization by Claviceps africana is related to ergot resistance in male-sterile sorghum lines. Plant Pathol 52:620–627

Kumar VK, Sharma H, Reddy KD (2006) Antibiosis mechanism of resistance to spotted stem borer, *Chilo partellus* in sorghum, *Sorghum bicolor*. Crop Protec 25:66–72

Kumar AA, Gorthy S, Sharma HC, Huang Y, Sharma R, Reddy BV (2014) Understanding genetic control of biotic stress resistance in sorghum for applied breeding. In: Wang Y-H, Upadhyay HD, Kole C (eds) Genetics, genomics and breeding of sorghum. CRC Press, USA, pp 198–225

Kumari A, Goyal M, Kumar R, Sohu R (2020) Morpho-physiological and biochemical attributes influence intra-genotypic preference of shoot-fly [*Atherigona soccata* (Rondani)] among sorghum genotypes. Protoplasma 1–16

Li J, Shi Y, Veeranampalayam-Sivakumar A-N, Schachtman DP (2018) Elucidating sorghum biomass, nitrogen and chlorophyll contents with spectral and morphological traits derived from unmanned aircraft system. Front Plant Sci 9:1406

Liu G, Godwin ID (2012) Highly efficient sorghum transformation. Plant Cell Rep 31:999–1007

Liu G, Campbell BC, Godwin ID (2014) Sorghum genetic transformation by particle bombardment. Methods Mol Biol 1099:219–234

Liu G, Gilding EK, Godwin ID (2015) A robust tissue culture system for sorghum [*Sorghum bicolor* (L.) Moench]. S Afr J Bot 98:157–160

López de Maturana E, Alonso L, Alarcón P, Martín-Antoniano IA, Pineda S, Piorno L, Calle ML, Malats N (2019) Challenges in the integration of omics and non-omics data. Genes 10:238

Lu J, Ehsani R, Shi Y, de Castro AI, Wang S (2018) Detection of multi-tomato leaf diseases (late blight, target and bacterial spots) in different stages by using a spectral-based sensor. Sci Rep 8:1–11

Luo H, Zhao W, Wang Y, Xia Y, Wu X, Zhang L, Tang B, Zhu J, Fang L, Du Z (2016) SorGSD: a sorghum genome SNP database. Biotechnol Biofuels 9:6

Luthra Y, Gandhi S, Joshi U, Arora S (1988) Total phenols and their oxidative enzymes in sorghum leaves resistant and susceptible to *Ramulisporasorghicola* Harris. Acta Phytopathol Entomol Hung 23:393–399

M Perez-de-Castro A, Vilanova S, Cañizares J, Pascual L, M Blanca J, J Diez M, Prohens J, Picó B (2012) Application of genomic tools in plant breeding. Curr Genom 13:179–195

Mace ES, Rami J-F, Bouchet S, Klein PE, Klein RR, Kilian A, Wenzl P, Xia L, Halloran K, Jordan DR (2009) A consensus genetic map of sorghum that integrates multiple component maps and high-throughput Diversity Array Technology (DArT) markers. BMC Plant Biol 9:13

Madhusudhana R (2015) Linkage mapping. In: Madhusudhana R, Rajendrakumar P, Patil JV (eds) Sorghum molecular breeding, pp 47–70. Springer, New Delhi

Mahdavi F, Sariah M, Maziah M (2012) Expression of rice thaumatin-like protein gene in transgenic banana plants enhances resistance to Fusarium wilt. Appl Biochem Biotechnol 166:1008–1019
Majumdar R, Rajasekaran K, Cary JW (2017) RNA interference (RNAi) as a potential tool for control of mycotoxin contamination in crop plants: concepts and considerations. Front Plant Sci 8:200
Malandrakis AA, Kavroulakis N, Chrysikopoulos CV (2019) Use of copper, silver and zinc nanoparticles against foliar and soil-borne plant pathogens. Sci Total Environ 670:292–299
Mareya CR, Tugizimana F, Piater LA, Madala NE, Steenkamp PA, Dubery IA (2019) Untargeted metabolomics reveal defensome-related metabolic reprogramming in *Sorghum bicolor* against infection by *Burkholderiaandropogonis*. Metab 9:8
Mareya CR, Tugizimana F, Di Lorenzo F, Silipo A, Piater LA, Molinaro A, Dubery IA (2020) Adaptive defence-related changes in the metabolome of *Sorghum bicolor* cells in response to lipopolysaccharides of the pathogen *Burkholderiaandropogonis*. Sci Rep 10:1–17
Mbuvi DA, Masiga CW, Kuria EK, Masanga J, Wamalwa M, Mohamed A, Odeny D, Hamza N, Timko MP, Runo SM (2017) Novel sources of witchweed (striga) resistance from wild sorghum accessions. Front Plant Sci 8:116
McCormick RF, Truong SK, Sreedasyam A, Jenkins J, Shu S, Sims D, Kennedy M, Amirebrahimi M, Weers BD, McKinley B (2018) The *Sorghum bicolor* reference genome: improved assembly, gene annotations, a transcriptome atlas, and signatures of genome organization. Plant J 93:338–354
Mehtre SP (2006) Genetic diversity analysis, QTL mapping and marker-assisted selection for shoot fly resistance in sorghum [*Sorghum bicolor* (L,) Moench]. Ph.D dissertation, Marathwada Agricultural University, Parbhani, Maharashtra, India
Meng R, Wang C, Wang L, Liu Y, Zhan Q, Zheng J, Li J (2020) An efficient sorghum protoplast assay for transient gene expression and gene editing by CRISPR/Cas9. Peer J 8:
Mengistu G, Shimelis H, Laing M, Lule D (2019) Assessment of sorghum genetic resources of Ethiopia for anthracnose (*Colletotrichum sublineolum* Henn.) resistance and agronomic traits. J Phytopathol 167:667–678
Menkir A, Ejeta G, Butler L, Melakeberhan A (1996) Physical and chemical kernel properties associated with resistance to grain mold in sorghum. Cereal Chem 73:613–617
Menz M, Klein R, Mullet J, Obert J, Unruh N, Klein P (2002) A high-density genetic map of *Sorghum bicolor* (L.) Moench based on 2926 AFLP, RFLP and SSR markers. Plant Mol Biol 48:483–499
Miller magazine (2020) World milling and pulses technologies refereed journal, 132. https://www.millermagazine.com/english/archive
Mittal M, Boora KS (2005) Molecular tagging of gene conferring leaf blight resistance using microsatellites in sorghum [*Sorghum bicolo*r (L.) Moench]. Indian J Exp Biol 43(5):462–466
Mizuno H, Kawahigashi H, Kawahara Y, Kanamori H, Ogata J, Minami H, Itoh T, Matsumoto T (2012) Global transcriptome analysis reveals distinct expression among duplicated genes during sorghum-*Bipolarissorghicola* interaction. BMC Plant Biol 12:121
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
Mohammed R, Are AK, Munghate RS, Bhavanasi R, Polavarapu KKB, Sharma HC (2016) Inheritance of resistance to sorghum shoot-fly, *Atherigona soccata*in sorghum, *Sorghum bicolor* (L.) Moench. Front Plant Sci 7:543
Mohan SM, Madhusudhana R, Mathur K, Chakravarthi D, Rathore S, Reddy RN, Satish K, Srinivas G, Mani NS, Seetharama N (2010) Identification of quantitative trait loci associated with resistance to foliar diseases in sorghum [*Sorghum bicolor* (L.) Moench]. Euphytica 176:199–211
Mughogho L, Pande S (1983) Charcoal rot of sorghum. In: Mughogho LK (ed) Sorghum root and stalk rots—a critical review: proceedings of the consultative group discussion on research

needs and strategies for control of sorghum root and stalk rot diseases, 27 Nov–2 Dec 1983, Bellagio, Italy. ICRISAT Patancheru, A.P. 502324, India, pp 11–24

Mundia CW, Secchi S, Akamani K, Wang G (2019) A regional comparison of factors affecting global sorghum production: the case of North America, Asia and Africa's Sahel. Sustainability 11:2135

Mushtaq M, Sakina A, Wani SH, Shikari AB, Tripathi P, Zaid A, Galla A, Abdelrahman M, Sharma M, Singh AK (2019) Harnessing genome editing techniques to engineer disease resistance in plants. Front Plant Sci 10:550

Muthukrishnan S, Jeoung J, Liang G, Krishnaveni S (2001) Transgenic sorghum plants constitutively expressing a rice chitinase gene show improved resistance to stalk rot [*Sorghum bicolor* (L.) Moench]. J Genet Breed 55:151–158

Nagabhushan (2014) QTL Identification and Marker assisted selection for sorghum downy mildew resistance in maize (*Zea mays* L.). Doctoral dissertation, University of Agricultural Sciences GKVK, Bangalore, India

Nagarajan K, Perumal K, Shanmugan N (1957) The red hairy caterpillar and its field scale control. Madras Agric J 4:150–153

Navi SS, Bandyopadhyay R, Reddy R, Thakur R, Yang X-B (2005) Effects of wetness duration and grain development stages on sorghum grain mold infection. Plant Dis 89:872–878

Ngugi H, King S, Holt J, Julian A (2001) Simultaneous temporal progress of sorghum anthracnose and leaf blight in crop mixtures with disparate patterns. Phytopathol 91:720–729

Padmaja P, Shwetha B, Swetha G, Patil J (2014) Oxidative enzyme changes in sorghum infested by shoot-fly. J Insect Sci 14(1):2–6

Parh D, Jordan D, Aitken E, Mace E, Jun-Ai P, McIntyre C, Godwin I (2008) QTL analysis of ergot resistance in sorghum. Theor Appl Genet 117:369–382

Park S-J, Huang Y, Ayoubi P (2006) Identification of expression profiles of sorghum genes in response to greenbug phloem-feeding using cDNA subtraction and microarray analysis. Planta 223:932–947

Parker C (2009) Observations on the current status of orobanche and striga problems worldwide. Pest Manag Sci 65:453–459

Passlow T (1965) Bionomics of sorghum midge [*Contarinia sorghicola* (Coq.)] in Queensland, with particular reference to diapause. Queensland J Agric Anim Sci 22:149–167

Paterson AH, Bowers JE, Bruggmann R, Dubchak I, Grimwood J, Gundlach H, Haberer G, Hellsten U, Mitros T, Poliakov A (2009) The *Sorghum bicolor* genome and the diversification of grasses. Nature 457:551–556

Patil S (2011) Genome-wide QTL mapping for post-flowering drought tolerance and validation of charcoal rot resistance QTLs NILs of sorghum. PhD Thesis. Univ Agric Sci, Dharwad, Karnataka, India

Paudyal S, Armstrong JS, Giles KL, Payton ME, Opit GP, Limaje A (2019) Categories of resistance to sugarcane aphid (Hemiptera: Aphididae) among sorghum genotypes. J Econ Entomol 112:1932–1940

Pazoutova S, Frederickson D (2005) Genetic diversity of *Claviceps africana* on sorghum and Hyparrhenia. Plant Pathol 54:749–763

Perumal R, Tomar SS, Bandara A, Maduraimuthu D, Tesso TT, Prasad PV, Upadhyaya HD, Little CR (2020) Variation in stalk rot resistance and physiological traits of sorghum genotypes in the field under high temperature. J Gen Plant Pathol 86:350–359

Poloni A, Schirawski J (2016) Host specificity in *Sporisorium reilianum* is determined by distinct mechanisms in maize and sorghum. Mol Plant Pathol 17:741–754

Prasada Rao K, Ramanatha Rao V (1995) The use of characterization data in developing a core collection of sorghum. Core Collections of Plant Genetic Resources IPGRI. John Wiley and Sons, New York, pp 109–115

Prom L, Perumal R, Erattaimuthu S, Little C, No E, Erpelding J, Rooney W, Odvody G, Magill C (2012) Genetic diversity and pathotype determination of *Colletotrichum sublineolum* isolates causing anthracnose in sorghum. Eur J Plant Pathol 133:671–685

Punnuri S, Huang Y, Steets J, Wu Y (2013) Developing new markers and QTL mapping for greenbug resistance in sorghum [*Sorghum bicolor* (L.) Moench]. Euphytica 191:191–203

Raghuwanshi A, Birch RG (2010) Genetic transformation of sweet sorghum. Plant Cell Rep 29:997–1005

Ramu P, Kassahun B, Senthilvel S, Kumar CA, Jayashree B, Folkertsma R, Reddy LA, Kuruvinashetti M, Haussmann B, Hash CT (2009) Exploiting rice–sorghum synteny for targeted development of EST-SSRs to enrich the sorghum genetic linkage map. Theor Appl Genet 119:1193–1204

Rao G, Patil S, Anahosur K (1993) Genetics of charcoal rot resistance in Rabi sorghum. Karnataka J Agric Sci 6:113–116

Rashid Z, Singh PK, Vemuri H, Zaidi PH, Prasanna BM, Nair SK (2018) Genome-wide association study in Asia-adapted tropical maize reveals novel and explored genomic regions for sorghum downy mildew resistance. Sci Rep 8:1–12

Rath D, Amlinger L, Rath A, Lundgren M (2015) The CRISPR-Cas immune system: biology, mechanisms and applications. Biochimie 117:119–128

Reddy KS (1988) Assessment of on-farm yield losses in sorghum due to insect pests. Int J Trop Insect Sci 9:679–685

Reddy BV, Bandyopadhyay R, Ramaiah B, Ortiz R (2000) Breeding grain mold resistant sorghum cultivars. Technical and institutional options for sorghum grain mold management. In: Proceedings of an international consultation. ICRISAT, Patancheru, India, pp 195–224

Reddy B, Ramesh S, Reddy P (2004) Sorghum breeding research at ICRISAT-goals, strategies, methods and accomplishments. Intl Sorghum Millets Newsl 45:5–12

Reddy PS, Rao V, Reddy BV, Ramesh S, Thakur R (2005) Grain mold resistance in advanced sorghum B-lines. Intl Sorghum Millets Newsl 46:29–32

Reddy PS, Fakrudin B, Punnuri S, Arun S, Kuruvinashetti M, Das I, Seetharama N (2008) Molecular mapping of genomic regions harboring QTLs for stalk rot resistance in sorghum. Euphytica 159:191–198

Reddy RN, Madhusudhana R, Mohan SM, Chakravarthi D, Seetharama N (2012) Characterization, development and mapping of Unigene-derived microsatellite markers in sorghum [*Sorghum bicolor* (L.) Moench]. Mol Breed 29:543–564

Reed J, Ramundo B, Claflin L, Tuinstra M (2002) Analysis of resistance to ergot in sorghum and potential alternate hosts. Crop Sci 42:1135–1138

Reynolds MP, Quilligan E, Aggarwal PK, Bansal KC, Cavalieri AJ, Chapman SC, Chapotin SM, Datta SK, Duveiller E, Gill KS (2016) An integrated approach to maintaining cereal productivity under climate change. Glob Food Secur 8:9–18

Ribaut J, De Vicente M, Delannay X (2010) Molecular breeding in developing countries: challenges and perspectives. Curr Opin Plant Biol 13:213–218

Riyazaddin M, Kavi Kishor PB, Ashok Kumar A, Reddy BV, Munghate RS, Sharma HC (2015) Mechanisms and diversity of resistance to sorghum shoot-fly, *Atherigona soccata*. Plant Breed 134:423–436

Rodriguez-Herrera R, Rooney W, Rosenow D, Frederiksen R (2000) Inheritance of grain mold resistance in grain sorghum without a pigmented testa. Crop Sci 40:1573–1578

Rodríguez-Herrera R, Waniska RD, Rooney WL (1999) Antifungal proteins and grain mold resistance in sorghum with non-pigmented testa. J Agric Food Chem 47:4802–4806

Roh JY, Choi JY, Li MS, Jin BR, Je YH (2007) *Bacillus thuringiensis* as a specific, safe, and effective tool for insect pest control. J Microbiol Biotechnol 17:547

Romana KK, Chander G, Deshpande S, Gupta R (2018) Genomic-assisted enhancement in stress tolerance for productivity improvement in sorghum. In: Biotechnologies of Crop Improvement, vol 3, pp 265–288. Springer, Cham, Switzerland

Sakiyama NS, Ramos HCC, Caixeta ET, Pereira MG (2014) Plant breeding with marker-assisted selection in Brazil. Crop Breed Appl Biotechnol 14:54–60

Salama EA, Kumaravadivel N, Mohankumar S, Muthukrishnan N (2020) Correlation study of traits associated with shoot-fly resistance in recombinant inbred lines (RILs) population of sorghum [*Sorghum bicolor* (L.) Moench]. Electron J Plant Breed 11:110–115

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

Santos Jd, Carmo Cd (1974) Evaluation of resistance to *Contarinia sorghicola* by sorghum lines from the Cameroon, Africa collection. Ceara, Brazil. Sorghum Newsl 17:10–11

Satish K, Srinivas G, Madhusudhana R, Padmaja P, Reddy RN, Mohan SM, Seetharama N (2009) Identification of quantitative trait loci for resistance to shoot-fly in sorghum [*Sorghum bicolor* (L.) Moench]. Theor Appl Genet 119:1425–1439

Satish K, Gutema Z, Grenier C, Rich PJ, Ejeta G (2012a) Molecular tagging and validation of microsatellite markers linked to the low germination stimulant gene (lgs) for Striga resistance in sorghum [*Sorghum bicolor* (L.) Moench]. Theor Appl Genet 124:989–1003

Satish K, Madhusudhana R, Padmaja P, Seetharama N, Patil J (2012b) Development, genetic mapping of candidate gene-based markers and their significant association with the shoot-fly resistance quantitative trait loci in sorghum [*Sorghum bicolor* (L.) Moench]. Mol Breed 30:1573–1591

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 Secur 4:519–537

Seifers DL, Perumal R, Little CR (2012) New sources of resistance in sorghum (*Sorghum bicolor*) germplasm are effective against a diverse array of *Potyvirus* spp. Plant Dis 96:1775–1779

Sharma H (1985) Strategies for pest control in sorghum in India. Intl J Pest Manag 31:167–185

Sharma H (1993) Host-plant resistance to insects in sorghum and its role in integrated pest management. Crop Protec 12:11–34

Sharma HC, Teetes GL (1995) Yield loss assessment and economic injury levels for panicle-feeding insects of sorghum. In: Nwanze KF, Youm O (eds) Panicle insect pests of sorghum and pearl millet: proceedings of an international consultative workshop, 4–7 Oct 1993, ICRISAT Sahelian Center, Niamey, Niger: (In En. Summaries in En, Fr, Es.), ICRISAT, Patancheru, Andhra Pradesh, India, pp 320

Sharma H, Vidyasagar P (1994) Antixenosis component of resistance to sorghum midge, *Contarinia sorghicola* Coq. in *Sorghum bicolor* (L.) Moench. Ann Appl Biol 124:495–507

Sharma HC, Taneja SL, Rao NK, Rao KP (2003) Evaluation of sorghum germplasm for resistance to insect pests. Information Bulletin No. 63. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India, pp 177

Sharma AD, Thakur M, Rana M, Singh K (2004) Effect of plant growth hormones and abiotic stresses on germination, growth and phosphatase activities in *Sorghum bicolor* (L.) Moench seeds. Afr J Biotechnol 3:308–312

Sharma H, Reddy BV, Dhillon M, Venkateswaran K, Singh B, Pampapathy G, Folkertsma R, Hash C, Sharma K (2005) Host-plant resistance to insects in sorghum: present status and need for future research. Intl Sorghum Millets Newsl 46:36–43

Sharma H, Dhillon M, Pampapathy G (2006) Multiple-resistance to sorghum shoot-fly, spotted stemborer and sugarcane aphid in sorghum. Intl J Trop Insect Sci 26:239–245

Sharma H, Dhillon M, Pampapathy G, Reddy B (2007) Inheritance of resistance to spotted stem borer, *Chilo partellus*, in sorghum, *Sorghum bicolor*. Euphytica 156:117–128

Sharma R, Rao V, Upadhyaya H, Reddy VG, Thakur R (2010) Resistance to grain mold and downy mildew in a mini-core collection of sorghum germplasm. Plant Dis 94:439–444

Sharma R, Upadhyaya H, Manjunatha S, Rao V, Thakur R (2012) Resistance to foliar diseases in a mini-core collection of sorghum germplasm. Plant Dis 96:1629–1633

Sharma HC, Bhagwat VR, Daware DG, Pawar DB, Munghate RS, Sharma SP, Kumar AA, Reddy BV, Prabhakar KB, Ambekar SS (2014a) Identification of sorghum genotypes with resistance to the sugarcane aphid (*Melanaphis sacchari)* under natural and artificial infestation. Plant Breed 133:36–44

Sharma I, Kumari N, Sharma V (2014b) Defense gene expression in *Sorghum bicolor* against *Macrophomina phaseolina* in leaves and roots of susceptible and resistant cultivars. J Plant Interact 9:315–323

Sharma HC, Bhagwat VR, Munghate RS, Sharma SP, Daware DG, Pawar DB, Kumar AA, Reddy BV, Prabhakar KB, Mehtre SP (2015) Stability of resistance to sorghum shoot-fly, *Atherigona soccata*. Field Crops Res 178:34–41

Shayanowako AT, Laing M, Shimelis H, Mwadzingeni L (2018) Resistance breeding and biocontrol of *Striga asiatica* (L.) Kuntze in maize: a review. Acta Agric Scand B 68:110–120

Shi TQ, Liu GN, Ji RY, Shi K, Song P, Ren LJ, Huang H, Ji XJ (2017) CRISPR/Cas9-based genome editing of the filamentous fungi: the state of the art. Appl Microbiol Biotechnol 101:7435–7443

Singh S, Bandyopadhyay R (2000) Grain mold. Compendium of sorghum diseases, pp 38–40

Singh BD, Singh AK (2015) Marker-assisted plant breeding: principles and practices. Springer, New Delhi, India

Singh B, Padmaja P, Seetharama N (2004a) Biology and management of the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Homoptera: Aphididae) in sorghum: a review. Crop Protec 23:739–755

Singh B, Padmaja P, Seetharama N (2004b) Stability of biochemical constituents and their relationships with resistance to shoot-fly, *Atherigona soccata* (Rondani) in seedling sorghum. Euphytica 136:279–289

Singh DK, Lee HK, Dweikat I, Mysore KS (2018) An efficient and improved method for virus-induced gene silencing in sorghum. BMC Plant Biol 18:123

Sinha K, Ghosh J, Sil PC (2017) New pesticides: a cutting-edge view of contributions from nanotechnology for the development of sustainable agricultural pest control. In: New pesticides and soil sensors, Elsevier, pp 47–79

Snowden J (1955) The wild fodder sorghums of the section Eu-sorghum. Biol J Linn Soc Lond 55:191–260

Song G, Jia M, Chen K, Kong X, Khattak B, Xie C, Li A, Mao L (2016) CRISPR/Cas9: a powerful tool for crop genome editing. Crop J 4:75–82

Stach JE, Good L (2011) Synthetic RNA silencing in bacteria–antimicrobial discovery and resistance breaking. Front Microbiol 2:185

Stenhouse JW, Bandyopadhyay R, Singh SD, Subramanian V (1997) Breeding for grain-mould resistance in sorghum. In: Proc Int Conf Genetic Improvement of Sorghum and Pearl Millet, 22–27 Sept 1996, Lubbock, Texas, pp 326–336

Subbarayudu B (2002) Shoot bug incidence on sorghum in South India. Intl Sorghum Millets Newsl 43:56–57

Subudhi PK, Nguyen HT (2000) Linkage group alignment of sorghum RFLP maps using a RIL mapping population. Genome 43:240–249

Tao Y, Jordan D, Henzell R, McIntyre C (1998) Identification of genomic regions for rust resistance in sorghum. Euphytica 103:287–292

Tao Y, Hardy A, Drenth J, Henzell R, Franzmann B, Jordan D, Butler D, McIntyre C (2003) Identifications of two different mechanisms for sorghum midge resistance through QTL mapping. Theor Appl Genet 107:116–122

Te-Beest D, Kirkpatrick T, Cartwright R (2004) Common and important diseases of grain sorghum. In: Espinoza L, Kelley J (eds) Grain sorghum production handbook. Univ Ark Coop Ext, USA MP297

Teetes GL, Becerra M, Peterson GC (1986) Sorghum midge (Diptera: Cecidomyiidae) management with resistant sorghum and insecticide. J Econ Entomol 79:1091–1095

Tenkouano A, Miller F, Frederiksen R, Rosenow D (1993) Genetics of non-senescence and charcoal rot resistance in sorghum. Theor Appl Genet 85:644–648

Tesso TT, Perumal R, Little CR, Adeyanju A, Radwan GL, Prom LK, Magill CW (2012) Sorghum pathology and biotechnology-a fungal disease perspective: Part II. Anthracnose, stalk rot, and downy mildew. Eur J Plant Sci Biotechnol 6:31–44

Tetreault HM, Grover S, Scully ED, Gries T, Palmer NA, Sarath G, Louis J, Sattler SE (2019) Global responses of resistant and susceptible sorghum (*Sorghum bicolor*) to sugarcane aphid (*Melanaphis sacchari*). Front Plant Sci 10:145

Tian T, You Q, Zhang L, Yi X, Yan H, Xu W, Su Z (2016) SorghumFDB: sorghum functional genomics database with multidimensional network analysis. Database 2016: baw099

Tsedaley B, Adugna G, Lemessa F (2016) Distribution and importance of sorghum anthracnose (*Colletotrichum sublineolum*) in southwestern and western Ethiopia. Plant Pathol J 15:75–85

Tuberosa R (2012) Phenotyping for drought tolerance of crops in the genomics era. Front Physiol 3:347

Tugizimana F, Djami-Tchatchou AT, Fahrmann JF, Steenkamp PA, Piater LA, Dubery IA (2019) Time-resolved decoding of metabolic signatures of in-vitro growth of the hemibiotrophic pathogen *Colletotrichum sublineolum*. Sci Rep 9:1–12

Tuinstra MR, Soumana S, Al-Khatib K, Kapran I, Toure A, van Ast A, Bastiaans L, Ochanda NW, Salami I, Kayentao M (2009) Efficacy of herbicide seed treatments for controlling striga infestation of sorghum. Crop Sci 49:923–929

Tyagi D, Wang H, Huang W, Hu L, Tang Y, Guo Z, Ouyang Z, Zhang H (2020) Recent advances in two-dimensional-material-based sensing technology toward health and environmental monitoring applications. Nanoscale 12:3535–3559

Upadhyaya H, Ortiz R (2001) A mini core subset for capturing diversity and promoting utilization of chickpea genetic resources in crop improvement. Theor Appl Genet 102:1292–1298

