Devender Sharma
Saurabh Singh
Susheel K. Sharma
Rajender Singh *Editors*

Smart Plant Breeding for Field Crops in Post-genomics Era

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Editors
Devender Sharma
Crop Improvement Division
ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan
Almora, Uttarakhand, India

Saurabh Singh
Department of Vegetable Science
Rani Lakshmi Bai Central Agricultural University
Jhansi, India

Susheel K. Sharma
Division of Plant Pathology
ICAR-Indian Agricultural Research Institute
New Delhi, India

Rajender Singh
ICAR-Central Potato Research Institute
Shimla, India

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Dedicated
To
Indian Farmers and Our Beloved Parents

Foreword

A systematic effort to improve the genetic potential of crops, i.e. conventional plant breeding, is not new—it began hundreds of years ago. It started with the selection and domestication of various crop species through the process of artificial selection which led to the development of crop plants fit for human consumption. Farmers have been altering the genetic makeup of crop plants/seeds through artificial selection and saving them for next year's planting since the dawn of agriculture. The inception of Mendelian genetics and its better understanding led plant breeders to select plants with desirable traits and improve crop plant varieties. The green revolution began in the 1940s and 1950s, brought up enhanced grain yield and saved the world from mass famine. The release of high-yielding crop varieties and hybrids has significantly increased food grain production worldwide. Farmers desire novel kinds that are ideal for domestic and international markets in climate change and new WTO regimes. The pre-genomic period consisted of conventional plant breeding efforts. Genome sequencing efforts dominate the genomic period and science is now moving towards extracting useful knowledge from the sequenced genomes in the post-genomic era. The book *Smart Plant Breeding for Field Crops in Post-genomics Era*, edited by Drs. Devender Sharma, Saurabh Singh, Susheel K. Sharma and Rajender Singh, aims to provide a comprehensive overview of important food crops, including new developments, emerging tools and techniques that supplement/complement conventional breeding methods to smart plant breeding from pre-genomic to post-genomic era. The first chapter involves various genomic approaches in cereals and the path forward in the post-genomics era. A further specific chapter on emerging molecular breeding strategies for rice drought and salinity tolerance has been included. SMART plant breeding strategies to develop climate-resilient cereals and improve terminal heat stress tolerance have been described in separate chapters. A chapter on the role of sugar signalling in mitigating abiotic stress and epigenetics in wheat improvement has been included. A chapter on accelerated plant breeding/speed breeding in maize through doubled haploid technology has been included. Besides chapters on finger millet, barnyard millet, pigeon pea, safflower and sesame have been included to cover the aspects of these crops. I feel this book will be very beneficial for students, researchers, scientists and policymakers in agriculture, plant science, plant physiology, biotechnology and

molecular biology for conducting research and different funding agencies for future strategic planning. I congratulate the editors of this book Drs. Devender Sharma, Saurabh Singh, Susheel K. Sharma and Rajender Singh for efforts in getting and compiling all the latest available information from the subject experts working in different areas.

Indian Council of Agricultural Research (ICAR) T. R. Sharma
New Delhi, India

Preface

The pre-genomic period consisted of genome sequencing efforts and science is now moving towards extracting useful knowledge from them in the post-genomic era, where we have more than 1000 genomes available. Sequencing has helped to uncover the secret significance of sequencing nucleotides and proteins. The main priority of breeding programmes is the improvement of agronomic traits, which shows complex quantitative inheritance. QTL identification followed by fine mapping and cloning of QTLs/candidate genes is central to trait analysis. Availability of reference/draft genome sequences and bioinformatics or analytical methods offers the opportunity for marker-assisted selection to accelerate plant breeding and genome-editing strategies. Post-genomic era mainly involves the interdisciplinary approaches of genomic annotations, computational genomics, structural and functional genomics. For instance, next-generation sequencing technologies have facilitated the availability of genome sequence assemblies, re-sequencing of several hundred lines, development of HapMaps, high-density genetic maps, high-density SNP arrays for faster mapping, Bulked Segregant RNA Seq (BSR-Seq) for gene discovery, QTL-Seq for gene identification QTL and mutation mapping techniques for gene identification (Mutmap) associated with several agronomic traits of cereal crops. Additionally, different online cereal genomic databases have been developed such as *Gramene* (comparative resource for cereal genomics), *GrainGenes* (*Triticeae* and *Avena*), *Maize GDB* (*Zea mays* ssp. *mays*) and *Phytozome* (*Sorghum bicolor* and *Oryza sativa*). These genomic resources provide valuable information on gene sequences, markers, QTLs, candidate genes, maps, proteins, diversity, pathway and ontology, which would enrich the crop improvement programmes. Interdisciplinary methods using emerging technology may currently lead to a new paradigm of plant breeding, with the increasing mass of genomic data and digitalized biological data.

With the increase in the world population, the production also needs to be doubled to meet the requirement. Amid UN's 17 sustainable development goals (SDGs), end hunger by achieving food security improved nutrition and promote sustainable agriculture are the major challenges to accomplish by 2030. A rise in the population and climate change has raised the problem of producing healthy food with low input and less impact on the environment. Around two-thirds of the world's population depends on rice, wheat and maize as the staple food crops. High in the

carbohydrate content, these crops are also a good source of essential micronutrients, amino acids, vitamins and antioxidants. The pandemic of Covid-19 is currently a significant threat in the world. In order to fight against viruses, it is important to achieve and maintain good health and nutritional status. The immune system is directly impacted by the nutrient status and nutrient intake to the body; therefore, in the present context, the only sustainable way of surviving is to improve the immune system. The novel genomic techniques and approaches of agronomy, conventional and molecular breeding (QTL mapping, association studies, candidate gene identification), omics, RNAi [through microRNA (miRNA), small interfering RNA (siRNA) and artificial microRNA (amiRNA)], antisense technology, genome editing (CRISPR/cas9, base editing) and epigenomics assist the crop improvement programmes to fulfil the UNs SDGs.

Previously published literature has sporadic information on the genomic resources, gene targets, approaches and available products in high yielding, early maturing, nutrient use efficiency and biotic/abiotic stress-tolerant crops. None of the available literature has specifically focused on plant breeding approaches during post-genome sequencing. Recent progress in genomics in the post-genomic era has provided new insights into the tools and technologies for making the plant breeding procedure more efficient and precise. In this volume, we tried to compile all the available information on the important food crops with the new developments, emerging tools and techniques to achieve the food and nutritional security for achieving the UN's SDGs. This volume has explored the influence of rapidly available sequencing data assisting in the next-generation breeding programmes. Consequently, this book would highlight the innovative next-generation plant breeding techniques for the full utilization of the genomic resources developed through high-throughput methods such as genotype by sequencing (GBS) for genomic analysis (SNPs, Single Nucleotide Polymorphism), whole-genome re-sequencing (WGRS), RNA seq for transcriptomic analysis (DEGs, Differentially Expressed Genes), transgenic breeding, genome editing, high-throughput phenotyping, reliable/precision phenotyping and genomic information-based analysis for maximizing the genetic gains in the cereal crops for ensuring the food security.

This book will contain the chapters on the enrichment of important cereals, millets (rice, wheat, maize, sorghum, barnyard millet, finger millet) through smart plant breeding techniques post-genomics era. This volume comprises chapters authored by various experts of different crops/aspects related to the post-genomic era's next-generation plant breeding techniques. The first chapter involves various technologies of the post-genomics era used to enhance productivity, resulting in sustainable yield. One chapter specifically dealing with the big genomic data in plant breeding has been included. Likewise, "Epigenetics" and "Genomic Selection" in the *Era* of Next-Generation Sequencing have been included. Two chapters on rice genomic resources and map-based cloning have been dealt. Separate chapters on wheat, maize, sorghum and other millets such as finger millet and barnyard millet have been included in the separate chapters.

We feel that this book will be very beneficial for students, researchers, scientists, policymakers working in the area of agriculture, horticulture, plant science, agronomy, plant physiology, food and nutrition, biotechnology, molecular biology, environmental science for conducting research and different funding agencies for future strategic planning. We express our greatest thanks to all the contributors for their untiring efforts to compile all the latest available information to make this volume a success.

This book will contain the chapters the influence of rapidly available sequencing data assisting in the next-generation breeding programmes of important cereals, millets (rice, wheat, maize, sorghum, barnyard millet and finger millet) through conventional, molecular breeding and advanced biotechnological tools.

Almora, Uttarakhand, India — Devender Sharma
Jhansi, India — Saurabh Singh
New Delhi, India — Susheel K. Sharma
Shimla, India — Rajender Singh

About the Book

In the post-genomics era, rapid evolution has occurred in the advancement of sequencing approaches and genome engineering. The revolution in genetic and genomics research, epigenomics, genomic selection, computational biology and bioinformatics, genome editing, speed breeding, doubled haploidy and other next-generation breeding methodologies has accelerated the plant breeding. This volume enumerates the latest applications of these post-genomic tools like genomics and genome editing, bioinformatics, genomic resources, epigenetics and smart breeding to tackle the challenges in field crop improvement. This volume is a fruitful and leading-edge resource for the researchers, students, scientists, teachers and private players interested in smart plant breeding tools for crop genetic improvement. This is a leading-edge volume highlighting the modern results in field crop breeding in the post-genomics era and forecasts crucial areas of future needs.

Contents

Editors and Contributors

About the Editors

Devender Sharma is currently working as a Maize Breeder [Scientist] at ICAR-VPKAS, Almora, Uttarakhand, India. He is the ICAR-Senior Research Fellowship recipient and completed his Ph.D. from GBPUAT Pantnagar, Uttarakhand, India. His main interest areas are the genetic improvement of cereal crops, doubled haploidy and pre-breeding. He is currently working on the biofortification of maize for nutritional quality using genomic tools. He has published over 22 peer-reviewed research papers, 11 book chapters and 12 popular articles. He is the consignee of the Young Scientist Award from UCOST, Dehradun. He is the recipient of Jagar Nath Raina Memorial All India Best Research Award-2020, in the recognition of his Doctoral research work. He is also the peer reviewer for reputed journals.

Saurabh Singh is currently working as Teaching cum Research Associate at RLBCAU, Jhansi, India. He completed his Ph.D. from ICAR-IARI, New Delhi, India. His main research interests are genetic improvement of vegetable crops using molecular breeding, genome editing and doubled haploidy. He has published research papers in many peer-reviewed journals. He has published 6 book chapters, 20 popular articles and one edited book. He is the recipient of Dr. B. R. Barwale Young Researcher Award by IAHS. He also holds the responsibility of independent peer reviewer for many journals.

Susheel K. Sharma is currently working as Scientist at ICAR Research Complex for NEH Region, Manipur Centre, Imphal, Manipur. He is recipient of University Gold Medal, Dr. J. S. Negi Gold Medal, ASPEE Gold Medal and Prakash Singha Gold Medal. He completed Ph.D. from ICAR-IARI, New Delhi. He is recipient of IARI-Best Student Merit Medal. He has 12 years of research experience in viral genomics and host–pathogen interactions studies.

Dr. Sharma has handled five externally funded projects as Principal Investigator funded by NASF, DBT and DST. He has published 45 research papers, 2 edited books, 11 technical bulletins and 17 book chapters. He is recipient of ISCA Young

Scientist Award, Fakhruddin Ali Ahmed Award from ICAR and many others in his credit are there.

Rajender Singh is currently working as Research Associate at ICAR-CPRI, Shimla, Himachal Pradesh, India. He completed his Ph.D. from Thapar University, Patiala and ICAR-DMR, Solan, Himachal Pradesh, India. He has also qualified ICAR-NET in Agricultural Biotechnology. He is the recipient of Junior Scientist of the Year award 2010 NESA, New Delhi. He has more than 11 years of experience in research. He has published research papers in many peer-reviewed journals. He is credited with publication of 11 book chapters, edited one. Previously, he was associated with research and development in edible fungi. Currently, his main research interest is technology management and licensing in potato research at ICAR-Central Potato Research Institute, Shimla.

Contributors

Sneha Adhikari ICAR-Indian Institute of Wheat and Barley Research, Regional Station, Shimla, Himachal Pradesh, India

K. Anjani ICAR-Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad, India

Alka Bharati ICAR-Central Agroforestry Research Institute, Jhansi, Uttar Pradesh, India

Mahendar S. Bhinda ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, Uttarakhand, India

Anju Biswas Department of Agronomy, University of Florida, Gainesville, FL, USA

Ajay Kumar Chandra Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi, India

Subhash Chand AICRP on Forage Crops and Utilization, ICAR-Indian Grassland and Fodder Research Institute, Jhansi, India
Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi, India

Rajat Chaudhary Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi, India

Sunita Choudhary International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Telangana, India

Amit Dadheech Department of Plant Breeding and Genetics, RCA, MPUA&T, Udaipur, India

Vishal Dinkar ICAR-Central Institute of Temperate Horticulture, Srinagar, India

Joginder Singh Duhan Chaudhary Devi Lal University (CDLU), Sirsa, Haryana, India

Bhagyasri Dulakakharia Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat, Assam, India

P. Duraimurugan ICAR-Indian Institute of Oilseeds Research, Hyderabad, India

Vijay Gahlaut Biotechnology Division, CSIR-Institute of Himalayan and Bioresource Technology, Palampur, Himachal Pradesh, India

Tinku Gautam Department of Genetics and Plant Breeding, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India

Raju Ghosh International Crops Research Institute for Semi-Arid Tropics, Patancheru, Telangana, India

S. K. Gupta International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Telangana, India

Nazarul Hasan ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, Uttarakhand, India

Indu Crop Improvement Division, ICAR-Indian Grassland and Fodder Research Institute, Jhansi, India

J. Jawahar-Lal ICAR-Indian Institute of Oilseeds Research, Hyderabad, India

Anjali Joshi Genetics and Tree Improvement Division, Arid Forest Research Institute, Jodhpur, Rajasthan, India

D. C. Joshi ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, Uttarakhand, India

Priyanka Joshi International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Telangana, India

M. Kasi Rao International Crops Research Institute for Semi-Arid Tropics, Patancheru, Telangana, India
University of Agricultural Sciences, Raichur, Karnataka, India

Srinivas Katravath International Crops Research Institute for Semi-Arid Tropics, Patancheru, Telangana, India
Professor Jayashankar Telangana State Agricultural University, Hyderabad, Telangana, India

Gurleen Kaur Horticultural Sciences Department, University of Florida, Gainesville, FL, USA

Ishveen Kaur School of Agriculture Environmental and Sustainability Sciences, University of Texas Rio Grande Valley, Edinburg, TX, USA

Shivreet Kaur Department of Plant Pathology, North Dakota State University, Fargo, ND, USA

Jana Kholova International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Telangana, India

Himabindu Kudapa International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Telangana, India

Amarjeet Kumar Department of Genetics and Plant Breeding, MTTC & VTC, Selesih, CAU, Imphal, Manipur, India

H. H. Kumaraswamy ICAR-Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad, India

Poonam Kumari CSIR-Institute of Himalayan Bioresource Technology (IHBT), Palampur, Himachal Pradesh, India

Santosh Kumar ICAR-Indian Agricultural Research Institute, Hazaribagh, Jharkhand, India

Khonang Longkho Department of Plant Breeding and Genetics, Assam Agricultural University, Jorhat, Assam, India

Swarup Nanda Mandal Department of Plant and Soil Science, Texas Tech University, Lubbock, TX, USA
Department of Genetics and Plant Breeding, Bidhan Chandra Krishi Viswavidyalaya, Burdwan, West Bengal, India

Vijay Kamal Meena Agriculture Research Sub-Station (Sumerpur), Agriculture University, Jodhpur, India

D. Naresh International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Telangana, India

Henry Ojulong International Crops Research Institute for the Semi-Arid Tropics, Nairobi, Kenya

Manoj Kumar Patel Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi, India

Surinder Paul Chaudhary Devi Lal University (CDLU), Sirsa, Haryana, India
ICAR-Indian Institute of Wheat and Barley Research (IIWBR), Karnal, Haryana, India
ICAR-National Bureau of Agriculturally Important Microorganism (NBAIM), Maunath Bhanjan, Uttar Pradesh, India
ICAR-Indian Grassland and Fodder Research Institute (IGFRI), Himachal Pasturelands, Palampur, Himachal Pradesh, India

Nitish Ranjan Prakash ICAR-Central Soil Salinity Research Institute, Regional Research Station, Canning Town, South 24 Parganas, West Bengal, India

H. D. Pushpa ICAR-Indian Institute of Oilseeds Research, Hyderabad, India

K. Ramesh ICAR-Indian Institute of Oilseeds Research, Hyderabad, India

K. T. Ramya ICAR-Indian Institute of Oilseeds Research, Hyderabad, India

Reena Rani Division of Plant Improvement and Pest Management, ICAR-Central Arid Zone Research Institute, Jodhpur, India

Sanket R. Rathi Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

A. L. Rathnakumar ICAR-Indian Institute of Oilseeds Research, Hyderabad, India

P. Ratnakumar ICAR-Indian Institute of Oilseeds Research, Hyderabad, India

Ashima Relan Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India

Dinesh Kumar Saini Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India

Sobhan Sajja International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Telangana, India

Sakthivel ICAR-Indian Institute of Oilseeds Research, Hyderabad, India

Prafull Salvi Agriculture Biotechnology Department, National Agri-Food Biotechnology Institute, Mohali, Punjab, India

Karansher Singh Sandhu Department of Crop and Soil Sciences, Washington State University, Pullman, WA, USA

Gautam Saripalli Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD, USA

Sayantan Sarkar Blackland Research and Extension Center, Texas A&M Agrilife Research, Temple, TX, USA

Ranjit Saroj Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi, India

Devender Sharma Crop Improvement Division, ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, Uttarakhand, India

Hemant Sharma Department of Genetics and Plant Breeding, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India

Mamta Sharma International Crops Research Institute for Semi-Arid Tropics, Patancheru, Telangana, India

Rajan Sharma International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Telangana, India

Vinay Sharma International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India
Department of Genetics and Plant Breeding, Ch. Charan Singh University, Meerut, India

Rajesh Kumar Singhal Crop Improvement Division, ICAR-Indian Grassland and Fodder Research Institute, Jhansi, India

Ashutosh Kumar Singh Center for Advanced Studies on Climate Change, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

Jawahar Singh Laboratorio De Genómica Funcional De Leguminosas, Facultad De Estudios Superiores, Iztacala, Universidad Nacional Autónoma De México, Tlalnepantla, Estado De Mexico, Mexico

Lovepreet Singh Department of Plant and Soil Sciences, Mississippi State University, Mississippi State, MS, USA

Sanchika Snehi Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

M. Sujatha ICAR-Indian Institute of Oilseeds Research, Hyderabad, India

Avijit Tarafdar International Crops Research Institute for Semi-Arid Tropics, Patancheru, Telangana, India

Helan B. Thomas ICAR-Indian Institute of Oilseeds Research, Hyderabad, India

Ratan Tiwari ICAR-Indian Institute of Wheat and Barley Research (IIWBR), Karnal, Haryana, India

B. Ushakiran ICAR-Indian Institute of Oilseeds Research, Hyderabad, India

Vishal Varshney Govt. Shaheed Gend Singh College, Charama, Chhattisgarh, India

Rahul K. Verma DBT-North East Centre for Agricultural Biotechnology, Jorhat, Assam, India

M. Vetriventhan International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Telangana, India

Revisiting the Genomic Approaches in the Cereals and the Path Forward

1

Ishveen Kaur, Ashima Relan, Dinesh Kumar Saini, Gurleen Kaur, Anju Biswas, Lovepreet Singh, Shivreet Kaur, and Karansher Singh Sandhu

Abstract

The important difficulties confronting humanity in the current era include combating global climate change, meeting human nutritional demands, and ensuring adequate energy sources. Cereal crops, which are grasses cultivated for their edible grains, are the primary dietary energy sources for humans and livestock and are produced in greater quantities than any other crop types. This chapter discusses the advancement and potential of various genomic tools for five main kinds of cereal: rice, maize, wheat, barley, and sorghum. We have discussed and

Ishveen Kaur and Ashima Relan contributed equally to this work.

I. Kaur
School of Agriculture Environmental and Sustainability Sciences, University of Texas Rio Grande Valley, Edinburg, TX, USA

A. Relan · D. K. Saini
Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India

G. Kaur
Horticultural Sciences Department, University of Florida, Gainesville, FL, USA

A. Biswas
Department of Agronomy, University of Florida, Gainesville, FL, USA

L. Singh
Department of Plant and Soil Sciences, Mississippi State University, Mississippi State, MS, USA

S. Kaur
Department of Plant Pathology, North Dakota State University, Fargo, ND, USA

K. S. Sandhu (✉)
Department of Crop and Soil Sciences, Washington State University, Pullman, WA, USA
e-mail: k.sandhu@wsu.edu

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speculated the advancements of genomics in plant improvement varying from transgenic cultivars, molecular markers and next-generation sequencing, linkage and association mapping, genome editing, pan-genome and super pan-genome sequencing, haplotype and optimal contribution selection, genomic and phenomics-assisted breeding, and finally merger of the domain of data science with plant genomics and breeding. The main success of each of these genomic tools is discussed for each crop, and why certain of them failed for specific crops is discussed with potential aspects to strengthen them with new tools. The chapter is divided into two sections. First, we have covered the traditionally used genomics. The other half shows the potential of novel genomic tools with the integration of data science. This chapter allows the reader to learn from the past inventions and failures to implement the new genomic tools with high precision and efficacy.

Keywords

Genomics · Genomic selection · Marker-assisted selection · Phenomics-assisted breeding

1.1 Introduction

The important difficulties confronting humanity in the current era include taking action to reduce global climate change, meeting the nutritional demands of humans, and ensuring adequate energy sources (Pimentel 2011). Cereal crops, which are grown for their edible grains, are the most important dietary energy sources for humans and cattle and are therefore produced in greater quantities than any other crop types (Papageorgiou and Skendi 2018). The term "cereals" refers to members of the Poaceae family and includes nine species: wheat, barley, oat, rye, rice, corn, pearl millet, sorghum, and triticale (a hybrid between wheat and rye). The top cereals cultivated in the world in 2020, ranked based on million thousand tons, are as follows: corn (1162), wheat (760), rice (756), barley (157), and oat (25.53) (https://knoema.com/atlas/topics/Agriculture).

By 2050, the world's population will have grown by 34% from its current level. To feed this larger, more urban population, food production must increase by 70%. Yearly cereal production will need to rise from 2.1 to over 3 billion tons, and annual meat production will need to rise by more than 200 to 470 million tons. (https://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf). Plant breeding has a long history of development from the artificial domestication of crop species. Plant cultivars and germplasm have been developed using traditional breeding methods with great success. Some of the most well-known examples include semi-dwarf, high-yielding cereal cultivars developed during the Green Revolution and hybrid rice developed in the 1970s (Nelson et al.

2019). Traditional breeding, on the other hand, continues to rely significantly on subjective assessment and empirical selection. Scientific breeding necessitates comparatively less subjectivity and more science, specifically practical and precise evaluation as well as efficient and effective selection.