Upadhyaya H, Pundir R, Dwivedi S, Gowda C, Reddy VG, Singh S (2009) Developing a mini core collection of sorghum for diversified utilization of germplasm. Crop Sci 49:1769–1780

Upadhyaya HD, Wang Y-H, Sharma R, Sharma S (2013a) Identification of genetic markers linked to anthracnose resistance in sorghum using association analysis. Theor Appl Genet 126:1649–1657

Upadhyaya HD, Wang Y-H, Sharma R, Sharma S (2013b) SNP markers linked to leaf rust and grain mold resistance in sorghum. Mol Breed 32:451–462

Van Rensburg N, Hamburg HV (1975) Grain sorghum pests: an integrated control approach. Entomological society of Southern Africa: proceedings of the first congress of the entomological society of Southern Africa, 1974, Stellenbosch. Entomol Soc South Afr, pp 151–162

Vanti GL, Nargund VB, Vanarchi R, Kurjogi M, Mulla SI, Tubaki S, Patil RR (2019) Synthesis of *Gossypium hirsutum*-derived silver nanoparticles and their antibacterial efficacy against plant pathogens. Appl Organomet Chem 33:e4630

Venkateswaran K (2003) Diversity analysis and identification of sources of resistance to downy mildew, shoot fly and stem borer in wild sorghums. PhD thesis. Department of Genetics, Osmania University, Hyderabad, Andhra Pradesh, India

Venkateswaran K, Elangovan M, Sivaraj N (2019) Chapter 2: origin, domestication and diffusion of *Sorghum bicolor*. In: Aruna C, Visarada KBRS, Bhat BV, Tonapi VA (eds) Breeding Sorghum for diverse end uses. Woodhead Publishing Series in Food Science, Technology and Nutrition, Elsevier, pp 15–31

Vikal Y, Kaur A, Jindal J, Kaur K, Pathak D, Garg T, Singh A, Singh P, Yadav I (2020) Identification of genomic regions associated with shoot-fly resistance in maize and their syntenic relationships in the sorghum genome. PLoSOne 15:e0234335

Visarada K, Kishore NS (2007) Improvement of Sorghum through transgenic technology. Information system for biotechnology news report (Virginia tech, US), Rajendranagar, India, pp 1–3

Visarada K, Padmaja P, Saikishore N, Pashupatinath E, Royer M, Seetharama N, Patil J (2014) Production and evaluation of transgenic sorghum for resistance to stem borer. Vitro Cell Dev Biol Plant 50:176–189

Wagh AD, Gahukar SJ, Gangurde SS (2016) Marker assisted selection for shoot-fly tolerance in sorghum, *Sorghum bicolor*. Biosci Biotechnol Res Commun 9:179–188

Wang ML, Zhu C, Barkley NA, Chen Z, Erpelding JE, Murray SC, Tuinstra MR, Tesso T, Pederson GA, Yu J (2009) Genetic diversity and population structure analysis of accessions in the US historic sweet sorghum collection. Theor Appl Genet 120:13–23

Wang F, Zhao S, Han Y, Shao Y, Dong Z, Gao Y, Zhang K, Liu X, Li D, Chang J (2013) Efficient and fine mapping of RMES1 conferring resistance to sorghum aphid *Melanaphis sacchari*. Mol Breed 31:777–784

Waniska R, Venkatesha R, Chandrashekar A, Krishnaveni S, Bejosano F, Jeoung J, Jayaraj J, Muthukrishnan S, Liang G (2001) Antifungal proteins and other mechanisms in the control of sorghum stalk rot and grain mold. J Agric Food Chem 49:4732–4742

Wegulo S, Carlson M (2011) Ergot of small grain cereals and grasses and its health effects on humans and livestock. University of Nebraska, Extension, EC1880

Weir TL, Park S-W, Vivanco JM (2004) Biochemical and physiological mechanisms mediated by allelochemicals. Curr Opin Plant Biol 7:472–479

Werner K, Friedt W, Ordon F (2005) Strategies for pyramiding resistance genes against the barley yellow mosaic virus complex (BaMMV, BaYMV, BaYMV-2). Mol Breed 16:45–55

Williams R, Rao K (1981) A review of sorghum grain moulds. Intl J Pest Manag 27:200–211

Witcombe J, Hash C (2000) Resistance gene deployment strategies in cereal hybrids using marker-assisted selection: gene pyramiding, three-way hybrids, and synthetic parent populations. Euphytica 112:175–186

Worrall EA, Hamid A, Mody KT, Mitter N, Pappu HR (2018) Nanotechnology for plant disease management. Agronomy 8:285

Xu GW, Magill C, Schertz K, Hart G (1994) A RFLP linkage map of *Sorghum bicolor* (L.) Moench. Theor Appl Genet 89:139–145

Yano M, Tuberosa R (2009) Genome studies and molecular genetics from sequence to crops: genomics comes of age. Curr Opin Plant Biol 12:103–106

Yazawa T, Kawahigashi H, Matsumoto T, Mizuno H (2013) Simultaneous transcriptome analysis of Sorghum and *Bipolaris sorghicola* by using RNA-seq in combination with de-novo transcriptome assembly. PLoS ONE 8:e62460

Yin K, Qiu J-L (2019) Genome editing for plant disease resistance: applications and perspectives. Philos Trans Roy Soc Lond B Biol Sci 374:1–8

Yin H, Song CQ, Suresh S, Kwan SY, Wu Q, Walsh S, Ding J, Bogorad RL, Zhu LJ, Wolfe SA (2018) Partial DNA-guided *Cas9* enables genome editing with reduced off-target activity. Nat Chem Biol 14:311

Yohannes T, Abraha T, Kiambi D, Folkertsma R, Hash CT, Ngugi K, Mutitu E, Abraha N, Weldetsion M, Mugoya C (2015) Marker-assisted introgression improves striga resistance in an Eritrean farmer-preferred sorghum variety. Field Crops Res 173:22–29

Yohannes T, Ngugi K, Emanuel A, Abraha T, Yao N, Asami P, Ahonsi M (2016) Genotypic variation for low striga germination stimulation in *Sorghum* (L.) moench landraces from eritrea. Amer J Plant Sci 7:2470–2482

Young W, Teetes G (1977) Sorghum entomology. Annu Rev Entomol 22:193–218

Zhang J, Khan SA, Heckel DG, Bock R (2017a) Next-generation insect-resistant plants: RNAi-mediated crop protection. Trends Biotechnol 35:871–882

Zhang Y, Ngu DW, Carvalho D, Liang Z, Qiu Y, Roston RL, Schnable JC (2017b) Differentially regulated orthologs in sorghum and the subgenomes of maize. Plant Cell 29:1938–1951

Zhao Y, Li X, Chen W, Peng X, Cheng X, Zhu S, Cheng B (2011) Whole-genome survey and characterization of MADS-box gene family in maize and sorghum. Plant Cell Tiss Org 105:159–173

Zhu H, Muthukrishnan S, Krishnaveni S, Wilde G, Jeoung JM, Liang GH (1998) Biolistic transformation of sorghum using a rice chitinase gene [*Sorghum bicolor* (L.) Moench-*Oryza sativa* L.]. J Genet Breed 52:243–252

Zou G, Zhai G, Feng Q, Yan S, Wang A, Zhao Q, Shao J, Zhang Z, Zou J, Han B (2012) Identification of QTLs for eight agronomically important traits using an ultra-high-density map based on SNPs generated from high-throughput sequencing in sorghum under contrasting photoperiods. J Exp Bot 63:5451–5462

# Chapter 6
# Genomic Designing for Biotic Stress Resistance in Pearl Millet [*Pennisetum glaucum* (L.) R. Br.]

**C. Tara Satyavathi, Supriya Ambawat, Subaran Singh, Charu Lata, Shalini Tiwari, and Chandra Nayaka Siddaiah**

**Abstract** Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a major staple crop of 90 million poor people and is grown on 27 million ha area in arid and semi-arid tropics of Asia and Africa. It is a mutipurpose crop with excellent nutritional and medicinal values. It is a rich source of energy and micronutrients like iron, zinc and vitamins and gluten free with low glycemic index. Pearl millet is affected by different biotic stresses such as fungal, bacterial and viral diseases as well as attack by major insects like shoot fly, stem borer, grasshopper, termite, white grub, grey weevil, cut worm etc. like other cereals resulting in yield losses to the tune of 10–60%. Thus, it is necessary to understand genetics of host plant resistance, pathogen variability and its mechanism of action using advanced tools. Further, there is a need to develop new insect and disease resistant genotypes using genomic tools there by reducing cost of cultivation, environmental pollution and reducing yield losses. There has been a lot of progress in pearl millet genetic improvement using genetic resource conservation and evaluation along with conventional and modern approaches to overcome biotic and abiotic stresses which helped in achieving high level of productivity, quality and profitability. Recently reported genome sequence information and several genomic studies signify the need to further exploit its beneficial attributes. Hence, use of

C. Tara Satyavathi (✉) · S. Ambawat
ICAR-AICRP on Pearl Millet, Jodhpur, Rajasthan 342304, India
e-mail: c.satyavathi@gmail.com

S. Singh
College of Horticulture, Maharana Pratap Horticultural University, Karnal, Haryana 132001, India

C. Lata
CSIR-National Institute of Science Communication and Information Resources, 14 Satsang Vihar Marg, New Delhi 110067, India

S. Tiwari
CSIR-National Botanical Research Institute, Rana Pratap Marg, Lucknow 226001, India

C. N. Siddaiah
Pearl Millet Pathology Laboratory, University of Mysore, Manasagangotri, Mysore, India

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021

C. Kole (ed.), *Genomic Designing for Biotic Stress Resistant Cereal Crops*,
https://doi.org/10.1007/978-3-030-75879-0_6

modern genomic tools and genomic designing approaches including, transcriptomics, proteomics, metabolomics, genome editing etc. is very much desired for gene identification, trait mapping to understand several complicated gene pathways and their interactions in order to better identify different genes governing biotic stresses.

**Keywords** Pearl millet · Biotic stress · Disease resistance · Downy mildew · Blast · Genomic designing

## 6.1 Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is the 6th most important and potential cereal to ensure food security for around 90 million poor people living in arid and semi-arid tropics of Asia and Africa. It is a dual purpose crop extensively used for food, bird feed, silage, hay, building material and fuel (Shivhare and Lata 2017). It has a very good nutritional profile as well as is a rich source of energy (361 kcal/100 g) and neutraceutical properties. It has 5–6% oil and possess high contents of micronutrients such as iron, zinc and vitamins (Malik 2015). It is gluten free with low glycemic index and thus extremely useful for persons suffering from celiac disease or diabetes.

Biotic stresses caused by organisms like virus, bacteria, fungi, insects, pest, birds, weeds are major constraints to agricultural production worldwide (Mishra et al. 2017). Pearl millet is also susceptible to these biotic stresses like other cereals. Since millets are primarily grown in dry climates, hence millets comparatively have a lower risk of biotic stress than other crops. However, estimated loss of grains in millets is enormous. Generally, in comparison to other infections, millets suffer more from fungal diseases (Das and Rakshit 2016). Different fungal infections like downy mildew, blast, rust, ergot and smut are believed to have a more serious effect on growth and yield of pearl millet in comparison to other pathogens (Sharma et al. 2020a; Shivhare and Lata 2017). Genetic resource conservation and evaluation along with conventional and modern approaches are used for national pearl millet improvement programme to overcome abiotic and biotic stresses to maintain higher productivity, quality and profitability. Like other cereals, pearl millet also is affected by various biotic stresses like fungal, bacterial and viral diseases as well as attack by major insects like shoot fly, stem borer, grasshopper, termite, white grub, grey weevil, cut worm etc. Worldwide, around 100 diseases have been accounted in pearl millet which are mainly caused by fungi, bacteria, viruses and nematodes leading to major loss to yield potential and quality reducing the market value. Downy mildew (*Sclerospora graminicola*), blast (*Magnoporthe grisea*), smut (*Moesziomyces penicillariae*), rust (*Puccinia substriata* var. indica) and ergot (*Claviceps fusiformis*) are major diseases of economic importance in pearl millet (Raj et al. 2014). Out of these, downy mildew or 'green ear' which is caused by *Sclerospora graminicola* (Sacc. Schroet.) is a major distressing disease resulting

into maximum yield losses of 10–60% (Kumar et al. 2012). Since few years, blast disease is also reported in many states of India making it a major disease affecting economic yield in Pearl millet.

With increasing environmental awareness, various viable and sustainable alternatives have been used to manage different plant diseases and insects (Kumar 2008). Use of resistant varieties is the most preferred strategy to increase and disease management in almost all crops. Extensive series of strategies are used for resistance to infection and diseases against different pathogenic organisms while several integrated pest management (IPM) modules are used to control insects. Effective screening methods, use of diverse germplasm, identification of resistant sources, understanding of genetics of resistance, knowledge of virulence variability, use of effective resistance breeding and monitoring of performance of cultivars at field level are some of the effective strategies used in resistance breeding to conquer the biotic stresses. Different breeding methods i.e. recombination/backcross, mutation as well as modern biotechnological approaches can prove useful for integration of resistance/tolerance genes and to obtain a number of inter specific crosses and identification of genomic regions. Conventional breeding has played a major role in gaining extensive success towards improvement of biotic stress resistance in pearl millet. In the past few years, molecular breeding and functional genomics were used in pearl millet to enhance yield in adverse conditions but still there are several possibilities to further improve this crop by using advanced genomic tools and approaches. Hence, it is necessary to understand genetics of host plant resistance, pathogen variability and the mechanism of action using genomic designing approaches to combat different biotic stresses.

## 6.2 Major Biotic Stresses in Pearl Millet

### *6.2.1 Major Diseases of Pearl Millet*

In Pearl millet, about 50 diseases caused by various biotic factors are accounted in India but very few are significant. These include downy mildew, blast, rust, ergot and smut which reduce grain yield leading to severe yield losses. In addition, ergot affects grain quality. Using resistant cultivars is the major cost-effective way to control diseases in pearl millet. Understanding epidemiology of diseases, screening techniques have been designed to easily discriminate between resistant and susceptible genotypes. Several selections from germplasm accessions have shown a high degree of stability for resistance over the years for various diseases in pearl millet.

#### 6.2.1.1 Downy Mildew

Downy mildew is an important disease of pearl millet caused by *Sclerospora graminicola*. *Sclerospora* spp. is originated in Africa (Brunken et al. 1977). It is supposed that area of origin of the host and the pathogen are same. Hence, downy mildew pathogen was linked to pearl millet since around 3500 BC which was also proved as the majority of recurrent sources of downy mildew resistance belong to Africa. There is also a possibility that this pathogen originated in indigenous Indian grasses and shifted to pearl millet at the time of its initiation from Africa. Shaw (1981) recommended a temperate origin for *S. graminicola* due to its circumpolar with *Setaria* species and thus it became adapted for plants thriving in tropical habitats, specifically for pearl millet. He preached that *S. graminicola* is primitive, but was circumpolar with *Paniceae* from Pleistocene times as it would have co-evolved at many places along with species of *Setaria*, *Chaetochloa*, *Pennisetum and Panicum*. Plants infected with *S. graminicola* are generally stunted and often undergo a transformation of flower organs into leaves (phyllody or witches' broom), resulting in serious yield loss.

Symptoms

The disease is known by two names, 'downy mildew' and 'green year' due to two types of symptoms that develop during systemic infection. The symptoms generally appear on the second leaf and subsequently all leaves and panicles also develop symptoms. Leaf symptoms first appear as chlorosis (yellowing) at the base of the leaf lamina and successively younger leaves show a progression of greater leaf area coverage by symptoms. Half-leaf symptoms characterized by a distinct margin between the diseased and non-diseased area towards the tip occur in pearl millet. Under conditions of high (>95%) relative humidity (RH) and moderate temperature (20–22°C), massive asexual sporulation occurs on infected chlorotic areas, generally on the abaxial surface of leaves, giving them a downy appearance.Severley infected plants are generally stunted and donot produce panicles. Green ear symptoms become visible at panicle emergence. Green ears develop because floral parts are transformed into leafy structures, which may vary in shape and size.The transformation may be partial or total, dependingon when the panicle is colonized by the pathogen. The leafy structure are chlorotic, and sometimes produce sporualtion. In latent infections, green ear is the only manifestaion of the disease. The oospores can remain viable in the soil for 8 months to 10 years or more causing primary infection in host plants. Sporangia are responsible for secondary spread of disease by producing both asexual (sporangia, zoospores) and sexual spores (oospores). Haustoria of the pathogen take nutrition from the host cell. The hyphae are developed in the tissues which can later produce numerous asexual spores on the lower surface of leaves. The pathogen starts sexual reproduction for producing oospores once the sporulation is over.

Symptoms of downy mildew

### Extent of Damage

Downy mildew or green ear disease is linked with pearl millet since long time (Butler 1907). Initially, the disease was limited only to the local cultivars and landraces. It was not epidemic until the introduction of $F_1$ hybrids. Later in 1970s and 1980s, downy mildew epidemics caused extensive yield losses in India and grain yield losses of up to 10–60% were reported. It can reduce yield up to a large extent and this was proved when grain production of pearl millet was reduced from 8 mt in 1970–71 to 5.3 mt in 1971–72 in India after cultivating popular hybrid HB 3. This reduction was very large and yield was even reduced by 60–70% in some fields. Genetically uniform single-cross $F_1$ hybrids become easily susceptible in comparison to heterogeneous open-pollinated varieties causing severe losses (Thakur et al. 2006).

### Chemical Control

Use of fungicides is not very popular in pearl millet as it is mainly cultivated in marginal conditions by resource poor farmers. Downy mildew pathogen is an oomycete organism and cannot be controlled by using normally recommended fungicides like other fungal diseases. Instead, treatment of seed with fungicide is more effective as it is possible to apply it easily before sowing. Thiram and Captan are the common fungicides used in pearl millet for seed treatment as they also act as seed protectants. Metalaxyl, a systemic fungicide, is very effective for downy mildew and has unique combinations of residual and systematic properties but it is a narrow range oomyceticide. This is extremely vigorous under both in vitro and in vivo for downy mildew pathogen in case of pearl millet. It acts by inhibiting

protein and ergosterol synthesis by interfering rRNA synthesis. It is systemic in nature and has defensive and remedial action and taken up through the stems, leaves and roots. It is accessible in form of Ridomil MZ 72, Apron 35 SD, Master 72% WP (Metalaxyl 8% + Mancozeb 64%). Upon treating seeds with Metalaxyl (35% WS) at 6 g/kg, disease is controlled effectively for first 35 DAS. Further, it was also observed that chitosan nanoparticles have higher degree of acetylation and induce resistance against pearl millet downy mildew (Siddaiah et al. 2018).

Biological Control

Bioagents such as *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus* species can be used to control downy mildew in an effective way. *Bacillus pumilus* INR 7, *Pseudomonas fluorescens* UOM SAR14, *Trichoderma harzianum* Th UOM 1and *Bacillus pumilus* SE 34 are some of the potential bioagents. Bioagents of *Trichoderma harzianum* (20 g/kg seeds) and *Pseudomonas fluorescens* and *Bacillus* species (10 g/kg seeds) were used for seed treatment as talc formulation (www.aicpmip.res.in, www.aicrp.icar.gov.in/pearl).

Integrated Disease Management

Integrated disease management (IDM) has become popular and implemented in many crops and it can be also useful for pearl millet. Thus, genetically uniform hybrids having plant resistance should be controlled using suitable management practices like crop rotation, prophylactic seed dressing chemicals which can broaden the economic value of hybrids (Hash et al. 1997, 1999; Witcombe and Hash 2000; Hash and Witcombe 2002). This strategy is also being followed by ICAR-AICRP on Pearl millet. IDM module having half dose of metalaxyl (3 g/kg seed), host plant resistance (moderate level), Chitosan (2.5 g/kg seed) and PGPR strain of *Bacillus pumilus* INR7 (8 g/kg seed) is being used or recommended for the management of this disease.

#### 6.2.1.2 Blast

Blast disease caused by *Magnaporthe grisea* is prevalent in pearl millet growing states of India since 1970. The disease incidence data from 2002–2016 shows that blast has become more widespread (www.aicpmip.res.in, www.aicrp.icar.gov.in/pearl) and its incidence increased in almost all pearl millet growing states like Rajasthan, Gujarat, Uttar Pradesh, Madhya Pradesh, Maharashtra, Delhi, Karnataka, Andhra Pradesh, Telangana and Tamil Nadu. The disease is reported by various testing centres of ICAR-AICRP on Pearl millet for over a decade and is observed on most of the entries evaluated in pathological trials. During 2016,

*Magnaporthe* blast incidence became very severe in the states of Rajasthan, Uttar Pradesh, Delhi and Maharashtra. Seed treatment with Carbendazim combined with Metalaxyl was recommended (Nayaka et al. 2017a). The disease becomes more severe under humid and warm conditions.

Extensive yield losses in pearl millet grain and forage were reported due to *Magnaporthe* blast and productivity and quality of the pearl millet crop was found to be negatively correlated with grain-plot yield, dry matter yield and digestive dry matter (Wilson and Hanna 1992; Wilson and Gates 1993; Timper et al. 2002). The data on evaluation of disease incidence, disease rating scale, severity, grain yield and fodder loss, photosynthetic efficiency of foliage, crop loss in terms of grain yield, effect on the metabolic activities, dry matter content loss etc. is also accessible (Satyavathi et al. 2019, 2020; Satyavathi 2020).

### Symptoms

*Magnaporthe* blast symptoms in pearl millet begin with tiny specks or lesions which broaden and turn necrotic, leading to widespread chlorosis and untimely drying of young leaves. Initially, lesions appear near the leaf tips or leaf margins or both and extend down towards the outer edges. Young lesions are usually pale green to greyish green and later turns yellow to grey. Foliage lesions are elliptical or diamond-shaped; approximately 2.5–3.5 × 1.5–2.5 mm. Centers of lesion are grey and water-soaked initially when fresh but later becomes brown surrounded by a chlorotic halo and ultimately turn necrotic appearing like concentric rings (Kato 2001). These symptoms appear from seedling to flowering stage on leaf, boot-leaf and stem.

Symptoms of blast

Chemical Control

*Magnaporthe* blast disease can be controlled by several fungicides and large scale foliar application is generally used at field level. Two sprays of carbendazim 0.05% (ICBR 1:3.85) or 1 g/l at 15 days intervals from the initiation of the disease are recommended to control the disease (Singh and Pavgi 1974). Many fungicides like benomyl, diclocymet, felimzone, metominostrobin, pyroquilon, carpropamid and iprobenfos are used against blast disease (Kato 2001). Tricyclazole (5-methyl-1,2,4-triazolo [3,4-b] [1,3] benzothiazole) which is target specific to *Magnaporthe* blast has been widely tested and recommended. It inhibits the biosynthetic pathway of melanin compound present in *M. grisea* conidia (Kurahashi 2001). Probenazole (3-allyloxy-1,2-benzothiazole1,1-dioxideor, 3-allyloxy-1,2-benz[d]isothiazole) is also useful in controlling blast disease as it activates plant defense system (Iwata 2001). Isoprothiolane (di-isopropyl 1, 3-dithiolan-2-ylidenemalonate) (Choline biosynthesis fungicides) is also recommended for blast disease and it acts by targeting fungal membrane phosphatidylcholine synthesis (Uesugi 2001). Azoxystrobin, a strobilurin fungicide inhibits fungal respiration by binding to the cytochrome b complex III at the Q0 site in mitochondrial respiration and provides protection against blast. In an experiment conducted in ICAR-AICRP on Pearl millet pathology trials, efficacy of different fungicides against blast in pearl millet revealed that spray application of Trifloxystrobin + Tebuconazole75WG @ 0.05% first at initiation of disease and 2nd spray at 15 days interval significantly reduced the blast incidence in grain and fodder pearl millet (www.aicpmip.res.in, www.aicrp.icar.gov.in/pearl).

Biological Control

Biocontrol agents such as *Trichoderma harzianum* and *Pseudomonas fluorescens* were used to control *Pyricularia* blast disease (Krishnamurthy and Gnanamanickam 1998), *Bacillus subtilis*, *Bacillus pumilus* also proved promising in controlling blast pathogen via biocontrol and induction of resistance (Yoshihiro et al. 2003). In addition, *Streptomyces* species were also found to be effective for blast disease management (Zarandi et al. 2009). Studies have also indicated that biological control agents like *T. harzianum*, *P. fluorescens*, *B. Subtilis* and *B. pumilus* could be quite useful in management of *Pyricularia* blast (www.aicpmip.res.in, www.aicrp.icar.gov.in/pearl).

#### 6.2.1.3 Rust

Rust in pearl millet is caused by *Puccinia substriata var. penicillariae.* (*Zimm.*). It is usually believed to be a less important disease in many pearl millet growing regions as compared to downy mildew, blast, smut and ergot as it appears subsequent to the grain-filling stage, leading to very less or no loss to the grain yield. It is

of key significance worldwide in forage hybrids as high rust severities may also lead to substantial losses of forage quality by reducing digestible dry matter yield. Symptoms of rust firstly come into sight on lower side of leaves in form of typical pustules of reddish brown powder (uredospores). Afterwards, dark brown teliospores are formed. Symptoms generally appear on upper surface of leaves and stem as well but they can also appear on both sides of the leaves. Large pustules are developed on leaf blades and sheaths of highly susceptible cultivars. Screening for rust is done by spraying uredospores collected from infector rows on 25–30 days old crop, at 25 and 35 DAS and extending of uredinia-containing leaves among test plants 25–30 days old.

Symptoms of rust

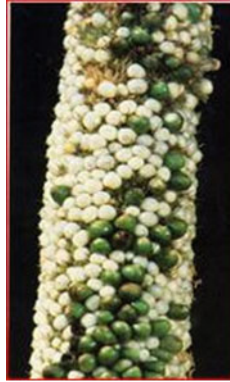
Disease Management

Resistant hybrids/varieties should be cultivated and sowing must be done when monsoon starts. Demolition of collateral hosts such as *Ischaemum pretosum* and *Panicum maximum* on the field bunds were also quite useful. Further, dusting with fine sulphur @ 17 kg as well as two sprays of 0.2% Mancozeb at the interval of 15 days is the useful strategy for disease control.

#### 6.2.1.4 Smut

Smut disease in pearl millet is caused by *Moesziomyces penicillariae* (Bref.). Economic yield losses to an extent of a 5–20% grain yield are reported. The disease ocurs during September/October and the early sown crop is usually escaped by this infection. Oval to conical sori of bright green colour are produced initially by the infected florets which later change to brown/black.

Disease screening is based on inoculation of panicles by injecting aqueous suspension of sporidia ($1 \times 10^6$/ml) in boot, covering the inoculated panicles with parchment paper/selfing bags. Providing high humidity (>80% RH) with the use of an overhead sprinkler, normally two times in a day, 30 min each at 10 am and 5 pm during normal days and removal of bags 15–20 days subsequent to inoculation and scoring of panicles for smut severity with the help of a standard smut severity assessment key are the steps followed during screening.



Symptoms of smut

Disease Management

Resistant hybrids/varieties must be cultivated. Spraying of panicle at boot leaf stage using Captafol followed by Zineb can reduce infection and helpful in disease management. In addition, smutted ears must be removed from the field to prevent proliferation of infection.

#### 6.2.1.5 Ergot

Ergot in pearl millet is caused by *Claviceps fusiformis* (Loveless). This disease is simply recognized as a honeydew material of creamish to light pink color oozing from infected florets having several conidia. The droplets get dried out in form of hard dark black structures after two weeks and protrude out from the florets instead of grain are known as sclerotia. Loss in grain yield depends on severity of infection because infected seed is completely altered into sclerotium. Conditions of weather during the flowering time determine the disease occurrence and its spread.

Symptoms of ergot

Disease screening is done by putting selfing bag on panicles at the boot-leaf stage which is a good practice. Further, inoculation with an aqueous conidial suspension ($1 \times 10^6$ conidia $ml^{-1}$) generated from honey dew of infected panicles should be done after 3–4 days by opening the bags slightly and spraying the panicles at full protogynous stage. In addition, overhead sprinklers must be used twice a day for 30 min each at 10 am and 5 pm for providing high humidity on normal days can be very effective and after two weeks of inoculation the bags are removed and ergot severity is scored with the help of a standard key.

Disease Management

Washing of seed in 2% salt water along with mechanical removal of sclerotia from seed are quite effective to manage the disease. Adjustment of sowing dates is important to prevent coincidence of ear emergence and more rainy days. Three foliar applications of Thiram 0.2% or Copper Oxychloride 0.25% or Ziram @ 0.2% are effective in disease management.

## 6.2.2 *Major Insect Pests of Pearl Millet*

Shoot fly, grey weevil and white grub are some of the major pearl millet insect pests. Insect pest incidence is relatively lesser in most pearl millet growing regions in India. Thus, formal breeding programs targeting resistance to insects are not yet available. The cultivars in the advanced stage of testing are screened against important insect pests. The cultural control measures have been worked and several recommendations have been generated to minimize the insect damage (www.aicpmip.res.in, www.aicrp.icar.gov.in/pearl).

#### 6.2.2.1 White Grub (*Holotrichia consanguinea*)

White grub is seen in pearl millet cultivating regions of Gujarat and Rajasthan states. Roots of the growing seedlings are attacked by the grubs and lead to shrinkage of the whole plants. Patchy gaps are created resulting into poor or uneven plant stand. Adults emerge from May to July with pre-monsoon/monsoon shower and feed on pearl millet flowers and grains in the milky stage. It causes mainly 5–25% damage in Rajasthan.

Major insect pests of pearl millet (*Source* www.aicpmip.res.in, www.aicrp.icar.gov.in/pearl)

Incidence of white grub can be reduced by using pigeon pea and sunflower for inter-cropping. Collecting and destroying adults just after first showers at the time of their visit to Neem/*Acacia* trees before mating is quite helpful. Carbofuran 3 G @ 12 kg/ha should be applied to seed furrows during sowing to control this insect. At the onset of monsoon or within 2–3 days of receiving first monsoon showers, the host trees should be sprayed with Carbaryl 0.2% or Chlorpyriphos 0.2%, which proved to be very useful (www.aicpmip.res.in, www.aicrp.icar.gov.in/pearl).

#### 6.2.2.2 Shoot Fly (*Atherigona approximata*)

Pearl millet shoot fly is seen in Tamilnadu and Gujarat. Larvae cause "dead heart" by cutting the growing point during seedling stage whereas they attack ear heads and cut down panicles during advanced stage. Late sown crop faces more infestation. Sowing the crop early with start of monsoon or generally within first 10–15 days of first showers, avoiding staggered sowing in the close proximity—are some of the good strategies to control this insect. Transplanting must be preferred for late sown crop but if direct seeding is taken up, 4 kg seed/ha along with thinning of affected seedlings must be followed up. The crop should be sprayed with 0.07% Endosulfan at 10 and 20 days after germination if incidence of shoot fly is heavy in endemic areas. 4% dust of Endosulfan is sufficient in areas with water scarcity (www.aicpmip.res.in, www.aicrp.icar.gov.in/pearl).

#### 6.2.2.3 Grass Hoppers [*Hieroglyphus nigrorepletus* (Bolivar)]

The grass hoppers lay **e**ggs at a depth of 75–200 mm in the soil. Adults and hoppers attack the foliage leading severe damage to the crop. Adults have short wings and are able to fly only to short distances. For pest management, weed free farming along with deep ploughing to expose "egg pods" are very effective. Crumbing of bunds, clean farming and dusting the field by 4% Endosulfan or Fenvalerate dust @ 25 kg/ha or 0.07% of Endosulfan are quite useful.

#### 6.2.2.4 Termites (*Odentotermes obesus*)

It is a social insect which flourishes in colonies in ground. It attacks young seedlings and mature plants. Infected plants shrivel and eventually die. After harvesting of the crop, deep ploughing along with collection and burning of plant refuge should be practiced to minimize termite effect. Well decomposed FYM and timely irrigation of the crop are very useful for termite management. Application of Chloropyriphos 20 EC @ 1.25 l/Endosulfan 35 EC @ 2.5 l in standing crop besides irrigation water is also recommended to manage termites (www.aicpmip.res.in, www.aicrp.icar.gov.in/pearl).

#### 6.2.2.5 Grey Weevil (*Myllocerus* spp.)

This insect is polyphagous in nature. Adult beetles eat green leaves and cause severe harm during infestation on seedlings. Malathion (5%), Methyl Parathion (2%) or Quinalphos (1.5%) @ 25 kg/ha should be dusted at the time of emergence for effective pest management.

#### 6.2.2.6 Ear Head Bug (*Calocoris angustatus*)

It is generally found in South India. Sap of tender grains is sucked by nymphs and adult bugs during milking stage making the seeds withery. Infestation of the pest can be controlled by early planting. Use of Endosulfan 4D @ 20 kg/ha or Carbaryl 50 SP @ 3 kg in 500 l of water/ha on pearl millet panicles has proven to be very useful against ear head bug.

#### 6.2.2.7 Stem Borers (*Chilo partellus*)

It is a nocturnal moth having dirty brown colour. Foliage is attacked by caterpillars and later they bore into the stem to cause “Dead heart” ultimately boring into ear heads.

#### 6.2.2.8 Hairy Caterpillar (*Amsacta moorei*)

This pest is normally found in Gujarat and semi-arid regions of Rajasthan. It attacks the crop in an intermittent way. Heavy defoliation of the plants is usually done by the larvae. Spraying of Endosulfan (35 EC @ 0.1%) and treatment with *Trichogramma chilonis* @ 75,000/ha/week are very useful to manage caterpillars.

#### 6.2.2.9 Blister Beetle (*Mylabris postulate*)

It mainly attacks different parts of plants. Grain formation is usually affected by the attack of adult beetle on flowers and tender panicles. Spraying the panicles with Endosulfan 4D @ 20 kg/ha or Carbaryl 50 SP @ 3 kg in 500 l of water/ha are quite effective to control beetles.

#### 6.2.2.10 Chaffer Beetle

Using light trap (200 W electric bulb/ha or petromax) up to 15 days at the time of 50% flowering stage or during the emergence of pest can be highly helpful.

#### 6.2.2.11 Cut Worm (*Agrotis ipsilon*)

Caterpillars usually attack the seedlings/cut seedlings on soil level. In case of severe attack, re-sowing is done. Spraying of Endosulfan (35 EC @ 0.1%) and dusting with *Trichogramma chilonis* @ 75,000 per ha/week are very effective.

The following recommendations are obtained from different entomological experiments conducted over years in ICAR-AICRP on Pearl millet against various pests (www.aicpmip.res.in, www.aicrp.icar.gov.in/pearl):

- Treatment of seed with imidacloprid 600 FS @ 8.75 ml/kg seed followed by dusting with fenvalerate 0.4% @ 20 kg/ha or spray of 5% NSKE after 35 days of germination was found to be economically viable and most effective for the management of shoot fly and stem borer in pearl millet. (Based on three years of testing in ICAR-AICRP on Pearl millet entomology experiments of 2009–2011)
- Treatment of seed with imidacloprid 600 FS @ 8.75 ml/kg seed followed by spraying with imidacloprid 17.8 SL 0.009% at 35 DAG was effective to control shoot fly and stem borer. Insecticide residues were also found to be beneath the detectable limit at 42 days after spraying. (Based on three years of testing in ICAR-AICRP on Pearl millet entomology experiments of 2012–14)
- Seeds of pearl millet can be stored for 6 months after mixing them with neem leaf powder @ 10 g/kg. With this treatment, lowest grain damage and lowest adult population of *Tribolium* spp. was observed. Viability of the seed was above MSCS level of 75%. (Based on three years of testing in ICAR-AICRP on Pearl millet entomology experiments of 2012–14)
- IPM module-III consisting of seed treatment with Imidacloprid 600 FS @ 8.75 ml/kg, installation of fish meal trap @ 10/ha and spraying of neem seed kernel extract 5% at ear head stage is recommended for the management of pest complex in pearl millet. (Based on three years of testing in ICAR-AICRP on Pearl millet entomology experiments of 2012–15)
- Seed treatment with Imidacloprid 600 FS @ 8.75 ml or Clothianidin 50 WDG @ 7.5 g/kg seed with sufficient quantity of water effectively controls the soil insect-pests (white grub and termite) infesting pearl millet. Treated seed should be sown within 2 h of treatment. (Based on three years of testing in ICAR-AICRP on Pearl millet entomology experiments of 2013–16)
- Use of diverse insecticides in pearl millet against shoot fly and stem borer revealed that seed treatment with clothianidin 50 WDG @ 7.5 g/kg seed along with spraying of fipronil 5 SC @ 0.01%, at 35 days after germination of crop, recorded lowest shoot fly incidence, highest grain and fodder yield. (Based on three years of testing in ICAR-AICRP on Pearl millet entomology experiments of 2015–18)
- Treatment of seed with imidacloprid 600 FS @ 8.75 ml/kg, fish meal trap @ 10/ha, removal of shoot fly dead hearts, spraying of dimethoate 30 EC 0.03% at 35 DAG exhibited lowest shoot fly % during ear head stage along with highest grain and fodder yield. It also depicted lowest white grub and termite % damage.

(Based on three years of testing in ICAR-AICRP on Pearl millet entomology experiments of 2016–18)

- Treatment of seed with imidacloprid 600 FS @ 8.75 ml/kg, fish meal trap @ 10/ha, spraying of novaluron 10 EC 0.01%, removal of shoot fly dead hearts at 35 DAG exhibited lowest stem borer % incidence and lowest *Helicoverpa* larval population during ear head stage. (Based on three years of testing in ICAR-AICRP on Pearl millet entomology experiments of 2016–18)

## 6.3 Genetic Resources of Resistance Genes

Researchers have gained success in identifying stable sources of germplasm of pearl millet for disease resistance including downy mildew, blast, rust, smut, ergot and a few of them were used in disease resistance breeding programs. Exploitation of germplasm of pearl millet for various biotic stresses is listed in Table 6.1. Pearl millet germplasm is available in ICRISAT, NBPGR and mini-core collection is also developed at ICRISAT. The 238 germplasm accessions of pearl millet mini-core of ICRISAT were screened under greenhouse conditions against five *M. grisea* pathotype-isolates (Pg118, Pg119, Pg56, Pg53 and Pg45). Resistance to multiple pathotypes (two or more) was recorded in several accessions, while three accessions (IP 7846, IP 11036, and IP 21187) exhibited resistance to four of the five blast isolates used for screening (Sharma et al. 2013). For blast and rust, the 305 *P. violaceum* accessions conserved in the genebank were assessed and resistant accessions were identified (Sharma et al. 2020b).

There is a need to explore additional sources of resistance through screening the pearl millet collections maintained at different breeding centres of pearl millet against major isolates/pathotypes.

In addition, genomic and genetic resources are also highly desired for mapping and mining of the blast resistance quantitative trait loci (QTLs) or genes. A pearl millet inbred germplasm association panel (PMiGAP) has been developed at ICRISAT from pearl millet core collection of over 2,000 accessions, breeding lines, cultivars and landraces representing main global diversity of pearl millet. It has been also sequenced recently using whole-genome re-sequencing (WGRS) strategy, generating over 25 million single nucleotide polymorphisms (SNPs). Phenotyping of the association mapping panel will be useful for precise genome-wide association mapping study (GWAS) and mining novel alleles for blast resistance at the blast hot-spot locations. Biparental and multiparental QTL mapping of the blast resistance genes can also be done for improvement. These available genetic and genomic resources can be further increased and utilized for mapping, mining and deployment of effective blast resistance (Sehgal et al. 2015). In addition, wild

**Table 6.1** Biotic stress resistant genotypes available in pearl millet

| Biotic stress | Genotypes | References |
|---|---|---|
| Downy mildew (*Sclerospora graminicola*) | ICML 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 ICMPE 13-6-30, 134-6-9, 134-6-34, 13-6-27, 37, 71; ICMA 92666, ICMB 92666, ICMA 91333, 91444, 91555 (resistant to downy mildew, smut and ergot), WGI 52, WGI 148, ICMR09999, ICMB 89111-P6 × ICMB 90111-P6 | Thakur et al. (1982), Willingale et al. (1986), Thakur and King (1988a, b), Thakur et al. (1992), Rai et al. (1998a), Khairwal and Yadav (2005), Satyavathi et al. (2016), Chelpuri et al. (2019) |
| Blast (*Magnaporthe grisea*) | IP 7846, IP 11036, IP 21187, IP 4291, IP 15256, IP 22449, IP 5964, IP 11010, IP 13636, IP 20577, IP 5964, IP 11010, IP 13636, IP 21525, IP 21531, 21536, 21540, 21594, 21610, 21640, 21706, 21711, 21716, 21719, 21720, 21721, 21724, 21987, 21988, 22160, IP 21544, IP 21720, IP 22269, IP 21544, ICMV 05555 × IP 21720, ICMB 94555, IP 21544, ICMB 97111, IP 21720; ICMB 02444, ICMB 02777, ICMB 06444, ICMB 93333, ICMB 96666, ICMB 97222, ICMB 99444, 863B, ICMR 06222, ICMB 95444 | Sharma et al. (2013, 2020b, c) |
| Rust (*Puccinia* spp.) | IP 16438, IP16762; P310-17, P1449-3; IP18292, IP18293, IP700651; ICML 12 to 16, 22; ICMP 312, 423, 85410; 7042S; 841A; IP 9, 55, 104, 262, 253, 346, 336, 498, 545, 558; landraces like Desi Bajri-Chomu, Dhodsar local, Ardi-Beniya Ka Bas, 81B-P6, ICMP 451-P8, IP 21629, 21645, 21658, 21660, 21662, 21711, 21974, 21975, and 22038 | Singh et al. (1997), Khairwal and Yadav (2005), Thakur et al. (2006), Sharma et al. (2007), Ambawat et al. (2016), Sharma et al. (2020b) |
| Smut (*Moesziomyces penicillariae*) | ICML 5 to 10; ICML 17 to 21; Tif leaf 3; Tift 3 (PI 547035) and Tift 4 (PI 547036); Tift 65 (resistant to leaf spot and rust) | Bourland (1987), Thakur and King (1988a), Wilson and Burton (1991), Burton and Wilson (1995), Hanna et al. (1997) |
| Ergot (*Claviceps fusiformis*) | ExB 46-1-2-S-2, ExB 112-1-S-1-1, ICMV 8282, 8283; ICMA 88006A and 88006B (resistant to downy mildew and smut); ICMA 91333 to 91555; ICML 5 to 10; SSC FS 252-S-4, ICMPS 100-5-1, | Thakur and King (1988c), Yadav and Duhan (1996), Rai et al. (1998b), Khairwal and Yadav (2005) |

(continued)

**Table 6.1** (continued)

| Biotic stress | Genotypes | References |
|---|---|---|
| | 700-1-5-4, 900-1-4-1, 900-3-1, 900-9-3, 1300-2-1-2, 1400-1-6-2, 1500-7-3-2, 1600-2-4, 1800-3-1-2, 2000-5-2; ICI 7517-S-1, ExB 132-2-S-5-2-DM-1, P-489-S-3; SSC 46-2-2-1, SC 77-7-2-3-1, SSC 18-7-3-1 | |

relatives of pearl millet germplasm can also be harnessed for germplasm enhancement and improving biotic stress tolerance in pearl millet (Sharma et al. 2020a, c).

## 6.4 Classical Genetics and Traditional Breeding

Plant pathological research in pearl millet started in India, when $F_1$ hybrids developed for commercial cultivation became susceptible for downy mildew in early 1970s. Hybrids are superior in comparison to open pollinated varieties in terms of uniformity in growth, short duration and grain yield leading to increased area under hybrid cultivation consequently favoring incidence of diseases. Downy mildew (DM) caused by an obligate parasite, *Sclerospora graminicola,* is economically most important disease of pearl millet in India and was first recorded by Butler in 1907. During 1971, popular hybrid HB 3 was widely affected by downy mildew epidemic and pathogenic variability for downy mildew was observed in 1973 when pearl millet hybrid NHB 3 was reported to be susceptible at Gulbarga and found to be resistant at Mysore (Bhat 1973; Shetty and Ahmad 1981). DM incidence is considered to vary in diverse hybrids and around 90% incidence was documented in farmers' fields (Thakur et al. 2003; Rao et al. 2007). A well defined program was started in the 1990s after observing increased incidences of pathogenic variability in *S.graminicola* and effect of downy mildew on hybrids.

During the past four decades, there has been lot of progress in development of extremely efficient lab and field screening techniques, development and identification of various downy mildew resistant cultivars including HHB 94, HHB 67 (improved), HHB 117, HHB 68, HHB 197, HHB 223, HC 4, HHB 256, HC 20, HC10 etc. Both conventional and molecular breeding approaches have been used successfully for DM resistance breeding program (Hash et al. 1999; Hash and Witcombe 2002). Three types of resistance to DM have been reported in pearl millet: complete resistance (Singh 1995), incomplete resistance (Singh et al. 1988) and recovery resistance (Singh and King 1988).

Conventional methods of breeding uses field screening or greenhouse procedures to integrate endurable levels of downy mildew resistance in parental lines, populations and open pollinated varieties having superior performance and quality

(Raj et al. 2014). Field screening relies on disease sick-plot, infector rows or perfo-irrigation system to create high humidity. It is refined time and again and is very effective if performed under appropriate management conditions. On the contrary, greenhouse screening method is independent of the season and thus can be operated throughout the year. It is also reproducible, easy, reliable, time-efficient and cost-effective. The entire procedure of maintenance of isolates, inoculation and incubation, inoculum multiplication has been refined very well and proved to be extremely useful to screen numerous breeding lines in shorter duration (Singh et al. 1993; Thakur et al. 2006).

Various methods like pure-line selection, pedigree selection, recurrent selection and backcrossing are used in breeding for resistance against various diseases. Breeding with downy mildew resistant seed parents like MS 5054A and MS 5141A was attained using backcrossing in the elite background of Tift 23 by IARI, New Delhi. On the other hand, induced mutations produced MS 5071 B from Tift 23 B while NHB series of hybrids having downy mildew resistance were produced using MS 5071 A (Kumar et al. 2012). Several effective resources and phenotypic screening techniques were developed and improved for screening and identification of virulence of downy mildew in pearl millet (Singh et al. 1997; Jones et al. 2002; Thakur et al. 2008), rust (Singh et al. 1997), smut (Thakur and King 1988b), ergot (Thakur et al. 1982) at ICRISAT and national research institutes like IARI, New Delhi and University of Mysore. A number of efforts have been put for identification of resistant local landraces and germplasm lines against the diseases using artificial epiphytotic conditions.

The importance of biological control for managing downy mildew disease has also been studied. Due to existence of pathogenic variability, concerns about fungicide resistance and lack of durable resistance, alternative and eco-friendly methods of DM control were searched. Activating plant's own defense mechanisms by specific biotic or abiotic elicitors was considered as a major step toward this. The effectiveness of cerebrosides (glycosphingolipids derived from different plant pathogens) against DM as resistance elicitors has been reported in pearl millet (Deepak et al. 2003). Further, detection of biochemical pathways and characterization of genes underlying resistance against different pathogens led to development of induced resistance (Shetty and Kumar 2000; Raj et al. 2005). In vitro culture techniques were also used for inducing desirable characterstics, genetic transformation and regeneration via embryogenesis (Srivastava and Kothari 2003; O'Kennedy et al. 2004).

Downy mildew resistance exhibits dominance over susceptible, additive or recessive traits but partial host plant resistance is governed by one or more genes as well as some modifiers (Hash and Witcombe 2001; Breese et al. 2002; Dwivedi et al. 2012). A total of 6 major pathotypes were accounted for downy mildew in India (Thakur et al. 2006). Several resistance genes have been successfully deployed to bestow near-complete resistance against specific races of pathogens using conventional breeding (Hovmøller et al. 1997; McDonald and Linde 2003; Hovmøller 2007). Significant success in breeding for DM resistance have been developed and exhibited by conventional pedigree breeding and several disease

resistant hybrids which have helped in controlling the widespread DM epidemics since 1990s (Khairwal et al. 2004; Thakur et al. 2006). Breeding for DM resistant pearl millet hybrids is one of the most cost effective disease management strategies and identification and use of new avirulence genes and their markers to develop biotic resistant varieties is quite essential. In addition, various advanced genomic and biotechnological tools have been used to identify resistant sources, QTLs and genes etc. for different biotic stresses (Shivhare and Lata 2017). Similarly, resistance to smut shows dominance and simple inheritance. Backcross breeding has been used to incorporate ergot resistant in both parent and pollinators. Thus, different combinations of practices of disease management proved useful in developing resistance against various diseases including host-plant resistance, chemical methods and cultural methods (Williams et al. 1981; Singh and Gopinath 1985).

## 6.5 Genetic Diversity Analysis

Development and use of various molecular markers like restriction fragment length polymorphism (RFLP), expressed sequence tag- simple sequence repeats (EST-SSRs), amplified fragment length polymorphism (AFLP), single strand conformational polymorphism-single nucleotide polymorphism (SSCP-SNP), conserved intron spanning primer (CISP) and diversity arrays technology (DArT) were used in pearl millet for genetic diversity, population structure analysis along with designing strategies for crop improvement with disease resistance at a faster rate and great precision (Hash et al. 2006; Jogaiah et al. 2014; Ambawat et al. 2016). However, few reports exist in pearl millet for identification and utilization of prospective genes against biotic stress resistance (Latha et al. 2006; Girgi et al. 2006). DNA marker-based genetic linkage mapping and detection of genomic regions will facilitate in identifying resistant sources and thus accelerate development of resistant sources. In addition to genetic markers, reference collections, minicore or core collections can be also very useful in pearl millet for identification of new resources for biotic stress resistance.

Genetic diversity and pathogen variability in isolates of *S. graminicola* were also studied with different molecular markers such as random amplified polymorphic DNAs (RAPDs) (Zahid 1997; Jogaiah et al. 2008), amplified fragment length polymorphism (AFLP) (Sivaramakrishnan et al. 2003; Pushpavathi et al. 2006), inter-simple sequence repeat (ISSR) (Jogaiah et al. 2008; Sudisha et al. 2009). Inter-simple sequence repeat (ISSR) primers were used by Sudisha et al. (2009) to illustrate pathogen variability; while 20 rapid amplified polymorphic DNA (RAPD) and 19 ISSR markers characterized 27 downy mildew isolates into 6 major pathotypes (Jogaiah et al. 2008). Likewise, 46 downy mildew isolates were grouped into 21 pathotypes based on latent period, disease incidence, virulence index and among these pathotype P11 was reported as the most virulent (Sharma et al. 2010). Similarly, after assessing 48 pearl millet inbred lines against nine diverse *S. graminicola* isolates collected from 5 diverse geographical locations of India, it was

established that gene pyramiding can increase resistance against different downy mildew isolates (Hash et al. 2006). Similarly, 39 native endophytic actinomycetes isolates from pearl millet roots were analyzed for their proteolytic action against downy mildew and it was proved that seven strains were able to repress sporangium formation in *S. graminicola* directly defining to be prospective and potential biocontrol agents (Jogaiah et al. 2016). Recently, 305 accessions of Pennisetum violaceum, a wild relative of pearl millet, were screened under greenhouse conditions against five pathotype-isolates of *M. grisea* and a local isolate of *P. substriata* var. indica to identify diverse sources of blast and rust resistance and based on the mean blast score (1–9 scale), 17 accessions (IP 21525, 21531, 21536, 21540, 21594, 21610, 21640, 21706, 21711, 21716, 21719, 21720, 21721, 21724, 21987, 21988, and 22160) were found resistant (score $\leq$ 3.0) to all five pathotypes and 24 accessions were resistant to four pathotypes of M. grisea (Sharma et al. 2013, 2020b).

## 6.6 Association Mapping Studies

Association mapping which is also called linkage disequilibrium (LD) mapping is a useful novel platform for allele mining. It can use different ancestral recombination events among germplasm collections or natural populations to trace marker-phenotype associations useful for crop improvement. It is less time consuming, efficient, less laborious and generates 1000s of recombinants giving rise to a diverse and large gene pool making it more useful as compared to QTL associated linkage mapping. It has been used in pearl millet as well as many other crops for detection of useful markers or genes related to biotic stress resistance (Senthilvel et al. 2010; Rajaram et al. 2013; Jogaiah et al. 2014). A PMiGAP comprising 346 lines was developed from 1000 landraces, parents of mapping population, diverse cultivars from different regions of Asia and Africa that can provide new insights for allele mining of favorable genes along with fine mapping of QTLs for important agronomic traits (Sehgal et al. 2015). Recently, a diversity panel consisting of 250 accessions collected from over 20 different countries was screened under natural epiphytotic conditions in five environments and a total of 43 resistant genotypes were found to have high and stable resistance against foliar blast disease of pearl millet (Sankar SM et al. 2021).