DNA-based molecular markers were first developed in the mid-1990s, and significant progress was achieved in developing molecular markers such as SSRs, AFLPs, DArT markers, and SNPs (Bohar et al. 2020; Sharma et al. 2021a, b). These markers were utilized to create molecular, genetic, and physical maps, as well as to perform single-marker analysis (SMA), interval mapping (IM), meta-QTL analysis, and association mapping studies (or genome-wide association studies, GWAS) in crops including cereals (Araus et al. 2008; Sharma et al. 2020; Saini et al. 2021a, 2022b) aiming for the identification of QTLs for marker-assisted selection (MAS). Although many reports are available on QTL analysis for different traits in cereals, only limited information has been utilized for MAS, leading to the selection of superior cereal cultivars in practical breeding programs.

Cereal crop genomes have been subjected to many evolutionary processes since diverging from a common ancestor 50–70 million years ago, resulting in variations in genome composition, complexity, and size. Over the last two decades, efforts to sequence the genomes of the major cereal crops have resulted in relatively contiguous, chromosome-scale genomic assemblies. Rice has the smallest genome (420 Mb), making it the first cereal genome to be constructed. However, genome sequencing progress has been hindered by the enormous complexity and size of genomes of some cereals, such as oat (12 Gb) and wheat (17 Gb) (Walkowiak et al. 2022). Reduced sequencing costs, combined with new technology developments such as ultra-long-read sequencing and improved genome assembly techniques, have recently enabled chromosome-scale assemblies in all cereals (Walkowiak et al. 2022). As a result, the genomics of cereal crops has entered a new era.

Genomic (or genome-wide) selection (GS) is a strategy that can overcome the constraints of MAS to improve complex quantitative traits. Despite identifying the specific QTLs, the goal of GS is to ascertain an individual's genetic potential. GS was first developed in livestock breeding as a method to predict breeding values (also known as genomic estimated breeding values, GEBVs) of individuals using simulated data and markers covering the entire genome (Meuwissen et al. 2001). In plants, GS has been shown to outperform MAS using the same economic investment, even at low accuracies (Cerrudo et al. 2018). The development of statistical approaches to properly predict marker effects and decreasing costs of genotyping using high-density SNP arrays led to the breakthrough of GS. Selection decisions based on GS data have been shown to improve selection accuracy and genetic improvement speed. Genomic predictions have been performed in cereals, including wheat (Saini et al. 2020; Sandhu et al. 2021a, b), rice (Spindel and Iwata 2018), maize (Fristche-Neto et al. 2018), and oats (Asoro et al. 2013). In hybrid breeding and inbred or doubled haploid lines, the potential of GS has been investigated (Zhao et al. 2015), with most authors concluding that prediction accuracies are sufficient to make GS more efficient than phenotypic selection.

Furthermore, combining next-generation sequencing (NGS) and high-throughput phenotyping technologies can discover new donors and alleles (haplotypes) linked with the traits of interest. Through haplotype-based breeding, superior haplotypes can be transferred into elite cultivars, assisting crop improvement and the production of climate-smart cultivars. Meuwissen et al. (2014) argued that employing haplotypes instead of single SNPs when constructing the association matrix could improve the accuracy of GS.

Major advances in genome editing technologies are expected to overcome the shortcomings and concerns associated with transgenic technology, allowing transgenic development to be replaced, at least for commercial purposes. The CRISPR/Cas9 technique has been efficiently and effectively utilized in important crops, specifically cereal crops owing to its wide acceptability, cost-effectiveness, enhanced and focused targeting, and less time required (Sharma et al. 2021a, b). Due to its rapid growth and potential implications, several review articles discussing genome editing and its relevance in various plants have recently been published (Ansari et al. 2020; Li et al. 2020a, b, c; Zhang et al. 2018). Genome editing, like MAS, will most likely not provide a solution because it is conditional on first detecting mutations or modifications with a large effect.

Here, we summarize current advances in genomics and their applications, focusing on cereal crops. In particular, we have discussed applications and advancements in interval mapping, a meta-analysis of QTLs, GWAS, GS, and genome editing. Finally, we provide a prospect for future cereal genomic research by integrating data science approaches with genomics, optimal contribution selection, and haplotype-based breeding for the development of climate-smart cereals.

1.2 Development and Use of Molecular Markers: A Beginning of the Genomic Era

Successful development of cultivars having various agronomic and nutritional qualities using conventional breeding is very tedious. Molecular marker technology has advanced and increased the efficiency of cereal breeding programs. Molecular markers, also known as DNA markers, are nucleotide sequences and have been used extensively to detect polymorphism at particular loci and whole genome levels. Owing to the advances in the area of molecular genetics, a wide range of molecular markers have been developed (Wani et al. 2020), which include restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats or microsatellites (SSR), inter simple sequence repeats (ISSR), cleaved amplified polymorphic sequences (CAPS), sequence characterized amplified region (SCAR), sequence-ragged sites (STS), sequence related amplified polymorphism (SRAP), diversity arrays technology (DArT), single-nucleotide polymorphism (SNP), etc. A systematic summary of the various molecular markers is shown in Fig. 1.1.

RFLPs, the "first-generation molecular markers," initiated the period of DNA marker technology in the 1980s. Back then, these markers were utilized for

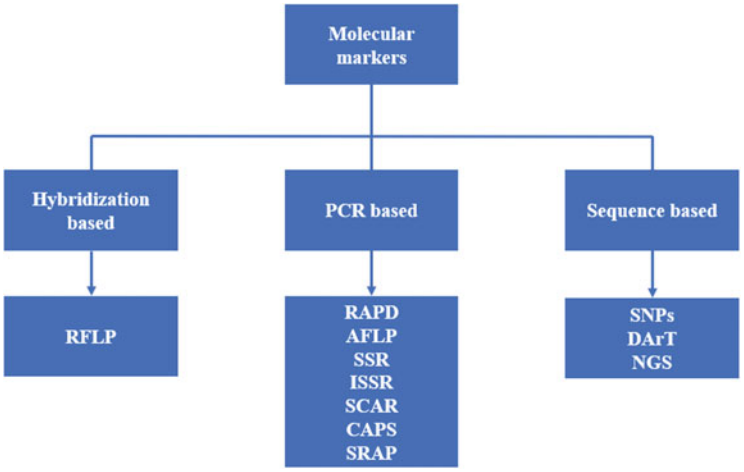

Fig. 1.1 A systematic overview of different molecular markers

developing genetic maps in cereals like maize, wheat, and rice. The slow and low-throughput nature of hybridization technology and tedious procedures rendered them useless in breeding projects. The advent of PCR during the 1980s led to the origination of various PCR-based markers. The first and the simplest of these is RAPD. It has been utilized extensively to tag genes controlling important traits, create linkage maps, and characterize genetic diversity in cereals. Another PCR-based marker, AFLP, which is a combination of RFLP and PCR technique, has been used for genetic map construction. SSRs were another notable development in molecular marker technology during the 1900s. Genetic maps in cereal crops like wheat, rice, and maize (Röder et al. 1998; Macaulay et al. 2001; Temnykh et al. 2001; Sharopova et al. 2002) have been developed using the SSR markers. However, they are time- and cost-inefficient. DArT, a microarray hybridization-based assay, has been considerably used for genetic mapping and bulked segregant analysis (BSA) in maize, rice, wheat, and sorghum. However, among all these markers, SNPs are the most advanced marker of choice in today's next-generation sequencing era. The smallest unit of DNA polymorphism (SNPs) has become progressively important in crop genetic studies due to its abundance, high-speed data generation, high throughput, and cost efficiency (Ganal et al. 2019). Approximately 20 million SNPs were identified in rice by aligning the reads from ~3000 rice genomes against the Nipponbare reference sequence (Alexandrov et al. 2015).

The availability of a wide range of SNP genotyping platforms is one of the critical components in the advantages of SNP markers for speed, high throughput, flexibility, and cost-effectiveness (Thomson 2014). In the past, identifying SNP markers for large-scale crop genotyping required considerable effort (Ganal et al. 2009). The increased demand for high-throughput SNP genotyping has led to the advent of various SNP genotyping technologies. The initial SNP genotyping depended on gel-based methods like the cleaved amplified polymorphic sequence (CAPS) marker

approach (Thiel et al. 2004), which is a combination of PCR and RFLP techniques requiring a very small quantity of DNA to detect polymorphism and allele-specific amplification methods (Drenkard et al. 2000). Some other available technologies are PCR-based fluorescently labeled high-throughput methods and high-resolution melting (HRM) curve analysis. Illumina's GoldenGate assay allows marker profiling at the genome-wide level. It has been used for conducting different genetic studies on wheat (Akhunov et al. 2009; Chao et al. 2010), barley (Rostoks et al. 2006; Close et al. 2009; Druka et al. 2011), and maize (Yan et al. 2010; Mammadov et al. 2012).

Some technological advancements transformed individual or multiplexed SNP marker genotyping. KASP™ (Kompetitive Allele-Specific Polymerase) chain reaction assay and TaqMan® (Martino et al. 2010) make individual marker analysis easy, accurate, and cost-effective. More than 4000 validated TaqMan and 8000 KASP assays have been developed and deployed in wheat SNP genotyping (www.cerealsdb.uk.net).

1.2.1 Array- and Sequencing-Based Genotyping Methods in Cereals

Fixed array-based genotyping platforms, such as Illumina Infinium (Mason et al. 2017) and Affymetrix/Axiom (Allen et al. 2017), provide the multiplexed marker analysis in a highly accurate manner. The former is based on a primer extension method, whereas the latter is an oligo-ligation assay-based system. The barley 9K Infinium array was the first genotyping array published for barley in 2012, consisting of 7842 markers (Comadran et al. 2012). A new 50K improved version of the barley genotyping array has also been developed (Bayer et al. 2017). Based on formerly detected and validated markers and the novel markers obtained from transcriptome sequencing and GBS studies, a 6K array has been developed for hexaploid oat (Tinker et al. 2014). In wheat, the 660K SNP array serves as a cost-effective and great potential array system for genetic improvement (Sun et al. 2020).

Another popular high-throughput genotyping platform is the next-generation sequencing (NGS)-enabled approach, genotyping by sequencing. GBS has been extensively deployed in small grain cereals for the last 5 years. GBS approach involves using restriction enzymes for digesting the whole genome into fragments, followed by multiplex sequencing using NGS technologies. The highly robust and multiplexed approach identifies and genotypes the SNPs simultaneously. "GBS" is a general term for any technique involving a sequencing approach for genotyping. Scheben et al. (2017) summarized 13 different GBS approaches used in plants, each having some distinguishing characteristics. Among these, there are certain techniques successfully deployed in cereals. This includes GBS (Elshire et al. 2011; Poland et al. 2012; Kim et al. 2016), diversity array technology sequencing (DArT-seq) (Li et al. 2015), sequence-based genotyping (SBG) (van Poecke et al. 2013), and restriction enzyme site comparative analysis (RESCAN) (Kim and Tai 2013). A two-enzyme modification of the original Elshire GBS protocol involving a single enzyme protocol has been used in wheat, barley (Poland et al. 2012), and oat

(Huang et al. 2014). Some other examples of using GBS to aid breeding efforts in cereal crops are as follows: maize (Gore et al. 2009; Elshire et al. 2011; Zhang et al. 2015; Wang et al. 2020), rice (Huang et al. 2009; Spindel et al. 2015), and sorghum (Morris et al. 2013). The recent decrease in NGS costs will pave a path forward for GBS to become a necessary tool in cereal breeding and research. The increasing availability of reference genomes for cereals will make GBS the choice approach regarding cost and throughput.

1.2.2 Sequencing of Cereal Genomes

Owing to the significant technological advancements along with the joint international efforts, there has been great progress in the construction of cereal genome assemblies which might be deployed in various genetic studies like large-scale diversity panel resequencing, scanning genomes for genes controlling salient traits. The small size and diploid nature of cereals like rice, maize, and sorghum have rendered their genome sequencing accessible. Rice, having the genome drafts of domesticated subspecies (ssp. *japonica* and *indica*) published in 2002 (Goff et al. 2002; Yu et al. 2002), became the first crop plant to be sequenced with a genome size of 420 megabases (Mb). Rice was followed later by sequencing the sorghum and maize genome (Paterson et al. 2009; Schnable et al. 2009). The large genome size and complex nature of the genomes hindered the sequencing of important cereals like wheat, oat, and barley. With the advent of NGS, there has been a great breakthrough in studying cereal genomes. The first draft assembly of barley (cultivar Morex) was published in 2012 (Mayer et al. 2012). With an enormous genome size of 16 gigabases (Gb), the first gold standard wheat genome sequence was published in 2014 using chromosome-sorted whole-genome shotgun sequences (International Wheat Genome Sequencing Consortium (IWGSC) et al. 2014). More freshly, a reference genome of the wheat cultivar Chinese Spring (RefSeq v1.0) was released by IWGSC in 2018 (International Wheat Genome Sequencing Consortium (IWGSC) et al. 2018).

1.2.3 Next-Generation Sequencing (NGS)

Genome sequencing technologies have led to the revelation of the crucial information masked in plant genomes. The first-generation sequencing technologies like Sanger sequencing and Maxam–Gilbert chemical cleavage pioneered the beginning of the genomic era. However, the demand for high-throughput information generation coupled with lower costs set off the development of second-generation sequencing technologies like Illumina Tech, 454 Pyrosequencing, and Ion Torrent. These approaches can be categorized into sequencing by synthesis (SBS) and sequencing by ligation (SBL). However, these short-read sequencing technologies (first and second generation) are not suited for wide-reaching projects as they yield short-reads in 50–1000 bp fragments. So this compelled the advent of third-generation

platforms, known as single-molecule sequencing technology. This technology includes sequencing platforms like Oxford Nanopore sequencing and PacBio (or single molecular real time; SMRT). These have considerable application potential and perform faster data reading. They can generate reads up to several kilobases, thus proving better resolution of exceedingly large genomes having long repetitive elements and copy number variations (CNVs). These NGS approaches allow the de novo genome assembly and resequencing of genomes. However, the reads produced through these third-generation sequencing technologies are still inadequate to cover some complex and repetitive genomic regions. The assembly problems can be overcome by Hi-C sequencing and optical mapping. Hi-C is an advanced version of the chromosome conformation capture (3C) coupled with NGS techniques. This method has been used in wheat and barley for producing physical mapping data to be deployed in various genome assembly projects (Padmarasu et al. 2019). The optical mapping follows a light microscope-based technology to physically track down a specific enzyme or sequence motif. Lately, optical mapping has been utilized to refine the wheat genome assembly by generating RefSeq v2.1 (Zhu et al. 2021).

1.3 Linkage-Based Mapping and Association Mapping: Getting Insights into the Genetic Architecture of Complex Traits in Cereals

The basic underlying idea behind linkage-based mapping (recombination-based mapping) and association mapping (linkage disequilibrium mapping) is to connect genotypic data with phenotypic data in a population that has a variation for the targeted trait to find genomic regions controlling that trait. Then using that information to develop improved lines for the trait of interest and develop new cultivars. The basic principle for constructing a linkage map is that the frequency of recombination among two markers estimates how far apart they are on a chromosome. To perform a linkage-based mapping, the requirements are appropriate mapping population, polymorphic marker genotyping, phenotypic data for the trait of interest, and software to do statistical analysis. The first genome map employing RFLP markers was described in maize crops (Helentjaris et al. 1986) and then reported in rice (McCouch et al. 1988). Hulbert et al. (1990) reported that the first linkage map in sorghum was of length 283 cM by employing 36 RFLP markers. In 1997, using a single F_2 population, the first high-density linkage map was created with 2275 markers in rice, covering a total length in Kosambi function of 1521.6 cM (Harushima et al. 1998).

Segregating populations that have been used in cereals for trait mapping are F_2 population in rice crop (Kumar et al. 2014), doubled haploid population in wheat crop (Liu et al. 2020), backcross population in wheat (Elouafi and Nachit 2004), recombinant inbred lines (RILs) in maize crop (Gonzalo et al. 2010), and near-isogenic lines (NILs) in maize crop (Szalma et al. 2007). Four populations of the multi-parent advanced generation inter-cross (MAGIC) type that harvest benefits from bi-parental populations and association panels have been used to discover new

Table 1.1 Software tools commonly used for QTL and association mapping in plants

Software resource	Authors and year
For QTL mapping	
MapMaker/QTL	Lincoln et al. (1993)
PLABQTL	Utz and Melchinger (1996)
QGene	Nelson (1997)
Map Manager QTX	Manly et al. (2001)
QTL Express	Seaton et al. (2002)
INTERQTL	Jannink and Wu (2003)
MCQTL	Jourjon et al. (2005)
R/QTLBIM	Yandell et al. (2007)
FlexQTL	Bink et al. (2008)
R/QTL	Broman et al. (2003)
MapQTL	van Ooijen (2009)
WinQTL Cartographer	Wang et al. (2012)
QGene	Joehanes and Nelson (2008)
For association mapping (GWAS)	
STRUCTURE	Pritchard et al. (2000)
Trait Analysis by aSSociation, Evolution and Linkage (TASSEL)	Bradbury et al. (2007)
EMMAX	Kang et al. (2010)
rrBLUP—R Package	Endelman (2011)
Genome Association and Prediction Integrated Tool (GAPIT)—R Package	Lipka et al. (2012)

QTL for resistance against powdery mildew disease in barley (Novakazi et al. 2020). The population which exploits both linkage and linkage disequilibrium is nested association mapping (NAM) population developed by Yu et al. (2008) in maize, and over 100 different phenotypes have been characterized from this population spanning from agronomic traits to ionomics profiles till now (Gage et al. 2020).

The methods for conducting linkage-based mapping can be categorized into four broad types. The first one is the single-marker analysis and it has been performed when there is no accessibility to the linkage map. The second one is interval mapping, and it can be classified into various subtypes such as simple interval mapping (SIM), in which there is no co-factor selection; composite interval mapping (CIM), which includes co-factor selection; multiple interval mapping (MIM), which is the two-locus analysis; and Bayesian interval mapping (BIM), which utilizes the prior information into data analysis. The third one is the meta-QTL analysis which brings results from various QTL studies performed for the same traits in the same crop to one ground and leads toward precise detection of QTL and candidate genes with high statistical power, as reported in various recent studies reported in wheat crop and other cereals (Kumar et al. 2021; Pal et al. 2021; Saini et al. 2021a, b, 2022a, b; Sandhu et al. 2021e). The fourth and last one is joint linkage and association mapping (JLAM), harvesting pros from linkage and association mapping. Various software tools used for QTL mapping are described in

Table 1.1. The bulk segregant analysis is mainly used for mapping qualitative traits but it has been used to map QTLs coupled with other techniques in various cereals like wheat (Shen et al. 2003), rice (Tiwari et al. 2016), and maize crop (Quarrie et al. 1999).

Even with the huge success of QTL mapping with tons of studies published from the past two decades and recent studies like Deng et al.'s (2022) in which they mapped a stable QTL for stripe rust resistance using 117 RILs by inclusive composite interval mapping in wheat, it has some limitations. GWAS overcomes two significant drawbacks of QTL mapping: we can detect only allelic diversity present in the segregating population parents from where it is derived and there is low mapping resolution because recombination happens only during population generation (Korte and Farlow 2013). Another major limitation in linkage mapping is the investment of resources and time to create an appropriate population (Nuzhdin and Turner 2013).

By employing the idea of linkage disequilibrium and utilizing historical recombination events, the association mapping tool is used for dissecting complex traits with high resolution (Nordborg and Tavaré 2002; Ersoz et al. 2007). Association studies in plants, especially cereals, got consideration due to ease of next-generation sequencing, high-throughput phenotyping, and advanced statistical tools. Moreover, many successful studies have been published in which gene loci have been identified as controlling quantitative traits (Alipour and Darvishzadeh 2019).

The genotypic data in GWAS is mainly ruled by single-nucleotide polymorphisms (SNPs) mainly obtained by the genotyping-by-sequencing (GBS) technique or array-based genotyping. While conducting GWAS, population structure and cryptic relatedness in diversity panels can result in false marker-trait associations (Yu et al. 2006). So, principal component analysis (PCA) (Price et al. 2006), running software such as STRUCTURE (Pritchard et al. 2000), including kinship matrix, is a common practice in GWAS. In cereals, many agronomically important traits have been dissected through GWAS (Huang et al. 2010; Tsai et al. 2020; Tao et al. 2020). Various commonly used software tools for plant GWAS are mentioned in Table 1.1.

Even though GWAS overcomes the limitations of QTL mapping, it comes with its challenges like confounding aroused by relatedness, genetic heterogeneity, epistasis, unexpected LD, low allele frequency, spurious associations, and heritability problem (Korte and Farlow 2013). GWAS and QTL mapping can be conducted together to defeat each other's shortcomings and to achieve better and more confident results. The genetic architecture of kernel test weight has been dissected by merging GWAS and QTL analysis in maize (Zhang et al. 2020a, b, c), and candidate genes have been identified for seed vigor in rice by combining GWAS, QTL mapping, and RNA-seq (Guo et al. 2019). Since the price of sequencing is reducing and is becoming more accessible so, in the future it can be expected that GWAS based on whole genome sequencing will replace GBS-based GWAS as Yano et al. (2016) discovered new genes in the rice crop controlling various agronomic traits by whole genome sequencing-based GWAS.

1.4 Marker-Assisted Selection in Cereals

Making a selection based on the molecular marker(s) for the allele of gene/QTL linked to a trait of interest rather than making a selection for the phenotype is called marker-assisted selection (MAS) (Singh and Singh 2015). The process of MAS is implemented after mapping genes and actual selections for developing a variety are made in the population. Various breeding schemes are used by applying MAS, like marker-assisted backcrossing (MABC) for resistance against diseases, yield, and various traits related to the quality of wheat crop (Salameh et al. 2011); marker-assisted recurrent selection (MARS) testified when maize is prone to drought stress for traits related to yield of the crop (Bankole et al. 2017); and other schemes such as breeding by design, pedigree MAS, single large-scale MAS, and marker-evaluated MAS. Although many varieties have been released through MAS in cereals, progress in mapping studies is enormous by comparison. Progress will be boosted by decreasing cost and improving efficiency through high-throughput genotyping and phenotyping and then it will be commonly applied in breeding programs, especially in developing countries (Koebner 2004).

1.5 Precision Breeding with Genome Editing Tools

Cereals, majorly rice, wheat, and maize, supply more than 42% of the calories taken by the entire world's population. Combating the changing climatic conditions while improving their nutritional content and maintaining their steady supply requires innovative and precise breeding strategies. Enhancing the genotypic value of a crop requires the variation that can be brought with existing variation in the gene pool or induced through mutagenesis or genome editing. Genome editing techniques having more promising advantages over random mutations like targeted and precise modification of plant genomes are becoming more prominent for crop enhancement (Puchta 2017). Genome editing is defined as the tool that can bring precise and specific alterations in the organism's genome with specialized nucleases (Weinthal and Gürel 2016).