## 6.7 Molecular Mapping of Resistance Genes and QTLs

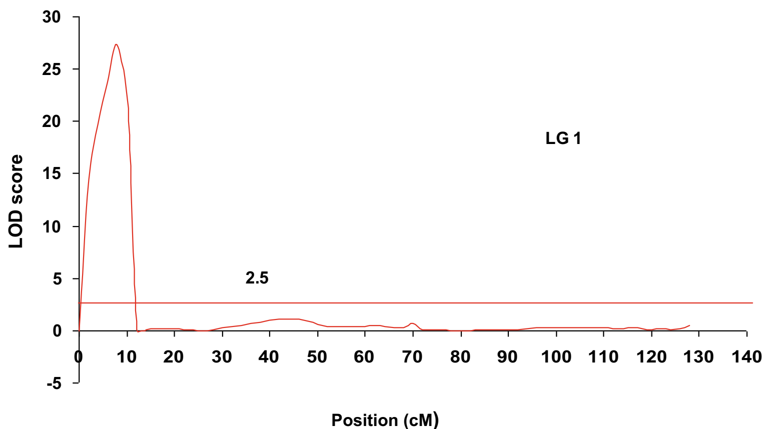
Numerous reports on DNA-marker based genetic linkage maps have been accounted in pearl millet which facilitated gene manipulation of disease resistance (Liu et al. 1994; Hash et al. 1995; Jones et al. 1995, 2002; Breese et al. 2002; Hash and Witcombe 2002; Ambawat et al. 2016). DNA markers have been established

for around 60 different putative DM resistance QTLs in pearl millet (Jones et al. 1995, 2002; Hash et al. 1999; Breese et al. 2002; Hash and Witcombe 2002; Breese et al. 2002; Gulia et al. 2007) rust and blast resistance (Morgan et al. 1998). Numerous mapped QTLs were found to be transmitted to backgrounds of elite inbred parental lines derived from a very well known single-cross hybrid HHB 67 (843A × H77/833-2) via RFLP-based MABC method. Different QTLs were identified for resistance against downy mildew in pearl millet (Jones et al. 2002; Breese et al. 2002; Gulia et al. 2007), rust and blast resistance (Morgan et al. 1998; Ambawat et al. 2016). The mapping population of the parents WGI 52 and WGI 148 developed for Delhi isolate (Sg 561) revealed seven linkage groups during linkage analysis and mapped 51 SSR loci at a minimum LOD score of 2.5 and a maximum recombination fraction of 0.5. The linkage map covered a map distance of 418.43 cM on the basis of Kosambi function with an average adjacent-marker interval length of 8.205 cM and two QTLs were identified. Further, a linkage map was also developed using the parents WGI 148 X ICMR09999 against the Rajasthan isolate (Sg 384) and the linkage analysis revealed seven linkage groups that mapped 48 SSR loci. The genetic linkage map of Pearl millet constructed using 48 SSR loci covered 228.2 cM of map distance with a marker density of 4.754 cM at a minimum LOD score of 2.5 and a maximum recombination fraction of 0.5 and four QTLs were identified. Using Gujarat Banaskanta isolate (Sg445), two QTLs identified for imparting resistance against Rajasthan isolate (Sg 384) using SSR markers (Satyavathi et al., unpublished data in DBT report 2016). Recently developed pearl millet whole genome sequence can provide several opportunities for crop improvement programs as it can be used to exploit various functional genes and assist in molecular breeding approaches (Varshney et al. 2017). Further, continued research is highly desired for pearl millet to map the different QTLs identified currently. A draft genome sequence of *S. graminicola pathotype*1 of 299,901,251 bp length was assembled having N of 17,909 bp with minimum of 1 Kb scaffold size and overall coverage of 40× (Nayaka et al. 2017b). It had 47.2% GC content with 26,786 scaffolds and scaffold size of 238,843 bp was found to be longest among these. Similarly, Prakash et al. ( 2019) sequenced genome of *Magnaporthe grisea* strain PMg_Dl and generated 13.1 Gb PE reads (number of reads, 43,962,401), 3.4 Gb mate-paired reads (number of reads, 17,160,010), and 1.1 Gb PacBio reads (number of reads, 148,768). Morgan et al. (1998) identified molecular markers for three rust loci and one *Magnaporthe* resistance locus in pearl millet. Further, RAPDs and RFLPs were used to screen three segregating populations and only one RAPD marker (OP-D11700, 5.6 cM) was found to be linked to *Magnaporthe* leaf spot resistance. Three molecular markers (SCAR-G8, OP-K19, and OPD11) were used to identify a plant carrying the Rr1 resistance gene from Tift 89D2, and *Pyricularia* resistance from *P. glaucum* sp. Monodii. This can be further used to develop more efficient markers for QTL locus, establish *Magnaporthe* resistance gene to a linkage group and identify *Magnaporthe* resistance loci. Advanced backcross QTL approach can be very helpful to incorporate quantitative blast resistance to popular hybrids to develop biotic stress resistant hybrids. Chelpuri et al. (2019) constructed a genetic linkage map comprised of 53 loci on 7

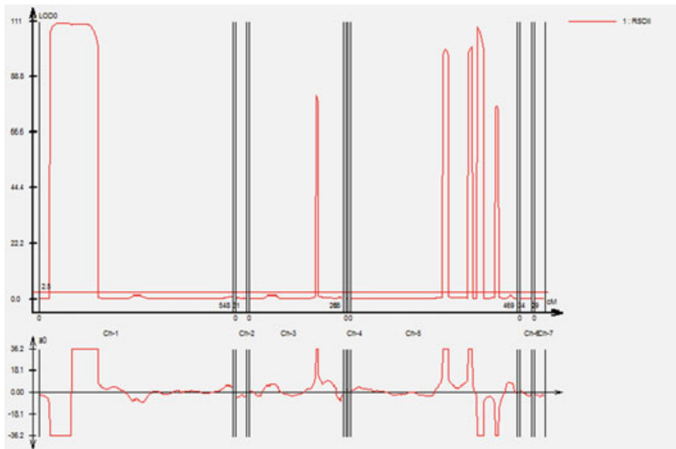
linkage groups (LGs) spanning 903.8 cM length and 18.1 cM as average adjacent marker distance. It was used further to identify 5 QTLs contributed by the resistant parent ICMB 90111-P6 showing large effect for resistance against 3 diverse pathotype isolates of *S. graminicola* collected from Haryana (Sg519), Rajasthan (Sg526) and Gujarat (Sg445). Isolate Sg445 gave one QTL while 4 QTLs were detected 2 each from Sg519 and Sg526 on LG4 with LOD scores varying from 5.1 to 16.0, elucidating a huge range (16.7–78.0%) of the phenotypic variation (R2). Table 6.2 depicts details of various QTLs linked with disease resistance in pearl millet.

**Table 6.2** QTLs associated with important traits under different diseases in pearl millet

| QTLs | Linkage group | Associated trait | References |
|---|---|---|---|
| Downy mildew (DM-QTL) | Linkage Group-1 and 4 | Downy mildew | Jones et al. (1995) |
| Downy mildew (DM-QTL) | Linkage Group-4 | Downy mildew | Gulia et al. (2007) |
| Downy mildew (DM-QTL) | Linkage Group-1, 2 and 3 | Downy mildew | Satyavathi et al. unpublished data in DBT report 2016 |
| Downy mildew (DM-QTL) | Linkage Group-1, 3 and 4 | Downy mildew | Chelpuri et al. (2019) |
| QTL | Linkage Group-1 | Rust | Ambawat et al. (2016) |
| QTL | – | Blast and rust | Morgan et al. (1998) |



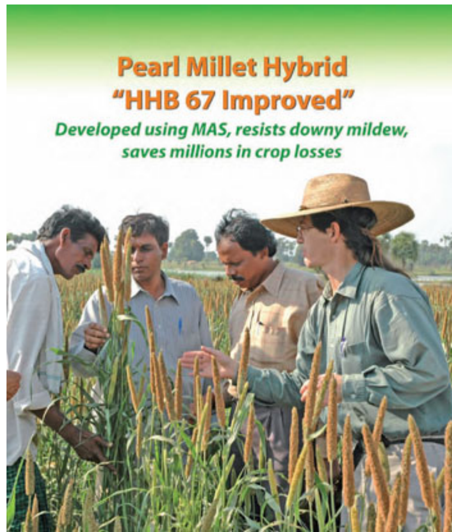

Logarithm of odds (LOD) profiles for LG1 for rust resistance QTLs segregating in the (81B-P6 X ICMP 451-P8)-based pearl millet RIL population (Adapted from Ambawat et al. 2016)



Seven QTLs for Downy mildew resistance for Rajasthan isolate detected by interval mapping on pearl millet chromosome 1, chromosome 3 and chromosome 5 in the $BC_2F_2$ population of the cross WGI 148 × ICMR 09999 [Adapted from Satyavathi et al. 2016]

## 6.8 Marker-Assisted Breeding for Resistance Traits

Marker-assisted backcross breeding was used to improve the capability and efficacy of breeding for downy mildew resistance (Hospital et al. 1992; Hash et al. 1999). Marker -assisted backcrossing was used first time to develop and commercialize downy mildew resistant version of HHB67 in field crops in public domain in India (Hash et al. 2006). Resistant donors can be crossed with a popular pearl millet variety and $BC_2F_3$ backcross lines can be analyzed for disease resistance, candidate defense genes and association mapping etc. Selections can be done on the basis of partial resistance at disease hotspots and genotyping for candidate defense genes bestowing partial resistance and gene pyramiding may be done for major genes for resistance. Previously, DArT platform has been developed using 95 different genotypes by following *Pst*I/*Ban*II complexity reduction which can be effectively used for genome organization and comparative genomic studies as cost of marker-assisted backcrossing (MABC) using these markers is lower in comparison to other markers (Supriya et al. 2011).




HHB 67-improved: a downy mildew resistant version of HHB67 developed by MAS

## 6.9 Map-Based Cloning of Resistance Genes

Various biotechnological tools for breeding have been developed and used during the last 10–15 years for pearl millet improvement as described by Bollam et al. (2018) and Ambawat et al. (2020). Several molecular maps, DArT libraries (Supriya et al. 2011; Ambawat et al. 2016), EST libraries (Senthilvel et al. 2008; Rajaram et al. 2013), bacterial artificial chromosome (BACs) library (Allouis et al. 2001), are now available and using these numerous quantitative trait loci (QTLs) linked to different diseases were identified as mentioned in Table 6.2 which may further be used in crop improvement programs.

## 6.10 Genomics-Aided Breeding for Resistance Traits

Sequence characterized amplified region (SCAR) markers derived from ISSRs were developed and pearl millet genotypes namely ICMR-01007 (P1) and ICMR-01004 (P2) and their population were screened for downy mildew resistance (Jogaiah et al. 2014). All these efforts would be highly useful to understand the genetics underlying resistance and efficacy of specific QTLs among diverse resistant lines and will be quite useful for resistance breeding. Transcriptomic analysis using NGS tool was also performed in pearl millet in order to discover the mechanisms underlying downy mildew resistance (Kulkarni et al. 2016). In this study, a total of 1000 and 1591 transcripts were found in inoculated/susceptible control, respectively. Further, 1396

up regulated and 936 down regulated transcripts were also identified among resistant inoculated/resistant control. This study revealed that in resistant genotypes in pearl millet, the up-regulation of genes of phenylpropanoid pathway along with induced impending hypersensitive response and systemic acquired resistance are the promising defense mechanisms against downy mildew infection. QTLs were identified for resistance to *Pyricularia* leaf spot disease using Genotyping by sequencing (GBS). A GBS platform having 83,875 SNP markers was deployed using 500 genotypes of pearl millet with *Pst*I-*Msp*I reduced representation libraries (Hu et al. 2015). Similarly, GBS platform was used and 333,567 sequence tags and 16,650 SNPs across the 7 chromosomes have been identified for leaf spot resistance (Punnauri et al. 2016). GBS was also used for identification of genomic regions associated with *striga* resistance (Moumouni et al. 2015). Other advanced technologies including high throughput sequencing, genome editing, gene silencing, insertional mutagenesis, targeted induced local lesion in genomes (TILLING) and transgenics can be crucial to improve our knowledge on complex disease resistance mechanisms.

## 6.11 Recent Concepts and Strategies Developed

Stress biology, genomics and bioinformatics collectively can help to develop stress resistant crops. For the production of superior crop varieties with stress tolerance and yield, various crop improvement strategies such as genetic maps, NGS, GWAS, GBS, expression profiling, synteny studies, QTL mapping, candidate gene identification and genetic engineering technologies have been used (Tiwari and Lata 2019). Genome editing has emerged as a very powerful instrument of functional genomics.

Globally researchers are working towards improving biotic stress resistance in peal millet through various approaches. The positive impacts of gene editing and nanotechnology on the improvement of biotic stress resistance in pearl millet are listed briefly below.

### *6.11.1 Genome Editing*

Recently, genome editing using targeted nucleases has been used as an important approach for improving various crops, promising major yield increases in the near future. In gene editing, techniques like clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR associated protein (Cas), zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) successfully revolutionized the gene alteration method via adding important traits or by eliminating undesirable traits, in plants for functional studies of genes and crop improvement (Tiwari and Lata 2019). These targeted nucleases open up new

prospects to develop improved crop varieties including pearl millet. Chanwala et al. (2020) conducted genome-wide study of WRKY transcription factors in pearl millet and speculated that *PgWRKYs* can be used to strengthen crops traits including biotic stress resistance using genome editing tools for improved production and future food safety. Gene editing technology definitely is a very promising technology for improving stress resistance in pearl millet.

### *6.11.2 Nanotechnology*

Besides gene editing, nanotechnology-based approaches for better crop growth and yield have also gained attention. Among various applications of nanotechnology, use of nanofertilizers plays an important role for precision farming. In pearl millet, chitosan nanoparticles (CNP) have been tested for their efficacy via nitric oxide generation against downy mildew disease (Siddaiah et al. 2018). Besides synthetic nanoparticles, usage of biogenic or green synthesized nanoparticles in pearl millet exists. The seed priming and foliar application of green synthesized zinc oxide (ZnO) nanoparticles (ZnNP) extracted from *Eclipta alba* decreases the incidence of downy mildew in pearl millet (*Pennisetum glaucum*) by inhibiting *Sclerospora graminicola* zoospore germination (Nandhini et al. 2019). In an earlier investigation, Nandini et al. (2017) controlled the extent of downy mildew disease in *P. glaucum* by applying the *Trichoderma*-mediated selenium nanoparticles (SeNP). Recently Nandini et al. (2020) also formulated trichogenic-lipid nanoemulsion and used it against pearl millet downy mildew disease that offers a newer way to manage biotrophic pathogens.

## 6.12 Genetic Engineering for Biotic Stress Resistance in Pearl millet

Downy mildew is one of the most devastating pearl millet diseases caused by *Sclerospora graminicola*, that attacks panicles causing extreme yield losses in Africa and India (Kumar et al. 2012). In a study, 529 accessions of wild *Pennisetum* species investigated in greenhouse and field-disease nurseries for downy mildew resistance, out of which 223 accessions were found disease free (Singh and Navi 2000). Recently, two downy mildew resistant varieties namely, SOSAT-C88 (LCICMV1) and SUPER SOSAT (LCICMV3) were released for North Eastern Nigeria (Ajeigbe et al. 2020). Researchers also successfully developed the transgenic lines of pearl millet to combat the adverse effect of downy mildew. A study documented the established transgenic pearl millet lines expressing *pin* gene,

**Table 6.3** Functional validation of biotic stress (downy mildew) responsive genes in pearl millet

| Gene | Gene function | Source | References |
|---|---|---|---|
| *afp* | Synthesis of antifungal protein AFP | *Aspergillus giganteus* | Girgi et al. (2006) |
| *NPR1* | Non-expresser pathogenesis related gene | *Brassica juncea* | Ramineni et al. (2014) |
| *CC-NBS-LRR* | Plant disease resistance | *Pennisetum glaucum* | Veena et al. (2016) |
| *EIRE*, *ELI-Box 3*, *BoxW1*, *WUN* motif, *WRKY* | Biotic stress resistance | *Pennisetum glaucum* | Chanwala et al. (2020) |

exhibits high resistance towards downy mildew pathogen (Latha et al. 2006). Similarly, Veena et al. (2016) generated transgenic via overexpressing coiled-coil-nucleotide-binding-site-leucine rich repeat (CC-NBS-LRR) gene from *P. glaucum*in response to downy mildew pathogen. In order to develop downy mildew resistant pearl millet crop researchers overexpressed *AFP* and *NPR1* gene from heterologous system *Aspergillus giganteus* and *Brassica juncea*, respectively (Ramineni et al. 2014; Girgi et al. 2006). Nowadays, blast has increased alarmingly in pearl millet (Nagaraja and Das 2016). Rapid spreading of this disease might be due to the reason that the initial infection most probably derives from weeds or other collateral hosts and also due to the rapid change in the pathogenicity of this fungus. Rust is another common leaf disease that decreases grain yield and adversely affects the biomass and quality of pearl millet (Sharma et al. 2020a). Apart from these foliar diseases, ergot (*Claviceps* sp.) and smut (*Moesziomyces penicillariae*) that are tissue-specific diseases (especially ovary), also infect pearl millet (Shivhare and Lata 2017). Efficient field screening methods are already available with ICRISAT (Das and Rajendrakumar 2016). Table 6.3 gives details of the genes involved in downy mildew resistance in pearl millet.

## 6.13 Role of Bioinformatics as a Tool

The availability of vast datasets through various 'omics' technologies can be effectively used to classify and functionally characterize candidate genes to be used for biotic stress resistance in genomics-assisted breeding or transgenic technology. After the whole genome sequencing of the pearl millet genome (Varshney et al. 2017), the task remains to classify thousands of genes crucial for the response and tolerance against both abiotic and biotic stresses. Additionally, for a better understanding of the population structure, genetic diversity, evolution, domestication and stress resistance of this essential crop, 994 pearl millet genotypes that included 963 inbred lines and single plants of 31 wild accessions were also resequenced. Other than the whole genome sequencing, transcript profiling through high throughput

NGS approaches and several bioinformatics approaches to understand various gene families were also taken up. De novo transcriptome sequencing was performed to explore the candidate genes involved in interaction between pearl millet and downy mildew pathogen (*Sclerospora graminicola* Sacc.) (Kulkarni et al. 2016). Further, comparative transcriptome analysis of a tolerant pearl millet genotype at two development stages and between two contrasting genotypes for terminal drought tolerance also led to the identification of several differentially expressed genes involved in salicylic and jasmonic acid pathways as well as flavonoid pathway which are well known to be associated with biotic stress resistance (Shivhare et al. 2020a, b). Zala et al. (2017) developed EST-SSR markers associated with resistance of downy mildew in *P. glaucum* and added them to the repository of molecular markers for pearl millet that can be employed for downy mildew resistance breeding. Details of all DNA-based molecular markers associated with biotic stress resistance in pearl millet are listed in Table 6.4. Genome-wide profiling of cytosine DNA methylation showed that salicylic acid induces defense pathways over seedling development in pearl millet (Ngom et al. 2017). Further, genome-wide identification and expression analysis of WRKY gene family in pearl millet led to the identification of various cis elements associated with biotic stress response such as EIRE, ELI-Box 3, BoxW1, WUN motif in the promoter regions of several WRKY genes highlighting the important role of WRKY genes in imparting biotic stress resistance to the crop (Chanwala et al. 2020). In addition, a compound called G_app7, purified from *Ganoderma applanatum* was found to be effective in

**Table 6.4** Summary of DNA based markers developed in pearl millet for biotic stress tolerance

| DNA markers | References |
|---|---|
| 53 SSRs loci were mapped on 7 LGs spanning 903.8 cM length with 18.1 cM as average adjacent marker distance; 5 co-localized QTLs were identified on LG 4 for DM resistance | Chelpuri et al. (2019) |
| SSRs were developed against DM resistance in *P. glaucum* and QTLs were mapped on LG 1 covering a total length of 101.1 cM and on LG 4 with a length of 91.4 cM | Taunk et al. (2018) |
| 2419 EST-SSRs markers were developed and 230 markers based on their function in downy mildew-pearl millet interaction were validated in 12 pearl millet genotypes which are parents of mapping population | Zala et al. (2017) |
| 229 DArT markers were developed and QTL linked with rust was identified on LG 1 in pearl millet | Ambawat et al. (2016) |
| 51 SSR loci were mapped and QTLs linked to downy mildew were identified | Satyavathi et al. (2016) |
| 333,567 sequence tags and 16,650 SNPs were identified across all 7 chromosomes for leaf spot resistance using GBS platform | Punnauri et al. (2016) |
| GBS has been also used for identification of genomic regions associated with *striga* resistance | Moumouni et al. (2015) |
| SCAR markers derived from ISSRs were developed for downy mildew resistance in pearl millet and used for QTL mapping | Jogaiah et al. (2014) |

inhibition of *S. graminicola*, as well as the seed treatment with G_app7 protects *P. glaucum* from downy mildew (Jogaiah et al. 2016). Overall, bioinformatics tools and softwares help us to identify genes, proteins and metabolites involved in different molecular and signaling network and also helps to decipher the molecular networking among them to reveal the possible mechanism within the cell.

## 6.14 Social, Political and Regulatory Issues

Pearl millet is a highly nutritious dual purpose cereal with several advantages. In spite of the inherent advantages, it has been paid little attention in comparison to other cereals. Changing climatic scenario, drudgery, market policy, rancidity, drought, diseases and insects are some of major key constraints for pearl millet production and promotion. A rapid advancement in the development of hybrids and varieties addressing these constraints is required taking into consideration farmer's practices and market acceptability. Host resistance against different diseases has been exploited to a large extent and presently the farmers' use resistant varieties. However, the shift in virulence pattern of the pathogen, especially in endemic areas is causing concern and need to be addressed. The long-lasting success and usefulness of disease resistance breeding depends on various factors such as availability and type of pathogen, virulence diversity, genetic resistance types, methods of screening, selection environment, utilization and deployment and monitoring resistance/virulence. Numerous factors such as inadequate accessibility of desired genes in cultivated gene pool, incompatibility barriers among different crosses of wild species and pearl millet, sterility among hybrids, lesser number of mapping reports on identification of avirulence genes, initiation of polyploidy to lessen the differences at ploidy level in order to obtain interspecific hybrids are some of the hindrances towards development of resistant hybrids.

Thus, there is a need to accelerate the development of a combination of pearl millet innovations to put into farmers hands that at finite will result in sustainable productivity enhancement of pearl millet for food security and future generation.

## 6.15 Future Perspectives

Substantial research has been done to understand host-pathogen relations, improving methods of disease screening, identification and use of sources of resistance and breeding for development of adequate parental lines and hybrids which are resistant to different diseases. Even then, biotic stresses are the main challenge for gaining high yield potential of hybrids and thus efforts are needed to develop breeding programs to deliver high yielding pearl millet cultivars resistant to diseases and insect pests with adapted and resilient farmer's practices and crop management strategies. Studying and transferring of different characters like

apomixis and perenniality for improving quality of fodder should be highly focused. Diversification of gene pools to reduce genetic vulnerability, identification of disease resistance genes/QTLs against specific isolates, developing near isogenic lines as host-differentials, exploiting wild germplam using pre-breeding and identification of genetic markers for avirulence are some of the other areas need to be focused. Further, gene pyramiding, molecular tagging of disease resistant genes, marker-assisted breeding, trait genetics, association mapping, transcriptomics and proteomics, high throughput assays and NGS techniques are some of the advanced tools and strategies highly desired to develop resistant varieties.

In addition, tissue culture technique and doubled-haploid breeding technology could also be helpful for studying genetic diversity and inheritance of resistance among hybrid parental lines to develop isolate specific resistant inbreds in a shorter duration. It is also necessary to identify and utilize sources of multiple disease resistance, collecting and characterizing different isolates, monitoring virulence and pathogen variability through on-farm surveys and virulence nurseries at different locations to overcome the biotic stresses. Use of recent phenotyping and genotyping approaches will be highly useful to exploit and evaluate natural genetic variations in the germplasm to identify/validate major quantitative traits loci (QTLs) to understand pathogen variability and development of resistance to major biotic stresses.

## References


AICRP on Pearl millet. www.aicpmip.res.in, www.aicrp.icar.gov.in/pearl

Ajeigbe HA, Angarawai II, Inuwa AH, Akinseye FM, Abdul AT (2020) Hand book on improved pearl millet production practices in North Eastern, Nigeria. International Institute of Tropical Agriculture, IITA Abuja Station, Kubwa, Abuja FCT, Nigeria, p 10

Allouis S, Qi X, Lindup S, Gale MD, Devos KM (2001) Construction of a BAC library of pearl millet, *Pennisetum glaucum*. Theor Appl Genet 102(8):1200–1205

Ambawat S, Senthivel S, Hash CT, Nepolean T, Rajaram V, Eshwar K et al (2016) QTL mapping for rust resistance in pearl millet using an integrated DArT and SSR-based linkage map. Euphytica 209:461–476. https://doi.org/10.1007/s10681-016-1671-9

Ambawat S, Subaran Singh S, Meena RC, Satyavathi CT (2020) Biotechnological applications for improvement of the pearl millet crop. In: Gahlawat SK, Punia S, Siroha AK, Sandhu KS, Kaur M (eds) Pearl millet: properties, functionality and its applications. Taylor & Francis (CRC Press), Boca Raton, Finland, pp 115–138. https://doi.org/10.1201/9780429331732-7

Bhat SS (1973) Investigation on the biology and control of *Sclerospora graminicola* on bajra. PhD thesis, University of Mysore, Mysore, Karnataka, India, 165 p

Bollam S, Pujarula V, Srivastava RK, Gupta RK (2018) Genomic approaches to enhance stress tolerance for productivity improvements in pearl millet. In: Biotechnologies of crop improvement, vol 3. Springer, Berlin/Heidelberg, Germany, pp 239–264

Bourland FM (1987) Registration of ICML11 rust resistant pearl millet germplasm. Crop Sci 27:367

Breese WA, Hash CT, Devos KM, Howarth CJ (2002) Pearl millet genomics-an overview with respect to breeding for resistance to downy mildew. In: Leslie JF (ed) Sorghum and millets diseases. Iowa State Press, Ames, Iowa, USA, pp 243–246

Brunken JN, de Wet JMJ, Harlan JR (1977) The morphology and domestication of pearl millet. Econ Bot 31:163–174
Burton GW, Wilson JP (1995) Registration of Tift 65 parental inbred line of pearl millet. Crop Sci 35:1244
Butler EJ (1907) Some diseases of cereals caused by *Sclerospora graminicola*. Memoirs of the Department of Agriculture. India Bot Ser 2:1–2
Chanwala J, Satpati S, Dixit A, Parida A, Giri MK, Dey N (2020) Genome-wide identification and expression analysis of WRKY transcription factors in pearl millet (*Pennisetum glaucum*) under dehydration and salinity stress. BMC Genom 21(1):1–16
Chelpuri D, Sharma R, Durga KK, Katiyar P, Mahendrakar MD, Singh RB, Yadav RS, Gupta R, Srivastava RK (2019) Mapping quantitative trait loci (QTLs) associated with resistance to major pathotype-isolates of pearl millet downy mildew pathogen. Eur J Plant Pathol 154 (4):983–994
Das IK, Rajendrakumar P (2016) Disease resistance in sorghum. In: Das IK, Padmaja PG (eds) Biotic stress resistance in millets. Academic Press, Cambridge, Massachusetts, pp 23–67
Das IK, Rakshit S (2016) Millets, their importance, and production constraints. In: Das IK, Padmaja PG (eds) Biotic stress resistance in millets. Academic Press, Cambridge, Massachusetts, pp 3–19
Deepak SA, Raj SN, Umemura K, Kono T, Shetty HS (2003) Cerebroside as an elicitor for induced resistance against the downy mildew pathogen in pearl millet. Ann Appl Biol 143:169–173
Dwivedi S, Upadhyaya H, Senthilvel S, Hash CT (2012) Millets: genetic, and genomic resources. In: Janick J (ed) Plant breeding reviews. Wiley, Hoboken, NJ, pp 247–375
Girgi M, Breese WA, Lörz H, Oldach KH (2006) Rust and downy mildew resistance in pearl millet (*Pennisetum glaucum* (L.) R. Br.) mediated by heterologous expression of the *afp* gene from *Aspergillus giganteus*. Transgen Res 15:313–324. https://doi.org/10.1007/s11248-006-0001-8
Gulia SK, Hash CT, Thakur RP, Breese WA, Sangwan RS (2007) Mapping new QTLs for downy mildew [*Sclerospora graminicola* (Sacc.) J. Schroet.] resistance in pearl millet [*Pennisetum glaucum*(L.) R. Br.]. In: Singh DS, Tomar VS, Behl RK, Upadhyaya SD, Bhale MS (eds) Crop production in stress environments-genetic and management options. Agrobios International, New Delhi
Hanna WW, Hill GM, Gates RN, Wilson JP, Burton GW (1997) Registration of tif leaf 3 pearl millet. Crop Sci 37:1388
Hash CT, Witcombe JR (2001) Pearl millet molecular marker research. Intl Sorghum Millets Newsl 42:8–15
Hash CT, Witcombe JR (2002) Gene management and breeding for downy mildew resistance. In: Leslie JE (ed) Sorghum and millets diseases. Iowa State Press, Ames, Iowa, USA, pp 27–36
Hash CT, Cavan GP, Bidinger FR, Howarth CJ, Singh SD (1995) Downy mildew resistance QTLs from a seedling heat tolerance mapping population. Intl Sorghum Millets Newsl 136:66–67
Hash CT, Witcombe JR, Thakur RP, Bhatnagar SK, Singh SD, Wilson JP (1997) Breeding for pearl millet disease resistance. In: Proceedings of international conference on genetic improvement of sorghum and pearl millet held from 22–27 September 1996 at Lubbock, Texas, USA, pp 337–373
Hash CT, Singh SD, Thakur RP, Talukdar BS (1999) Breeding for disease resistance. In: Khairwal IS, Rai KN, Andrews DJ, Harinarayana G (eds) Pearl millet breeding. Oxford & IBH, New Delhi, India, pp 337–379
Hash CT, Sharma A, Kolesnikova-Allen MA, Singh SD, Thakur RP, Raj AGB, Ratnajirao M NV, Nijhawan DC, Beniwal CR, Sagar P, Yadav HP, YadavYP, Srikant, Bhatnagar SK, KhairwalI S, Howarth CJ, Cavan GP, Gale MD, Liu C, Devos KM, Breese WA, Witcombe JR (2006) Teamwork delivers biotechnology products to Indian small-holder crop-livestock producers : Pearl millet hybrid “HHB 67 Improved” enters seed delivery pipeline. J SAT Agric Res 2: 16-20

Hospital F, Chevalet C, Mulsant P (1992) Using markers in gene introgression breeding programmes. Genetics 132:1199–1210
Hovmøller MS (2007) Source of seedling and adult plant resistance to *Puccinia striiformis* f. sp. *tritici* in European wheats. Plant Breed 126:225–233
Hovmøller MS, Ostergard H, Munk L (1997) Modelling virulence dynamics of air-borne plant pathogens in relation to selection by host resistance. In: Crute IR, Holub E, Burdon JJ (eds) The gene-for-gene relationship in plant parasite interactions. CAB International, Wallingford, Oxon, UK, pp 173–190
Hu Z, Mbacké B, Perumal R, Guèye MC, Sy O, Bouchet S, Prasad PVV, Morris GP (2015) Population genomics of pearl millet (*Pennisetum glaucum* (L.) R. Br.): comparative analysis of global accessions and Senegalese landraces. BMC Genomics 16(1):1048
Iwata M (2001) Probenazole a plant defence activator. Pestic Outlook 12:2831
Jogaiah S, Soshee AK, Hunthrike SS (2008) Characterization of downy mildew isolates of *Sclerospora graminicola* by using differential cultivars and molecular markers. J Cell Mol Biol 7:41–755. https://doi.org/10.1094/PHYTO.1998.88.4.292
Jogaiah S, Sharathchandra RG, Raj N (2014) Development of SCAR marker associated with downy mildew disease resistance in pearl millet (*Pennisetum glaucum* L.). Mol Biol Rep 41:7815–7824. https://doi.org/10.1007/s11033-014-3675-7
Jogaiah S, Shetty HS, Ito SI, Tran LP (2016) Enhancement of downy mildew disease resistance in pearl millet by the G_app7 bioactive compound produced by *Ganoderma applanatum*. Plant Physiol Biochem 105:109–117. https://doi.org/10.1016/j.plaphy.2016.04.006. Epub 2016 Apr 4
Jones ES, Liu CJ, Gale MD, Hash CT, Witcombe JR (1995) Mapping quantitative trait loci for downy mildew resistance in pearl millet. Theor and Appl Genet 91:448–456
Jones ES, Breese WA, Liu CJ, Singh SD, Shaw DS, Witcombe JR (2002) Mapping quantitative trait loci for resistance to downy mildew in pearl millet: field and glasshouse screens detect the same QTL. Crop Sci 42:1316–1323
Kato H (2001) Rice blast control. Pesticide Outlook 23–25. Japan Crop Protection Association (JCPA), Japan
Khairwal IS, Yadav OP (2005) Pearl millet (*Pennisetum glaucum*) improvement in India-retrospect and prospects. Indian J Agric Sci 75:183–191
Khairwal IS, Rai KN, Yadav OP, Bhatnagar SK (2004) Pearl millet cultivars. Mandor, Jodhpur, Rajasthan, India: All India Coordinated Pearl Millet Improvement Project, Indian Council of Agricultural Research, New Delhi, India, p 22
Krishnamurthy K, Gnanamanickam SS (1998) Biological control of rice blast by pseudomonas fluorescens strain Pf7-14: evaluation of a marker gene and formulations. Biol Control 3:158–165
Kulkarni KS, Zala HN, Bosamia TC, Shukla YM, Kumar S, Fougat RS, Patel MS, Narayanan S, Joshi CG (2016) De novo transcriptome sequencing to dissect candidate genes associated with pearl millet-downy mildew (*Sclerospora graminicola* Sacc.) interaction. Front Plant Sci 7:847. https://doi.org/10.3389/fpls.2016.00847
Kumar A (2008) Biocontrol of plant diseases: need to tap the options. J Arid Legumes 5:99–108
Kumar A, Manga VK, Gour HN, Purohit AK (2012) Pearl millet downy mildew: challenges and prospects. Annu Rev Plant Pathol 5:139–177
Kurahashi Y (2001) Melanin biosynthesis inhibitors (MBIs) for control of rice blast. Pestic Outlook 12:32–35
Latha AM, Rao KV, Reddy TP, Reddy VD (2006) Development of transgenic pearl millet (*Pennisetum glaucum* (L.) R. Br.) plants resistant to downy mildew. Plant Cell Rep 25:927–935. https://doi.org/10.1007/s00299-006-0141-6
Liu CJ, Witcombe JR, Pittaway TS, Nash M, Hash CT, Busso CS, Gale MD (1994) An RFLP-based genetic map of pearl millet (*Pennisetum glaucum*). Theor Appl Genet 89:481–487
Malik S (2015) Pearl millet-nutritional value and medicinal use. Intl J Adv Res Innov Ideas Educ 1:414–418

McDonald BA, Linde C (2003) The population genetics of plant pathogens and breeding strategies for durable resistance. Euphytica 124:163–180

Mishra MK, Srivastava M, Singh G, Tiwari S, Niranjan A, Kumari N, Misra P (2017) Over expression of *Withania somnifera* SGTL1 gene resists the interaction of fungus *Alternaria brassicicola* in *Arabidopsis thaliana*. Physiol Mol Plant Pathol 97:11–19

Morgan RN, Wilson JP, Hanna WW, Ozias-Akins P (1998) Molecular markers for rust and *Pyricularia* leaf spot disease resistance in pearl millet. Theor Appl Genet 96:413–420. https://doi.org/10.1007/s001220050757

Moumouni KH, Kountche BA, Jean M, Hash CT, Vigouroux Y, Haussmann BI, Belzile F (2015) Construction of a genetic map for pearl millet, *Pennisetum glaucum* (L.) R. Br., using a genotyping-by-sequencing (GBS) approach. Mol Breed 35(1) 5–10

Nagaraja A, Das IK (2016) Disease resistance in pearl millet and small millets. In: Das IK, Padmaja PG (eds) Biotic stress resistance in millets. Academic Press Cambridge, Massachusetts, pp 69–104

Nandhini M, Rajini SB, Udayashankar AC, Niranjana SR, Lund OS, Shetty HS, Prakash HS (2019) Biofabricated zinc oxide nanoparticles as an eco-friendly alternative for growth promotion and management of downy mildew of pearl millet. Crop Protec 121:103–112

Nandini B, Hariprasad P, Prakash HS, Shetty HS, Geetha N (2017) Trichogenic-selenium nanoparticles enhance disease suppressive ability of trichoderma against downy mildew disease caused by *Sclerospora graminicola* in pearl millet. Sci Rep 7(1):1–11

Nandini B, Puttaswamy H, Prakash HS, Adhikari S, Jogaiah S, Nagaraja G (2020) Elicitation of novel trichogenic-lipid nanoemulsion signaling resistance against pearl millet downy mildew disease. Biomolecules 10(1):25

Nayaka SC, Shetty HS, Satyavathi CT, Yadav RS, Kavi Kishor PB, Nagarajud M et al (2017a) Draft genome sequence of *Sclerospora graminicola* the pearl millet downy mildew pathogen. Biotechnol Rep 16:18–20

Nayaka SC, Srivastava RK, Udayashankar AC, Lavanya SN, Prakash G, Bishnoi HR, Kadvani DL, Singh OV, Niranjana SR, Prakash HS, Satyavathi CT (2017b) *Magnaporthe* blast of pearl millet in India—present status and future prospects. All India Coordinated Research Project on Pearl Millet (Indian Council of Agricultural Research), Mandor, Jodhpur, India, 51 p

Ngom B, Sarr I, Kimatu J, Mamati E, Kane NA (2017) Genome-wide analysis of cytosine DNA methylation revealed salicylic acid promotes defense pathways over seedling development in pearl millet. Plant Signal Behav 12(9):1356967

O'Kennedy MM, Smith G, Botha FC (2004) Improved regeneration efficiency of a pearl millet [*Pennisetum glaucum* (L.)] R. Br.] breeding line. S Afr J Bot 70:502–508

Prakash G, Kumar A, Sheoran N, Aggarwal R, Satyavathi CT, Chikara SK, Ghosh A, Jain RK (2019) First draft genome sequence of a pearl millet blast pathogen, *Magnaporthe grisea* strain PMg_Dl, obtained using PacBio single-molecule real-time and Illumina NextSeq 500 sequencing. Microbiol Resour Announc 8:e01499-18. https://doi.org/10.1128/MRA.01499-18

Punnauri SM, Wallace JG, knoll JE, Hyma KE, Mitchell SE, Buckler ES, Varshney K, Singh BP (2016) Development of a high-density linkage map and tagging leaf spot resistance in pearl millet using genotyping-by-sequencing markers. Plant Genome 9 (2):1–13

Pushpavathi B, Thakur RP, Chandrashekar Rao K, Rao VP (2006) Characterization of *Sclerospora graminicola* isolates from pearl millet for virulence and genetic diversity. Plant Pathol J 22:28–35

Rai KN, Talukdar BS, Rao AS (1998a) Registration of pearl millet parental lines ICMA 92666 and ICMB 92666 with multiple disease resistance. Crop Sci 38:575

Rai KN, Thakur RP, Rao AS (1998b) Registration of pearl millet parental lines ICMA 88006 and ICMB 88006. Crop Sci 38:575–576

Raj SN, Shetty NP, Shetty HS (2005) Synergistic effects of trichoshield on enhancement of growth and resistance to downy mildew in pearl millet. Biol Control 50:493–509

Raj K, Arya RK, Kumar R (2014) Pearl millet improvement for disease resistance. Forage Res 40 (3):133–146

Ramineni R, Sadumpati V, Khareedu VR, Vudem DR (2014) Transgenic pearl millet male fertility restorer line (ICMP451) and hybrid (ICMH451) expressing *Brassica juncea* nonexpressor of pathogenesis related genes 1 (BjNPR1) exhibit resistance to downy mildew disease. PLoS ONE 9(3):90839

Rao VP, Kadwani DL, Sharma YK, Sharma R, Thakur RP (2007) Prevalence of pearl millet downy mildew, *Sclerospora graminicola* in Gujarat and pathogenic characterization of its isolates. Indian J Plant Protec 35:291–295

Sankar SM, Singh SP, Prakash G, Satyavathi CT, Soumya SL, Yadav Y, Sharma LD, Rao AR, Singh N and Srivastava RK (2021) Deciphering Genotype-By- Environment Interaction for Target Environmental Delineation and Identification of Stable Resistant Sources Against Foliar Blast Disease of Pearl Millet. Front. Plant Sci. 12:656158. https://doi.org/10.3389/fpls.2021.656158

Satyavathi CT, Sharma R, Singhal T (2016) Generation of mapping populations and identification of QTL(S) for downy mildew resistance in pearl millet [*Pennisetum glaucum* (L.) R. Br.] (BT/PR-11035/AGR/02/622/2008), Project Report submitted to DBT, GOI (unpublished data)

Satyavathi CT (2020) Proceeding of 55th annual group meet, ICAR-AICRP on pearl millet, Jodhpur, Rajasthan, India

Satyavathi CT, Khandelwal V, Beniwal BR, Ambawat S, Kumar A, Kumar M, Meena RC, Nayaka SC, Vishnoi JP, Juneja RK et al (2019) Summary of research experiments 2018–19. ICAR-AICRP on Pearl Millet, Jodhpur, Rajasthan, India

Satyavathi CT, Khandelwal V, Beniwal BR, Ambawat S, Kumar A, Kumar M, Meena RC, Nayaka SC, Vishnoi JP, Juneja RK et al (2020) Summary of research experiments 2019-20. ICAR-AICRP on Pearl Millet, Jodhpur, Rajasthan, India

Sehgal D, Skot L, Singh R, Srivastava RK, Das SP, Taunk J, Sharma PC, Pal R, Raj B, Hash CT, Yadav RS (2015) Exploring potential of pearl millet germplasm association panel for association mapping of drought tolerance traits. PLoS One 10(5):e0122165

Senthilvel S, Jayashree B, Mahalakshmi V, Kumar PS, Nakka S, Nepolean T, Hash CT (2008) Development and mapping of simple sequence repeat markers for pearl millet from data mining of expressed sequence tags. BMC Plant Biol 8(1):119

Senthilvel S, Nepolean T, Supriya A, Rajaram V, Kumar S, Hash CT et al (2010) Development of a molecular linkage map of pearl millet integrating DArT and SSR markers. In: Proceedings of the plant and animal genome 18 conference, San Diego, CA, p 368

Sharma YK, Yadav SK, Khairwal IS (2007) Evaluation of pearl millet germplasm lines against downy mildew incited by *Sclerospora graminicola* in western Rajasthan. J SAT Agri 3:2

Sharma R, Rao VP, Varshney RK, Prasanth VP, Kannan S, Thakur RP (2010) Characterization of pathogenic and molecular diversity in *Sclerospora graminicola*, the causal agent of pearl millet downy mildew. Arch Phytopathol Plant Protec 43:538–551. https://doi.org/10.1080/03235400801939896

Sharma R, Upadhyaya HD, Manjunatha SV, Rai KN, Gupta SK, Thakur RP (2013) Pathogenic variation in the pearl millet blast pathogen *Magnaporthe grisea* and identification of resistance to diverse pathotypes. Plant Dis 97(2):189–195. https://doi.org/10.1094/PDIS-05-12-0481-RE

Sharma S, Sharma R, Govindaraj M, Mahala RS, Satyavathi CT, Srivastava RK, Gumma MK, Kilian B (2020a) Harnessing wild relatives of pearl millet (*Pennisetum glaucum* LR Br) for germplasm enhancement: challenges and opportunities. Crop Sci. https://doi.org/10.1002/csc2.20343

Sharma R, Sharma S, Gate VL (2020b) Tapping *Pennisetum violaceum*, a wild relative of pearl millet (*Pennisetum glaucum*), for resistance to Blast (caused by *Magnaporthe grisea*) and rust (caused by *Puccinia substriata* var. indica). Plant Dis 104(5):1487–1491

Sharma S, Sharma R, Pujara M, Yadav D, Yadav Y et al (2020c) Use of wild *Pennisetum* species for improving biotic and abiotic stress tolerance in pearl millet (*Pennisetum glaucum* L.). Crop Sci. https://doi.org/10.1002/csc2.20408

Shaw EG (1981) Taxonomy and evolution. In: Spencer DM (ed) the downy mildews. Academic Press, London, UK, pp 17–29
Shetty HS, Ahmad R (1981) Physiologic specialization in *Sclerospora graminicola*. Indian Phytopathol 34:307–309
Shetty HS, Kumar VU (2000) Biological control of pearl millet downy mildew: present status and future prospects. In: Upadhyay R, Mukerji KG, Chamola BP (eds) Biocontrol potential and its exploitation in sustainable agriculture, vol 1. Springer, Heidelberg, Germany, pp 251–265
Shivhare R, Lata C (2017) Exploration of genetic and genomic resources for abiotic and biotic stress tolerance in pearl millet. Front Plant Sci 7:2069. https://doi.org/10.3389/fpls.2016.02069
Shivhare R, Asif MH, Lata C (2020a) Comparative transcriptome analysis reveals the genes and pathways involved in terminal drought tolerance in pearl millet. Plant Mol Biol 103:639–652. https://doi.org/10.1007/s11103-020-01015-w
Shivhare R, Lakhwani D, Asif MH, Chauhan PS, Lata C (2020b) De novo assembly and comparative transcriptome analysis of contrasting pearl millet (*Pennisetum glaucum* L.) genotypes under terminal drought stress using illumina sequencing. Nucleus. https://doi.org/10.1007/s13237-020-00324-1
Siddaiah CN, Prasanth KVH, Satyanarayana NR, Mudili V, Gupta VK, Kalagatur NK, Satyavati T, Dai XF, Chen JY, Mocan A, Singh BP (2018) Chitosan nanoparticles having higher degree of acetylation induce resistance against pearl millet downy mildew through nitric oxide generation. Sci rep 8(1):1–14
Singh SD (1995) Downy mildew of pearl millet. Plant Dis 79:545–550
Singh DS, Pavgi MS (1974) Evaluation of some fungicides against *Pyricularia penniseti* causing brown leaf spot of bajra. Hindustan Antibiot Bull 16(2–3):97–100 (PMID: 4807862)
Singh SD, Gopinath R (1985) A seedling inoculation technique for detecting downy mildew resistance in pearl millet. Plant Dis 69:582–584. https://doi.org/10.1094/PD-69-582
Singh SD, King SB (1988) Recovery resistance to downy mildew in pearl millet. Plant Dis 72:425–428
Singh SD, Navi SS (2000) Genetic resistance to pearl millet downy mildew II. Resistance in wild relatives. J Mycol Plant Pathol 30(2):167–171
Singh SD, Williams RJ, Reddy PM (1988) In isolation of downy mildew resistant lines from a highly susceptible cultivar of pearl millet. Indian Phytopathol l41:450–456
Singh SD, King SB, Werder J (1993) Downy mildew disease of pearl millet. Inf Bull No 37, 36 (International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India)
Singh SD, Wilson JP, Navi SS, Talukdar BS, Hess DE, Reddy KN (1997) Screening techniques and sources of resistance to downy mildew and rust in pearl millet. Inf Bull No 48 36:110 (International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India)
Sivaramakrishnan S, Thakur RP, Kannan S, Rao VP (2003) Pathogenic and genetic diversity among Indian isolates of *Sclerospora graminicola*. Indian Phytopathol l56:392–397
Srivastava S, Kothari SL (2003) Assessment of somaclonal variations in two lines of pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Indian J Genet 63:295–298
Sudisha J, Kumar SA, Thakur RP, Rao VP, Shetty HK (2009) Molecular characterization of *Sclerospora graminicola*, the incitant of pearl millet downy mildew using ISSR markers. J Phytopathol 157:748–755. https://doi.org/10.1111/j.1439-0434.2009.01547
Supriya A, Senthilvel S, Nepolean Eshwar K, Rajaram V, Shaw R, Hash CT, Kilian A, Yadav RC, Narasu ML (2011) Development of a molecular linkage map of pearl millet integrating DArT and SSR markers. Theor Appl Genet 123:239–250
Rajaram V, Nepolean T, Senthilvel S, Varshney RK, Vadez V, Srivastava RK, Shah TM, Supriya A, Kumar S, Kumari BR, Bhanuprakash A (2013) Pearl millet [*Pennisetum glaucum* (L.) R. Br.] consensus linkage map constructed using four RIL mapping populations and newly developed EST-SSRs. BMC Genomics 14(1):159

Taunk J, Sehgal D, Yadav NR, Howarth C, Yadav RC, Yadav RS (2018) Mapping of easy to screen SSR markers for selection of RFLP markers-bracketed downy mildew resistance QTLs in pearl millet. Eur J Plant Pathol 151:401–411. https://doi.org/10.1007/s10658-017-1381-8
Thakur RP, King SB (1988a) Registration of four ergot resistant germplasms of pearl millet. Crop Sci 28:382. https://doi.org/10.2135/cropsci1988.0011183X002800020057x
Thakur RP, King SB (1988b) Registration of six smut resistant germplasms of pearl millet. Crop Sci 28:382–383
Thakur RP, King SB (1988c) Smut disease of pearl millet. ICRISAT, ICRISAT Infor Bulletin, Patancheru, p 25
Thakur RP, Williams RJ, Rao VP (1982) Development of resistance to ergot in pearl millet. Phytopathology 72:406–408. https://doi.org/10.1094/Phyto-72-406
Thakur RP, King SB, Rai KN, Rao VP (1992) Identification and utilization of smut resistance in pearl millet. ICRISAT, Patancheru, Hyderabad, India
Thakur RP, Rao VP, Amruthesh KN, Shetty HS, Datar VV (2003) Field surveys of pearl millet downy mildew-effects of hybrids, fungicide and cropping sequence. J Mycol Plant Pathol 33:387–394
Thakur RP, Shetty HS, Khairwal IS (2006) Pearl millet downy mildew research in India: progress and perspectives. J SAT Agric Res 2:6
Thakur RP, Rai KN, Khairwal IS, Mahala RS (2008) Strategy for downy mildew resistance breeding in pearl millet in India. J SAT Agric Res 6:1–11
Timper P, Wilson JP, Johnson AW, Hanna WW (2002) Evaluation of pearl millet grain hybrids for resistance to *Meloidogyne* spp. and leaf blight caused by *Pyricularia grisea*. Plant Dis 86:909–914
Tiwari S, Lata C (2019) Genome engineering in rice: applications, advancements and future perspectives. In: Singh SP, Upadhyay SK, Pandey A, Kumar S (eds) Molecular approaches in plant biology and environmental challenges. Springer, Singapore, pp 323–337
Uesugi Y (2001) Fungal choline biosynthesis a target for controlling rice blast. Pestic Outlook 12:2627
Varshney RK, Shi C, Thudi M, Mariac C, Wallace J, Qi P, Zhang H, Zhao Y, Wang X, Rathore A, Srivastava RK (2017) Pearl millet genome sequence provides a resource to improve agronomic traits in arid environments. Nat Biotechnol 35(10):969–976. https://doi.org/10.1038/nbt.3943
Veena M, Melvin P, Prabhu SA, Shailasree S, Shetty HS, Kini KR (2016) Molecular cloning of a coiled-coil-nucleotide-binding-site-leucine-rich repeat gene from pearl millet and its expression pattern in response to the downy mildew pathogen. Mol Biol Rep 43(3):117–128
Williams RJ, Singh SD, Pawar MN (1981) An improved field screening technique for downy mildew resistance in pearl millet. Plant Dis 65:239–241
Willingale J, Mantle PG, Thakur RP (1986) Post-pollination stigmatic constriction, the basis of ergot resistance in selected lines of pearl millet. Phytopathology 76:536–539. https://doi.org/10.1094/Phyto-76-536
Wilson JP, Burton GW (1991) Registration of Tift 3 and Tift 4 rust resistant pearl millet germplasms. Crop Sci 31:1713
Wilson JP, Hanna WW (1992) Disease resistance in wild *Pennisetum* species. Plant Dis 76 (11):1171–1175
Wilson JP, Gates RN (1993) Forage yield losses in hybrid pearl millet due to leaf blight caused primarily by *Pyricularia grisea*. Phytopathology 83:739–743
Witcombe JR, Hash CT (2000) Resistance gene deployment strategies in cereal hybrids using marker-assisted selection: gene pyramiding, three-way hybrids, and synthetic parent populations. Euphytica 112:175–186
Yadav MS, Duhan JC (1996) Screening of pearl millet genotypes for resistance to smut. Plant Dis Res 11:95–96
Yoshihiro T, Mitsuo H, Hayato H, Futoshi K (2003) Biological control of rice blast disease by *Bacillus subtilis* IK-1080. Ann Phytopathol Soc Japan 69:85–93
Zahid M (1997) Genetical variation in the downy mildew fungus, *Sclerospora graminicola*. PhD thesis, School of Biological Sciences, University of Wales, UK, 213 p

Zala HN, Kulkarni KS, Bosamia TC, Shukla YM, Kumar S, Fougat RS, Patel A (2017) Development and validation of EST derived SSR markers with relevance to downy mildew (*Sclerospora graminicola* Sacc.) resistance in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. J Plant Biochem Biotechnol 26(3):356–365

Zarandi EH, Bonjar GH, Dehkaei FP, Moosavi SAA, Farokhi PR, Aghighi S (2009) Biological control of rice blast (*Magnaporthe oryzae*) by use of *Streptomyces sindeneusis* isolate 263 in Greenhouse. Amer J Appl Sci 6:194–199

# Chapter 7
# Genomic Designing for Biotic Stress Tolerance in Foxtail Millet (*Setaria italica* L.)

**Sumi Rana, Lydia Pramitha, Pooja Rani Aggarwal, and Mehanathan Muthamilarasan**

**Abstract** Global food insecurity has become one of the significant issues in recent years. The research community has begun to rely on nutritionally rich millets to resolve the issue since they are considered hardy crops and have a higher potential to cope with biotic and abiotic stresses. Foxtail millet (*Setaria italica*) has gained popularity as a model crop due to its small diploid genome, short life-cycle, in-breeding nature, and resilience to various climatic conditions. Domestication of foxtail millet took place about 8,000 years ago. It is known as the oldest cultivated crop in the world. Because of its climate resilience feature, foxtail millet is considered tolerant to most of the biotic and abiotic stresses. However, the cultivated foxtail millet does encounter several pathogens in the natural conditions, fungi and virus in particular, which causes significant yield losses in foxtail millet production worldwide. With the availability of genome sequence information, research in foxtail millet has accelerated, as many genes responsible for better agronomic traits have been identified. In this regard, genes and molecular pathways underlying abiotic stress response have been studied extensively in foxtail millet. However, the investigation of biotic stress response in foxtail millet is still at an early stage. Given this, the chapter briefs the patho-stresses known to affect foxtail millet yield and genes identified from the crops to understand the biotic stress-induced response. Further, the chapter highlights the use of genome editing tools like TALENs, ZFNs, CRISPR/Cas9, etc., that could be used to enhance the agronomic traits of the crop.