The genome editing methods include meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) which have been employed in cereals (Zhu et al. 2017). These techniques work on the principle of the formation of double-strand breaks (DSB) at target loci and initiating their repair mechanisms (Matres et al. 2021). There are two endogenous repair mechanisms: a fallible nonhomologous end joining (NHEJ) pathway, which creates random insertions or deletions (Feng et al. 2013), and a homology-directed repair (HDR) pathway, which utilizes a template DNA strand so is more precise and leads to gene replacement or gene knock-in (Baltes et al. 2014).

ZFNs and TALENs comprise a sequence-specific DNA binding domain and a nonspecific DNA cleavage domain, producing a double-stranded break at a given target site (Bortesi and Fischer 2015). In contrast, CRISPR/Cas9 has RNA (sgRNA)

guided Cas9 nuclease, generating DSB at target loci (Jinek et al. 2012). Stumbling blocks like complexity and the high cost of protein domain assembly have limited the usage of ZFNs and TALENs for crop improvement through genome editing. They also suffer from the limitation of illegitimate interaction between domains which results in off-target cleavage of DNA (Jones 2015). On the other hand, CRISPR, having advantages of simplicity and less cost of construction, fewer off-target mutations, and multiplexed mutations, is gaining more limelight than other genome editing techniques (Kumar et al. 2019).

With the advantage of small genome size, rice is among the early cereal crops edited via different editing techniques (Matres et al. 2021). The first method employed in rice for genome editing was the HR-mediated positive-negative selection (PNS) technique for altering the *Waxy* gene (Terada et al. 2002). Another laborious method based on homologous recombination (HR) was used in cereals for targeted mutagenesis before the development of the abovementioned designer nucleases (Cotsaftis and Guiderdoni 2005), which later was succeeded by an elegant and less tedious solution wherein a target site for the yeast harboring endonuclease *I-SceI* was introduced through random insertion into the genome (D'Halluin et al. 2008). This method was first employed in maize to confer herbicide tolerance. Later, it was used to mutate the cytosine demethylation gene (*ROS1*) in rice which is hard to mutate through conventional mutagenesis (Ono et al. 2012). This method could induce the target mutation in the genome of maize, but the full potential of genome editing was only realized with techniques targeting endogenous loci. The method was first reported to be used in maize using ZFNs, where they were targeted to disrupt the INOSITOL PHOSPHOKINASE-1 (*IPK1*) locus with the knock-in of the herbicide-resistant gene (Shukla et al. 2009). *OsCKX2* (cytokinin oxidase 2) gene was first mutated in rice using ZFNs, increasing grain number and total yield (Li et al. 2012). Large genome size and recalcitrance toward genetic transformation are the major hurdles in wheat for genome editing. *Agrobacterium*-mediated and particle bombardment are the only methods used to date in wheat to introduce genome editing components in immature embryos. Resistance against imidazolinone herbicide was achieved in bread wheat using ZFNs targeting *AHAS* gene (acetohydroxyacid synthase) (Ran et al. 2018) with a 2.9% recovery rate in transgenic plants.

I-CreI-derived meganuclease named LIG3::4 was the first ENM used in maize to target an upstream region of the LIGULELESS1 (*LG1*) gene (Gao et al. 2010). With the first and foremost use of TALENs, stable and heritable mutations were induced in the GLOSSY2 (GL2) locus of maize (Char et al. 2015). Using TALENs, genetically engineered lines harboring monoallelic or biallelic mutations were obtained at a frequency of as high as ~10% in maize (Matres et al. 2021). Plants resistant against *Xanthomonas oryzae* that causes bacterial blight were obtained using TALENs (Li et al. 2012). Heritable mutations were induced in rice by disrupting the bacterial blight susceptibility gene, *Os11N3* (*OsSWEET14*). The first genome editing event in barley was accomplished using TALENs, which were targeted to the promoter site of the phytase gene (*HvPAPhy-a*) (Wendt et al. 2013). TALENs were used in wheat to

modify *TaMLO* genes which led to the induction of horizontal resistance against powdery mildew in wheat (Wang et al. 2014).

The development of the CRISPR/Cas9 platform and its advantages over other methods like multiplex editing and DNA-free editing with the introgression of Cas9/gRNA ribonucleoprotein (RNPs) (2016) has paved the way of massive genome editing in cereals. The co-transformation of rice protoplasts first achieved genome modification in rice using CRISPR/Cas9 with sgRNA to target a specific site, Cas9 protein to generate breaks, and single-stranded DNA oligos as the template strand for the repair of breaks (Shan et al. 2013). The targeted genes for genome modification were *OsPDS* (phytoene desaturase) and *OsBADH2* (betain aldehyde dehydrogenase 2); mutations at 9.4% and 7.1%, respectively, were obtained. The first and foremost use of CRISPR/Cas9 for multiplex editing in maize was delineated by in which they targeted five loci, namely, the upstream region of *LG1*, two male fertility genes (*MS26* and *MS45*), and two acetolactate synthase genes (*ALS1* and *ALS2*). The DNA constructs were introduced in the maize embryos using the particle bombardment method of gene insertion. The technique was ten times more efficient than the available EMNs. In another study, the maize embryos were bombarded with the pre-assembled constructs of Cas9/gRNA ribonucleoproteins (RNPs) to achieve the knock-out mutations at four loci (*LG1*, *MS26*, *MS45*, and *ALS2*) (Svitashev et al. 2016). The initial validation of the CRISPR/Cas9 system in wheat was done with the knock-out of *TaMLO* (Shan et al. 2013), *TaPDS*, and *TaINOX* (Upadhyay et al. 2013) loci. In consecutive studies, resistance against powdery mildew was achieved with the knock-out of all the three homeoalleles of the *TaMLO* locus (Wang et al. 2014). The system has also been used to establish single base editing (C to T substitution) in the *LOX2* gene of wheat protoplasts (Zong et al. 2017). Under the control of the TaU3 promoter, knock-out of three different genes, viz., *TaMLO*, *TaGW2*, and *TaLpx-1*, were targeted for multiplex genome modification in bread wheat by making use of CRISPR/Cas9 system (Wang et al. 2018). Genome editing in sorghum was first affirmed with the use of CRISPR/Cas9 system targeting the *DsRED2* gene (Jiang et al. 2013). Subsequently, monoallelic frameshift mutations were regenerated in the *Sb-CENH3* gene following the CRISPR/Cas9 system using *Agrobacterium*-mediated transformation (Che et al. 2018). In barley, mutations in *HvHPT* and *HvHGGT* genes using CRISPR/Cas9 were created to enhance the tocopherol (vitamin E) in barley grains (Zeng et al. 2020a, b). These and other coeval studies have revealed the CRISPR/Cas9 as an effective and efficient technique for genome modification in cereals (Feng et al. 2016).

Several traits are taken in herbicide tolerance; physiological, morphological, and biotic and abiotic stress-related traits; and nutritional improvement, which have been modified successfully following genome editing approaches. A few examples of such traits are mentioned in Table 1.2.

Table 1.2 Representative examples of genome editing in cereals

Crop	Explant used	Target gene	Genome editing method	Transformation method	Reference
Rice	Embryogenic cell culture	*Os11N3*	TALEN	*Agrobacterium*-mediated transformation	Li et al. (2012)
	Callus	*CAO1* and *LAZY1*	CRISPR	*Agrobacterium*-mediated transformation	Miao et al. (2013)
	Embryogenic callus	*OsBADH2*	TALEN	*Agrobacterium*-mediated transformation	Shan et al. (2015)
	Callus	*ALS*	CRISPR	Particle bombardment	Sun et al. (2016)
	Callus	*OsSPS1* and *OsSPS11*	CRISPR	*Agrobacterium*-mediated transformation	Hashida et al. (2016)
	Callus	*OsSWEET14*	TALEN	*Agrobacterium*-mediated transformation	Blanvillain-Baufumé et al. (2017)
	Callus	*SSIVa*	ZFN	*Agrobacterium*-mediated transformation	Jung et al. (2018)
	Mature embryos	*RL1*, *BU1*, and *BC1*	TALEN CRISPR	*Agrobacterium*-mediated transformation	Ruan et al. (2018)
	Immature embryos	*OseIF4G*	CRISPR	*Agrobacterium*-mediated transformation	Macovei et al. (2018)
	Callus	*OsGS3*, *OsGW2*, and *OsGn1a*	CRISPR	*Agrobacterium*-mediated transformation	Zhou et al. (2019)
	Callus	*SRL1* and *SRL2*	CRISPR	*Agrobacterium*-mediated transformation	Liao et al. (2019)
	Callus	*OsF'H*, *OsDFR*, and *OsLDOX*	CRISPR	*Agrobacterium*-mediated transformation	Jung et al. (2019)
	Callus	*OsNAC2*	CRISPR	*Agrobacterium*-mediated transformation	Mao et al. (2020)
	Callus	*FWL* genes	CRISPR	*Agrobacterium*-mediated transformation	Gao et al. (2020)
	Callus	*OsPIN5b*, *GS3*, and *OsMYB30*	CRISPR	*Agrobacterium*-mediated transformation	Zeng et al. (2020a, b)

(continued)

Table 1.2 (continued)

Crop	Explant used	Target gene	Genome editing method	Transformation method	Reference
Maize	Embryogenic cell culture	*IPK1*	ZFN	Whisker-mediated transformation	Shukla et al. (2009)
	Immature embryos	Upstream of *LG1* promoter	ENM (based on I-CreI)	*Agrobacterium*-mediated transformation	Gao et al. (2010)
	Protoplasts	*ZmIPK*, *ZmIPK1A*, *ZmMRP4*, and *ZmPDS*	TALEN and CRISR/Cas9	PEG-mediated transformation	Liang et al. (2014)
	Immature embryos	*LIG*, *ALS2*, *MS26*, and *MS45*	CRISPR	Particle bombardment	Svitashev et al. (2016)
	Protoplasts	*Zmzb7*	CRISPR	PEG-mediated transformation	Feng et al. (2016)
	Immature embryos	*AGROS8*	CRISPR	Biolistic-mediated transformation	Shi et al. (2017)
	Immature embryos	*MTL*	TALEN	*Agrobacterium*-mediated transformation	Kelliher et al. (2017)
	Immature embryos	*zyp1* and *zb7*	CRISPR	*Agrobacterium*-mediated transformation	Feng et al. (2018)
	Immature embryos	*LIG*, *MS26*, and *MS45*	CRISPR	Biolistic-mediated transformation	Young et al. (2019)
	Immature embryos	20 genes	CRISPR	*Agrobacterium*-mediated transformation	Doll et al. (2019)
	Immature embryos	*gl2*	CRISPR	*Agrobacterium*-mediated transformation	Lee et al. (2019)
	Immature embryos	*ZnSMC3*	CRISPR	*Agrobacterium*-mediated transformation	Zhang et al. (2020a, b, c)
	Immature embryos	*ZmPHYC1* and *ZmPHYC2*	CRISPR	*Agrobacterium*-mediated transformation	Li et al. (2020a, b, c)
	Immature embryos	*ZmFCP1*, *ZmCLE7*, and *ZmCLE1E5*	CRISPR	*Agrobacterium*-mediated transformation	Liu et al. (2021)

(continued)

Table 1.2 (continued)

Crop	Explant used	Target gene	Genome editing method	Transformation method	Reference
Bread wheat	Cell suspension	*INOX* and *PDS*	CRISPR	*Agrobacterium*-mediated transformation	Upadhyay et al. (2013)
	Protoplast	*TaMLO*	TALEN and CRISPR	Biolistic transformation	Wang et al. (2014)
	Protoplast Immature embryos	*TaGASR7*	CRISPR	PEG-mediated transformation Biolistic transformation	Zhang et al. (2016)
	Microspores	*DsRed*, *TaLox2*, and *TaUbiL1*	CRISPR	Electroporation	Bhowmik et al. (2018)
	Protoplast	*TaGW2*, *TaLpx-a*, and *TaMLO*	CRISPR	Biolistic transformation	Wang et al. (2018)
	Immature scutella	*alpha-gliadins*	CRISPR	Biolistic transformation	Sánchez-León et al. (2018)
	Immature embryos	*TaALS* and *TaACC*	CRISPR	Biolistic transformation	Zhang et al. (2019)
	Microspores	*IPK1*	ZFNs	CPP transfection	Bilichak et al. (2020)
	Embryos	*TaPDS*	CRISPR	Particle bombardment	Kim et al. (2021)
Durum wheat	Immature scutella	*CM3* and *CM6*	CRISPR	Biolistic transformation	Camerlengo et al. (2020)
Barley	Immature embryos	*HvPM19*	CRISPR	*Agrobacterium*-mediated transformation	Lawrenson et al. (2015)
	Immature embryos	Promoter of *HvPAPhy_a*	CRISPR and TALEN	*Agrobacterium*-mediated transformation	Holme et al. (2017)
	Immature embryos	*Nud*, *HvCKX1* and *HvCKX3*	CRISPR	*Agrobacterium*-mediated transformation	Gasparis et al. (2018)
	Immature embryos	*Ptst1* and *Gbss1a*	CRISPR	*Agrobacterium*-mediated transformation	Zhong et al. (2019)
	Immature embryos	*HvCslF3*, *HvCslF6*, *HvCslF9*, and *HvCslH1*	CRISPR	*Agrobacterium*-mediated transformation	Garcia-Gimenez et al. (2020)
	Immature scutella	*HvHPT* and *HvHGGT*	CRISPR	*Agrobacterium*-mediated transformation	Zeng et al. (2020a, b)
	Immature embryos	*Hor3*	CRISPR	*Agrobacterium*-mediated transformation	Li et al. (2020a, b, c)

(continued)

Table 1.2 (continued)

Crop	Explant used	Target gene	Genome editing method	Transformation method	Reference
	Microspore derived callus	*HvPDS*	CRISPR	*Agrobacterium*-mediated transformation	Han et al. (2021)
Sorghum	Immature embryos	*k1C* gene family	CRISPR	*Agrobacterium*-mediated transformation	Li et al. (2018)
	Immature embryos	*CAD* and *PDS*	CRISPR	Biolistic bombardment	Liu et al. (2019)
	Immature embryos	*Wus2*	CRISPR	*Agrobacterium*-mediated transformation	Che et al. (2022)

1.6 Expansion of Gene Pool with Pan-Genome

The genomic and transcriptomic variations can help in understanding the phenotypic variation. Most of the whole-genome variations in plants are based on sequences from one reference genome (Hirsch et al. 2014) with a focus on single nucleotide polymorphisms (SNPs) and insertions and deletions (indels) in exon regions (Monat et al. 2019). It was first noticed in bacteria that an individual genome represents only a proportion of genes in each species (Monat et al. 2019). This observation gave rise to the concept of a pan-genome which consists of core and dispensable genes. The core genome represents genes shared by all individuals of a given species, and the dispensable genome represents the rest. To capture the dispensable genome, many individuals should be evaluated along with the core genome to obtain the maximum genomic variation by creating the pan-genome (Brunner et al. 2005). A pan-genome represents each species' complete set of genomic regions, representing a genetic variant without reference bias (Eizenga et al. 2020). Because of the high cost of data generation, it took almost 10 years for the construction of plant pan-genomes even after the findings of bacterial pan-genome (Bayer et al. 2020). Then the first publication with the term "pan-genome" came in 2007, with short transposable regions in rice and maize (Morgante et al. 2007).

Three general approaches are used to construct the pan genomes: the first method uses whole-genome assembly and comparison for multiple individuals. Next, an iterative assembly and presence and absence variation (PAV) calling approach complemented it. Genomics reads from a set of individuals are aligned against a reference, and all the non-aligned reads are assembled and added to the pan-genome reference (Bayer et al. 2020). The graph-based pan-genome assembly was recently introduced, which constructs a graph representing genomic diversity (Guarracino et al. 2021). A graphical representation of a pan-genome generated from de novo

assembly alignment is more effective than a variant calling file (Hickey et al. 2020; Paten et al. 2017).

Many studies have now documented pan-genomes in cereals such as bread wheat (*Triticum aestivum*) (Montenegro et al. 2017), barley (*Hordeum vulgare* ssp. *vulgare*) (Ma et al. 2019), maize (*Zea mays*) (Hirsch et al. 2014), and rice (*Oryza sativa*) (Sun et al. 2017; Zhao et al. 2018) showing dispensable genes to constitute 20–50% of the pan-genome. The QTL or GWAS approaches using a single reference genome do not represent a trait's variant if not present in the reference. For example, a GWAS study identified a maize gene responsible for resistance to sugarcane mosaic virus with B73 but not the PH207 reference because the gene was absent in the latter's assembly (Gage et al. 2019). Another report based on 503 maize inbred lines showed 8681 transcript assemblies not found in reference B73 (Hirsch et al. 2014). A wheat gene, *Lr*49, responsible for rust resistance, showed significant structural variation between varieties, resulting in reference bias (Nsabiyera et al. 2020).

With the rapid growth in pan-genome research, there has been a substantially increased interest in understanding dispensable genomes in cereal crops. For example, rice pan-genome research examined gene variation from a collection of 1083 *Oryza sativa* and 446 wild *O. rufipogon* accessions and reported 10,783 newly identified genes that were at least partially missing in the reference assembly (Zhao et al. 2018). These genes are associated with submergence tolerance (*Sub1A*) and phosphorus deficiency tolerance, consistent with earlier observations based on three rice accessions (Schatz et al. 2014).

The pan-genome concept has been extended to a pan-transcriptome, indicating that the variations are not limited to gene content (Jin et al. 2016). Transcriptome profiling using RNA-Seq can capture mRNAs, noncoding RNAs, and small RNAs. In recent years, publicly available transcriptome data have allowed the creation of a pan-transcriptome to capture most of the expressed genes in any species (Ma et al. 2019).

There is still limited availability of high-quality complete and well-annotated genome sequences for understudied or non-model crops. Beyond the advancements in pan-genome studies, there are technical difficulties in storing and visualizing pan-genome data. Overall, pan-genomic studies have the potential for a much broader understanding of crop genetic diversity with improved infrastructure and method development.

1.7 Haplotype-Based Breeding and Optimum Contribution Selection

Three major overarching challenges that modern agriculture has to combat are the changing climate, increasing crop productivity to feed the increasing population, and ensuring the nutritional demands of every section of the population (Prosekov and Ivanova 2018; Barrett 2021; Kilian et al. 2021) This necessitates the expansion of outputs through intensive crop breeding programs which tend to explore natural

variation, to produce next-generation smart crops encompassing all the desirable attributes (Swarup et al. 2021; Yu and Li 2021). However, the conventional breeding methods usually take a long time and are generally more expensive, with no assurance of a desirable crop being produced at the end (Shivakumar et al. 2018; Bhat and Yu 2021). Intensive breeding programs are deployed to alleviate these challenges, followed by high-throughput gene sequencing and precision agriculture, which offer fast and timely solutions to these overarching problems. One such technique deployed is haplotype breeding, which exploits functional allelic diversity responsible for genetic diversity among populations (Zhang et al. 2021). A haplotype is basically when two or more alleles or, more specifically, SNPs present on the same chromosome which are inherited together depending on linkage disequilibrium between them (Coffman et al. 2020; Li et al. 2021) such that a maximum of 1–3% diversity is allowed just to account for genotype errors. These SNPs are used by breeders fastening the target approach analysis to identify haplotypes within cultivar rather than sequencing the entire genome. Nearly all the traits responsible for genetic variation are due to different polymorphisms (single-nucleotide polymorphisms (SNP), insertion, deletions) and copy number variation leading to 99% variation within species/populations (Varshney et al. 2014; Bailey-Serres et al. 2019). For instance, Jensen et al. (2020) reported the identification of 1974 haplotype markers in sorghum with 0.57–0.73 genome selection (GS) prediction accuracy for agronomic and yield characteristics. Screening variation through haplotype analysis has led to an enormous improvement in crop breeding programs with drastically reducing time, inputs, and hence cost of production.

Moreover, the desirable genes of interest are introgressed from diverse germplasm to modern cultivars through haplotype breeding (Varshney et al. 2014; Bailey-Serres et al. 2019), or superior haplotypes are crossed together to produce elite cultivar via genome additivity responsible for crop improvement and adaptation (Mason and Snowdon 2016; Qian et al. 2017). For instance, haplotype analysis of thousand double haploid lines of three maize landraces revealed superior phenotype performance and stability of lines carrying haplotype compared to other breeding lines, thus further corroborating the importance of haplotype breeding in crop improvement (Qian et al. 2017). Haplotype analysis allows researchers to sample a selection from haplotype variants rather than genotyping the entire germplasm, extensively using specific target genes (Wu et al. 2018; Rodriguez et al. 2020). Generally, a map of a haplotype genome known as HapMap is developed to trace genes and describe the common patterns of genetic variation among individuals (Bohra et al. 2019). Thus, haplotype breeding urges the integration of genomics along with phenotypic data to eliminate any undesirable effect due to linkage or multiple effects of the same gene (Bhat et al. 2021). It is followed by screening thousands of lines/accession to locate haplotype variation for a successful breeding program. Due to its overarching benefits, it is extensively used in breeding programs of important cereals such as wheat, rice, and barley.

Abbai et al. (2019) reported the recognition haplotype of 21 genes across the 3K rice genome. Similarly, haplotypes for deep water adaptation, direct seeded rice, salinity tolerance, grain cooking, and eating quality have been identified. Further,

QTL and haplotype analysis performed by Zhang et al. (2020a, b, c) revealed Os09g0535500 as the promising cultivar in gene *WTG9* for grain width and thickness—useful traits for rice grain quality and yield. Most crops are allopolyploids, yet the direct effect of polyploidy is still not clearly understood. Haplotype breeding holds immense importance in the case of self-pollinating crops such as hexaploid bread wheat, where genetic diversity is often limited due to pure line breeding (Meyer and Purugganan 2013). For instance, Brinton et al. (2020) identified five haplotypes of *RHT-B1* and four haplotypes of *RHT-D1* (semi-dwarfing reduced height genes) in 15 sequenced cultivars, suggesting a higher number of haplotypes across commonly cultivated cultivars which also means narrow genetic variability of modern wheat post-Green Revolution. Similarly, prediction accuracies up to "$r = 0.74$" have been achieved in the case of haplotype-based selection of oats for heading date.