---

Sumi Rana and Lydia Pramitha contributed equally.

---

S. Rana · P. R. Aggarwal · M. Muthamilarasan (✉)
Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, Telangana 500046, India
e-mail: muthu@uohyd.ac.in

L. Pramitha
School of Agriculture and Biosciences, Karunya Institute of Technology and Sciences, Coimbatore, Tamil Nadu 641114, India

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021

C. Kole (ed.), *Genomic Designing for Biotic Stress Resistant Cereal Crops*,
https://doi.org/10.1007/978-3-030-75879-0_7

**Keywords** Foxtail millet · *Setaria italica* · Genetics · Genomics · Genome editing · Genomic designing · Climate-resilience · Biotic stress tolerance

## 7.1 Introduction

Disease incidence is among the most significant features that limit the productive potential of crop plants. Fungal pathogens incite the majority of plant diseases. Fungi affect a broad spectrum of plants, which also includes cereal plants. Disease response to the pathogen varies among individuals of the same species, owing to their genetic makeup and adaptability. Plant immunity and vulnerability are modulated by the interaction between host and pathogen, consequently activating a cascade of signaling responses during certain stress conditions. Activation of these responses regulates the expression of genes specific to the stresses condition. Study of the molecular mechanisms and the underlying genetic determinants are of utmost importance to unravel the genes and pathways targeted for combating the disease.

Millets are annual crops known for bearing small seeds with high nutrition content and their climate-resilient nature. It is believed that their domestication took place in Asia, mainly in China and a few other parts of the globe. They could be easily grown in semi-arid and warm areas with minimal nutrient requirements and are consumed as food and fodder. These are considered as nutri-cereals that are from the family Poaceae and include millets, namely pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracana*), foxtail millet (*Setaria italica*), little millet (*Panicum sumatrense*), tef (*Eragrostis tef*), guinea millet (*Brachiaria deflexa*), Proso millet (*Panicum miliaceum*) and so on (Dwivedi et al. 2012). Fluctuating climatic conditions and burgeoning population have already raised the concern of food security. Besides, plants cannot move and are constantly exposed to the numerous biotic and abiotic stresses present in nature that eventually led to productivity loss. Abiotic stresses include salinity, heat, cold, drought, etc., whereas biotic stresses include living organisms like bacteria, viruses, pests, insects, fungi, and other predators of plants. These biotic stresses have become a serious threat not only for the yield of crops but also for the security of global food availability. Researchers are looking up to the orphaned crops like millets, owing to their climatic resilient nature, to cope with global food security issues.

Foxtail millet holds the second position among the major millets, wherein pearl millet holds the first position with respect to global productivity. Domestication of foxtail millet took place in China about 8,700 years ago. It is included in the “Five grains of China” due to its significant impact on Chinese civilization and is considered one of the major staple crops for a long time (Austin 2006). It is cultivated worldwide in semi-arid, dry, tropical, and subtropical sections for grains and forage. Currently, India is among the major producers of millets globally, followed by Niger and China (FAOSTAT 2018). Foxtail millet has a diploid genome ($2n = 2x = 18$) of about 423 Mb with a low amount of repetitive DNA. It undergoes self-pollination and has a very rapid life-cycle. Being a $C_4$ crop, it has

excellent photosynthetic efficiency and produces high yield even in minimal nutrient containing soil and low precipitation. Foxtail millet also has high water use efficiency (WUE) and exhibits remarkable tolerance against abiotic stresses. Owing to these properties and their relatedness to the $C_4$ grasses, foxtail millet is regarded as a model to study other $C_4$ biofuel grasses (Muthamilarasan and Prasad 2015). It is rich in nutritional content from other cereals as it has a higher content of antioxidants, proteins, crude fat, dietary fibers, and minerals with a significantly less glycemic index (Muthamilarasan et al. 2016). Therefore, it has gained popularity among plant researches around the globe other than China due to its agricultural and economic importance.

Foxtail millet has received major attention for research due to its potential to withstand most of the biotic and abiotic stresses and has been acknowledged as a climate-resilient crop as well (Lata et al. 2010; Muthamilarasan and Prasad 2015). However, the production of cultivated foxtail millet is limited by few pathogens. In brief, diseases like blast (*Pyricularia setariae*), rust (*Uromyces setaria*), smut (*Ustilago crameri*), udbatta (*Ephelis oryzae*) and downey mildew (*Sclerospora graminicola*) are reported rarely in foxtail millet. These diseases are not prevalent in larger zones, whereas they emerge in particular locations due to the conducive environment, season, and excessive fertilizer application. However, they are easily managed by cultural and chemical measures (http://www.aicrpsm.res.in/). Regarding pests, foxtail millet has an inherent non-preference stature as its host-plant resistance. Thus, not much yield is affected due to pests in foxtail millet. The predominant pests in foxtail millet include shoot fly (*Atherigona athripalpis*), flea beetle (*Chaetocnema basalis*, Baly. *Madurasia* sp.), armyworm (*Mythimna separata*. Wlk), leaf roller (*Marasmia trapezalis*. Wlk.), stem borer (*Chilo partellus*, Swim.), surface grasshopper (*Chrotogonus* sp.), ant and leaf minor. Among these pests, flea beetle has a moderate effect in reducing the yield. These can be effectively controlled by utilizing the existing variability in the gene pools (http://www.aicrpsm.res.in/). Here, we summarize the biotic stresses affecting foxtail millet growth and productivity. Further, genomics and genetic analysis, expression studies to identify genes related to stress response are outlined. Also, the importance of genome editing tools has been highlighted that might be useful in the future for crop improvement.

## 7.2 Biotic Stresses Affecting Foxtail Millet Cultivation

Foxtail millet is a crop with wild behavioral traits and is less prone to diseases and pests. However, this has originated from *Setaria viridis*, a wild green foxtail millet with weedy features. This has minimized seed shattering and higher-yielding ability. The hairy leaves, spined stem, bristled inflorescence, tillering nature and shorter duration creates a persistent natural barrier for the infestation of pests and diseases.

Currently, one of the significant constraints in foxtail millet yield is blast disease caused by *Pyricularia grisea*. The infected plant develops disease symptoms such as circular spots of about 2–5 mm with a dark brown perimeter on the leaf along with a straw-colored leaf blade center. The disease is at its peak during increased moisture content affecting the plant's growth and development at all the developmental stages, thereby affecting both the grains and forage production of foxtail millet (Gaikwad and D'Souza 1986). Other than blast, the common disease affecting foxtail millet crop is the bacterial blight caused by *Xanthomonas* sp. The pathogen also carries wheat curl mite and wheat streak mosaic virus (Baltensperger 2002). In 2010, Mirzaee et al. reported for the first time that foxtail millet is infected by the fungus *Bipolaris australiensis*, which causes brown spots in leaf and sheath, leading to severe lodging in the plants. In China, sheath blight caused by *Waitea cicinata* was detrimental for foxtail millet, leading to severe yield loss. Infected plants tend to lodge when the disease is at the peak (Li et al. 2014). Rust is also a common disease in foxtail millet, where the causative agent is *Uromyces setaria italica*. The symptoms include the appearance of tiny uredosori on either face of the leaf. During acute infection, leaves exhibit premature drying leading to about 10–30% yield loss (Li et al. 2015). In addition, oomycetes, commonly known as water molds, are filamentous eukaryotic microorganisms that infect Gramineae crops and cause downy mildew disease. Further, *Sclerospora graminicola* (Sacc.) is also known to infect foxtail millet, causing vein chlorosis. Accumulation of the pathogen in the panicle shows a peculiar behavior wherein a leafy structure is observed in the floral organs, commonly termed as witche's brooms. The process is called phyllody (Jegera et al. 1998; Das et al. 2016). One of the seed borne panicle disease named udbatta caused by the fungus *Ephelis oryzae* has been observed to affect foxtail millet productivity. The infected panicle appears like an incense stick as the spike becomes cylindrical, compact, and silver in color (Nagaraja and Das 2016). In Korea, it has been reported that foxtail millet is infected by *Rice stripe virus* (RSV) and *Rice black-streaked dwarf virus* (RBSDV). In 2017, the first report was published on *Barley virus G* (BVG) infecting foxtail millet in Korea that showed symptoms of yellow stripes and the formation of mosaic pattern (Oh et al. 2017). In 2020, Dwarf disease caused by *Barley yellow striate mosaic virus* was reported for the first time in China. The infection showed high head sterility with about a 10% reduction in the yield (Shen et al. 2020). A list of microorganisms infecting foxtail millet is given in Table 7.1.

Few pests can infect foxtail millet periodically and cause significant damage. *Atherigona atripalpis*, a shoot fly, is one of the major pests that infect foxtail millet and produces symptoms of dead heart. During dry states, *Agrotis ipsilon*, a cutworm, can cause acute damage by cutting the seedlings of foxtail millet that appears like ruminant grazing (Das and Rakshit 2016). At the age of a month, foxtail millet plants can be occasionally invaded by stem borers like *Chilo partellus* and pink borers like *Sesamia inferens* (Kundu and Kishore 1971). Along with a rapid life cycle that aids in escaping from biotic stress, Foxtail millet also has a very diverse collection of germplasm. Thus, cultivars showing natural resistance to these biotic stresses can be selected using proper screening methods.

**Table 7.1** List of pathogens that interact with foxtail millet to develop diseases

| Causative agent | Symptoms in foxtail millet | Reference |
|---|---|---|
| *Bipolaris australiensis* | Brown spots on leaves and sheath | Mirzaee et al. (1998) |
| *Pyricularia grisea* (Blast fungus) | Circular spot with dark brown periphery | Gaikwad and D'Souza (1986) |
| *Xanthomonas* sp (Bacterial blight) | Necrotic lesions on plant | Baltensperger (2002) |
| *Waitea cicinata* | Sheath blight | Li et al. (2014) |
| *Uromyces setaria italic* (Rust) | Tiny uredosori on leaves | Li et al. (2015) |
| *Sclerospora graminicola* (Sacc.) (Downy mildew) | Vein sclerosis, Witch's broom | Jegera et al. (1998), Das et al. (2016) |
| *Ephelis oryzae* (Udbatta) | Spike takes a cylindrical shape and appears silver coloured | Nagaraja and Das (2016) |
| *Barley virus G* | Yellow stripes and mosaic pattern | Oh et al. (2017) |
| *Barley yellow striate mosaic virus* (Dwarfing) | Dwarfing and head sterility | Shen et al. (2020) |
| *Ustilago crameri* (Smut) | Appearance of black colored spore balls in ear | Kumar (2011) |

## 7.3 Molecular Mapping of Resistance Genes and QTLs for Biotic Stress

The genus *Setaria* comprises 125 species, of which five species are categorized in the primary and secondary gene pool. The remaining species are yet to be studied for their key traits (Lata et al. 2013). Foxtail millet contains the highest phenol content in most of the accessions. The phytochemical compositions of foxtail cultivars also presented a higher chlorogenic acid, catechin, naringenin, hesperetin, and quercetin (Ghimire et al. 2019). These components have an essential role in developing resistance towards biotic stresses. Molecular characterization of these compounds in foxtail millet in the future will revolutionize the breeding program in millet crops. The genetic diversity of resistance (R) genes in the foxtail millet revealed 242 CNL genes, which is thrice the number of R genes present in other cereals (Andersen and Nepal 2017) and mapped on chromosome 8. These gene products are known as effector molecules. The phylogeny-based comparison between foxtail millet and rice revealed the similarity of this region with Os11. To date, there is no study available for mapping the tolerant/resistant genes in foxtail millet, highlighting the importance of exploring this particular area of millet research in the near future.

Foxtail millet is a well-known crop for its phenomic diversity and morphological characters. The advent of markers led to establishing the correlation between these phenotypic variations to the geographical locations. Restriction fragment length polymorphism (RFLP) markers were used to generate the first linkage map with

markers signifying agronomical features in foxtail millet (Botstein et al. 1980). Preceding these studies, simple sequence repeat (SSR) and expressed sequence tag (EST) markers were predominantly used for grouping the germplasm accessions to clusters for diversity (Chander et al. 2017). Recent techniques of genotyping by sequencing (GBS) using single nucleotide polymorphisms (SNPs) were used to dissect the nucleotide variations for chlorophyll pigmentation (Jaiswal et al. 2019). Further, these techniques have to be employed to identify the molecular phenomenon behind the biotic stress tolerance in foxtail millet.

Recombinant inbred lines and $F_2$ populations are the major mapping populations developed in foxtail millet to identify the quantitative trait loci (QTLs) linked to agronomic parameters. These populations were used to improve the yield plateau of foxtail millet successfully. The sets of mapping populations could further be developed for studying the QTLs linked to major biotic stresses in foxtail millet (Fang et al. 2016; Wang et al. 2019).

## 7.4 Marker-Assisted Breeding for Resistance Traits

Marker-assisted breeding techniques are empowering tools to improve the crops in a short breeding cycle. These markers could be developed from identifying marker-trait association from an association mapping technique. These techniques were utilized to understand the linkage disequilibrium (LD) values for morphological variations in core collections and germplasms of foxtail millet (Jia et al. 2013). Formulated core and reference set populations for foxtail millet could be later characterized to identify key traits responsible for biotic stress tolerance. The major races, viz. indica, maxima, and moharia were studied for their variations in abiotic stress conditions (Krishnamurthy et al. 2007, 2016) and could be further characterized for their biotic stress resistance. No such studies linked to patho-stress was performed in India. However, the Chinese cultivars for rust tolerance, categorized by Diao and Jia (2017) could be used as effective donors to develop rust resistant lines in endemic regions. The distinctness, uniformity and stability (DUS) descriptors used as screening traits by Banu et al. (2018) could be further stratified to accomplish indicator traits required for biotic tolerance in foxtail millet cultivation.

Foxtail millet is a model crop due to its lesser genomic complexities and hardy stature, which imparts biotic stress tolerance to the crop. The key traits linked to biotic stress tolerance has to be characterized to accelerate the gene introgression and pyramiding studies in other related cereals and millets. This could also introduce resistant cultivars to the endemic regions that induces pest and disease attacks in the future (Fig. 7.1).

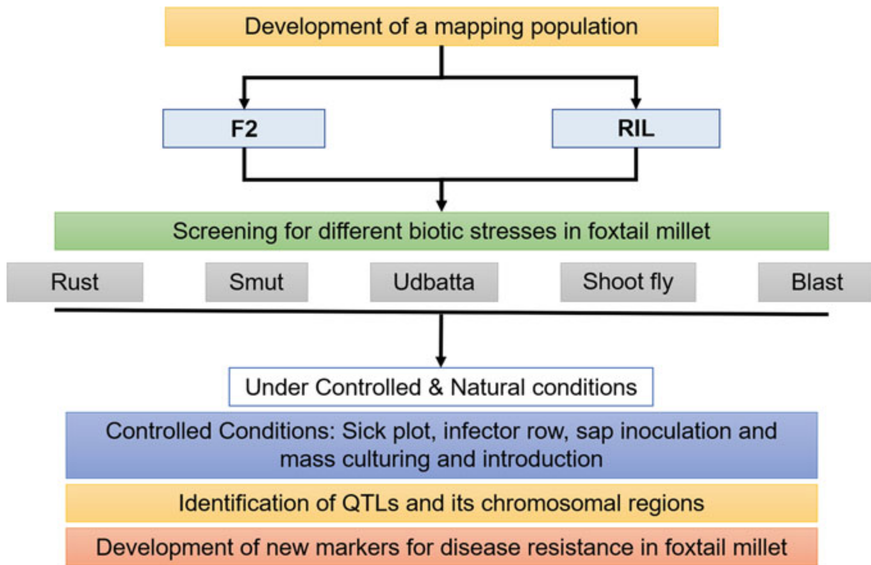



**Fig. 7.1** Schematic illustration of marker assisted selection for biotic stress tolerance in foxtail millet

## 7.5 Genomics-Aided Breeding for Resistance Traits

### *7.5.1 Details of Genome Sequencing*

Draft genome sequence of foxtail millet cv. ‘Yugu1’ and ‘Zhang gu’ were decoded by two independent research groups (Bennetzen et al. 2012; Zhang et al. 2012), which paved the way to explore the determinants of stress response at the genomics level. Bennetzen et al. (2012) generated ~400 Mb assembly (396.7 Mb of sequence in nine chromosomes and about 4.2 Mb in 327 scaffolds), representing ~80% of the genome. Further, the genetic basis of foxtail millet adaptation to various stresses was investigated. Several genes belonging to various classes of stress-responsive proteins such as AMP-dependent synthetase/ligase, NADH oxidase, lipid transfer protein, multi-antimicrobial extrusion protein, aldo/keto reductase and glutathione S-transferase were identified in the study. These genes may have a potential role in the abiotic as well as biotic stress response in foxtail millet. In parallel, the draft genome of cv. ‘Zhang gu’ have been sequenced with whole-genome shotgun combined cum next-generation sequencing (NGS). Approximately 423 Mb assembly representing ~86% of genome size, with repeat sequence comprising ~46% were reported in the study (Zhang et al. 2012). Annotation was done using gene ontology that revealed that many genes related to stress response were identified that might have involvement in foxtail millet adaptation to a myriad of biotic stresses.

### 7.5.2 Gene Annotation

Following genome sequencing, gene annotation is imperative to provide researchers tools for biological research. Approximately 40% of the 'Yugu1' genome was found to be composed of transposable elements. In addition, 48 families of miRNA transcripts were also discovered in the 'Yugu1' genome. Whole-genome annotation precited 24,000–29,000 protein-coding genes, of which 10,059 were single intron genes. Further, a comparison of homologous gene sets and alignment of ESTs between *Setaria* and switchgrass revealed that both were diverged around 3–7 Myr ago (Bennetzen et al. 2012). In 'Zhang gu' assembly, approximately 46% of the genome was composed of transposable elements. Of these, both class I (31.6%) and class II (9.4%) transposable elements were identified. A total of 38,801 genes were predicted in the 'Zhang gu' genome with an average transcript length of 2,522 bp. Further, 1,367 pseudogenes were also identified in the study. In addition, several non-protein coding genes such as 99 rRNA genes, 704 tRNA genes, 159 miRNA genes, and 99 snRNA genes were also predicted in 'Zhang gu' genome (Zhang et al. 2012). Functional annotation of both foxtail millet strains was performed for further gene discovery and pathway identification, which in turn highlighted the set of genes that might have a role in stress adaptation.

### 7.5.3 Impact on Germplasm Characterization and Gene Discovery

The molecular basis of genetic adaptation and genes having a potential role in abiotic stress response has been studied extensively in foxtail millet. However, germplasm characterization for identifying foxtail millet genotypes/cultivars exhibiting differential response to very few pathogens has been performed in the past. Earlier, 20 cultivars of foxtail millet were tested for blast resistance against 11 Japanese *Setaria* isolates of blast fungus (Nakayama et al. 2005). Further, evaluation of resistance against blast among 155 accessions of foxtail millet has been performed at ICRISAT, Patancheru, India against *Magnaporthe grisea* Patancheru isolate (Sharma et al. 2014). Bioagents such as *Bacillus cereus* and *B. subtilis*, and fungicides such as mancozeb, combinations of carbendazim + mancozeb, carboxin + thiram, tebuconazole + trifloxystrobin have used to inhibit the growth of leaf blast pathogen, *P. grisea* in vitro (Konda et al. 2016).

After the accessibility of genome annotation, gene family analysis is one the most informative approach for gene discovery within the species or in comparison with other species. Several studies reported the genome-wide identification of gene families involved in multiple biological processes in foxtail millet, including transcription factors. In brief, 147 NAC (Puranik et al. 2013), 171 AP2/ERF (Lata et al. 2014), 209 MYB, 149 bHLH (Wang et al. 2018a, b), 124 C2H2-ZF (Muthamilarasan et al. 2014), 110 WRKY (Muthamilarasan et al. 2015), 44 SCL

(Liu et al. 2017), 35 Dof (Zhang et al. 2017), 47 HD-ZIP (Chai et al. 2018) and 27 Trihelix transcription factors (Wang et al. 2018a, b). In addition, RNA silencing complex proteins viz. Dicer-like (8), Argonaute (19), and RNA-dependent RNA polymerase (11) have also been identified (Yadav et al. 2015). Of these, few genes have shown altered expression in response to abiotic stress. Also, heat shock proteins, such as HSP100, HSP90, HSP70, HSP60 and sHSP, were found to be responsive during abiotic stress (Singh et al. 2016). Future investigation of these gene families might provide an idea about their involvement during the biotic stress response. Smut disease causes severe yield loss in foxtail millet. Jigu20 is one of the cultivars resistant to smut, and Chanhnong35 is a susceptible one. Hao et al. studied the RNA-seq of these cultivars to identify the differentially expressed genes post smut infection. In the infected resistant cultivar (Jigu20), an upregulation was observed in the *SiRPM1* resistant gene (R gene) along with *SiHSP* and *SiSGT1* (signaling molecules). The study also identified few putative genes that might confer resistance against smut viz *SiCDPK*, *SiCHI*, *SiBGL*, *SiHSP*, *SiRbohF*, *SiPAL*, and *SiRPM1* (Hao et al. 2020). Zhu et al. performed in silico genomic study of foxtail millet cv. Yugu1 and Zhang gu to identify the nucleotide-binding site (NBS) disease resistance gene wherein they observed 281 NBS coding genes in Zhang gu and 269 in Yugu1. Out of the total sequences identified, 72 were identical, while the rest 164 showed a similarity of 90% (Zhu et al. 2014).

### 7.5.4 Application of Structural and Functional Genomics in Genomics-Assisted Breeding

Several transcriptomics-based studies have been performed in the post-genome era in foxtail millet at different developmental stages and/or during abiotic stress response. Based on the genomics and expression-based studies, advances were made to generate large-scale genomic resources in foxtail millet. The research community is using these resources to decipher physiological and molecular causes of tolerance against abiotic stress factors, namely drought, heat, and salinity. Evaluation of differential responsive cultivars during stress and identification of stress-linked QTLs is followed by NGS based genomics-assisted breeding (GAB) for developing stress-tolerant cultivars. This strategy has to be utilized in foxtail millet to develop biotic stress-resistant cultivars by genomics-assisted breeding in the future.

## 7.6 Gene Editing Strategies Developed in Foxtail Millet

Genetic manipulation has received attention in the past decade with the advancement in genome editing tools and technologies. The liberty to add, subtract, replace or even delete single or multiple bases has become possible with programmable nucleases. Clustered regularly interspaced short palindromic repeats (CRISPR)/ CRISPR-associated 9 (Cas9) nucleases are among the most advanced and robust tools based on nucleases. In addition, meganucleases (MN), transcription activator-like effector nucleases (TALENs), and zinc finger nucleases have also gained significant importance in the past. These nucleases basically introduce a double-stranded break in the DNA followed by repair using non-homologous end joining or microhomology-mediated end joining methods. Although the CRISPR/ cas9 technique has become very popular these days, its efficiency entirely depends on the optimization when used for a particular crop system. The efficiency also depends on the nuclease design and features, target site chromatin state, transformation strategy, target site repair, and outcome. Genome editing in foxtail millet using CRISPR/Cas9 and other tools is still at the budding. Editing a particular gene or genomic region to provide resistance against biotic stress has not been reported in foxtail millet yet. Lin et al. (2018) have mutated the *PDS* gene using CRISPR/ Cas9 that showed about 10% mutagenesis with 51% transfection efficiency (Lin et al. 2018). In *Setaria viridis* (Green foxtail), CRISPR/Cas9 was used to knock out *SvLES1* (*Less Shattering 1*), *Drm1a*, and *Drm1b* (Domain rearrange methylase) genes (Huang et al. 2019; Weiss et al. 2020). The use of editing tools, including CRISPR/Cas9 is yet to be done in foxtail millet for studying genes involved in biotic stress resistance.