The primary and broader goal of genetics is to exploit the genetic variation among species to produce smart crops with efficient productivity by understanding the effects of DNA sequence variation on plant traits (Sella and Barton 2019). Haplotype breeding acts as a powerful tool in this regard as it is more reliant than SNP-based GS selection as they are multi-allelic in nature and highly polymorphic which reduces the chances of false positives and negatives drastically in haplotype-based selection as compared to SNP-based selection (Browning and Yu 2009; Tsuji et al. 2018; Yuan and Biswas 2019). Haplotype-based selection has further helped identify rare alleles and epistatic interaction, thus widening the horizons of the plant breeding program. This will help breeders make intelligent decisions based on additive and epistatic effects (Zeng et al. 2019). However, there is limiting knowledge on different haplotypes involved in phenotypic selection. There is also a need for advancement in third-generation sequencing, which produces longer reads, thus encompassing more than a single variant enabling direct haplotype construction (Maestri et al. 2020; Delaneau et al. 2019). Although current statistical tools such as WhatsHap, HapCUT2, HapTree, Whap polyphase, Falcon phase, Hifiasm, SDip, POLYTE, DESMAN, fastPHASE, MetaMaps, and ProxiMeta have tremendously improved the haplotype analysis (Varshney et al. 2016), further research is warranted for advances in various computational tools for haplotype analysis to fully exploit the potential of haplotype breeding (Garg 2021). Thus, haplotype-based breeding will lead to the precise parental selection and the production of elite cultivars, thus maximizing genetic gains and broadening the existing germplasm resources and widening the scope for improved genetics (Mayer et al. 2020; Brinton et al. 2020).

1.8 Enhancement of Genetic Gain with Genomic and Phenomics-Assisted Breeding

Agronomic and quality traits are crucial in cereal crops, and breeders have developed improved varieties using phenotyping selection. The genetic gain is relatively low in the phenotypic selection due to low heritability, complex genetic constitution, and

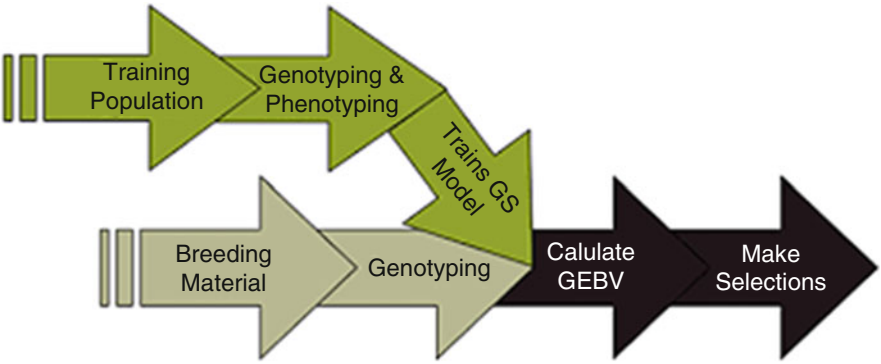

Fig. 1.2 Different steps involved in genomic selection (GS) (adapted from Heffner et al. 2009)

high interaction between genotype and environment (Jia et al. 2018). According to Jia et al. (2018), breeding value (BV) cannot be measured directly in a plant; this is a significant issue in plant breeding. It is almost impossible to measure the BV accurately using phenotypic data only. In recent years, the utilization of genotyping information has become highly prioritized in plant breeding. Marker-assisted selection (MAS) can be an option to incorporate into phenotypic data to increase the accuracy of the BV. Marker-assisted selection considers QTL associated with the markers, which is the superiority of MAS over the phenotypic selection, but this marker effect is not enough to explain complex traits (Hayes and Goddard 2010; Meuwissen et al. 2001). Therefore, linkage disequilibrium (LD) markers associated with QTLs are needed to understand desired traits with high prediction accuracy.

The deployment of genotyping information has become highly spotlighted in plant breeding since the high-throughput genotyping method is low cost. Genomic selection (GS) showed a great potential to increase the precision of BV (Meuwissen et al. 2001). GS enables the speedy selection of improved genotypes and speeds up the breeding cycle (Crossa et al. 2017). A huge amount of genomic information can be found from this process, and it considers all genes, either small or large, associated with the targeted traits in LD, thus achieving a high accuracy genomic estimated breeding value (GEBV). According to Meuwissen et al. (2001), the accuracy could be as high as 0.85. Still, it varies from crop to crop, ranging from 0.05 to 0.08 depending upon traits, statistical methods, and experimental design (Meuwissen et al. 2001). GS can accelerate genetic gain for the trait of interest. Thus, we meet the demand of different cereal productions.

The GS model aims to predict the GEBV. This method is comprised of two populations: a training population and a testing population. Phenotyping and genotyping are done on the training population to make the prediction model to obtain GEBV of individuals or family pool in the testing population (Fig. 1.2) that has been genotyped only; phenotypic data does not require data in the testing population. GS uses fewer resources because it does not require extensive phenotyping and can quickly improve complex traits with low heritability and

reduce total breeding costs. GS can also apply for simple traits with higher heritability and prediction accuracy (Crossa et al. 2017).

This approach is being utilized to improve quantitative traits, comprised of high-density markers, high-throughput genotyping, phenotypic data, genomic prediction, and marker data. Pedigree information can be used as a data source to verify the model or the prediction. GS can provide more genetic gain than other nongenomic methods and can be measured early in the plant's life (Lin et al. 2014). However, the application of GS can be affected by two main factors in plant breeding: (1) genotyping cost and (2) proper guidelines in which stage of plant breeding uses GS for efficient results (Crossa et al. 2017).

Nowadays, GS is becoming a promising tool for cereal crops in developing different traits, either alone or combined with phenomics. To our knowledge, this method is extensively used in wheat, whereas it is gaining popularity with other cereal crops such as rice, maize, sorghum, barley, and oats for grain yield (Marulanda et al. 2016). GS is a promising tool to increase the genetic gain of a complex trait like grain yield. Bassi et al. (2016) described the breeding scheme of GS for wheat as the breeding scheme of GS is not clear to everyone. Some GS selection studies have been done in the breeding of wheat (Arruda et al. 2015, 2016; Battenfield et al. 2016; Bentley et al. 2014; Guzman et al. 2016; Haile et al. 2018; He et al. 2016; Huang et al. 2016; Lozada et al. 2019; Michel et al. 2018; Rutkoski et al. 2011; Todorovska et al. 2009; Yao et al. 2018), rice (Grenier et al. 2015; Huang et al. 2019; Wang et al. 2021; Xu et al. 2014, 2021), maize (Marulanda et al. 2016; Shikha et al. 2017; Zhang et al. 2017; Zhao et al. 2012), and sorghum (de Oliveira et al. 2018; Fernandes et al. 2018; Morris et al. 2013; Prasad et al. 2021) for grain yield, quality, biotic-abiotic stress, and other traits.

Phenotyping plays a crucial role in plant breeding because the precise and speedy acquisition of phenotypic data helps explore the association between genomic and phenotypic information. Traditional phenotyping methods, such as chlorophyll content, leaf color, leaf area index (LAI), plant height, biomass, and yield, depend on manual sampling, which is laborious and time-consuming. The utilization of remote sensing is a game-changer in precision agriculture (Maes and Steppe 2019). This process collects information from the object in a nondestructive way. It offers unprecedented spectral, temporal, and spatial resolution to provide comprehensive vegetation data with multi-angular observations (Maes and Steppe 2019). The advancement in recent decade and the steep rise of unmanned aerial vehicles (UAVs) or drones have revealed a new era in remote sensing. It is becoming popular in agricultural research. Remote sensing can monitor high-throughput plant physiology in a nondestructive way. Recent advances in remote sensing have increased application in the field and controlled growing conditions (Araus and Cairns 2014; Leinonen and Jones 2004; Möller et al. 2007; Swain and Zaman 2012) which brings significant consequences for crop improvement. High-throughput phenotyping (HTP) using UAVs has captivated the interest of plant breeders worldwide because this approach aims at predicting complex traits along with genomic selection (Sandhu et al. 2021a). Some studies (Crain et al. 2018; Sun et al. 2019) have been done to combine GS with HTP for cereals and other crops to increase the prediction

of its accuracy. Despite some challenges in HTP, RS data can give an accurate selection from phenotyping (Biswas et al. 2021). It can be a great addition to GS for predicting any traits more accurately, thereby enhancing genetic gain, which is the eventual goal of plant breeders.

1.9 Integrating Data Science Approaches into Genomics

Since the completion of the Arabidopsis (*Arabidopsis thaliana*) genome sequencing project in 2001, there has been an unprecedented proliferation of genome sequence information from other plants. Genome sequencing capabilities have increased exponentially compared to computing power. Extraction of useful information using genomics from plants not only requires fast computers but also smart algorithms. Furthermore, these improvements are greater for animals and have not reached a comparable level in plants. With the rapid development of high-throughput sequencing tools and cost reduction, there has been a plethora of genotyping information. This has resulted in a problem of "large p and small n," and data science offers the potential to deal with this. Data science is being applied for identifying causal genes, making predictions for plant performances before planting them in the field, comparing ancestral divergence of plant species, and storing data to make it available for public use.

Analyzing and understanding data is critical for new inventions and findings. Data science is a multidisciplinary field encompassing computer science, statistics, mathematics, data visualization, domain knowledge, the craft of problem development, artificial intelligence, and machine and deep learning (Sandhu et al. 2022a). Experience in all these domains helps data scientists work on genomics to craft a problem and systematically engineer the solutions. Our era has witnessed tremendous development in plant genomics, resulting from developing a high-throughput genotyping platform with a meager cost, which is even reduced further by new inventions (Kaur et al. 2021). Although genomic data is increasing, it is imperative to develop and integrate some data mining and analysis tools for predicting and explaining the information contained in the sequences. There is a considerable gap between the flow of information between the genome sequence and terminal plant phenotype. Recent inventions in association analysis and prediction of plant phenotypes result in lowering the bridge between these two domains. The association analysis involves looking at variation in the genome sequence and linking it to the actual plant phenotype using various mixed linear models and machine and deep learning models. The prediction of plant phenotypes involves using whole-genome sequence information to predict the real phenotypes by training the model on the dataset from previous years using machine and deep learning models (Sandhu et al. 2022b).

Machine learning (ML) is a division of artificial intelligence that is getting attention from plant scientists to exploit massive data in plant genomics (Sandhu et al. 2020, 2021c, d). With the increase in genomic datasets, there is a problem with extracting useful information without good algorithms. In this regard, ML is a

technical basis for digging into the extraction of useful information from the genomic dataset. ML can be categorized into supervised and unsupervised learning models (Sandhu et al. 2022a). Supervised learning uses a labeled dataset where we always know the output values. On the one hand, unsupervised learning methods use the unlabeled dataset for the work. ML has an ample prospectus in plant genomics and has shown its power for analyzing and dissecting the complex datasets in the plants (Sandhu et al. 2021a). ML has demonstrated its application for predicting various traits in wheat and maize before phenotyping plants in the field and provides the best alternative for the plant breeders for increasing the genetic gain per unit time. Similarly, various ML models have been developed to analyze genomic data to identify the causal gene responsible for the associated phenotype.

A major development in the field of ML includes learning information from the data without being explicitly trained using the deep neural networks and is known as deep learning (DL). The critical difference between ML and DL is they are more flexible and have a much higher capacity (Sandhu et al. 2021a). There are millions of trainable parameters to train the model, and the optimum choice depends upon the dataset used. DL models automatically learn the information from the dataset without any handcrafting. DL models improve the prediction abilities of the models, requiring the collection of large datasets for training the models. The starting point of DL models includes the use of neurons which learns the information from the input dataset, and weight is associated with each neuron and ultimately performing a nonlinear transformation to provide an output value. The output of each neuron acts as input for the next layer's neurons, which eventually results in the creation of dense neural networks. Recently, various studies have used DL models in plant genomics and some good opinion papers discussing the future use of DL models in genomics (Sandhu et al. 2020, 2021c, d).

1.10 Conclusion

Modern advances in genome sequencing, assembly, and functional annotation, as well as advanced bioinformatics and computational techniques, have made it easier to understand the structure and information contained in cereal genomes. As a result, the precision of genomic mapping regions regulating different traits of agronomic importance has also increased. This has necessitated the use of genomic-assisted breeding for the genetic improvement of cereals for different agronomic traits. Further, CRISPR has become one of the most flexible genetic engineering tools in recent decades, having been used for various genome editing applications in cereals. In comparison to traditional procedures and transgenic technologies, CRISPR-based genome editing techniques are more cost-effective, faster, and accurate in attaining targeted cereal improvement. Still, genome editing confronts multiple challenges in its application; these challenges must be overcome to support the effective utilization of these genome editing techniques for crop development with long-term prospects. Genomic prediction is a useful technique for plant breeders since it uses markers that span the entire genome to predict GEBVs of individuals. However, the best way to

apply GS is still a topic of discussion. The best way to use GS in plant breeding efforts may be to combine different strategies. Machine learning, deep learning, and high-throughput crop phenotyping have become increasingly important to improve gene function prediction and relate genotypes to phenotypes. Pan-genomics will also help us decode crop genetic diversity and identify new crop alleles. These genomic approaches would be critical in developing climate-resilient, high-yielding, and nutritionally enhanced cereal crops for the world's rapidly rising population.

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SMART Plant Breeding from Pre-genomic to Post-genomic Era for Developing Climate-Resilient Cereals

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Sneha Adhikari, Anjali Joshi, Ajay Kumar Chandra, Alka Bharati, Sayantan Sarkar, Vishal Dinkar, Amarjeet Kumar, and Ashutosh Kumar Singh

Abstract

The world is facing unprecedented repercussions of climate change or global warming. Rising temperature makes glaciers melt, causing flooding and erosion, which undermines food production. Various technologies, including soil management, crop diversification, rainwater harvesting, farm machinery, livestock and fishery interventions, and weather-based agro advisories, assist in adapting the climate changes for crop production. Plant breeding has played a pivotal role in human history by revolutionizing agriculture to feed the ever-growing population. Recent advancements in omics platforms have enabled breeders to gain

S. Adhikari
ICAR-Indian Institute of Wheat and Barley Research, Regional Station, Shimla, Himachal Pradesh, India

A. Joshi
Genetics and Tree Improvement Division, Arid Forest Research Institute, Jodhpur, Rajasthan, India

A. K. Chandra
Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi, India

A. Bharati
ICAR-Central Agroforestry Research Institute, Jhansi, Uttar Pradesh, India

S. Sarkar
Blackland Research and Extension Center, Texas A & M Agrilife Research, Temple, TX, USA

V. Dinkar
ICAR-Central Institute of Temperate Horticulture, Srinagar, Jammu and Kashmir, India

A. Kumar (✉)
Department of Genetics and Plant Breeding, MTTC & VTC, Selesih, CAU, Imphal, Manipur, India

A. K. Singh
Center for Advanced Studies on Climate Change, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

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better insight into crop physiology and underlying genetic mechanisms. A better understanding of the structure, function, regulation, and interaction of genetic factors is possible due to the advent of high-throughput genome sequencing platforms, precise phenotyping, advanced computing, and data analysis platforms. Breeding for high yield with sustainable use of scarce resources in a diverse environment urgently demands the amalgamation of these throughput technologies. Wild species, wild relatives, and landraces are the storehouse of various desirable traits and cornerstones of breeding programs. Conventional breeding methods played a tremendous role in crop improvement, but it is challenging to achieve climate resiliency demand by depending on traditional methods alone. The present chapter discusses the classical breeding methods and advancements in genomics, genome sequencing, transgenics, genome editing, and related breeding methodologies such as marker-assisted selection and incorporation of phenomics, data analytics, and artificial intelligence for the rapid development of climate-resilient cereal crops. The chapter briefly presents the success achieved through holistic SMART-breeding approaches in cereal crops from the pre- to post-genomic period.

Keywords

SMART plant breeding · Cereals · Post-genomic era · Climate-resilient

2.1 Introduction

Plant species and agriculture are indispensable in human evolution, migration, and civilization. Human depends on various plant species and crops for food, medicine, shelter, fire, and other needs (Purugganan 2019). Cereals have been principal human food and have markedly influenced human civilization. Even in modern times, cereals are a major nutrition source worldwide, particularly in developing nations. Cereals fulfill ~60% of the total calorie demands in developing countries, while it can be ~80% in the poorest countries. However, in developed nations, >70% of cereal production is fed to animals, while humans consume the rest (Awika 2011; Olugbire et al. 2021). Major cereals, including wheat, rice, and maize, contribute 48% of the total calories and 42% of the total protein requirement in developing nations. Cereal grains comprise ~75% carbohydrates (mainly starches, 10,000–15,000 kJ/kg of energy) and about 6–15% protein, which varies with crop species. Cereals are a rich source of amino acids and vitamins such as niacin, riboflavin, thiamine, vitamin B complex, vitamin E, fiber, iron, magnesium, and trace minerals that are important for human and animal health (Papanikolaou and Fulgoni 2017; Laskowski et al. 2019). FAO (2017) projected that for global food security, cereals will continue to play a critical role till 2050 by contributing nearly half of the daily protein and calorie intake in both low- and middle-income countries. Presently, around 80% of the world's cereal grains are contributed by Asia and America (Jeyasri et al. 2021). Continuously shrinking arable land and increasing

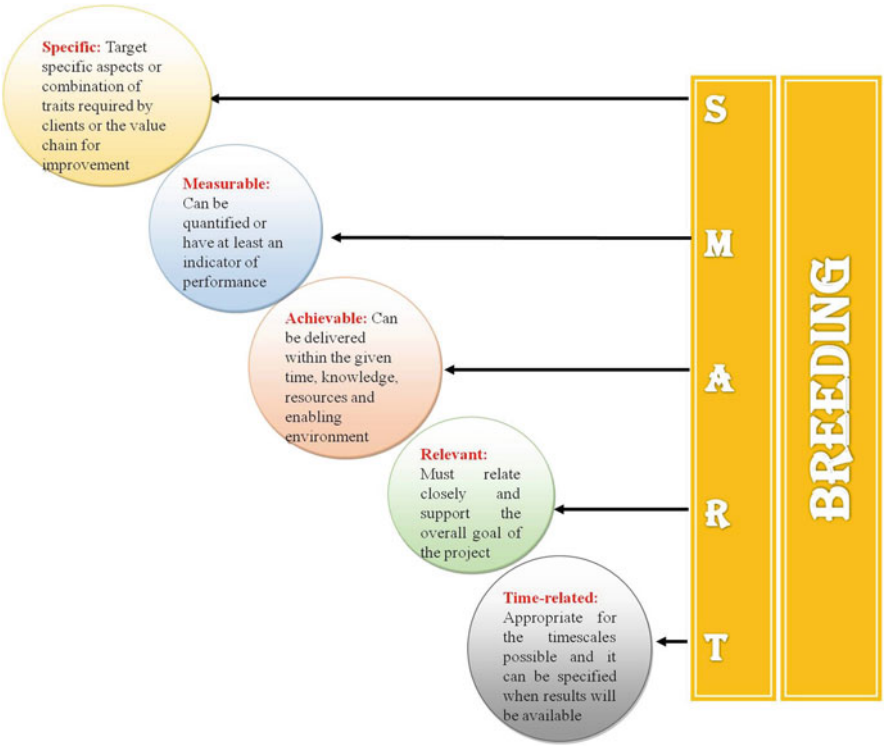

Fig. 2.1 Definition of terms SMART breeding objectives

human population make it difficult to meet the projected demand to feed ~10 billion people by 2050 via traditional agriculture (Hickey et al. 2019).

Moreover, global warming led to changes in the rainfall patterns, rising global temperature, and melting glaciers resulting in unprecedented drought and flood, heat waves, and chilling stress across the globe. Likewise, soil degradation via salinity, alkalinity, acidity, toxic metals, erosion, poor soil carbon status, and elevated CO_2 levels causes a severe impact on agriculture production (Leisner 2020). Changing climate favors plant pathogens, the evolution of pests, and frequent disease/pest outbreaks. Climate change is also expected to cause biodiversity loss, especially in marginal environments. It has been estimated that the yield loss is due to several abiotic stresses, i.e., drought (17%), salinity (20%), heat stress (40%), low-temperature stress (15%), and other factors (8%) (Athar and Ashraf 2009). The ability of the plant to survive or recover from adverse climatic conditions is called climate resiliency. Breeding genetically superior climate-resilient varieties is one of the most adaptable, economical, and sustainable methods to cope with environmental stresses and ensure food security (Falconer and Mackay 1996) (Fig. 2.1).

Breakthrough advancement in molecular biology, biotechnology, and omics platforms led to the generation of tremendous genomic information, i.e., structural, functional, and evolutionary. Their interactions of different genes/QTLs and regulation, high-throughput, and robust phenotyping and genotyping platforms are critical for speeding up breeding programs for screening and breeding climate SMART cultivars (Wang 2007; Gobu et al. 2020; Kushwaha et al. 2021). The idea of SMART breeding is a need-based combination of traditional breeding methods and modern biotechnological tools (genomics, phenomics, proteomics, metabolomics, and ionomics) for developing climate-SMART crop cultivars with enhanced production capabilities. Certain new breeding approaches helpful in the SMART include rapid advancement of generation by *speed breeding* protocols, rapid achievement of homozygosity via *doubled haploid* (DH) techniques, and *marker-assisted selection* (MAS) for environment-independent and multi-trait selection, which have resulted in shortened breeding duration.

2.2 Morphological, Physiological, and Biochemical Alteration in Response to Abiotic Stresses in Cereals

Various abiotic stresses, including moisture stress, thermal stress, soil salinity, nutrient deficiency, and toxicity, inflict plant morphology and physiology mostly via perturbed osmotic and ionic balance. Abiotic stresses disrupt numerous developmental processes in cereals, including seed germination, vegetative growth, tillering, dry matter accumulation, photosynthate partitioning, reproductive organ development, reproductive processes, grain filling, and grain quality (Manickavelu et al. 2006; Britz et al. 2007; Sehgal et al. 2018; Sharma et al. 2018, 2021). In response to abiotic stress stimuli, the earliest events in plants include a rise in ABA level, increased concentration of cytosolic Ca^{2+} ions, and activation of kinases and phosphorylases, which bring numerous biochemical and molecular changes such as osmotic adjustments and gene regulation (Baxter et al. 2014).

2.2.1 Morphological Changes

2.2.1.1 Plant Establishment

Seed germination is the first step toward plant establishment that is highly dependent on moisture and temperature. Drought has a negative effect on germination percentage and time (Mut et al. 2010). Most cereal seeds show poor seedling emergence when water potential decreases (Kim and Jeon 2009). Wheat and oat seed dormancy can be accentuated by high soil temperature, commonly referred to as high-temperature germination sensitivity (Lei et al. 2013). In contrast, to minimize postharvest dormancy, rice can be exposed to dry heat (50–55°C) for 3 days which is a common practice at IRRI with respect to all *japonica* and *indica* cultivars (Krishnan et al. 2011). Under saline conditions, germination percentage, radicle length, hypocotyl length, dry weight, and seedling fresh and dry weight decrease

significantly (Akbari et al. 2007). The detrimental effect of salinity on seed germination of different crops, including rice (Xu et al. 2011), wheat (Akbarimoghaddam et al. 2011), and maize (Carpici et al. 2009; Khodarahmpour et al. 2012), is mainly due to hampered water imbibition by seed which can be attributed to low osmotic potential of germination media (Khan and Weber 2008), altered activities of the enzyme involved in nucleic acid metabolism (Gomes-Filho et al. 2008), altered hormonal balance and protein metabolism (Khan and Rizvi 1994; Dantas et al. 2007), and restricted utilization of seed reserves (Othman et al. 2006). Germination is oxygen-dependent, and cereal seed rot in a waterlogged state.

2.2.1.2 Root Architecture

Plant growth and productivity depend on water and nutrient uptake and plant interactions with microbiota, particularly those that prevail in the rhizosphere. Stresses are first sensed by roots which affect root architecture (root length, spread, number, and length of lateral roots) and rhizosphere microbial community, which ultimately affect water and nutrients absorption (Huang et al. 2012). Plants alter their root length and root surface area to facilitate the absorption of less mobile elements in water-deficient soils (Khan et al. 2016). Root elongation in rice is hampered due to poor meristematic activity in response to drought stress (Slayter 1973). Waterlogging stress affects the growth and development of plant roots and causes root decay. In many crops, waterlogging replaces basal roots and induces the development of adventitious roots, which is responsible for waterlogging tolerance in plants (Malik et al. 2001; Steffens et al. 2006). Flood-tolerant rice develops more aerenchyma to facilitate aeration between roots and shoots and develops gas films to facilitate O_2 and CO_2 entry from the surrounding water (Panda and Barik 2021; Pedersen et al. 2009).

2.2.1.3 Vegetative Growth

Disrupted water and heat stress intake by higher plants cause frequent stomata closure, may induce leaves wilting, and negatively affect both cell elongation and expansion, thereby causing diminished growth and development of plants (Kapoor et al. 2020; Yadav et al. 2020). Reduction in leaf area on account of drought stress is a stress avoidance strategy to reduce water loss by transpiration (Kapoor et al. 2020; Xu et al. 2010). High heat reduces the photosynthetic rate in rice and wheat flag leaves (Feng et al. 2014). Perturbed photosynthesis rate affects plant height (shoot length), number and size of leaves, stem thickness, and root characteristics under drought, reducing plant biomass on a fresh and dry weight basis (Abobatta 2019). In contrast, flood-tolerant rice lines show accelerated internodal elongation to keep some shoots above water level (Panda and Barik 2021). Salinity-induced loss of leaf turgor and closure of stomata reduce leaf growth and area, thereby reducing the overall rate of photosynthesis (Munns and Tester 2008). Both root and shoot cell expansion is hampered due to low turgor pressure (Munns et al. 2000; Fricke et al. 2004). Salinity also causes premature leaf senescence, chlorosis, and necrosis and reduces cell metabolism (Al-Shareef and Tester 2019).

Salinity stress in rice reduces leaf area index, plant height, and the number of tillers (Hasanuzzaman et al. 2009). Cold stress (chilling and freezing) injury in plants adversely affects the vegetative and reproductive stages of the plant; however, the latter is more susceptible (Nishiyama 1995). Changes in morphological traits include delayed seedling emergence, reduced seedling vigour, and reduced leaf initiation and growth, inducing the development of necrotic lesions in leaves and stem and also leading to disordered cell division in roots and reduced root elongation and enlargement. Due to this, nutrient and water uptake of roots decline to reduce nutrient use efficiency (Grossnickle 2005; Farooq et al. 2009; Mukhopadhyay and Roychoudhury 2018).

2.2.1.4 Reproductive Organs

The effect of water deficit on yield and yield components at different growth stages has been reported in numerous studies (Farooq et al. 2011). Drought stress is particularly detrimental during the reproductive stage of the cereal crops, mainly due to hindrance in nutrient uptake from dried soil, which adversely affects the development of flower buds (Abobatta 2019; Kapoor et al. 2020). Abiotic stresses may cause delayed flowering, reduced flowering duration, delayed anthesis, reduced floret fertility, abnormal ovary development, poor pollination and fertilization, and consequently reduced seed set and productivity (Aghamolki et al. 2014; Fu et al. 2016; Fahad et al. 2017a, b). Anthers and pollens are more susceptible to high heat than ovules (Harsant et al. 2013). Floret sterility can be attributed to decreased anther dehiscence, reduced pollen shedding, poor pollen grains germination on the stigma, and slow elongation of pollen tubes (Fahad et al. 2017a, b). In rice, under high heat, tight closure of the locules leads to poor anther dehiscence and low pollen production and, thus, causes sterility (Matsui and Omasa 2002). Likewise, in maize, high temperatures reduce pollen germination ability and pollen tube elongation (Barnabás et al. 2008). Chilling temperature delays flowering, induces abscission of flowers, causes pollen sterility, distorts pollen tube, and induces ovule abortion which ultimately caused poor fruit set and seed development and lowers yield (Thakur et al. 2010; Zinn et al. 2010; Arshad et al. 2017). Cold stress in rice causes ~30–40% reduction in total yield (Andaya and Mackill 2003).

2.2.1.5 Seed Setting and Grain Quality

Partitioning plant biomass under drought conditions is one of the key aspects of drought tolerance, determining the plant's productivity (Kage et al. 2004). Exposure to water-deficient conditions or suboptimal temperatures is often associated with slowing down plant metabolic activities, causing a significant reduction in the expression of economically important traits (Coskun et al. 2016). Yield component traits such as the number of spikes per plant, grain per spike number, grain test weight, and grain shape and size are drastically affected under stress. Water deficiency also causes abortion of pistil florets, leading to reduced seed set, disturbed assimilate partitioning, and compromised efficiency of sucrose and starch synthesis enzymes, leading to smaller grains (Farooq et al. 2009; Nuttall et al. 2017). Drought stress during the vegetative growth of maize (during V1 to V5) leads to a significant

reduction in grain yield, increases the period of vegetative growth, reduces the reproductive growth period, and reduces photosynthesis leading to accelerated leaf senescence during grain filling, thereby affecting kernel weight and reducing total maize yield by 20–30%. Strong heat waves may scorch the twigs and leaves and plant wilts, leaf senescence, discoloration of leaves, poor grain filling, and shriveled grains (Fahad et al. 2017a, b). High temperature (>34 °C) during the grain filling period in wheat induces senescence (Lobell et al. 2012). However, heat stress during the ripening stage in rice does not significantly affect the yield and yield-contributing traits (Aghamolki et al. 2014). High salt conditions also modulate grain texture in cereals (Raza et al. 2019; Jamshidi and Javanmard 2018). Salt stress increases the grain protein content in cereals such as durum wheat, maize, and barley while reducing the carbohydrate content in maize and barley (Houshmand et al. 2014; Jamshidi and Javanmard 2018; Li et al. 2019).

2.2.2 Physiological and Biochemical Changes

2.2.2.1 Photosynthesis

Stress-induced stomatal closure is the first obvious change in crop plants. Closure of stomata increases plant canopy and internal temperature and may lead to oxidative damage. Under moisture-deficit conditions (drought and salinity), decreased turgor pressure along with ABA signals from the root reduces stomatal conductance. ABA buildup in the roots results in a rise in leaf ABA. Stomatal closure reduces transpiration water loss in plants to maintain the cellular water potential. But this checks carbon dioxide intake and, consequently, decreases photosynthesis. The high heat effect on photosynthetic apparatus can be attributed to elevated ROS (Pintó-Marijuan and Munné-Bosch 2014). Plant pigments such as carotene, xanthophyll, and chlorophylls constitute the light-harvesting complex (LHC). LHC protects photosynthetic apparatus (PS I and PS II) against intense light-induced oxidative damage via dissipation of excess light as heat called nonphotochemical quenching (NPQ) (Müller et al. 2001). Thus, reduced photosynthesis under drought, heat, and salt stress can be attributed to their damaged photosynthetic pigments and reduced light absorption (Pintó-Marijuan and Munné-Bosch 2014). The impaired function of photosystems, ETS, and photophosphorylation reduces the production of ATP and NADPH, ultimately leading to diminished CO_2 reduction (Hu et al. 2022). Elevated salt concentration in the cell, particularly Na^+, impairs chlorophyll biosynthesis and/or elevates pigment degradation (Ashraf and Harris 2013). Chlorophyll accumulation under salinity stress has been suggested to indicate plant tolerance capacity (Athar et al. 2015). Thermal (heat and cold) and heavy metal (such as arsenic) stresses reduce chlorophyll biosynthesis due to perturbed enzyme activity such as 5-aminolevulinate dehydratase (ALAD), which catalyzes the first step in the pyrrole biosynthetic pathway (Jain and Gadre 2004). Chlorophyll content, a reliable measure of cereal drought tolerance, declines under moisture deficit. The concentration of chlorophyll b is more affected than chlorophyll a (Ashraf and Harris 2013).

Drought and heat stress drastically affect the PS II efficiency, which is an outcome of poor CO_2 intake, disturbed electron transport chain, photophosphorylation, and dissociation of Ca^{2+} and Mg^{2+} ions from enzymes leading to their inactivation (Fahad et al. 2017a, b). PS II activity has been suggested as a good physiological criterion for selecting drought-tolerant genotypes in cereals (Jumrani and Bhatia 2019). Protein D1 in the reaction center of PS II is highly vulnerable to photodamage (Cortleven et al. 2019). Heat stress disturbs the regeneration of RuBP. Synthesis of small subunits of RUBISCo enzyme is deceased (Fahad et al. 2017a, b). RUBISCo activity is suppressed under severe drought, which is a chief cause of decreased photosynthesis. Parry et al. (2002) reported that under drought and light stress, RUBISCo activity could be suppressed due to inhibitors such as 2-carboxyaribinitol 1-phosphate (2CA1P).

2.2.2.2 Yield and Quality

Critical components for cereal crop yield, such as grains per spike, spike length, and the number of spikelets per spike, are negatively affected by moisture, temperature, and other stresses (Yang et al. 2018). Reduced yield and yield-related traits during abiotic stresses have been attributed to pollen abortion, reduced photosynthesis, and assimilate partitioning (Barnabás et al. 2008). In rice, tillering was sensitive to elevated night temperatures (Fahad et al. 2017a, b). Salt-sensitive basmati rice cultivars showed reduced activity of starch synthase enzyme in pollen which decreased pollen viability drastically (Khan and Abdullah 2003). Mayer et al. (2014) correlated the heat-induced reductions of kernel weight with shorter grain-filling periods in maize. The drought at the pre-anthesis stage shortens the anthesis time, whereas the post-anthesis drought contracts the grain filling duration in triticale (Estrada-Campuzano et al. 2008). Likewise, intensity, duration, and a combination of stresses are critical for the extent of yield losses. Temperature between 30 and 40 °C leads to about a 30% reduction in the accumulation of starch in wheat grains. An increase in protein content and a decline in gluten quality and content were also observed in durum wheat grains in response to dry conditions (Li et al. 2013a, b; Magallanes-López et al. 2017).

Economic yield parameters such as protein, oil, mineral content, and other biochemical parameters are drastically affected under stress. Several enzymes are involved in grain filling and starch metabolism, such as adenosine diphosphate (ADP)-glucose pyrophosphorylase (AGPase), aldolase, acid invertase, sucrose synthase (SuSy), glucokinase, soluble starch synthase (SSS), and starch branching enzyme (SBE), which show reduced activity under drought and heat stress in cereals like maize and wheat (Duke and Doehlert 1996; Ahmadi and baker 2001; Yang et al. 2018). Heat stress suppresses the enzymes related to starch synthesis and increases alpha-amylase activity, leading to poor starch filling and causing chalky grains in rice (Hakata et al. 2012; Phan et al. 2013). Heat stress represses zein accumulation during endosperm development in maize, during early stages via repressing zein synthesis, and at later stages via zein protein degradation (Monjardino et al. 2005). Heat stress reduces maize kernel oil content, which is mainly associated with lower embryo oil concentrations and kernel weight (Mayer et al. 2014). Antioxidant

gamma-oryzanol content is reduced in rice bran under drought conditions (Kumar et al. 2014a, b).

2.2.2.3 Osmotic Adjustment

Water relations of the plant system are disturbed due to changes in leaf turgor pressure, canopy temperature, transpiration rate, and changed stomatal conductance. Decreased water potential reduces water loss. Wheat maintains osmotic adjustments longer post-anthesis, showing that plants spend more energy, thus saving water, post-anthesis than pre-anthesis (Verbeke et al. 2022). Canopy temperature increases due to reduced transpiration cooling and cellular water potential. Cellular temperature rise can prove fatal under moisture-deficit conditions (Hu et al. 2022). Various organic and inorganic solutes accumulate in a cell called osmoregulation to counteract the loss of turgor pressure in response to drought, salt, and temperature stress. Organic solutes include sugars (sucrose, glucose, and fructose), organic acids, free amino acids, proline, and glycine-betaine, and inorganic solutes include K^+, Mg^{2+}, Cl^-, and NO_3^- (Turner 2018). Polyols such as alditols (e.g., sorbitol and mannitol) and cyclitols or inositol (e.g., myo-inositol, galactinol, etc.) accumulate in plants under moisture, thermal, and salt stress (Merchant and Richter 2011; Szepesi 2020). To protect cells from dehydration injury, plants accumulate LEA proteins (hydrophillins), osmotins, dehydrins, as well as HSPs (molecular chaperones) that are upregulated to stabilize membranes and protein motifs (Nagaraju et al. 2019; Priya et al. 2019).

2.2.2.4 Plant Nutrition

Nutrient excess and deficiency are extremely harmful to crop productivity and induce different symptoms depending on the nutrient involved. Sometimes the excess of one nutrient also affects the uptake of the other one and leads to the development of deficiency symptoms. The root structure also tends to change when crops are grown in nutrient-deficient soils inducing elongation of roots or enhancing root area so that the crop plants can better access nutrients leading to a higher root-to-shoot ratio (Morgan and Connolly 2013). Nutrient stress also limits plant growth and adversely affects produce quantity and quality. Crop plant nutrient relations are also disturbed under various abiotic stresses. Nutrients such as nitrogen, magnesium, calcium, and silicon diffuse along with water which is affected by moisture deficit (Fahad et al. 2017a, b). Though nutrient uptake under drought varies with crop species, nitrogen uptake is generally increased, phosphorus uptake is declined, and potassium uptake remains unaffected. High-temperature stress reduces the nutrient-absorbing proteins in the root and changes nutrient uptake patterns (Giri et al. 2017).

Heat stress and heavy metal negatively affect nitrate reductase enzyme activity, decreasing crop plant nitrogen utilization capacity (Onwueme et al. 1971; Singh et al. 2019). Plant nutrient deficiency perturbs nearly all the physiological processes depending on the nutrient, whereas an excess of any element in plants can be toxic. Nutrient deficiencies are common in different kinds of soils, such as Fe, Zn, Cu, and Mn deficiencies in calcareous and limed soils; Ca, Mg, P, and Mo deficiencies in

acidic soils; and Fe, Mn, and Zn deficiencies in alkaline soils (Osman 2012). Soil nutrient status affects a plant's ability to absorb and transport minerals. Mn uptake by plants can be reduced due to high levels of Fe, Zn, Cu, and Mo in soil, whereas high nitrate and sulfate content promote the process. Likewise, excess P may induce deficiency of K and micronutrients, particularly of Fe and Zn. Under waterlogging conditions, Mn^{2+} can initially be reduced to Mn^{+}, which is unavailable to plants (Osman 2012). Under salt stress, Na^{+} and K^{+} compete to be absorbed by roots (Zhu 2003). Compartmentation of excess Na^{+} in the vacuole and high salt accumulation in the root system are important parameters for salinity tolerance (Zhu 2003). Crop plant root-microbe interactions play an important role in nutrient absorption by crop plants. Rhizosphere microorganisms such as endophytes, arbuscular mycorrhizal fungi (AMF), and plant growth-promoting rhizobacteria (PGPR) have proven role in plant nutrition and stress tolerance. These plant microbes assist in N_2-fixation; acquire nutrients; secrete phytohormones like auxins, gibberellins, and cytokinins; produce antioxidants and osmolytes; and enhance heavy metal tolerance via transportation, intra- and extracellular entrapment, complex formation, and redox homeostasis (Inbaraj 2021).

2.2.2.5 Phytohormones

Plant endogenous hormone levels change in response to stresses and inflict morphological and physiological changes in plants to cope with the prevailing stress. Root ABA rises upon sensing drought and salt-induced moisture deficit, which triggers various plant responses via root-to-shoot communication. Elevated ABA level in plant system signals several biochemical changes, including an influx of Ca^{2+} in the cytosol, activation of membrane-localized anion channels, K^{+} efflux, and elevated H_2O_2 production (Ali et al. 2020). In wheat, elevated ABA reduces stomatal conductance and plant transpiration rates (Innes et al. 1984). ABA-dependent pathways involve ABA-responsive genes for the abiotic response. ABA promotes K^{+} ion efflux from the guard cells leading to stomata closure and impeding plant growth (Vishwakarma et al. 2017). Similarly, under different stresses, phytohormones such as ABA, cytokinin, GA, ethylene, and other chemical factors are implicated in the root-shoot signaling and physiological changes.

Generally, cytokinin hormone level changes in response to temperature, drought, osmotic, salt, high light, and nutrient stress (Todaka et al. 2017; Cortleven et al. 2019). Drought-induced cytokinin synthesis in the transgenic rice plants promoted sink strengthening through a cytokinin-dependent coordinated regulation of carbon and nitrogen metabolism (Reguera et al. 2013). Cytokinin has a role in tolerance against high light stress by maintaining the D1 protein level in PS II and promoting antioxidant-based protection in chloroplasts (Cortleven et al. 2019). However, more research is needed to decipher the role of cytokinin under temperature stress. Under flood stress, the endogenous concentration of ethylene hormone increases in rice plants due to poor diffusion, which leads to leaf chlorosis and excessive elongation (Sarkar et al. 2006). Ultraviolet radiations (UV-B) increased the ethylene levels in plants and have a role in tolerance against drought and submergence conditions (Cortleven et al. 2019). UV-B reduces the gibberellin synthesis in rice (Lin et al.

2002). The saline environment results in unfavorable metabolic changes in seeds, such as K^+ efflux, higher solute leakage, and reduced alpha-amylase activity due to decreased bioactive gibberellin content (Liu et al. 2018).

2.2.2.6 Reactive Oxygen and Nitrogen Species

Oxidative burst (rapid increase in ROS) is one of the foremost events on different abiotic stresses (Baxter et al. 2014). Under abiotic stresses, metabolic shifts in mitochondria and chloroplast lead to elevated ROS production and oxidative stress (Gill and Tuteja 2010). ROS triggers a cascade of stress signaling in plants. ROS can inflict damage by increasing lipid peroxidation, which affects membrane fluidity and permeability, directly modifies amino acids and protein degradation, and causes DNA fragmentation via creating strand breaks, depurination, depyrimidination, and protein-DNA crosslinking, which ultimately leads to cell death (Carvalho 2008; Tripathi et al. 2020; Juan et al. 2021). Thylakoid membrane fluidity and permeability are drastically affected by high heat (Hu et al. 2022). To maintain ROS homeostasis cells, plants produce enzymatic (SOD, POD, CAT, APX, MDHAR, DHAR, GR, GPX) and nonenzymatic (amino acids, GSH, α-tocopherol, carotenoids, phenolics, flavonoids, and amino acid cum osmolyte proline) ROS scavengers (Das and Roychoudhury 2014).

Under different biotic and abiotic stresses, plants synthesized various RNS, which include radicals ($NO^{\cdot}$, and $NO_2^{\cdot}$, $NO_3^{\cdot}$) and non-radicals (HNO_2, NO^+, NO^-, $ONNO^-$, N_2O_3 and N_2O_4) (del Río 2015). RNS exerts "nitrosative stress" in plants (Turkan 2018). However, the synthesis and signaling mechanism are poorly understood. For details see del Río (2015), Turkan (2018), and Yu et al. (2014). Under the submerged condition, ROS and RNS accumulation in rice roots cause PCD and the formation of lysigenous aerenchyma (Basu et al. 2020).

2.2.2.7 Transcription Factors

Plant perception of abiotic stress generates various kinds of signals, bringing plant responses via on and off gene transcription. Kinases, phosphatases, TFs, *cis*-regulatory elements, epigenetic modifications, and post-transcriptional and post-translational modifications are key regulatory players and processes under stress and can be targeted for engineering tolerance to multiple abiotic stresses in cereals (Kushwaha et al. 2021). TFs are key players in the genetic regulation of plant responses. Several TFs have been identified in genome-wide studies and functionally characterized through cereal crops' transgenic or mutant base studies. *ABF*, *NAC*, *MYB*, and *MYC* TFs (ABA-dependent pathway) and *DREB2* TFs (ABA-independent pathway) regulate drought response (Baldoni et al. 2015; Haak et al. 2017; Yoon et al. 2020). Likewise, *HSFs* and *HSPs* are important in heat stress regulation, *DREB1/CBF* TFs are major regulators of cold response, and *ERF*-type TFs are well-established regulators of flooding and hypoxia tolerance (Xu et al. 2006; Haak et al. 2017).

2.3 Progress in Temporal Perspective

2.3.1 Pre-genomic Era of Abiotic Stress Tolerance Breeding in Cereals

The pre-genomic era of abiotic stress tolerance breeding mainly constituted the application of conventional breeding techniques, including domestication, introduction selection, hybridization (pedigree method, backcross method, recurrent selection, diallel selective mating, pre-breeding), and mutation breeding. The success of breeding programs for the development of stress-tolerant genotypes depends on numerous factors such as screening techniques, underlying mechanisms, source of the trait/gene, heritability of the trait(s), gene action, and its relationship with other agronomically important traits.

2.3.1.1 Pre-breeding

The traits contributing to abiotic stress tolerance can be sourced either from the cultivated or wild gene pool. Continuous breeding activities have led to the exhaustion of genetic variability among the cultivated germplasm (Rauf et al. 2010). However, a wide diversity is still present in underutilized germplasm, including landraces and wild relatives of the crops. A large collection of landraces has been known to possess traits/genes for abiotic stress tolerance, readily using diversity for breeders (see Table 2.1). Crop wild relatives (CWRs) include various traits/genes responsible for abiotic stress tolerance (see Table 2.2); however, they may have a poor agronomic background and some incompatibility barriers restricting their use. *Pre-breeding* involves the identification of desirable traits in wild germplasm and transfer of such traits into the genetic background of cultivated germplasm and produces intermediate germplasm that is easily crossable with the cultivated germplasm and can be used as a donor for future breeding programs. Thus, pre-breeding increases the usability of wild alleles. Many abiotic stress resistance traits have been introduced in cultivated rice from *Oryza nivara*. Introgression from *O. rufipogon* and *O. longistaminata* tends to increase aluminum and drought tolerance in rice crops, respectively. Two salinity tolerance genes (*Nax1* and *Nax2*) have been introgressed from *Triticum monococcum* into a durum wheat variety Tamaroi, leading to the development of salt-tolerant wheat lines.