## 7.7 Brief on Genetic Engineering for Resistance Traits

### *7.7.1 Biotic Stress Resistance*

Puranik et al. (2013) have reported that the NAC gene family can provide resistance against biotic and abiotic stress and shows a crucial role in development, secondary cell wall formation, cell cycle control, and senescence in plants. At various developmental stages in foxtail millet, expression of LEA (late embryogenesis abundant) (Wang et al. 2014), LTP (lipid transfer protein) (Pan et al. 2016) and HSP (heat shock proteins) (Singh et al. 2016) was observed in different tissues including inflorescence, seeds, leaves, stem and roots indicating towards their involvement in several biotic and abiotic stress response. ADP-ribosylation factors (ARFs) also play a crucial role in conferring tolerance to biotic and abiotic stress. In 2015, Li et al. studied the differentially expressed genes in contrasting cultivars of foxtail millet, cv. Shilixiang (resistant) and c. Yugu-1, against *Uromyces setariae-italica*. WRKY70, PER, PAL, SGT, and MKK1/2 showed higher expression levels

in the resistant cultivar. Post 24 h of infection, increased expression of GLU, GST, HSP90 and RPM1/RPS2 were observed in the cv. Shilixiang (Li et al. 2015). Further, microRNAs are known to play a significant part in conferring stress response in various crops. Several miRNAs were found to be upregulated in wheat leaves during powdery mildew disease, such as miR393, miR827, and miR444 (Xin et al. 2010). In brinjal, miRNAs, namely m0001 and m0002 provide resistance against *Verticillium dahlia* infection (Yang et al. 2013). *Exserohilum turcicum* infection in maize leads to upregulation of miR811 and miR845 in the resistant cultivar (Wu et al. 2014). Similar studies can be performed to ascertain the role of different genes and miRNAs in providing biotic stress resistance in foxtail millet.

### *7.7.2 Achievements of Transgenic Research in Foxtail Millet*

Studies conducted in response to multiple stresses have identified few candidate genes in foxtail millet so far. The development of an efficient transformation method is imperative for the generation of transgenics with an enhanced or silenced expression of the selected candidate genes. Few studies have reported the method for stable transformation of *Setaria* in the past decade (Martins et al. 2015; Saha and Blumwald 2016; Van Eck 2018; Rathinapriya et al. 2019; Nguyen et al. 2020; Santos et al. 2020; Sood et al. 2020). In addition to these transformation methods, the foxtail mosaic virus (FoMV)-induced gene silencing (VIGS) and virus-mediated overexpression (VOX) vector based on Foxtail mosaic virus (genus Potexvirus) has also been developed in *Setaria* (Liu et al. 2016; Bouton et al. 2018). Using these protocols, the generation of transgenic plants has been performed if foxtail millet, mainly to study genes identified during the abiotic stress response. For example, overexpression of *SiASR4* showed drought and salt tolerance via the ABA-dependent pathway in foxtail millet (Li et al. 2017). In another study, plants overexpressing *SiLTP* showed increased resistance while RNAi plants exhibited sensitivity to drought and salt stress (Pan et al. 2016). An in-depth analysis of genes involved in biotic stress response is achievable by utilizing these methods in the future.

## 7.8 Role of Bioinformatics as a Tool

### *7.8.1 Databases*

Following genome and transcriptome-based studies, several databases have been developed to serve as valuable resources for foxtail millet research. The Foxtail millet Marker Database (FmMDb; http://www.nipgr.res.in/foxtail.html) provides

access to genomic-, genic-SSRs, and ILP markers linking basic and applied sciences in foxtail millet (Bonthala et al. 2013). Foxtail millet microRNA Database (FmMiRNADb: http://59.163.192.91/FmMiRNADb/index.html) provided markers, secondary structure, and putative targets information for 355 mature miRNAs (Khan et al. 2014). Foxtail millet transcription factor database FmTFDb (http://59.163.192.91/FmTFDb/index.html) comprises 2,297 putative TFs belonging to 55 families (Bonthala et al. 2014). In 2015, SIFGD (http://structuralbiology.cau.edu.cn/SIFGD/) was established, combining the information from various data sources for functional analysis of foxtail millet genes (You et al. 2015). Further, Yadav et al. (2015) developed Foxtail millet Transposable Element-based Marker Database (FmTEMDb; http://59.163.192.83/ltrdb/index.html) from 30,706 TEs and 20,278 TE-based markers.

### *7.8.2 Integration of Different Datasets*

In addition to the databases designed for *Setaria*, other data sources such as Plantgbd, Phytozome, and Gramene also provide integrated information related to foxtail millet (Duvick et al. 2008; Goodstein et al. 2012; Gupta et al. 2016). Also, SIFGD (http://structuralbiology.cau.edu.cn/SIFGD/) was developed in 2015 by combining the information from various data sources, viz. Beijing Genomics Institute, NCBI, and Phytozome (You et al. 2015). Few of the studies have investigated differentially expressed genes and stress-responsive pathways during biotic stress in foxtail millet. For example, identification of DEGs during *Uromyces setariae-italicae* infection in foxtail millet to understand rust response (Li et al. 2015), and *S. graminicola* infection (Li et al. 2020). However, no databases are available until date, highlighting the genes or pathways linked to biotic stress response in foxtail millet. Though, information available in the above-mentioned databases can be translated to study molecular mechanism underlying biotic stress-induced response in *Setaria.*

## 7.9 Conclusions and Future Perspectives

It is evident that the screening and identification of genotypes exhibiting tolerance and susceptibility to various pathogens in foxtail millet has just begun in the past few years. Elucidation of genetic determinants underlying the contrasting disease responses in foxtail millet is at a nascent stage now. Thus, identifying marker gene/s associated with biotic stress could be a primary step for future breeding programs in foxtail millet. Genetic approaches targeting the candidate gene offer a powerful alternative to facilitate crop improvement by providing effective biotic stress resistance. Different omics technologies can be integrated to develop elite cultivars tolerant against biotic stress (Fig. 7.2). Thus, this chapter aims to brief the research

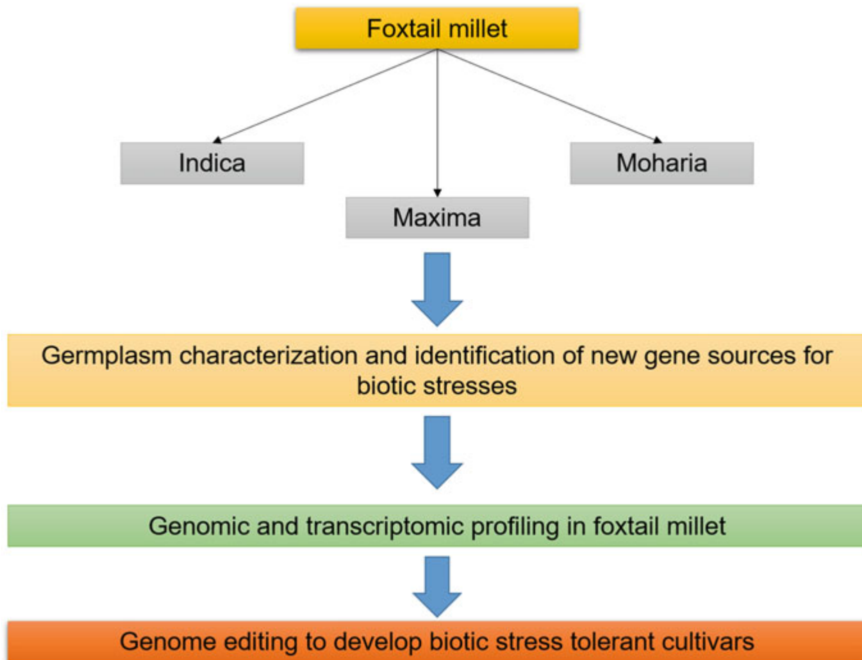



**Fig. 7.2** Strategies to incorporate biotic stress tolerance using the germplasm available for foxtail millet

work done to understand biotic stress response in foxtail millet and identify genetic determinants underlying the trait. Furthermore, the identification of selected candidate genes, followed by their targeted sequencing, may provide information regarding the SNPs linked to tolerance trait. Identified SNPs (allele-specific) will serve as diagnostic markers or selectable markers for marker-assisted breeding (MAS) to generate patho-stress-tolerant varieties. Functional characterization of selected candidate genes would be the next step to determine their precise role in stress response and improved tolerance.

**Acknowledgements** The authors' research in millet genetics and genomics is supported by the DST INSPIRE Grant (File No. DST/INSPIRE/04/2016/002341) and DST-SERB ECRA Grant (File No. ECR/2017/001526) awarded by the Department of Science and Technology, Govt. of India, and Science and Engineering Research Board, Govt. of India.

## References

Andersen EJ, Nepal MP (2017) Genetic diversity of disease resistance genes in foxtail millet (*Setaria italica* L). Plant Gene 10:8–16

Austin DF (2006) Foxtail millets (Setaria: Poaceae) - Abandoned food in two hemispheres. Econ Bot 60(2):143–158

Baltensperger DD (2002) Progress with proso, pearl, and other millets. In: Janick J, Whipley A (eds) Trends in new crops and new uses. ASHS Press, Alexandria, pp 100–103

Banu H, Gowda J, Gowda MC (2018) Characterization and identification of DUS traits in reference set of foxtail millet germplasm (*Setaria italica* (L) Beauv). Intl J Curr Microbiol Appl Sci 7(12):175–183

Bennetzen JL, Schmutz J, Wang H, Percifield R, Hawkins J et al (2012) Reference genome sequence of the model plant Setaria. Nat Biotechnol 30:555–561

Bonthala VS, Muthamilarasan M, Misra G, Prasad M (2013) FmMDb: a versatile database of foxtail millet markers for millets and bioenergy grasses research. PLoS ONE 8(8):e71418

Bonthala VS, Muthamilarasan M, Roy R, Prasad M (2014) FmTFDb: a foxtail millet transcription factors database for expediting functional genomics in millets. Mol Biol Rep 41:6343–6348

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

Bouton C, King RC, Chen H, Azhakanandam K, Bieri S et al (2018) Foxtail mosaic virus: a viral vector for protein expression in cereals. Plant Physiol 177:1352–1367

Chai W, Si W, Ji W, Qi Q, Zhao M, Jiang H (2018) Genome-wide investigation and expression profiling of HD-zip transcription factors in foxtail millet (*Setaria italica* L.). Biomed Res Int

Chander S, Bhat KV, Kumari R, Sen S, Gaikwad AB, Gowda MVC, Dikshit N (2017) Analysis of spatial distribution of genetic diversity and validation of Indian foxtail millet core collection. Physiol Mol Biol Plants 23(3):663–673

Das IK, Rakshit S (2016) Millets, their importance, and production constraints. Biotic stress resistance in millets, Academic Press, pp 3–19. https://doi.org/10.1016/B978-0-12-804549-7.00001-9

Das IK, Nagaraja A, Tonapi VA (2016) Diseases of millets—a ready reckoner. Indian Institute of Millets Research, Rajendranagar, Hyderabad 500030, Telangana, p 67. ISBN: 81-89-335-59-6.7

Diao X, Jia G (2017) Foxtail millet breeding in China. In: Doust A, Diao X (eds) Genetics and genomics of Setaria. Springer, Cham, pp 93–113

Duvick J, Fu A, Muppirala U, Sabharwal M, Wilkerson MD et al (2008) PlantGDB: a resource for comparative plant genomics. Nucleic Acids Res 36:959–965

Dwivedi S, Upadhyaya H, Senthilvel S, Hash C, Fukunaga K, et al (2012) Millets: genetic and genomic resources. In: Janick J (ed) Plant Breeding Reviews, vol 35. Wiley, USA, pp 247–375

Fang X, Dong K, Wang X, Liu T, He J, Ren R, Huang M (2016) A high density genetic map and QTL for agronomic and yield traits in foxtail millet [*Setaria italica* (L) P Beauv]. BMC Genom 17(1):336

FAOSTAT (2018) Food and agricultural organization of the United Nation. FAO statistical database. http://faostat.fao.org

Gaikwad AP, D'souza TF (1986) Studies on the blast disease of rala. J Maharashtra Agri Univ 11 (1):108–109

Ghimire BK, Yu CY, Kim SH, Chung IM (2019) Assessment of diversity in the accessions of *Setaria italica* L based on phytochemical and morphological traits and ISSR markers. Molecules 24(8):1486

Goodstein DM, Shu S, Howson R, Neupane R, Hayes RD et al (2012) Phytozome: a comparative platform for green plant genomics. Nucleic Acids Res 40:1178–1186

Gupta P, Naithani S, Tello-Ruiz MK, Chougule K, D'Eustachio P et al (2016) Gramene database: navigating plant comparative genomics resources. Curr Plant Biol 7–8:10–15

Hao L, Liu J, Zhang A, Han Y, Guo E, Yi H (2020) Differential gene expression in foxtail millet during interaction with the smut fungus *Ustilago crameri*. Physiol Mol Plant Pathol 110:101459. https://doi.org/10.1016/j.pmpp.2020.101459

Huang P, Mamidi S, Healey A, Grimwood J, Jenkins J, et al (2019) The Setaria viridis genome and diversity panel enables discovery of a novel domestication gene. bioRxiv 744557

Jaiswal V, Gupta S, Gahlaut V, Muthamilarasan M, Bandyopadhyay T, Ramchiary N, Prasad M (2019) Genome-wide association study of major agronomic traits in foxtail millet (*Setaria italica* L) using ddRAD sequencing. Sci Rep 9(1):1–11

Jegera MJ, Gilijamsea E, Bockb CH, Frinking HD (1998) The epidemiology, variability and control of the downy mildews of pearl millet and sorghum, with particular reference to Africa. Plant Pathol 47:544–569

Jia G, Huang X, Zhi H, Zhao Y, Zhao Q, Li W, Zhu C (2013) A haplotype map of genomic variations and genome-wide association studies of agronomic traits in foxtail millet (*Setaria italica*). Nat Genet 45(8):957–961

Khan Y, Yadav A, Bonthala VS, Muthamilarasan M, Yadav CB, Prasad M (2014) Comprehensive genome-wide identification and expression profiling of foxtail millet [*Setaria italica* (L.)] miRNAs in response to abiotic stress and development of miRNA database. Plant Cell Tiss Org Cult 118:279–292

Konda S, Nagaraja A, Nagamma G, Sangeetha PS, Patil S, Dev D, Anjum SS (2016) In vitro evaluation of bio-agents and fungicides against leaf blast (Pyricularia setariae) in foxtail millet [*Setaria italica* (L.) Beauv.]. J Pure Appl Microbiol 10(1):489–495

Krishnamurthy L, Serraj R, Rai KN, Hash CT, Dakheel AJ (2007) Identification of pearl millet [*Pennisetum glaucum* (L) RBr] lines tolerant to soil salinity. Euphytica 158(1–2):179–188

Krishnamurthy L, Upadhyaya HD, Kashiwagi J, Purushothaman R, Dwivedi SL, Vadez V (2016) Variation in drought-tolerance components and their interrelationships in the core collection of foxtail millet (*Setaria italica*) germplasm. Crop Pasture Sci 67(8):834–846

Kumar B (2011) First record of smut disease of foxtail millet caused by *Ustilago crameri* Korn. J Mycol Plant Pathol 41:459–461

Kundu GG, Kishore P (1971) New record of parasites of *Sesamia inferens W.* and *Atherigona nudiseta R.* infesting minor millets. Indian J Otolaryngol 33:466–467

Lata C, Sahu PP, Prasad M (2010) Comparative transcriptome analysis of differentially expressed genes in foxtail millet (*Setaria italica* L.) during dehydration stress. Biochem Biophy Res Commun 393:720–727

Lata C, Gupta S, Prasad M (2013) Foxtail millet: a model crop for genetic and genomic studies in bioenergy grasses. Crit Rev Biotechnol 33:328–343

Lata C, Mishra AK, Muthamilarasan M, Bonthala VS, Khan Y, Prasad M (2014) Genome-wide investigation and expression profiling of AP2/ERF transcription factor superfamily in foxtail millet (*Setaria italica* L.). PLoS ONE 9(11):e113092

Lin CS, Hsu CT, Yang LH, Lee LH, Fu JY et al (2018) Application of protoplast technology to CRISPR/Cas9 mutagenesis: from single-cell mutation detection to mutant plant regeneration. Plant Biotechnol J 16:1295–1310. https://doi.org/10.1111/pbi.12870

Liu N, Xie K, Jia Q, Zhao J, Chen T et al (2016) Foxtail mosaic virus-induced gene silencing in monocot plants. Plant Physiol 171:1801–1807

Liu H, Qin J, Fan H, Cheng J, Li L, Liu Z (2017) Genome-wide identification, phylogeny and expression analyses of SCARECROW-LIKE(SCL) genes in millet (*Setaria italica*). Physiol Mol Biol Plants 23:629–640

Li ZY, Wang N, Dong ZP, Dong L, Bai H, Quan JZ, Liu L (2014) First report of sheath blight caused by Waitea circinata affecting foxtail millet (*Setaria italica*) in China. Plant Dis 98:1442

Li ZY, Wang N, Dong L, Bai H, Quan JZ, Liu L, Dong ZP (2015) Differential gene expression in foxtail millet during incompatible interaction with *Uromyces setariae-italicae*. PLoS ONE 10 (4):e012382 5. https://doi.org/10.1371/journal.pone.0123825

Li J, Dong Y, Li C, Pan Y, Yu J (2017) SiASR4, the target gene of SiARDP from Setaria italica, improves abiotic stress adaption in plants. Front Plant Sci 7:1–17

Li R, Han Y, Zhang Q, Chang G, Han Y, Li X, Zhang B (2020) Transcriptome profiling analysis reveals co-regulation of hormone pathways in foxtail millet during *Sclerospora graminicola infection*. Intl J Mol Sci 12, 21(4):1226

Martins PK, Ribeiro AP, Cunha BA, Kobayashi AK, Molinari HBC (2015) A simple and highly efficient *Agrobacterium*-mediated transformation protocol for *Setaria viridis*. Biotechnol Rep 6:41–44

Mirzaee MR, Zare R, Nasrabad AA (2010). A new leaf and sheath brown spot of foxtail millet caused by Bipolaris australiensis. Australas Plant Dis Notes 5(1):19–20

Muthamilarasan M, Prasad M (2015) Advances in Setaria genomics for genetic improvement of cereals and bioenergy grasses. Theor Appl Genet 128:1–14. https://doi.org/10.1007/s00122-014-2399-3

Muthamilarasan M, Bonthala VS, Mishra AK, Khandelwal R, Khan Y, Roy R et al (2014) C2H2 type of zinc finger transcription factors in foxtail millet define response to abiotic stresses. Funct Integr Genom 14:531–543

Muthamilarasan M, Bonthala VS, Khandelwal R, Jaishankar J, Shweta S et al (2015) Global analysis of WRKY transcription factor superfamily in setaria identifies potential candidates involved in abiotic stress signaling. Front Plant Sci 6:1–15

Muthamilarasan M, Mangu VR, Zandkarimi H, Prasad M, Baisakh N (2016) Structure, organization and evolution of ADP-ribosylation factors in rice and foxtail millet, and their expression in rice. Sci Rep 6:1–13. https://doi.org/10.1038/srep24008

Nagaraja A, Das IK (2016) Disease resistance in pearl millet and small millets. Indian Institute of Millets Research, Rajendranagar, Hyderabad, Telangana, India, p 67. ISBN: 81-89-335-59-6.7

Nakayama H, Nagamine T, Hayashi N (2005) Genetic variation of blast resistance in foxtail millet (*Setaria italica* (L.) P. Beauv.) and its geographic distribution. Genet Resour Crop Evol 52:863–868

Nguyen DQ, Van Eck J, Eamens AL, Grof CPL (2020) Robust and reproducible *Agrobacterium*-mediated transformation system of the C4 genetic model species *Setaria viridis*. Front Plant Sci 11:1–12

Oh J, Park CY, Min HG, Lee HK, Yeom YA, Yoon Y, Lee SH (2017) First report of barley virus G in foxtail millet (*Setaria italic*a) in Korea. Plant Dis 101(6):1061

Pan Y, Li J, Jiao L, Li C, Zhu D, Yu J (2016) A non-specific *Setaria italica* lipid transfer protein gene plays a critical role under abiotic stress. Front Plant Sci 7:1–15

Puranik S, Sahu PP, Mandal SN, Bonthala VS, Parida SK, Prasad M (2013) Comprehensive genome-wide survey, genomic constitution and expression profiling of the NAC transcription factor family in foxtail millet (*Setaria italica* L.). PLoS ONE 8(5):e64594

Rathinapriya P, Satish L, Rameshkumar R, Pandian S, Rency AS, Ramesh M (2019) Role of activated charcoal and amino acids in developing an efficient regeneration system for foxtail millet (*Setaria italica* (L.) Beauv.) using leaf base segments. Physiol Mol Biol Plants 25:533–548

Saha P, Blumwald E (2016) Spike-dip transformation of *Setaria viridis*. Plant J 86:89–101

Santos CM, Romeiro D, Silva JP, Basso MF, Molinari HBC, Centeno DC (2020) An improved protocol for efficient transformation and regeneration of *Setaria italica*. Plant Cell Rep 39:501–510

Sharma R, Girish AG, Upadhyaya HD, Humayun P, Babu TK, Rao VP, Thakur RP (2014) Identification of blast resistance in a core collection of foxtail millet germplasm. Plant Dis 98 (4):519–524

Shen HR, Dong ZP, Wang YF, Quan JZ, Bai H, Li ZY (2020) First report of dwarf disease in foxtail millet (*Setaria italica*) caused by Barley Yellow Striate Mosaic Virus in China. Plant Dis 104(4):1262. https://doi.org/10.1094/PDIS-10-19-2056-PDN

Singh RK, Jaishankar J, Muthamilarasan M, Shweta S, Dangi A, Prasad M (2016) Genome-wide analysis of heat shock proteins in C4 model, foxtail millet identifies potential candidates for crop improvement under abiotic stress. Sci Rep 6:1–14

Sood P, Singh RK, Prasad M (2020) An efficient *Agrobacterium*-mediated genetic transformation method for foxtail millet (*Setaria italica* L.). Plant Cell Rep 39:511–525

Van Eck J (2018) The status of *setaria viridis* transformation: *Agrobacterium*-mediated to floral dip. Front Plant Sci 9:1–5
Wang M, Li P, Li C, Pan Y, Jiang X, et al (2014) SiLEA14, a novel atypical LEA protein, confers abiotic stress resistance in foxtail millet. BMC Plant Biol 14(1):1–16
Wang P, Wang H, Wang Y, Ren F, Liu W (2018a) Analysis of bHLH genes from foxtail millet (*Setaria italica*) and their potential relevance to drought stress. PLoS ONE 13:1–16
Wang Z, Zhao K, Pan Y, Wang J, Song X et al (2018b) Genomic, expressional, protein-protein interactional analysis of Trihelix transcription factor genes in *Setaria italica* and inference of their evolutionary trajectory. BMC Genom 19:1–12
Wang Z, Wang J, Peng J, Du X, Jiang M, Li Y, Yong J (2019) QTL mapping for 11 agronomic traits based on a genome-wide Bin-map in a large F2 population of foxtail millet (*Setaria italica* (L) P Beauv). Mol Breed 39(2):18
Weiss T, Wang C, Kang X, Zhao H, Elena Gamo M, et al (2020) Optimization of multiplexed CRISPR/Cas9 system for highly efficient genome editing in Setaria viridis. Plant J 104(3):828–838
Wu F, Shu J, Jin W (2014) Identification and validation of miRNAs associated with the resistance of maize (Zea mays L.) to Exserohilum turcicum. PLoS ONE 9(1):e87251
Xin M, Wang Y, Yao Y, Xie, C, Peng H, et al (2010) Diverse set of microRNAs are responsive to powdery mildew infection and heat stress in wheat (Triticum aestivum L.). BMC Plant Biol 10 (1):1–11
Yadav CB, Bonthala VS, Muthamilarasan M, Pandey G, Khan Y, Prasad M (2015) Genome-wide development of transposable elements-based markers in foxtail millet and construction of an integrated database. DNA Res 22:79–90
Yang L, Jue D, Li W, Zhang R, Chen M, et al (2013) Identification of MiRNA from eggplant (Solanum melongena L.) by small RNA deep sequencing and their response to Verticillium dahliae infection. PLoS ONE 8(8):e72840
You Q, Zhang L, Yi X, Zhang Z, Xu W, Su Z (2015) SIFGD: Setaria italica functional genomics database. Mol Plant 8:967–970
Zhang G, Liu X, Quan Z, Cheng S, Xu X et al (2012) Genome sequence of foxtail millet (*Setaria italica*) provides insights into grass evolution and biofuel potential. Nat Biotechnol 30:549–554
Zhang L, Liu B, Zheng G, Zhang A, Li R (2017) Genome-wide characterization of the SiD of gene family in foxtail millet (*Setaria italica*). BioSystems 151:27–33
Zhu YB, Xie XQ, Li ZY, Bai H, Dong L, Dong JG (2014) Bioinformatic analysis of the nucleotide binding site-encoding disease-resistance genes in foxtail millet (*Setaria italica* (L.) Beauv.). Genet Mol Res 13(3):6602–6609. https://doi.org/10.4238/2014.august.28.5

# Chapter 8
# Genomic Designing for Biotic Stress Resistance in Finger Millet

**B. Kalyana Babu and Rashmi Chauhan**

**Abstract** Finger millet is highly nutra-cereal crop having rich sources of fiber, calcium, and proteins. In India, crop is grown mainly as rainfed crop which is known as poor man's crop. Till recently, very less genomic information is available, however which was filled with recent whole genome sequence of finger millet. The crop finger millet is widely adapted to harsh climatic conditions which are also a drought tolerant crop. Despite its well-known use as key cereal crop, mainly for deprived persons in the dry climatic areas, it is ignored in genetic enhancement programmes. Genomic studies revealed several significant QTLs for blast resistance, drought tolerance, and other quality parameters. The present chapter described in brief on the genomics improvement of finger millet for various biotic, abiotic and nutritional parameters. Comparative genomics also studied in brief in comparison with related crops like pearl millet and rice to identify the key homolog and orthologous genes. Genetic engineering approaches were mainly targeted for improvement of drought tolerance and blast resistance which are major factors in finger millet improvement. The present chapter gives a clear picture on various aspects of phylogeny, origin, genetics, molecular and genetic engineering approaches for finger millet genetic enhancement.

**Keywords** Finger millet · Blast resistance · Association mapping · Comparative genomics · Quantitative trait loci

---

B. Kalyana Babu (✉)
ICAR—Indian Institute of Oil Palm Research, West Godavari District, Pedavegi, Andhra Pradesh 534450, India

R. Chauhan
G. B. Pant University of Agriculture and Technology, Pant nagar, Uttarakhand, India

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021

C. Kole (ed.), *Genomic Designing for Biotic Stress Resistant Cereal Crops*,
https://doi.org/10.1007/978-3-030-75879-0_8

## 8.1 Introduction

Finger millet, is from the genus *Eleusine*, subspecies *coracana*, family Poaceae belongs to Eragrostideae tribe. Once, it was thought that Uganda and its neighbouring part was origin of *Eleusine coracana* and brought to India, most likely around 3,000 years ago (FAO 2007). Finger millet also called as *Ragi* in India. *Ragi* also grown in some regions of Tibet, Burma, Nepal, Sumatra, Malaysia, Philippines, Java, Iran, and Afghanistan. Due to continuous natural and forcible selection by the human beings, the secondary origin of center was developed which presently being called as India.