Collection and evaluation of the germplasm against abiotic stress is a prerequisite for breeding. In case resistant/tolerant lines are identified, only such lines with desirable characteristics are carried further. Suppose the desirable variability is not present in the local germplasm. In that case, a breeder may resort to introducing the exotic germplasm with desirable characteristics after its thorough evaluation in the new area. Submergence tolerance varieties of rice, namely, BR-8, BR-9, BR-34, Sugandha, Rajshree, and T-141, were released by the pure line selection within local landraces (Mallik 1995; Mallik et al. 2002). Rice varieties Damodar (CSR-1), Dasal (CSR-2), and Getu (CSR-3) were tolerant to saline conditions and were obtained by pure line selection from cultivars growing in the Sundarbans of West Bengal. Deepwater rice varieties Jaladhi-1 and Jaladhi-2 were obtained by sampling from

Table 2.1 Landraces with abiotic stress tolerance in cereals

Crop species	Landraces	Country of origin	Abiotic stress tolerance	References
Wheat	Grinias Zakinthou, Skilopetra Ptolemaidas	Greek	Drought	Adhikari et al. (2022)
	IC 321987, IC 322005, IC 138852, IC 138870, Dharwad Dry	India		
	AUS28451, Bolani, WC-47572, WC47574, WC4953S, Madhavi	Iran		
	NPGR 7504	Nepal		
	Sorık	Turkey		
	Karak	Jordan		
	Leweucei and Mateteleki	Egypt		
	Hindi 62, IC 28661, IC 57586, IC 78856, IC 28938B, IC 36761A and IC 78869A	India	Heat and drought	
	CWI 59788, CWI 60155, and CWI 60391	Mexico	Heat	
	Ardito and Magueija	Portugal		
	Kharchia	India	Salinity	
	Shorawaki, Pasban 9, 10790, 10828, 10823, 4098805	Pakistan		
	Sakha-92	Egypt		
	Atlay2000, UZ-11CWA-8			
	Gandum Siahloshe Zamistani Aubi (AUS-14740), Gandum Kofari (AUS-14752)	Afghanistan		
	Timilia	Italy		
	Norsi	Palestine		
	G61450	Australia	B toxicity	
	Batini	Oman	Multiple abiotic stresses	Ayadi et al. (2020)
Rice	Kasalath	India	P deficiency	Wissuwa et al. (2002)
	Nagina 22	India	Heat	Bahuguna et al. (2015)
	Dhalputtia (FR13A)	India	Submergence	Mickelbart et al. (2015)

(continued)

Table 2.1 (continued)

Crop species	Landraces	Country of origin	Abiotic stress tolerance	References
	Kinandang Patong		Drought	
	Aus 257, Aus Bak Tulsi, Azucena, Basmati 370, Dular, Kalia, Kali Aus, Lal Aus, and N22	India	Drought	Dwivedi et al. (2016)
	FR13A, Goda Heenathi, Thavalu, Kurkaruppan	India	Submergence	Nachimuthu et al. (2017)
	Khao Hlan On, Ma-Zhan Red, Khaiyan, Kalonji, Kharsu, and Nanhi		Submergence	
	Nona Bokra and Pokkali	India	Salinity	Marone et al. (2021)
	Hasawi, Capsule, Changmaogu, Horkuch		Salinity	Rahman et al. (2021)
	Hijoldigha, Laxmidigha, Kartiksail, Khoiyamtor, Lalmohan, Shishumati		Submergence	
	PD 27 (Khoda)	India	Submergence	http://www.nbpgr.ernet.in:8080/registration/InventoryofGermplasm.aspx
	AC-42087, Kalaketki	India	Submergence	
	Andekarma (JBS-420), Khadara (PD 33), Atrianga (RM 5/232), Kalaputia (PCP-01), Gangasiuli (PB-265), Mahulata (PB-294), Kusuma (PD 75)	India	Submergence	
	Kalakeri	India	Drought, P deficiency	
	Wazuhophek	India	P deficiency	
	Kolajoha, Chettivirippu (AC 39394), Talmugur (AC 43228), KORGUT, Kalanamak 3119,	India	Salinity	
	Sal kaiin (PB-78), Brahman Nakhi (DPS-3)	India	Drought	

(continued)

Table 2.1 (continued)

Crop species	Landraces	Country of origin	Abiotic stress tolerance	References
Barley	Scots Bere	Scotland	B toxicity and acidity	Stølen and Andersen (1978)
	Abyssinia	Ethiopia	Salt	Abo-Elenin et al. (1981)
	Sahara 3763	Algeria	B toxicity	Nable (1988)
	Dayton	USA	Al toxicity	Minella and Sorrells (1992)
	Sahara 3771	Algeria	Salinity	Rivandi et al. (2011)
	Arta	Syria	Drought and heat	Rollins et al. (2013)
	TX9425	China	Salinity	Fan et al. (2015)
	Arabi Abiad and Arabi Aswad	Syria	Drought	Kalaji et al. (2018)
	Naigou	China	Flooding	Liu et al. (2019a)
Pearl millet	Iniadi	West Africa	Drought	Andrews and Kumar (1996)
	CZMS 44A (landrace 3072); IP 8210; landraces 220, 184, 235, 238		Drought	Karthika and Govintharaj (2022)
	IP 3201; IP 19877; 9444, Nandi 32, ICMB 05666; ICMB 92777; ICMB 02333		Heat	Karthika and Govintharaj (2022)
	93613, KAT/PM-2, Kitui, Kitui local, 93612; 10876, 10878, 18406, 18570; IP 3757, 3732; Birjand pearl millet; IP 6112; IP 3616, 6104, 6112; ZZ ecotype		Salinity	Karthika and Govintharaj (2022)
Maize	Cateto	South America	Al toxicity	Liu et al. (2003)
	Bolita, Breve de Padilla, Conica, Conica Nortena	Global collection	Alkalinity	Prasanna (2012)
	La Posta Sequia, Nal Tel, Oloton	Global collection	Acidity	
	Tuxpeño	Global collection	Drought	
	L25, L14, L1, and L3		Drought	Andjelkovic et al. (2014)
	Palomero Toluqueño	Mexico	Cold, heat, and salt	Aguilar-Rangel et al. (2017)
		Brazil	P-deficiency	Spolaor et al. (2018)

(continued)

Table 2.1 (continued)

Crop species	Landraces	Country of origin	Abiotic stress tolerance	References
	Amarelao, Caiano, and Caiano 2			
	TZm-1167, TZm-1162, TZm-1472, TZm-1508 and TZm-1506	West Africa	Drought and heat	Nelimor et al. (2020)
	GalTrini, SITexas	Mexico	Drought	Hernández et al. (2021)
Sorghum	ICSV111, Teshale Meko		Salt	Mola (2021)
	Valsangh maldandi local, Vadgaon dagdi maldandi, Tongraligaon maldandi, Tongraligaon dagdi, Sultanpur local dagdi, Sultanpur maldandi, Harni jogdi (dagdi), Harni jogdi; Chungi maldandi, Musti local (Maldandi), Chungi kuch-kachi, Baddi jowar, Chakur maldandi, and Sai jonna; EJN 4 (IC 585174)		Drought	Karthika and Govintharaj (2022)
	DeKalb 28E		Heat	
Sugarcane	Katha (Coimbatore), Kewali-14-G, Khatuia-124, Kuswar, Lalri, Nargori, Pathri, Khakai, Panshahi, Reha, and Uba	India	Salinity	Shrivastava et al. (2017)
	Hemja, Khari, Khagari, and Ikri	India	Drought, submergence	
Finger millet	GP # 3, 111, 153; IE2301 and IE5201		Heat	Karthika and Govintharaj (2022)
	GPU 48, Indaf 5, Co 12, Trichy 1, IE #518, 2034, 2217, 2790, 2872, 3045, 3077, 3391, 3470, 3973, 4073, 4329, 4671, 4673, 4757, 4789, 4795, 4797, 5066, 6154, 6165, 6326		Salinity	

(continued)

Table 2.1 (continued)

Crop species	Landraces	Country of origin	Abiotic stress tolerance	References
Foxtail millet	BSi-1, EM 15/BSi 467, EM 8/BSi 467, Tie Gu 7, Jinan 8337		Drought	
	IC-403579′ (IC-4)		Heat	
	ISe #254, 869, 1851, 96, 388, 480, 995, 1629, 969, 1888, Honggu, Xiaohuanggou, and Sanbianchou, ICERI 5, ICERI 6		Salinity	

Kalakhersail and Baku, respectively, while Jalaprabha, Neeraja, and Dinesh were selected from a composite, a landrace, and progenies of Jaladhi-2/Pankaj, respectively. Hangseswari was also a deepwater-tolerant rice variety obtained by pure line selection (Dana et al. 2013). A rainfed rice variety Mahsuri was introduced in India from Malaysia and rose to prominence in eastern India mainly due to good grain quality and lodging resistance (Rao Balakrishna and Biswas 1979).

2.3.1.2 Pedigree Method

Pedigree breeding is another conventional breeding method for crops that involves the selection of superior genotypes from segregating generations of a cross. Selection is continuously made up to F_7 or F_8 generations until the genotypes become stable. During the entire process, the records of the ancestry of the selected plants are maintained. The method is generally followed in instances when certain desirable traits are distributed in parental lines and have to be assembled. In self-pollinated species, the pedigree method of breeding is used for the development of new plant varieties, while in cross-pollinated species, it leads to the development of inbred lines, which are ultimately used as a parental line for hybrid production. The pedigree method is generally used for the improvement of oligogenic traits and is advantageous as the selection is practiced at every level, which provides ample opportunities for the breeder to exercise his skill and judgment; the breeder can isolate transgressive segregants and ensures judicious utilization of the meager resources as inferior germplasm is rejected at an early stage of breeding. However, this method is expensive, laborious, and time-consuming and demands more attention from a breeder. This method has been used to develop drought-tolerant lines (Tammam et al. 2004).

Pedigree-bulk, a modification of the pedigree method, is equally effective as the pedigree method but utilizes fewer resources. It involves bulking up to F_4 or F_5 generation, following which individual panicle is selected, and generations advanced as a pedigree method. For traits with high heritability, individual plant selection can be imposed in early segregating generations. This method is generally adopted in

Table 2.2 Crop wild relatives with abiotic stress tolerance in cereals

Crop species	CWR	Abiotic stress tolerance	References
Wheat	*T. monococcum*	Heat, salt	Vierling and Nguyen (1992); James et al. (2011)
	Ae. Uniarisfata	Al toxicity	Miller et al. (1997)
	T. urartu, T. boeticum, T. dicoccoides	Drought	Valkoun (2001)
	Ae. geniculata	Drought	Zaharieva et al. (2001)
	T. bessarabicum, T. elongatum, and Thinopyrum ponticum	Salinity	Witcombe et al. (2008)
	T. turgidum ssp. *dicoccoides*	Drought	Krugman et al. (2011)
	Ae. tauschii	Drought, salinity	Sohail et al. (2011)
	Ae. crassa	Drought	
	Leymus mollis	Salinity	Habora et al. (2012)
Rice	*Oryza glaberrima*	Drought	Sarla and Mallikarjuna Swamy (2005)
	O. longistaminata	Drought	Brar (2005)
	O. nivara, *O. rufipogon, O. rhizomatis, O. eichingeri*	Submergence	Niroula et al. (2012)
	Porteresia coarctata	Salinity, submergence	Rohini et al. (2014)
	O. australiensis, O. meridionalis	Drought	Singh et al. (2016a, b)
	O. rufipogon, O. glaberrima	Fe-toxicity, P-deficiency, Soil acidity	
	O. punctata, O. rhizomatis	Drought	
	O. rufipogon	Cold, Al toxicity	
	O. officinalis	Drought	Szareski et al. (2018)
	O. grandiglumis	Submergence	
	O. glamaepatula	Submergence	
Maize	*Zea mays* subsp. *huehuetenangensis*	Submergence	Mano and Omori (2013)
	Tripsacum dactyloides	Salinity	Hossain et al. (2016)
	Z. mays subsp. *mexicana*	Drought	Gonzaalez et al. (2018)
	Eastern gamagrass	Drought, salinity, acidity, submergence	Mammadov et al. (2018)
	Z. parviglumis	Drought	Kumar et al. (2020a, b); Adhikari et al. (2021a, b); Sahoo et al. (2021)
Barley	*Hordeum spontaneum*	Salinity, drought, Al toxicity	Shavrukov et al. (2010); Kalladan et al. (2013)

(continued)

Table 2.2 (continued)

Crop species	CWR	Abiotic stress tolerance	References
	H. marinum	Salinity, submergence	Alamri et al. (2013)
	H. chilense	Drought	Zhang et al. (2016)
	H. jubatum	Salinity	Kharub et al. (2017)
Sorghum	*S. leiocladum*	Cold	Fiedler et al. (2016)
	S. brachypodum and *S. macrospermum*	Drought	Cowan et al. (2020)
	S. bicolor subsp. *verticilliflorum*	Drought, heat	Ananda et al. (2020)
Sugarcane	*Saccharum spontaneum*	Drought, submergence, cold	Shrivastava et al. (2017)
	S. robustum	Salinity, submergence	
	S. sinense	Salinity	
	Erianthus spp.	Drought, cold, salinity	
	Narenga spp.	Drought	
	Miscanthus spp. *miscanthus nepalensis*	Cold	

case the land or labor facility is inadequate or the environment required to make a selection, particularly for stress-resistant traits, is unavailable. Unlike the pedigree method, it can also be used to improve traits with low heritability, requires less labor, and is less expensive. This method has been used for rice salt tolerance breeding at IRRI. Wheat variety "Veery" and the lines derived from it, including Attila, Baviacora, Kauz, and Pastor, showed high nutrient (N and P) efficiency and tolerance to multiple abiotic stresses, heat, drought, etc. Wheat genotypes 6, 27, and 31 derived using this method at ICARDA also showed high drought tolerance (Meena et al. 2017).

2.3.1.3 Shuttle Breeding

Shuttle breeding involves using diverse ecological environments to develop improved varieties possessing higher adaptability. Here, alternate generations are grown in different environments, enhancing selection efficiency. It was initiated for the first time in the 1940s to develop and select wheat populations in two other locations in Mexico, which not only led to the faster advance of wheat generations but also helped in the identification of genotypes with broader adaptation and performance stability (Mwadzingeni et al. 2017; Mondal et al. 2020). The shuttle breeding approach was used by the Government of Brazil and the CIMMYT in 1974 to develop aluminum toxicity-resistant wheat varieties. Development of several submergence-tolerant rice cultivars, viz., Jagabandhu, Kishori, Upahar, Varshadhan, Bhudev, Prafulla, NDR-8002, CR-978-8-2, Cr-2003-2, and CR-2003-3, is the result

of the Eastern India Rainfed Lowland (EIRL) shuttle breeding program (Singh et al. 1998; Mallik et al. 2002). Rice varieties CSR-23, CSR-27, and FR13A possessing salt and submergence tolerance, respectively, were also produced by shuttle breeding (Mishra 1994). Improved rice lines CRLC-899 and CR-2003-2 were identified by shuttle breeding and showed tolerance to waterlogged conditions.

2.3.1.4 Backcross Method

Certain high-yielding elite varieties are susceptible to adverse climatic conditions. The process of backcross breeding has been used to transfer stress-tolerant traits from a donor parent into such varieties (Hospital 2005; Reyes-Valdés 2000). The backcross method involves hybridization of the donor parent with the recipient parent, usually an adapted variety, followed by selection for the donor trait in the progeny. It is followed by recurrent hybridization of the selected progeny with the recipient parent and selection for the donor trait so as to recover the entire recurrent parent genome. About six to eight backcrosses are required to recover the recurrent parent genome (Hasan et al. 2015). The process is advantageous as it does not require multilocation testing of the improved lines, because only a few traits are improved and the adaptability and performance of the recurrent parent are not modified as such, requires a small population, and is the only conventional breeding method for transfer of dominant or recessive genes. However, backcross breeding is ineffective for traits with low heritability, may lead to linkage drag, and is time-consuming and laborious as multiple backcrosses must be made to recover recurrent parent background. When a variety is improved for a particular trait, it may get replaced by a different superior variety. Dudely (1984) also proposed that backcrossing is particularly beneficial when one parent has more favorable alleles, the level of dominance is high, and the parents are diverse. The method was used to transfer Al tolerance from the "Carazinho" wheat variety to the "Egret" variety and to improve drought tolerance in rice by IRRI (Fisher and Scott 1987; Lafitte et al. 2006). Three elite rice lines and 203 donors were used in the backcross breeding program at IRRI to develop promising lines with tolerance to complex traits such as Zn deficiency, low temperature, submergence, and salinity stresses (Ali et al. 2006).

2.3.1.5 Recurrent Selection

Recurrent selection is a breeding scheme to assemble desired alleles in a population. It is a cyclical improvement method constituting of selection of the superior individuals followed by intermating and evaluation. Recurrent selection is an important population improvement method, and as it involves frequent crossing, it helps in breaking down undesirable linkages and maintains high genetic variability in the population. However, the end product of this method is an improved population, not a variety. The lines from the improved population are further used in the hybridization program. In addition, it involves frequent crossing and selection, which is labor intensive. The recurrent selection programs at CIMMYT led to the production of drought-tolerant improved populations, viz., DTP white, DTP yellow, La Posta Sequía, and Tuxpeño Sequía, that served as source germplasm in a hybrid breeding program, and the lines derived from these populations possessed tolerance

to low nitrogen, heat, drought, soil acidity, and waterlogging stresses (Prasanna et al. 2021). Edmeades et al. (1992) reported that eight cycles of recurrent selection in tropical maize improved the drought tolerance resulting in a yield increase of 500–800 kg/ha.

2.3.1.6 Selective Mating

Breeding in self-pollinated crops leads to a narrowing of the genetic background of the progenies as, at the most, four parents can be involved in the case of double-crossing hybrid. To broaden the genetic base of the developed cultivar, break tight linkage, and increase parental control, a process of diallel selective mating system (DSMS) is being employed by IRRI, which involves the recurrent selection of the desired individuals followed by intermating of the selected individuals to increase the probability of getting the desired genotype. This method leads to genotypes developing resistance to multiple abiotic stresses with wider adaptability. The breeding material generated by using this scheme in IRRI has led to the development of rice lines possessing tolerance to submergence, salinity, Fe toxicity, and Zn deficiency (Singh et al. 2009; Meena et al. 2017).

2.3.1.7 Mutation Breeding

Mutation breeding is the most popular approach to induce variability in both cultivated and wild germplasms. Mutation breeding involves the application of mutagens, either physical (gamma-ray, X-ray, fast neutron, etc.) or chemical (EMS, azides, nitrous acid, etc.) to plant parts to create mutants with desirable traits. It involves employing mutagen to induce mutation and screening (in vitro and/or in vivo) of the mutagenized plant progenies for desirable traits. Induced mutations may create novel alleles which do not exist naturally or are rare. However, mutations are generally recessive and deleterious and often occur in low frequency. They need to screen a large population to identify desirable mutants, making it a costly and labor-intensive process. Desirable mutants for quantitative traits are seldom achieved with mutation breeding. Mutation breeding has been used to develop the first salt-tolerant rice cultivar CSR-10, which was formed by the pedigree method using Jaya as a male parent and a female parent derived from γ-ray irradiated seeds of a cross CSR-1/IR-8. Two lowland rainfed rice cultivars, namely, Jagannath and Biraj, were also derived by mutation breeding from T141 and OC-1393, respectively (Rao Balakrishna and Biswas 1979). Maize hybrids, Kneja HP 556, Kneja 509, Kneja 570, Kneja 674, Kneja 682, Kneja 712, and Kneja 641, possessing tolerance to drought and soil acidity, respectively, were also produced by mutation breeding. A cold-tolerant barley cultivar IZ Bori (Kt3026) was also produced by sodium azide-induced mutagenesis (Tomlekova 2010). Apart from this, rice varieties BINAdhan-9, Mohan, NIAB-IRRI-9, Atomita 2, and A-20 were tolerant to salinity; wheat variety Changwei 19 was resistant to salinity and alkalinity; wheat variety Jiaxuan 1 was tolerant to salinity, alkalinity, and cold; wheat variety Albidum 12, 1161, 503 were tolerant to low temperature; wheat variety Changwei 51503 was tolerant to low temperature, salinity, alkalinity, and drought; and barley variety Akdeniz M-Q-54 was tolerant to low temperature and were all produced by mutation breeding.

2.3.2 Genomic Era of Abiotic Stress Tolerance Breeding in Cereals

Genomics involves crop genome analysis for identifying, quantifying, and comparing sequences, gene expression, function, and regulation. Genomic studies detect variation at the DNA level and aim to characterize genes, molecular pathways, and their regulation under plant abiotic stress response (Pourkheirandish et al. 2020). The most significant developments in plant breeding during the genomic era can be attributed to genome sequencing and molecular marker technology advancement. These advancements enabled breeders to design numerous approaches for precise identification, characterization, and quantification of genetic variation, gene discovery, allele mining, candidate gene identification, and gene transfer/pyramiding to improve multiple stress tolerance traits simultaneously (Kushwaha et al. 2021).

2.3.2.1 QTL Analysis

Quantitative trait loci (QTLs) are crop genotype's genomic regions associated with a phenotypic trait. QTL analysis involves detecting the loci influencing a quantitative trait, their location, number of QTLs involved, their effect size, and interaction between different QTLs with background genome and environment. Molecular markers can be identified to be closely linked to QTLs governing stress tolerance and can be used for marker-assisted selection (MAS). For decades, many efforts have been made for QTL identification and mapping associated with abiotic stresses in agricultural crops that have facilitated the conventional breeding approaches in achieving abiotic stress-tolerant genotypes (Table 2.3). For example, 16 QTLs in the rice F_2 populations generated from crossing two contrasting rice genotypes were identified and mapped using polymorphic SSR markers under salt stress. Likewise, 85 different QTLs were mapped to 12 haploid sets of rice chromosomes under

Table 2.3 QTL mapping in major cereal crops under different abiotic stress

Stress	QTL detection	Reference
Drought	3 QTLs linked with SSR markers in wheat	Maccaferri et al. (2016)
Drought	9 QTLs were identified under moisture stress	Hu et al. (2021)
Drought	QTL *qSDW3* associated with stem dry weight	Sabar et al. (2019)
Salinity	*Saltol* locus delimited within 10.7–12.2 Mb interval on the short arm of chr-1 of rice	Bonilla et al. (2002)
Cold	QTL *qCTS12a* identified on chr-12 of rice	Andaya and Mackill (2003)
Cold	4 QTLs identified on chr-3 in maize	Jin et al. (2021)
Heat	2 QTLs detected on 3B and one QTL on chr-1D	Sharma et al. (2017)
Heat	4 QTLs identified for root length in rice by using SNPs marker	Kilasi et al. (2018)
Heat	6 QTLs identified in maize	Inghelandt et al. (2019)
Cd-toxicity	36 QTLs identified for root-shoot length, shoot-root dry weight, and total dry weight in rice	Shilin et al. (2021)

salinity using the SNP markers. *Saltol* QTL in the basmati rice variety is also well-characterized via marker-assisted backcrossing. In maize, 15 salinity stress-associated QTLs were identified on different chromosomes of $F_{2:3}$ populations.

MAS has generated several new varieties as well as improved versions of existing varieties of cereal crops, including Swarna-Sub1, Improved Pusa Basmati 1, Pusa Basmati 1728, Pusa Basmati 1637, Pusa Samba 1850, Pusa Samba 1850, and Improved Samba Mahsuri in rice (Neeraja et al. 2007; Gopalakrishnan et al. 2008; Madhavi et al. 2016; Singh et al. 2017; Krishnan et al. 2019); HUW510 in wheat (Vasistha et al. 2017); HHB67 Improved in pearl millet (Rai et al. 2008); and Pusa Vivek QPM-9 Improved in maize (Gupta et al. 2009). However, MAS is very effective for the oligogenic trait but impractical for polygenic traits (Bernardo 2008). Further, abiotic stress tolerance-related traits are polygenic in nature. To overcome this issue, new selection tools called genome-wide association study (GWAS) and genomic selection (GS) have been proposed which can detect all QTLs associated with targeted trait and can potentially facilitate selection for minor gene-governed traits based on *net genetic merit* of an individual obtained using the effects of dense markers distributed across the genome (Meuwissen et al. 2001).

2.3.2.2 Genome-Wide Association Study (GWAS)

GWAS investigates the presence of genome-wide variation in different lines and establishes an association between genomic variation and desired trait(s). It generally emphasizes SNP and trait associations. GWAS is based on different factors such as GWAS designs, techniques used for genotyping, statistical models for data analysis and interpretation, and follow-up of association results (Bush and Moore 2012). GWAS has a broad range of applications in crop improvement, among which studying abiotic stress is the most important. There are many studies available where GWAS has been used to study abiotic stresses. Examples are drought tolerance (Verslues et al. 2014; Thoen et al. 2017), salinity stress tolerance (Kumar et al. 2015b; Shi et al. 2017a, b; Thoen et al. 2017), heat stress tolerance (Lafarge et al. 2017; Thoen et al. 2017; Sharma et al. 2020), and boron (B) toxicity (de Abreu Neto et al. 2017). Based on GWAS, Kumar et al. (2015a, b) identified a novel and major QTL Saltol and other minor QTLs associated with salinity tolerance at the rice seedling stage. Likewise, GWAS identified candidate genes for spikelet sterility and traits potentially affecting the fertilization process within a genomic block associated with anthesis in rice (Lafarge et al. 2017). Further, Shi et al. (2017a, b) identified 11 loci in rice significantly associated with salt tolerance response at the seed germination stage.

2.3.2.3 Genomic Selection (GS)

GS is a MAS method for detecting marker-trait associations where whole genomic variants are quantified into phenotypic terms and a selection index is developed based on the marker additive effects (i.e., marker breeding values). It requires two types of populations: training and breeding population. The training population develops both phenotyping and genotyping data. A densely saturated linkage map

(preferably SNPs) brackets the whole genome in small intervals, assuming each interval harbors a QTL that affects the trait. The effects associated with each interval are estimated using genotype and phenotype data in the training population. The effects of each locus are used to calculate the genomic estimated breeding value (GEBV) based on genotype and phenotype data (Meuwissen et al. 2001). Thus, even when the contribution of any marker loci is minimal, it can be captured. In subsequent generations, these GEBVs are used to develop selection strategies in breeding populations based on genotype data.

The GS can be used to select the high breeding value of individuals rapidly from early-generation populations without extensive phenotyping in each generation. Many attempts have been made for cereal improvement via GS. The effectiveness of GS has been studied first in wheat, rice, maize, and barley. GS is mainly used to predict the additive effects in germplasm, whereas nonadditive effects are generally ignored (Robertsen et al. 2019). The potential of GS has been explored in several crops and traits. However, the optimal strategy and stage for implementing GS in a plant-breeding program are still uncertain. The accuracy of GS is affected by the data used in the GS model, size of the training population used, germplasm diversity, marker density, and pedigree information of germplasm. Model selection is a critical step. Under severe drought, multi-trait models are effective, whereas, under normal drought, random regression is preferred over repeatability and multi-trait models. Selection model prediction can be more accurate (up to 70%) in wheat when high-throughput secondary traits (i.e., yield-related traits) are considered than primary traits (i.e., per se performance) for screening heat- and drought-tolerant lines (Rutkoski et al. 2016; Sun et al. 2017). The accuracy of genomic prediction can be improved under multi-environment models compared to single-environment models in rice and wheat trails under drought stress (Sukumaran et al. 2018; Bhandari et al. 2019).

2.3.2.4 Speed Breeding

Speed breeding is a manipulation of environmental conditions under which crop genotypes are grown for the acceleration of flowering and seed set to advance the generation of breeding as quickly as possible. This method reduces breeding time and resources through rapid generation advancement. Various selection methods can be integrated into speed breeding, such as single seed descent (SSD), single pod descent, single plant selection, clonal selection, PBS (pollen-based selection), and MAS to shorten the breeding cycle and for efficient resource use. Speed breeding results in ~3–9 generations per year compared to 1–2 generations per year achieved with conventional breeding methods. As a result, speed breeding provides opportunities to develop homozygous and stable genotypes quickly and facilitates rapid generation advancement. It will accelerate the development and release of new varieties. Also, speed breeding technology can be combined with MAS, high-throughput phenotyping, and transgenic technologies for multiple trait selection (Pandey et al. 2022).

Speed breeding protocols have accelerated the pace of varietal development programs with less time, space, and resource investment during generation

advancement and selection cycles. Furthermore, integration of speed breeding with conventional MAS, PBS, GS, and genome editing (GE) approaches can enhance the generation and effective selection of elite genotypes with novel trait combinations, such as higher yield with multiple stress tolerance. For example, Watson et al. (2018) successfully recapitulated the phenotypes associated with the EMS-induced mutation of the awn suppressor *B1 locus9* and the Green Revolution *Reduced height (Rht)* genes in wheat cv. Norin 10 in the controlled environment room conditions within limited time.

2.3.3 Post-genomic Era of Abiotic Stress Tolerance Breeding in Cereals

2.3.3.1 Transgenics

Recombinant DNA technology led to the development of transgenic plants which are indispensable in candidate gene identification and functional validation experiments. Several transgenic varieties of crop plants have been released for commercial use in different countries. Although transgenic crops for human consumption have been debatable, their potential application and importance cannot be ignored. Candidate genes identified through various molecular approaches like QTL analysis, GWAS, genomic selection, and genome editing identification can be used to create a set of transgenic lines with abiotic stress tolerance in cereals (Noman et al. 2017) (Table 2.4).

2.3.3.1.1 Drought Stress

Several genes conferring resilience to water-deficit stress are identified and cloned. Drought-responsive TFs, such as *NAC*, *MYB*, *DREB1A*, etc., can control drought stress tolerance and activate drought inducible genes (Sharma et al. 2019). NAC family genes such as rice TF *OsNAC6* change root structure, increase the quantity of roots, and promote drought tolerance. In rice, overexpression of *OsNAC5* increases root diameter, which leads to higher drought tolerance and grain yield. Overexpression of *Arabidopsis* TF *AtNAC2* resulted in increased tolerance to moisture deficit, making it a potential candidate gene for water stress tolerance in major crops (Patil et al. 2014). Plant responses to environmental stressors may also be regulated by microRNAs. Drought stress causes plants to upregulate or downregulate the expression of specific miRNAs and synthesize novel miRNAs. Using high-throughput sequencing platforms, several drought-responsive miRNAs have been identified in various plants, including *O. sativa*, *A. thaliana*, wheat, soybeans, and barley (Yu et al. 2019). Kinase *SnRK2s* phosphorylate the important ion channels *KAT1* and *SLAC1* and promote stomatal opening under moisture deficit. *SnRK2* can also phosphorylate and upregulate *AREB/ABFs* (ABA-responsive protein) and *bZIP* TFs to activate the ABA signaling cascades and bring drought stress response. Transgenic plants with an ABA-independent TF, *DREB1A*, improve water consumption efficiency in plants (Fujita et al. 2013).

Table 2.4 List of studies targeting abiotic stress tolerance in cereals through various breeding methodologies

Technology/ technique used	Crop species	Target trait/improved trait	References
Pre-genomic era			
Conventional breeding	Rice	Salinity	Gazal et al. (2018)
	Maize	Drought	
Pre-breeding	Rice	Salt	Puram et al. (2018)
	Wheat	Drought	Valkoun (2001)
	Wheat	Heat, drought	Singh et al. (2018); Sukumaran et al. (2021)
Mutation breeding	Rice	Salinity	Negrão et al. (2011)
	Rice	Cold	Awan (1991)
	Rice	Drought	Naredo et al. (2009)
	Barley	Drought	Cseri et al. (2011)
	Sugarcane	Drought	Hartati et al. (2021)
Genomic era			
MAS	Rice	Salinity	Ren et al. (2005)
	Rice	Submergence	Septiningsih et al. (2009)
	Rice	Drought	Gandhi (2007)
	Rice	Cold	Liu et al. (2007)
	Wheat	Salinity	Byrt et al. (2007)
	Wheat	Drought, heat	Wei et al. (2009); Jain et al. (2014)
	Maize	Drought	Ribaut and Ragot (2007)
Speed breeding	Rice	Salinity	Rana et al. (2019)
QTL mapping	Rice	Drought	Dixit et al. (2020); Selamat and Nadarajah (2021)
	Rice	Salinity tolerant	Rahman et al. (2021)
	Rice	Heat, cold, submergence	Choudhary et al. (2019)
	Wheat	Drought	Mondal et al. (2020)
	Wheat	Heat	Mondal et al. (2020)
	Wheat	Heat, drought	Liu et al. (2019b)
	Wheat	Drought, cold, flooding, Al toxicity, B toxicity	Langridge et al. (2006)
	Maize	Drought	Xiao et al. (2005); Nelson et al. (2007); Hao et al. (2008); Gazal et al. (2016)
	Maize	Submergence	Qiu et al. (2007); Mano et al. (2005, 2009)
	Barley	Drought, cold, B toxicity,	Langridge et al. (2006)
	Barley	Drought, cold, submergence, salinity	Li et al. (2013a, b)
	Sugarcane	Drought	Sharma (2009)
	Sorghum	Drought	Sanchez et al. (2002)

(continued)

Table 2.4 (continued)

Technology/ technique used	Crop species	Target trait/improved trait	References
	Sorghum	Drought, salinity, cold	Maharajan et al. (2021)
GWAS	Rice	Drought	Pantalião et al. (2016)
	Rice	Chilling	Schläppi et al. (2017)
	Rice	Salt	Nayyeripasand et al. (2021)
	Wheat	Drought	Paliwal et al. (2012)
	Wheat	Drought	Sehgal et al. (2017)
	Wheat	Heat, drought	Mondal et al. (2020)
	Wheat	Salt	Hu et al. (2020a, b)
	Maize	Heat, drought	Yuan et al. (2019)
	Maize	Heat	Seetharam et al. (2021)
	Maize	Drought, submergence, salinity	Shikha et al. (2021)
	Sorghum	Drought, Al toxicity	Kulwal (2016)
		Salinity, cold	Deshpande et al. (2016)
	Barley	Drought	Jabbari et al. (2018)
		Salinity	Fan et al. (2016); Mwando et al. (2020)
	Pearl millet	Drought, salinity	Shivhare and Lata (2017)
RNA sequencing	Finger millet	Salt	Rahman et al. (2014)
	Sorghum	Low and high nitrogen conditions	Gelli et al. (2016)
Genomic selection	Wheat	Heat, drought	Juliana et al. (2019)
	Maize	Drought	Shikha et al. (2017)
Post-genomic era			
Transgenics	Rice	Salinity	Kishitani et al. (2000)
	Rice	Drought	Wang et al. (2005)
	Rice	Drought, salinity	Prashanth et al. (2008)
	Rice	Photooxidative stress	Melchiorre et al. (2009)
	Wheat	Drought	Abebe et al. (2003); Pellegrineschi et al. (2004)
	Rice, wheat	Drought	Xue et al. (2016)
	Rice, oat	Drought	Xu et al. (1996); Oraby et al. (2005)
	Barley	Cold, drought, frost	El-Hashash and El-Absy (2019)
	Sugarcane	Drought	Marshall (2014)
	Sugarcane	Drought and salinity	Kumar et al. (2014a, b)
	Sugarcane	Drought, cold, salinity	Devarumath et al. (2019)
	Maize	Drought	Wang et al. (2008); Zhang et al. (2010); Amara et al. (2013)
	Sorghum	Drought and salinity	Dalal (2016)

(continued)

Table 2.4 (continued)

Technology/ technique used	Crop species	Target trait/improved trait	References
	Pearl millet	Drought, heat, and salinity	Shivhare and Lata (2017)
Genome editing (CRISPR/ Cas9)	Rice	Salinity	Kaur et al. (2022)
	Rice	Cold, salinity, drought	
	Maize	Drought	
	Wheat	Abiotic stress	Kim et al. (2018)
	Maize	Drought	Shi et al. (2017a, b)
	Sugarcane	Drought and chilling resistance	Chen et al. (2017)
	Sorghum	Drought	Maharajan et al. (2021)

2.3.3.1.2 Salinity Stress

Numerous structural genes, regulatory genes, and regulatory sequences play a role in plant salinity stress response using biotechnological methods. For instance, *high-affinity potassium transporter (HKT)* (K^+ transporter family) regulates Na^+/K^+ transport in higher plants. Wheat *TaHKT2;1* is the first studied plant HKT gene. HKT genes have been implicated in the segregation of Na^+ from crop leaves. It was reported that *SbHKT1;4* in *S. bicolor* and *HvHKT1* and *HvHKT2* in barley regulate Na^+/K^+ transport and that HKTs play a substantial role in salt tolerance (Han et al. 2018). The salt is overly sensitive; *SOS1* plays an important function in Na^+ efflux and helps Na^+ toxicity reduction. SOS1 is mostly found in the cell's cytosol, along with other Na+ sensors, which subsequently serve as Na^+ transporter. Based sequence similarity with *Arabidopsis* (*AtSOS1*) and *OsSOS1* of *O. sativa* was extensively studied. The *OsSOS1* encodes a putative Na^+/H^+ antiporter that facilitates Na^+ flux during salt stress in roots, similar to *AtSOS1*. Likely, the CBL interacting protein kinases, *OsCIPK24* and *OsCBL4*, improved *OsSOS1* transport in rice cells by reducing Na^+ ion accumulation during salt stress (Martínez-Atienza et al. 2007). The SOS signaling system played a significant role in salinity stress resistance in dicot and monocots. Hence, one can conclude that plant biotechnology plays a significant role in candidate genes discovery.

2.3.3.1.3 Temperature Stress

Transgenic technology has helped identify and characterize genetic factors regulating temperature stress (cold and heat) tolerance in various crops. A family of TFs discovered in *Arabidopsis* dehydration-responsive element binding factors (DREBs) also called C-repeat binding factors (CBFs) are known to encode *cold-regulated (COR)* family proteins (Wang et al. 2014). *Arabidopsis* has three CBF/DREB1 genes, viz., *CBF3/DREB1a, CBF1/DREB1b*, and *CBF2/DREB1c*. *CBF1/DREB1b* and *CBF1/DREB1b* overexpression improved cold stress resistance in *Arabidopsis* by enhancing COR gene expression and sugar and proline accumulation at non-acclimating temperatures. For example, CBF/DREB1 TFs regulate many potential genes implicated in low-temperature adaptation. The rice and

Arabidopsis CBF/DREB1-dependent cold response pathway was demonstrated to have a major role in freezing tolerance during cold acclimation (Zhang et al. 2013). Post-transcriptional regulation via miRNAs can also play an important role in stress responses, growth, and development. Many cold-responsive miRNAs have been discovered in plants such as *Arabidopsis*, rice, wheat, and tomatoes, including *miR319*, *miR396*, and *miR397* (Yu et al. 2019).

High temperature (heat stress) due to global warming is one of the major concerns. Heat stress poses deleterious impacts on the physiology as well as biochemical activity of model plants like *Arabidopsis*. Over the past few decades, several potential heat sensors and heat shock proteins (HSPs) involved in the cross-talk of chaperones, phytohormones, and secondary metabolites during stress response have been discovered as a result of genetic engineering. Finally, several candidate genes and miRNAs have been extensively explored and found to be putatively involved in the mitigation of adverse effects arising due to temperature stress on crop improvement.

2.3.3.1.4 Heavy Metal Stress

The metal tolerance protein (*MTP*) family, also known as cation diffusion facilitators (*CDFs*), has been found in many taxa, including plants, mammals, fungi, and bacteria. In rice and *Arabidopsis*, several *MTP* genes have been identified. The first *CDF* gene in Arabidopsis has been identified as the *ZAT1 (Zinc Transporter 1)* gene, which was later annotated as *AtMTP1* (*Metal Tolerance Protein 1*) (Gustin et al. 2011). The *AtMTP1* gene is constitutively expressed in both the roots and the shoot tissues of *Arabidopsis* and improves Zn tolerance. Plants also regulate the metal uptake and accumulation of metals by differential and dynamic expression of auxin-related genes such as *PIN*, *PAT1*, *YUCCA*, *GH3*, *ABCB*, *CYP79B2*, and *CYP79B3* family (Jalmi et al. 2018). Congruently, the Cu^{2+} toxicity is caused by alterations in cytokinin and auxin accumulations via mitotic activity in root tissues of *Arabidopsis* (Hu et al. 2013).

2.3.3.2 Genome Editing

Genetic variation is essential for crop improvement through conventional methods, MAS, or *cis*-genesis and transgenesis. Often, variation for targeted trait can be poor in cultivated and wild gene pools. Induced mutations are random and highly time-consuming and transgenics suffer from environmental and nations policy concerns. Under such situations, modern targeted genome editing tools like TALEN and CRISPR/Cas systems are quick, easy, highly efficient, and precise tools for the generation of targeted genetic mutations/variations (InDels, gene replacement and epigenetic changes) at multiple loci simultaneously (Tang et al. 2017; Kushwaha et al. 2021).

Genome editing tools, particularly CRISPR/Cas systems, have dramatically accelerated crop breeding. Advancement in plant genome editing has recently been revealed. Generally, most phenotypic traits are controlled by a single gene and are referred to as single-gene traits. These genes often alter a specific property during the mutation process without compromising other agronomic traits, making

genome-editing technologies more useful for crop improvement. CRISPR/Cas systems have shown great potential for cereal improvement and pave a new path to improve production potential via better mineral accumulation, tolerance to biotic and abiotic factors, quality trait improvement, and accelerated domestication of wild species (Chandra et al. 2020).

A multi-genome editing toolbox is recently developed using a Cas9 binary vector and gRNA module vectors. This will make it easier to employ CRISPR/Cas9 in different plant systems for high-throughput multiplex plant genome editing. In a nutshell, the only prerequisite for plant genome editing in the face of abiotic stresses is the introgression of cas9 and sgRNA into host cells via genetic transformation (Xu et al. 2016). The efficacy of various viral-mediated Cas9/sgRNA for efficient plant genome editing has been recently reported in several studies using direct delivery of the cabbage leaf virus (Calcv) and tobacco rattlesnake virus (Trv). By fusing inactivated dCas9 into the effect domain, CRISPRi (CRISPR interference) and CRISPRa (CRISPR activation) in plants have been found to regulate the transcription of target genes in plants.

Moreover, dCas9 can be used with the epigenetic effector domain for chromatin modulation and transcriptional gene regulation (Ansari et al. 2020). The dCas9 has been effectively utilized to modulate target gene expression in functional genomics for various synthetic biological applications (Ali et al. 2015). Using CRISPR/Cas9 for genome editing, significant successes have been recorded in different plants (*Arabidopsis*, rice, wheat, maize, tobacco, tomato, etc.) over the past two decades. However, more efforts are needed to enhance and improve the CRISPR/Cas9 technology to produce more easy and accessible methodologies for researchers to impact agricultural production under growing limiting environmental conditions (Table 2.5).

2.4 Phenomics and Artificial Intelligence

High-throughput (HT) techniques involve using advanced technologies for faster and more accurate data collection, extraction, and analysis (Gehan and Kellogg 2017; Sarkar and Jha 2020). For agriculture, the research includes measuring a large area several times over a season (temporal variability). It contains small phenotypic variations within the same field (spatial variability) (Fahlgren et al. 2015). Determining spatiotemporal variations within the field can help to select genotypes with desirable traits within a large pool. This HT phenotyping process involves the remote collection of data, also known as remote sensing, and is the crucial first step (Sadeghpour et al. 2017; Oakes et al. 2019).