The inflorescence is called as digitate or sub digitate, where as spikes are stout and having curved. The grain is in globose structure where its colour generally in reddish, blackish or sometimes whitish also (Phillips 1972). The grain of finger millet is mostly black in colour. The inflorescence branches are long slender, outward curve nature exists mostly in the maturity times. The *recluse* has open fingers with short length, but no curves observed in the branches of finger millet. The *laxa* race long fingers which are open in nature, where spikelets are settled on shallow portions of inflorescence.

The primary, secondary and tertiary centres of origin were not clearly demarcated in oil palm due to insufficient data. The phylogenetic and systematic relations also not clearly determined. However, the germplasm of finger millet divided into three categories. The secondary gene pool consisted of diploid genomes, whereas tertiary gene pool having *Eleusine*. The literature clearly indicated that variation persisted in cultivated germplasm of finger millet in Africa (Primary center) and also in secondary center of origin (India). The high amount of genetic variation is important for germplasm collection, preservation and for exploration studies. For this purpose wild and cultivated finger millet are important which may be efficiently used for these purposes. The wild millet migrated from Africa to other regions of the world like American continent and Asian sub continents. In Africa, weeds similar to finger millet were emerged due to natural pollination occurred between wild and cultivated millet. This hybrid was almost similar to the *E. coracana*, however it is a weed with no productivity. This has paved the way for generating new forms which are intermediate in nature. These are very restricted to the strongly related cereal crop. The secondary gene pool consists of *indica*, *floccifolia* and *tristachya* species, whereas *intermedia*, *kigeziensis* belongs to tertiary gene pool.

## 8.2 Biotic Stresses in Finger Millet

The interaction of avirelence (*Avr*) genes and resistance (*R*) genes will determine the resistance mechanism of disease in most of the crop plants. This type of resistance mechanism is mostly supported by hypersensitive response which is key

to the control of the growth and wide spread of the pathogen (Carine et al. 2008). Panwar et al. (2010) identified the relationship of *Magnaporthe grisea* NBS sequences through functional molecular markers. These markers are part of the genes related to the NBS-LRR proteins in finger millet. These genes are large diverse gene family which are having distinctive N-terminal domain. They conducted this study in a large sample of finger millet for unravelling the genetic relationship of disease resistance genes. The results showed that these NBS-LRR sequences are highly conserved among the related germplasm which helped in molecular dissection of germplasm of finger millet. Like this few reports are supported this evidence where RAPD markers were used on forty five blast pathogen isolates. They found that finger print profile was produced for nearly 25–30% linkage distance. This resulted in forming two major clusters based on RAPD molecular classification. From crops like rice, Arabidopsis and wheat, nearly 48–50 resistance (*R*) genes were isolated and cloned through transposon tagging as reported by Okuyama et al. (2011). The amino acid motifs are highly conserved among these species have been studied widely and used to characterize NBS-LRR connected genes (Meyers et al. 2003). The amount of polymorphism was observed between 71.4 and 85%. With an aim to conserve the NBS-LRR regions of cloned resistance genes, Reddy et al. (2011) secluded these *R* gene homologous genes in finger millet. These were isolated using EST based primers which amplify the conserved NBS regions. They cloned nearly 107 NBS-LRR sequences. Out of the 107, high amount of similarity was found for 41 known *R* genes which they named as EcRGHs (*Eleusine coracana* resistance gene homologs). However, nearly 10 cloned sequences showed similarity to pollen related proteins which were named as EcPSiPs (*Eleusine coracana* pollen signalling proteins).

## 8.3 Genetic Resources of Resistance Genes

In India, the main organization conserving the crop germplasm or gene pool is ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi. It is a repository source of all crop germplasm including finger millet. It is estimated that nearly 10,507 germplasm is under long term conservation to cater the needs of the finger millet breeding programmes. Out of these 10,507 gene pool, nearly 4,500 are of exotic origin and non-Indian origin. This shows the importance of gene pool in the ongoing research activities. Most of these collections are from locally collected sources however included 117 accessions belong to exotic origin. Sood et al. (2016) observed that these collections were having mostly wild species belonging to *intermedia*, *kigeziensis*, *semisterlis* and *jaegeri*. Along with this wild species, 154 advanced varieties, and 64 pre breeding material also included. Some of these germplasm also being maintained at AICSMIP center at Bangalore as a short and medium term storage which is being used in research purposes of the needs of India. It also helping the research needs of most of the national scholars for their post graduate studies. Along with NBPGR, ICRISAT also maintaining the genetic

resources of finger millet in a large scale. The ICRISAT is having a global depository of nearly 6,000 accessions belongs to different origin of countries (mainly exotic origin) having 105 wild one, 5,600 land races, 140 advanced breeding materials and 50 pre breeding germplasm (Sood et al. 2016). All these collections throughout the world makes a rich source of finger millet germplasm which provides long term storage. These collections are a major source of gene pool across the world. At global level, other than ICRISAT, USDA-ARS in Griffn, Georgia also maintaining finger millet core germplasm of 770 accessions which consists of around 20 wild species. Other countries like Zimbabwe, Uganda, Bhutan, Srilanka and Kenya also maintaining 1,158, 1,155, 84, 393 and 1,902 gene pool respectively (The Global Crop Diversity Trust 2012). All these germplasm collections lead to development of core collections which captured the maximum genetic diversity which is nearly representing 10% of the whole germplasm (Upadhyaya et al. 2006). This core collection is presently being used by several researchers in all the breeding and molecular breeding programmes.

## 8.4 Classical Genetics and Traditional Breeding for Biotic Stress Resistance

Finger millet is known as poor man's crop which serves the needs of underprivileged persons of most of the Asian countries. Since long time, it is ignored from the main research activities as compared to rice, and maize (Upadhyaya et al. 2006). Because of its nutritional properties, wide acceptance has been taken place now a days in the developing and under developed countries where developed countries already utilizing its sources. However, till 2010, less work in the improvement of finger millet using conventional and molecular breeding approaches. The phenotypic characterization of finger millet like other crops also faced some restrictions for improving the complex quantitative traits. The phenotypic traits are highly influence by environmental conditions and conventional breeding offer limited success to improve those traits. In such cases, conventional breeding together molecular techniques will help in improving the finger millet improvement in a short time, precisely.

After the availability of Sanger sequencing on a commercial scale, the molecular biology lead to whole genome sequencing (WGS) in essential crops where it is economically feasible. These techniques surpass the disadvantages of biochemical and morphological markers like environmental factor influence, developmental stages and inadequate number (Winter and Kahl 1995). These modern biological tools like molecular markers are more stable and are large number. The finger millet DNA content was determined using Feulgen micro-spectrophotometry technique by Hiremath and Salimath (1991). Later in 1997, Mysore and Baird found the 2C value of finger millet as 3.36–3.87 pg (1 pg = 980 Mbp) which is more than the genome size of rice. Several works found that near about 45% of the finger millet genome

had repeated sequences in DNA which also having 18% with single copy (Gupta and Ranjekar 1981). Out of the total repetitive sequences, 20% were belonged to long-range interspersion pattern, whereas short-period interspersion patterns represent 60%. They proposed based on the evidences that, crops having more than 2.5 pg of DNA content responsible for short-period interspersion pattern, while less than 2.5 pg of DNA content responsible for diverse genome organization.

## 8.5 Marker-Assisted Breeding for Resistance Traits in Finger Millet

Disease resistance mechanism of any species like finger millet need to be studied thoroughly at molecular level for identifying the genes linked to those traits. The modern tools like molecular markers offers a vast range of applications and tools to dissect the loci involved in disease or pest resistance. The DNA based markers are presently being widely used in marker assisted breeding programmes to improve the traits (Babu et al. 2007). This gives more accuracy towards tracking the significant QTLs in comparison to conventional breeding (Ceasar et al. 2018). Research work done for the investigation of genetic diversity and quantitative trait loci mapping in finger millet are discussed below.

### *8.5.1 Genetic Diversity Analysis*

The whole living organisms are made up of infinite genetic diversity (Narain 2000). As we know that in the earth, no two sexually reproducing organisms are similar. This is due to changes occurred in the hereditary mechanism while reproduction. The high amount of heterozygosity is the major driving factor in creating large genetic divergence in several populations which is a basic determinant of breeding knowledge and programmes (Durand et al. 2010). Earlier, assessment of variations at genetic level was done at morphological basis and also by biochemical parameters. However, these were inhibited due to several factors which were discussed in the above section. These were undergone by using modern molecular tools like genomics and transcriptomes for the evaluation of genetic variation. To induce the desirable variations in the economic traits, different genetic resources and technologies were used. A pervasive investigation of feasible genetic materials and utilization of naturally occurring changes can establish to be a helpful basis of genomic information. As per the collections made at several institutes, this rich gene pool present in the wild species and land races can be used in essential traits like blast resistance to develop the high yielding and blast resistance cultivars. Very less research has been done at molecular level for diversity studies and mapping studies using several molecular markers like RAPD, RFLP (Salimath et al. 1995),

micro satellite markers (Dida et al. 2007; Babu et al. 2014a, b, c, d), and biochemical markers (Hilu 1995). Recently, little work was done on several aspects of finger millet improvement using molecular techniques (Kumar et al. 2012; Panwar et al. 2010; Babu et al. 2007; Dida et al. 2007). In comparison to other major crops like rice, wheat and maize, scanty of genome information available for finger millet crop.

### 8.5.2 Association Mapping Studies

Worldwide, plant breeders are aiming to create and make use of natural variation by the way of changing the genetic code in DNA sequences. Since long back, linkage based mapping is the most widely used methodology using F2:3, RIL, BC populations by crossing diverse parents for a trait with known relation. However, later in the animal sciences, linkage disequilibrium (LD) and association mapping (AM) are being popular for gene identification because of their alternate advantages in addition to the linkage based mapping. Association mapping detects significant loci by combination of genotypic and phenotypic data using linkage disequilibrium in the population estimated. The concept of AM was first developed for human genetics which is now extended to animals and plants. It is useful for detection of new loci or QTLs based on the lineage, strength of correlation between genetic markers and traits of interest. This is particularly useful where highly dense markers are available like SNP markers. Through parental linkage mapping studies, one can depend only on recombination events which were come across throughout the mapping populations under study. The main advantage of association mapping is the much higher mapping resolution, and it will also consider the evolutionary history of the population in comparison with family mapping. In addition, it also has advantage of identifying unlimited QTLs for any quantitative trait. The advantages of association mapping over traditional QTL mapping are broader genetically different reference population, higher resolution mapping, utilization of ancestral origin data, and it is cheaper and saves time (Sood et al. 2016). There is a report on identifying major QTLs for important economic traits in finger millet. They used genomic SSRs which identified five SSRs linked to some traits at a p value of less than 0.001 (Babu et al. 2014b). Out of these QTLs, the one linked to basal tiller number was linked tightly by the UGEP81 marker at p of 0.001 which explained 10.8% of phenotypic variance. Likewise 50% flowering trait was associated with UGEP77 and UGEP90 markers by explaining phenotypic variance of 10 and 8.7% respectively. Flag leaf width and height of the plant were found to linked to FM9 genic SSR marker.

### 8.5.3 Molecular Mapping of Resistance Genes and QTLs

The major disease leaf blast, in finger millet is caused by *Pyricularia grisea* (Cke.) Sacc. affects yield loss to a great extent and also other biotic factors like Striga also causes yield loss. These two are major biotic factors causing reduction in production and productivity varies from 25 to 90% in severe cases. Several workers made for identifying resistance genes for leaf blast by comparative genomic approaches using genic or functional EST based markers (Sood et al. 2016). Seventy percent of the *R* genes (transcription factors) consist of NBS-LLR domain proteins. In few reports, these NBS regions showed maximum similarity with rice blast resistance genes like *PiKh* and *Pi21*. From these reports, it may be evident that blast resistance genes of rice might be having similarity with finger millet NBS genes might be playing vital role in marker assisted selection of blast resistant cultivars (Babu et al. 2014c; Kumar et al. 2016). Some SSR markers (EST-SSR4, FMBLEST5) which were found to be potential were designed in the conserved region of NBS-LRR and EST sequences (Babu et al. 2014a; Sood et al. 2016). It is also found that blast resistant Pi genes showed similarity *In silico* level by comparing the finger millet NBS-LRR and Pi gene sequences (Babu et al. 2014c). Several workers (Saha and Rana 2016) isolated R gene homologs from the conserved regions of cloned *R* genes (Babu et al. 2014a; Reddy et al. 2011). Then they developed flanking SSR markers for the resistant genes. These markers were used for delineation of susceptible and resistant genotypes (Saha and Rana 2016) which were called EcRGHs.

The other major biotic constraint is *Striga* spp. In African countries, where finger millet is a major staple crop. It affects almost 25–85% yield losses (Atera and Itoh 2011; Sood et al. 2016). Even in severity, it was reports that 100% crop damage by the Striga (Haussmann et al. 2000). Development of Striga resistant cultivars is the most possible objective of the breeders and researchers throughout the world. Development of genetic resistance to the Striga is the only feasible method to restrict it which is also eco friendly technique. Searching of the potential candidate genes for Striga resistance also may be useful, may sometimes available in the wild finger millet crops. This type of resistance to Striga also reported in other crops like rice and other millets (Oswald 2005; Harahap et al. 1993; Ejeta and Gressel 2007). Taking lead from these crops may help in developing the Striga resistant finger millet crop. Association mapping studies were available for identifying QTLs for traits like morphological traits like days to flowering, plant height and flag leaf blade width. Four significant SSR marker associations were found out of 100 SSR markers using core collection of finger millet germplasm (Babu et al. 2014b, d). They also rice markers which were reported to be linked for blast resistance in rice (Babu et al. 2014e, 2016). The genic marker FMBLEST32 and RM 262 were found to be associated with blast disease resistance of finger millet at a *p*-value of 0.007 and $R^2$ of 10 and 8%, respectively. Finger blast was associated with UGEP81 at p of 0.009 and with 7.5% of phenotypic variance. However, same trait was linked with multi markers like UGEP56, UGEP8, UGEP65, and UGEP31 (Bharathi 2011).

So, the notable use of mapping populations for the benefit of getting better idea on marker association and broad revision of these allied QTL would be useful for authentication of the multi-trait QTLs which were found till now.

## 8.6 Genetic Engineering for Resistance Traits in Finger Millet

Though some work was done on the linkage and association mapping, considerably genetic engineering works were initiated in finger millet well before the genomics (Gupta et al. 2011). They used GUS reporter gene for genetic engineering and transformation work using five promoters of genes like *uql*, *Actl*, *RbcS* via (Ft) gene promoter. They observed efficient GUS expression under Rbc S and CaMV35S promoters (Gupta et al. 2011). This work will be very much useful for beginners who can directly use these promoters for efficient GUS expression in finger millet transformation studies. The first report of Latha et al. (2005) in finger millet used *pin* gene encoding for fungicidal PIN protein of prawn for leaf blast resistance. These are basic requirements for efficient transgenic development and transformation protocol which are involved in cisgenics development for improvement of yield and nutritional parameters. Lot of progress also had been made using bombardment of callus and *Agrobacterium* approaches (Ceasar and Ignacimuthu 2009, 2011; Kothari et al. 2005). Along with transformation works, genomics may play a significant role in resistance development of finger millet.

## 8.7 Brief Account on Social, Political and Regulatory Issues

Millets are facing a lot of social and regulatory issues in developing countries like India, due to more urbanization. In the last 40 years (1960–2010), millets faced a bad situation in terms of decreasing area and also production. Small millets area decreased nearly 80%, whereas 23% decrease in area was observed for pearl millet. Also there is steep decreasing trend in overall utilization of consumption of millets for household and other purposes. There are very limited schemes available for encouraging the millets area and production which are not well reached to the farmers (Dhan Foundation 2012). They were became eye wash to the poor farmers. Of course, from government side, no major policies or schemes were initiated, not as like mission mode on oil seed crops. By looking at all these things, more awareness, and funding for research need to be allotted for millets development. The important and notable schemes for millet crop improvement are Rashtriya Krishi Vikas Yojana, Coarse Cereals based Cropping Systems Areas, Macro Management of Agriculture, and Integrated Cereals Development Programmes

(Dhan Foundation 2012). A large variation also persisted between states to utilize these funds. Some states like Karnataka utilized the benefits of these schemes, however other could not utilize them properly and diverted for other uses. Though there are number of policy initiative as mentioned above, full fledge efforts were not done by the government organization, NGOs or any other societies for millets improvement. It also emphasized that the involvement of public also matters a lot for promoting the consumption of millet. Only cardiac and sugar patients consuming in one other form due to compulsion or else its consumption is almost less. The still most awaited pending bill called National Food Security Bill may give better results in changing the current scenario to a maximum extent (Dhan Foundation 2012).

## 8.8 Future Perspectives

Though recently, the draft genome sequence of finger millet available, genomics has not been fully explored in finger millet to the extent to use in marker assisted breeding programmes and also for introgression of desirable genes. So, the genome of finger millet may be used as a basic platform for comparative genomics and to identify significant genes for economic traits. Computational genomics also plays important role in improving the oil palm genomic information. Along with this basic research focused policies need to put forth for upliftment of finger millet area and consumption. Awareness need to be brought out on the nutrional importance of millets especially finger millet.

## References

Atera E, Itoh K (2011) Evaluation of ecologies and severity of Striga weed on rice in sub-Saharan Africa. Agric Biol J Amer 2:752–760. https://doi.org/10.5251/abjna.2011.2.5.752.760

Babu BK, Senthil N, Gomez S, Biji K, Rajendraprasad N, Kumar S et al (2007) Assessment of genetic diversity among finger millet [*Eleusine coracana* (L.) Gaertn.] accessions using molecular markers. Genet Resour Crop Evol 54:399–404. https://doi.org/10.1007/s10722-006-0002-8

Babu BK, Agrawal PK, Pandey D, Kumar A (2014a) Comparative genomics and association mapping approaches for opaque2 modifier genes in finger millet accessions using genic, genomic and candidate gene-based simple sequence repeat markers. Mol Breed 34:1261–1279. https://doi.org/10.1007/s11032-0140115-2

Babu BK, Agrawal PK, Pandey D, Jaiswal JP, Kumar A (2014b) Association mapping of agro-morphological characters among the global collection of finger millet genotypes using genomic SSR markers. Mol Biol Rep 41:5287–5297. https://doi.org/10.1007/s11033-014-3400-6

Babu BK, Agrawal PK, Pandey D, Sood S, Chandrashekara C, Kumar A (2014c) Molecular analysis of world collection of finger millet accessions for blast disease resistance using functional SSR markers. SABRAO J Breed Genet 46:202–216

Babu BK, Dinesh P, Agrawal PK, Sood S, Chandrashekara C, Bhatt JC et al (2014d) Comparative genomics and association mapping approaches for blast resistant genes in finger millet using SSRs. PLoS ONE 9:e99182. https://doi.org/10.1371/journal.pone.0099182

Babu BK, Pandey D, Agrawal PK, Sood S, Kumar A (2014e) *In silico* mining, type and frequency analysis of genic microsatellites of finger millet (*Eleusine coracana* (L.) Gaertn.): a comparative genomic analysis of NBS–LRR regions of finger millet with rice. Mol Biol Rep 41:3081–3090. https://doi.org/10.1007/s11033-014-3168-8

Babu BK, Sood S, Agrawal PK, Chandrashekara C, Arun Kumar R, Kumar A (2016) Molecular and phenotypic characterization of 149 finger millet accessions using microsatellite and agro-morphological markers. Proc Natl Acad Sci India Sect B Biol Sci. https://doi.org/10.1007/s40011-015-0695-6

Bharathi A (2011) Phenotypic and genotypic diversity of global finger millet (*Eleusine coracana* (L.) Gaertn.) composite collection. Dissertation/doctoral thesis, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Carine AT, Wang BB, Majesta SO, Shweta D, Zhu HY, Bruce R, Nevin DY, Steven BC (2008) Identification and characterization of nucleotide binding site-leucine-rich repeat genes in the model plant *Medicago truncatula*. Plant Physiol 146:5–21

Ceasar SA, Ignacimuthu S (2009) Genetic engineering of millets: current status and future prospects. Biotechnol Lett 31:779–788. https://doi.org/10.1007/s10529009-9933-4

Ceasar SA, Maharajan T, Ajeesh Krishna TP, Ramakrishnan M, Victor Roch G, Satish L, Ignacimuthu S (2018) Finger millet [*Eleusine coracana* (L.) Gaertn.] improvement: current status and future interventions of whole genome sequence. Front Plant Sci 9:1054. https://doi.org/10.3389/fpls.2018.01054

Dhan Foundation (2012) Supporting millets in India, policy, review and suggestions for action. http://www.dhan.org/smallmillets/

Dida MM, Srinivasachary Ramakrishnan S, Bennetzen JL, Gale MD, Devos KM (2007) The genetic map of finger millet, *Eleusine coracana*. Theor Appl Genet 114:321–332

Durand J, Catherine B, Emilie C, Frigerio JM et al (2010) A fast and cost effective approach to develop and map EST-SSR markers: oak as a case study. BMC Genom 11:570–583

Ejeta G, Gressel J (2007) Integrating new technologies for Striga control towards ending the Witch-Hunt. World Scientific Publishing, Singapore

FAO (2007) FAOSTAT database. http://www/fao.org

Gupta VS, Ranjekar PK (1981) DNA sequence organization in finger millet (*Eleusine coracana*). J Biosci 3(4):417–430

Gupta N, Gupta AK, Singh NK, Kumar A (2011) Diffrential expression of PBF Dof transcription factor in different tissues of three finger millet genotypes differing in seed protein content and color. Plant Mol Biol Rep 29:69–76. https://doi.org/10.1007/s11105-010-0208-y

Harahap Z, Ampong-Nyarko K, Olela JC (1993) *Striga hermonthica* resisatnce in upland rice. Crop Protec 12:229–231. https://doi.org/10.1016/0261-2194(93)90114-X

Haussmann BIG, Hess DE, Welz HG, Geiger HH (2000) Improved methodologies for breeding Striga-resistant sorghums. Field Crops Res 66:195–211. https://doi.org/10.1016/S0378-4290(00)00076-9

Hilu KW (1995) Evolution of finger millet: evidence from random amplified polymorphic DNA. Genome 38:232–238

Hiremath SC, Salimath SS (1991) Quantitative nuclear DNA changes in Eleusine (Gramineae). Plant Syst Evol 178:225–233

Kothari SL, Kumar S, Vishnoi RK, Kothari A, Watanabe KN (2005) Applications of biotechnology for improvement of millet crops: review of progress and future prospects. Plant Biotechnol 22:81–88. https://doi.org/10.5511/plantbiotechnology.22.81

Kumar A, Sharma N, Panwar P, Gupta A (2012) Use of SSR, RAPD markers and protein profiles based analysis to differentiate *Eleusine coracana* genotypes differing in their protein content. Mol Biol Rep 39:4949–4960. https://doi.org/10.1007/s11033-011-1291-3

Kumar A, Kalyana Babu B, Yadav S, Agrawal PK (2016) Allele mining for resistance gene analogs (RGAs) in crop plants: a special emphasis on blast resistance in finger millet (*Eleusine coracana* L.). Indian J Genet 76:1–9. https://doi.org/10.5958/0975-6906.2016.00001.8

Latha AM, Rao KV, Reddy VD (2005) Production of transgenic plants resistant to leaf blast disease in finger millet (*Eleusine coracana* (L.) Gaertn.). Plant Sci 169:657–667. https://doi.org/10.1016/j.plantsci.2005.05.009

Meyers BC, Kozik A, Griego A, Kuang H, Michelmore RW (2003) Genome-wide analysis of NBS–LRR encoding genes in Arabidopsis. Plant Cell 15:809–834

Narain P (2000) Genetic diversity-conservation and assessment. Curr Sci 75(2):170175

Okuyama Y, Kanzaki H, Abe A, Yoshida K, Tamiru M, Saitoh H, Fujibe T, Matsumura H, Shenton M, Galam DC, Undan J, Ito A, Sone T, Terauchi R (2011) A multifaceted genomics approach allows the isolation of the rice Pia-blast resistance gene consisting of two adjacent NBS-LRR protein genes. Plant J 66:467–479

Oswald A (2005) Striga control-technologies and their dissemination. Crop Prot 24:333–342. https://doi.org/10.1016/j.cropro.2004.09.003

Panwar P, Saini RK, Sharma N, Yadav D, Kumar A (2010) Effciency of RAPD, SSR and cytochrome P450 gene based markers in accessing genetic variability amongst finger millet (*Eleusine coracana*) accessions. Mol Biol Rep 37:4075–4082. https://doi.org/10.1007/s11033-010-0067-5

Phillips SM (1972) A survey of the Eleusine Gaertn. (Gramineae) in Africa. Kew Bull 27:251–270

Reddy BL, Srinivas Reddy D, Lakshmi Narasu M, Sivaramakrishnan S (2011) Characterization of disease resistance gene homologues isolated from finger millet (*Eleusine coracana* L. Gaertn). Mol Breed 27(3):315–328

Saha D, Rana RS (2016) Cloning and analysis of the NBS-LRR gene family in finger millet [Eleusine coracana (L.) Gaertn.]. Plant Knowl J 5:1–8. Available online at: http://www.sciencej.com/saha_5_1_2016_1_8.pdf

Salimath SS, de Oliveira AC, Godwin ID, Bennetzen JL (1995) Assessment of genome origins and genetic diversity in the genus Eleusine with DNA markers. Genome 38:757–763. https://doi.org/10.1139/g95-096

Sood S, Kumar A, Kalyana Babu B, Gaur VS, Pandey D, Kant L, Pattnayak A (2016) Gene discovery and advances in finger millet [Eleusine coracana (L.) Gaertn.] genomics—an important nutri-cereal of future. Front Plant Sci 7:1634. https://doi.org/10.3389/fpls.2016.01634

The Global Crop Diversity Trust (2012) Global strategy for the ex situ conservation of finger millet and its wild relatives. Rome. Available online at: https://www.croptrust.org/wp-content/uploads/2014/12/Finger-MilletStrategy-FINAL-14May2012.pdf

Upadhyaya HD, Gowda CLL, Pundir RPS, Reddy VG, Singh S (2006) Development of core subset of finger millet germplasm using geographical origin and data on 14 quantitative traits. Genet Res Crop Evol 53:679–685. https://doi.org/10.1007/s10722-004-3228-3

Winter P, Kahl G (1995) Molecular marker technologies for plant improvement. World J Microbiol Biotechnol 11(4):438–448