Aerial sensors such as multispectral and hyperspectral cameras mounted on an automated *unmanned aerial vehicle* (UAV) can be used for remote sensing (Fig. 2.2). Aerial and proximal imagery in different visible and invisible wavelengths from the electromagnetic spectrum is collected to determine the extent to which different wavelengths are reflected by the plants (Kim et al. 2021). This reflected part of the electromagnetic spectrum is known as reflectance (Ladoni et al. 2010). Based

Table 2.5 Crop varieties/breeding lines developed through various breeding methodologies between pre- and post-genomic eras

Crop	Technology	Varieties/breeding lines developed	Target trait	Reference
Wheat	Conventional method	W4909, W4910, Kharchia-65, kri-210	Salinity	Kumar et al. (2015a, b)
	Back crossing	Chuanmai 42	Submergence	Villareal et al. (2001)
	Mutation breeding	Jauhar-78, Kiran-95	Salinity	Mir et al. (2020)
	QTL mapping and MAS	Yield QTL (*Qyld.csdh.7AL*) transferred into four wheat cultivars, viz., HUW468, HUW234, DBW17, and K307	Drought	Gautam et al. (2021)
		Introgression of QTL *GY-d, SpDM*, and *HI* for drought tolerance in variety Inbar	Drought	Choudhary et al. (2019)
		Transfer of QTL *TaALMT1* for Al toxicity tolerance in wheat cv. Kumpa-INIA	Al toxicity	
Rice	Conventional breeding	Dinalaga, IRAT106, Tre Smeses, Yunlu 99, Huhan3, Sookha dhan1, Sookha dhan2, IAC47	Drought	Mahajan and Kapoor (2019)
		Sahbhagi Dhan, DRR Dhan 43, DRR Dhan 44, CR Dhan 201, CR Dhan 202, CR Dhan 203, CR Dhan 204, CR Dhan 205, Tripura Hakuchuk 1, Tripura Hakuchuk 2, Swarna Shreya, BRRI Dhan 56, BRRI Dhan 57, BRRI Dhan 66, BRRI Dhan 71, Inpago 7, Inpago 8, Inpago 9, Inpago LIPI Go 1, Inpago LIPI Go 2, Inpago LIPI Go 4, M'ziva, Yeanelo 1, Yeanelo 2, Yeanelo 4, Yeanelo 5, Yeanelo 6, Yeanelo 7, Myaungmya May, Tarahara 1, Hardinath 2, Hardinath 3, Upia 1, Upia 2, Upia 3, Sahod Ulan 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 20, Katihan 1, Katihan 2, Katihan 3, Katihan 4	Drought	Vinod et al. (2019)
		Pureline selections from traditional cultivars: Pokkali, Nona Bokra, and Kala-rata, Damodar (CSR1), Dasal	Salinity	Gazal et al. (2018)

(continued)

Table 2.5 (continued)

Crop	Technology	Varieties/breeding lines developed	Target trait	Reference
		(CSR2), CSR3. CSR10, CSR 13, CSR 23, CSR 27, CSR 30, CSR 36		
	Back crossing	GSR5A, GSR5, GSR8, GSR12, NSCIC Rc480	Drought	Ali et al. (2017)
	QTL mapping and MAS	MAS 946-1	Drought	Gandhi (2007)
		MABB is being employed to efficiently transfer the Pokkali seedling stage salinity-tolerant *Saltol* QTL into popular varieties such as IR64, BR11, BR28, Swarna	Salinity	Gazal et al. (2018)
		MABB for *Saltol* and other QTLs transfer in some popular rice varieties like ADT45, CR1009, Gayatri, MTU1010, PR114, Pusa 44, Sarjoo 5	Salinity	Singh et al. (2016a, b)
		QTL *Sub1A* responsible for submergence tolerance has been integrated by MABB Swarna-Sub1, BR11-Sub1, CR1009-Sub, Sambha Mahsuri-Sub	Submergence	Neeraja et al. (2007); Dar et al. (2021)
		Birsa Vikas Dhan 111	Drought	Nachimuthu et al. (2017)
		Using QTLs, several commercial high-yielding varieties, viz., IR64, Swarna, Vandana, Sabitri, Samba Mahsuri, TDK1, and Anjali, were improved	Drought	Nachimuthu et al. (2017)
		IR64 Sookha 1, DRR Dhan 42, Tripura Khara Dhan 1, Tripura Khara Dhan 2	Drought	Vinod et al. (2019)
		Sookha dhan4	Drought	Vinod et al. (2019)
	Transgenic	Heat-tolerant basmati rice was developed using *Arabidopsis thaliana Athsp101* gene into Pusa basmati 1 by *Agrobacterium* mediated transformation	Heat	Agarwal et al. (2003)
		Barley gene *HVA7* conferring drought tolerance, rice	Drought and salinity	Xu et al. (1996)

(continued)

Table 2.5 (continued)

Crop	Technology	Varieties/breeding lines developed	Target trait	Reference
		suspension cells transformed via biolistic-mediated method		
	Speed breeding	YNU31-2-4	Salinity	Rana et al. (2019)
	Mutation breeding	Kashmir Basmati	Cold	Awan (1991)
		EMS-induced mutants of Nagina 22	Heat	Poli et al. (2013)
	MABC	Birsa Vikas Dhan 111 (PY 84)	Drought	Shashidhar et al. (2012)
	MAS	BT4-74-8	Cold tolerance	Gazal et al. (2016)
		Batur, Dodokan, Situ Bagendit	P-deficiency	Gazal et al. (2016)
		DRR dhan-42, Yaenelo 4, Yaenelo 5, Yaenelo 7	Drought	Sandhu et al. (2020)
		CR dhan-801, Bahuguni dhan-1, Bahuguni dhan-2	Drought, Submergence	
	Wide hybridization and MAS	Co-31	Drought	Singh et al. (2016)
		Arizona Rice-1, Arizona Rice-2	Heat	
		BRRIdhan55	Salinity	
		AS996	Acidity	
Maize	Conventional breeding	ZM 309, ZM 401, ZM 423, ZM 521, ZM 623, ZM 625, ZM 721, KDV1, KDV 4, KDV 6, WS103, Melkassa 4, WH 403, WH 502, WH 504, ZMS402, ZMS 737	Drought	Gazal et al. (2018)
	Doubled haploid (DH)-based breeding	CML566, CML567, CML568, CML569, CML570, CML584, CML603	Drought	Prasanna et al. (2021)
	Mutation breeding	NH219	Heat	Nachimuthu et al. (2017)
Barley	Conventional breeding	PL 419, K 560, Getanjali (K1149), K 603, RD 2624, JB58, RD 2660, BHS352, BHS380, BHS400, VLB118	Drought	Kharub et al. (2017)
		NDB1445, RD2794	Salinity	
	Mutation breeding	Phenix, Furat 3	Drought	El-Hashash and El-Absy (2019)
		Dobrynia-3, IZ Bori, Janus, Taran	Cold	

(continued)

Table 2.5 (continued)

Crop	Technology	Varieties/breeding lines developed	Target trait	Reference
Sugarcane	Conventional breeding	Co 87044 (Uttara), CoH 119, CoPK 05191, CoC 01061	Drought	Shrivastava et al. (2017)
		Co 8371 (Bhima), Co 87025 (Kalyani), Co 87268 (Moti), CoLk 94184 (Birendra), BO 146, CoPant 90223 (pant 90223), Co 98014 (Karan-1), Co 0118 (Karan-2), Co 0238 (Karan-4), Co 0239 (Karan-6)	Drought, submergence	
		Co 94008 (Shyama), Co 99004 (Damodar), Co 2001–13 (Sulabh), Co 2001–15 (Mangal),	Drought, salinity	
		CoPant 97222	Submergence, salinity	
		Katha, Kewai-14-G, Khatuia-124, Kuswar, Lalri, Nargori, Pathari, IJ 76-422, NG 77–55, NG 77–160, NG 77-167, 57 NG201, NG77-237,28NG251, Khakai, Panshahi, Reha, Uba	Salinity	Meena et al. (2020)
		BO 34, BO 70, BO 128, CoLk 94,184 (Birendra), CoLk 8102, CoLk 8001, Co 210, Co 285, Co 6907, Co 7717, Co 8371, Co 86011, Co 87268, Co 89029, Co 98014, Co 0124, Co 0232 (Kamal), Co 0233 (Kosi), Co 0238, CoPant 90223, CoPk 05191 (Pratap Ganna-1), CoPk 05191 (Pratap Ganna-1)	Drought, submergence	
		BO 106, Co 8145, Co 88019, Co 94008, Co 99004, Co 2001–13, Co 2001–15, Co 0238, Co 0118 and Co 05011, Co 09004, CoM 0265, CoM 7125	Drought, salinity	
		BO 99, BO 128 (Pramod), Co 395, Co 453, Co 87263, CoPant 97222, 93227, CoSnk 05103, CoSnk 05104	Submergence, salinity	
Finger millet	Conventional method	RAU 8, GN 3, Suraj, Saptagiri, Katumani, Dalle-1, Okhle-1, Kabre Kodo-1, Kabre Kodo-2, Sailung Kodo-1	Drought	Mirza and Marla (2019)

(continued)

Table 2.5 (continued)

Crop	Technology	Varieties/breeding lines developed	Target trait	Reference
		FM/ST/01	Drought	Saleem et al. (2021)
		PRM6107, PR202	Drought	
		Trichy 1	Salinity	
Foxtail millet	Conventional method	TNAU 186, AK 132–1	Drought	Moharil et al. (2019)
		523-P1219619, Damaomao	Heat	Saleem et al. (2021)
Pearl millet	Conventional method	CZP9802, Okashana 1	Drought	Shivhare and Lata (2017)
		HTP-94/54	Drought	Bisht et al. (2019)
		PRLT2/89-33	Drought	Saleem et al. (2021)
		H77/833-2, G73-107, CVJ-2-5-3-1-3, Togo-II, 99HS-18	Heat	Bisht et al. (2019)
		HASHAKI I	Salinity	Shivhare and Lata (2017)
		AVKB-19	Salinity	Saleem et al. (2021)
Sorghum	Conventional method	PI 510898, IS 1212, and PI 533946	Drought	Kulwal (2016)
		E 182 (IC 568399), E 183 (IC 568400), E 184 (IC 568401), E 160 (IC 568377), E 161 (IC 568378), E 162 (IC 568379), E 163 (IC 568380), ERN 26 (IC 568541), ERN 27 (IC 568542), and ERN 28 (IC 568543)	Salinity	Karthika and Govintharaj (2022)

on this reflectance, various methods have been developed for data acquisition, band selection, model estimation, and remote sensing data verification (Sarkar 2021). Multispectral imagery captures this reflectance in several wavelengths or bands to be analyzed in a lab. Apart from reflectance, aerial imagery can be used to determine the colors of vegetation using red-green-blue (RGB) color space models (Kushwaha et al. 2021). A color space model is a way human eyes can visualize color through its attributes such as hue angle and brightness (Schanda 2007; Lee et al. 2020). The color space models used for crop phenotyping are CIE-Luv and CIE-Lab. Here, L in Luv and Lab represents luminance, whereas u and v in Luv and a and b in Lab represent chrominance. Chrominance ranges from red (+a) to green (−a) and from yellow (+b) to blue (−b). Other indices such as green area (GA) include pixels ranging from 60° to 120° hue angle, and greener area (GGA) includes pixels from

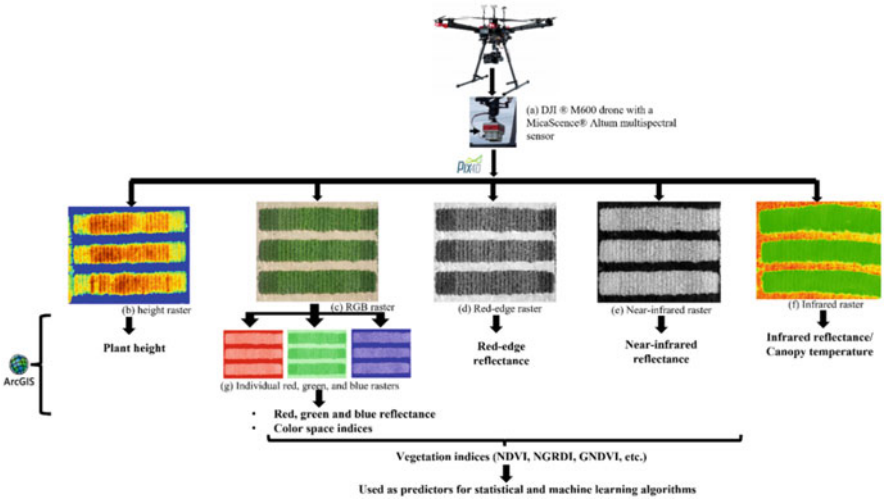

Fig. 2.2 Schematic diagram of using aerial imagery for high-throughput phenotyping (Balota et al. 2021)

80° to 120° on CIE-Lab (Schanda 2007). These spectral reflectances and RGB color space models are converted to arithmetic ratios known as spectral or color space indices. Studies have shown that these indices are heritable ($H^2 > 0.7$) and could be used not only as a proxy for phenotypic traits but as phenotypic traits themselves (Balota et al. 2021). For example, QTL analysis of durum wheat showed that 46 significant QTLs affected NDVI across platforms, several of which affect leaf chlorophyll content, leaf greenness, leaf rolling, and biomass under terminal drought stress (Condorelli et al. 2018). Thermal long-wave infrared (TIR) imagery can also be calibrated and used to estimate the canopy temperature of plants (Pineda et al. 2020). Aerial thermography can be used to measure the leaf or canopy temperature of field crops as a stress phenotyping trait for virtually any crop. However, using remote sensing data for phenotyping requires managing huge volumes of spectral data (also known as big data), analyzing statistical data, and interpreting the results to create machine learning (ML) algorithms.

The development in remote sensing in recent years has coincided with the development of computers with powerful processors for iterative statistical analysis. The indices are used as predictors for statistical and ML algorithms using stepwise multiple linear regression (MLR), partial least square regression (PLSR), multivariate adaptive regression splines, principal component regression, spatial pyramid matching (SPM), support vector machines (SVMs), and artificial neural networks to create HT phenotyping models (Shen et al. 2020; Song et al. 2021). These phenotyping models train a "computer" to predict results based on previous data, a step toward artificial intelligence (AI) in phenomics. Several remote sensors are being enabled with network connectivity and a computational model to improve the training process to create, transfer, and execute data among them with minimum

human intervention. This process is known as the *Internet of Things* (IoT), and it integrates the physical and digital world to improve speed and accuracy (Biswas et al. 2018). IoT can be used to create a framework that collects data automatically (known as data mining) and analyze them based on pre-trained ML and AI models. Such IoT-generated data can be converted to knowledge for SMART decision-making, such as selecting a stress-tolerant genotype based on HT phenotyping. Several studies estimated yield by identifying panicles of rice, wheat, and sorghum crops using computer vision and convoluted neural network (CNN) from RGB images (Xiong et al. 2017; Hasan et al. 2018; Ghosal et al. 2019). Likewise, Wang et al. (2019) reported the identification of the flowering stage of wheat crop from digital images using CNN architecture. The CNN architecture used a *training-validation-testing* approach to predict awn phenotype among several wheat lines. Sadeghi-Tehran et al. (2017) used SPM and SVM as learning models to identify and differentiate between flowering and heading stages in wheat. These studies on wheat demonstrated that deep learning using breeder-trained models from aerial or proximal images could accurately classify important morphological traits for drought phenotyping in cereals. In all the studies presented above, the sensors can be integrated using IoT to provide a continuous stream of spectral data and perform data mining and ML in real time. This automated process would mean a constantly learning model using AI and better phenomics predictions. The spectral sensors can also be integrated with soil moisture sensors, weather stations, and smartphones for automated agronomic decision-making (Jayaraman et al. 2016).

Aerial images can also be used to create a 3D model built by structure from motion (SfM) photogrammetry (Micheletti et al. 2015a, b). SfM photogrammetry uses multiple overlapping digital images acquired from multiple viewpoints. A software algorithm then identifies common feature points using computer vision across the overlapped image sets. The common points identified are used to determine spatial data of the point's elevation in an arbitrary 3D coordinate system. The algorithm then uses AI to transform these elevation points (also known as point clouds) into the coordinate system, which is then intensified to generate high-resolution 3D models (Rothermel et al. 2012; Remondino et al. 2014). Aerial imagery and SfM photogrammetry have been successfully used to estimate plant height, canopy width, crop architecture, crop growth rate, and aboveground biomass in wheat, corn, sorghum, and barley (Freeman et al. 2007; Bendig et al. 2013; Holman et al. 2016; Watanabe et al. 2017; Demir et al. 2018; Wang et al. 2018; Yuan et al. 2018). SfM photogrammetry and IoT can be used to create real-time 3D crop models to increase the frequency of spatiotemporal data collection. Recently, evapotranspiration rates in 48 chickpea genotypes were forecasted using ML and data-mining tools such as SVM, ANN, and Random Forests (RF) by 3D scanning around 5000 plants every 2 h (Kar et al. 2021). ML and AI approach for such big data require cutting-edge technologies such as larger storage devices, state-of-the-art software, faster computing processors, and fast Internet connection for IoT. These state-of-the-art software programs run using high-processing power computers resulting in automated decision-making. This gives an edge to the HT system by making data extraction faster and more accurate. Therefore, advanced technology in

the form of AI and ML for data mining and decision-making using AoT is the backbone of HT phenotyping technology.

2.5 Conclusion

The world crop husbandry is facing the challenge of high yield under the changing climate scenario across the globe. Under climate change, along with biotic stress, i.e., minor disease and pest become major and abiotic stress, i.e., heat stress, moisture stress, chilling stress, waterlogging stress, metal toxicity, salinity, and acidity which are the major challenges in front of a plant breeder to breed climate-resilient varieties. For this, conventional breeding approaches were very much successful during the pre-genomic era as well as the post-genomic era and were still relevant. After the discovery of genomics and molecular biology, the dynamics of understanding the crop physiology and biochemical process became known and allowed us to utilize this knowledge to develop new and improved varieties. A better understanding of the structure, function, regulation, and interaction of genetic factors is possible due to the advent of high-throughput genome sequencing platforms, precise phenotyping, advanced computing, data analysis platforms, and artificial intelligence. Gradually new breeding techniques, i.e., marker-assisted selection, QTL mapping, GWAS, transgenics, speed breeding, and genome editing techniques, have been developed to speed up the varietal development process and make available climate-SMART high-yielding varieties to the farmers.

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Rice Drought Tolerance: Emerging Molecular Breeding Strategies in the *Post-genomic Era*

3

Bhagyasri Dulakakharia, Khonang Longkho, Vinay Sharma, and Rahul K. Verma

Abstract

Global food security is threatened owing to the rapid change in climatic conditions. Rice, the predominant cereal crop, faces brutal drought severity, where the development of tolerant rice varieties becomes cumbersome with traditional breeding methods. Nevertheless, with the development of advanced technologies, we are leaping into the era of molecular breeding. Therefore, breeding drought-tolerant rice cultivars is possible. In recent times, one aspect of advancement has been using DNA-based molecular markers closely linked to the economically desired trait or trait of interest, or QTLs, to develop drought-tolerant cultivars. And the process of marker-assisted selection (MAS) enables the transfer of desirable stocks of genes with drought-driven characters into a single genotype. One major setback in traditional breeding is the longer breeding cycle. Therefore, the emerging new techniques like rapid generation advancement (RGA) and speed breeding have the onus to accelerate plant development and generation turnover, thereby reducing the varietal breeding time and enhancing

Bhagyasri Dulakakharia, Khonang Longkho, and Vinay Sharma contributed equally to this work.

B. Dulakakharia
Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat, Assam, India

K. Longkho
Department of Plant Breeding and Genetics, Assam Agricultural University, Jorhat, Assam, India

V. Sharma
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India

Department of Genetics and Plant Breeding, Ch. Charan Singh University, Meerut, India
e-mail: s.vinay@cgiar.org

R. K. Verma (✉)
DBT-North East Centre for Agricultural Biotechnology, Jorhat, Assam, India

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the genetic gain. Moreover, the fastest-growing accessibility of genome sequencing has motivated genomics-assisted breeding (GAB) approaches such as NGS-based genotyping and haplotype-based breeding. Thus, the key to tackling the world's escalating population with billions of mouths to feed is smart breeding strategies, which are the need of the hour in this post-genomic era. Here, we discuss traditional and emerging advanced breeding strategies used to develop climate-smart rice cultivars.

Keywords

Marker-assisted selection (MAS) · NGS-based genotyping · Haplotype · Rapid generation advancement (RGA) · QTL

3.1 Introduction

Climate change, a global phenomenon, is unpredictably uprooting the agriculture scenario. The uncertain consequences of climate change bring abrupt changes in the quantity and quality of the earth's water level, directly hampering crop productivity. The agriculture sector is known to be the most vulnerable sector to changing climate, which results in severe and prolonged drought periods due to less precipitation, thereby changing the character of vegetation and cropping patterns of a particular country. In addition, unpredictable extreme events such as floods, drought, cold and heat waves, etc. hamper food security and threaten the livelihood of billions of people, making agricultural workers less productive (Sharma et al. 2022). Lately, the whole world has faced a challenging situation of the global pandemic, which by far completely disrupted our comfortable lifestyle making the whole world on standby, but, when observed, the global warming situation didn't stop; strangely enough, the year 2020 was recorded as the hottest in the recent times. Now, as a matter of ongoing climatic conditions, global food security is at stake. The developing countries face more hardship than the rest of the world as it lacks the technical and financial capability to respond to increased variability and causes lows returns from agricultural exports (Karki and Gurung 2012). Thus, the agricultural crop production systems become extremely difficult with the changing temperature and rainfall patterns, often leading to sudden outbreaks of pests and diseases, which reduces the yield tremendously (Bhattacharya 2019). Rice, a major cereal crop, is hugely impacted due to scanty and erratic rainfall as a consequence of global warming. Rice is semi-aquatic and flourishes well in a good amount of rainfall.

However, due to the changing climate, its growth and productivity are strongly affected by low soil moisture. Being one of the predominant cereal crops and consumed as stable food by more than 50% of the population, changes in the ecosystem are causing yield decline. In Asia alone, about 34 million ha of rainfed lowland and 8 million ha of upland rice are subject to frequent drought stress

(Vikram et al. 2011a, b). During yield loss, all other agronomic characteristics like plant height, number of tiller/plant, number of panicle/plant, number of spike/panicle, number of grains, grain weight, etc., are affected (Denčić et al. 2000) because of phenotypic adaptation. Out of all the abiotic stresses, drought conditions cause a huge yield loss in rice plants which can affect at any stage of the growth period and, in extreme conditions, cause the plant to die. Generally, the susceptibility of rice plants to low moisture conditions is due to the small root system, thin cuticle, and quick stomata closure (Singhal et al. 2016). The term 'drought' refers to lack or devoid of moisture for an extended period of time, which in turn has the tendency to cause a deficit of moisture in the soil. It can be defined as the inadequacy of available water, which includes the quantity of precipitation and soil moisture distributed during the life cycle of a crop plant, which restricts the expression of the full genetic potential of the plant. Drought stress accounts for about 25–30% yield loss in the rice plant, which further could be more if a means to tackle it cannot be implemented. Therefore, there is a need to breed drought-tolerant varieties. The conventional breeding methods and the development of the dwarfing gene (*sd1*) as a consequence of the green revolution could sustain the world's population for quite some time, but the need for higher yield did not stop here. Generally, the traditional breeding methods of introduction, hybridization, pedigree selection, recurrent selection, and backcross were used to develop various biotic and abiotic stress varieties. However, these methods are time-consuming, laborious, and expensive and the major drawback is the genetic drag it produces as a result of crossing. Breeding for a drought-tolerant variety has its own set of challenges because drought is a complex quantitative trait governed by various physiologically, biochemically, and genetically mechanisms.

Drought tolerance can be the capacity of a plant to produce a higher yield under water-deficit soil conditions. Several factors in plants are responsible for better drought stress response, such as plant variety, age of the plant, stage of growth, plant genotype, and drought intensity (Le Gall et al. 2015). To become self-sustainable in rice crop production by 2050, the focus should be to develop a variety of resistance to both biotic and abiotic stresses and high yield and nutrient quality (Chukwu et al. 2019). This is possible by genomic tools such as marker-assisted breeding (MAB), QTL mapping, haplotype-based breeding, speed breeding, and RGA (describe in detail below). The current high-tech transgenic approaches and genome editing tools like CRISPR-Cas9 can also be used to develop a drought-tolerant cultivar in the post-genomic era. Therefore, breeding a climate-resilient variety becomes a need to withstand the testing environmental change.

3.2 Rice Drought Stress Response

Drought tolerance (DT) is a complex polygenic trait whose tolerance mechanism depends on the action and reaction of diverse morphological, biochemical, and physiological responses (Mitra 2001). DT is the tendency of the plant to withstand drought conditions and produce more yield (Sharifunnessa and Islam 2017). The rice

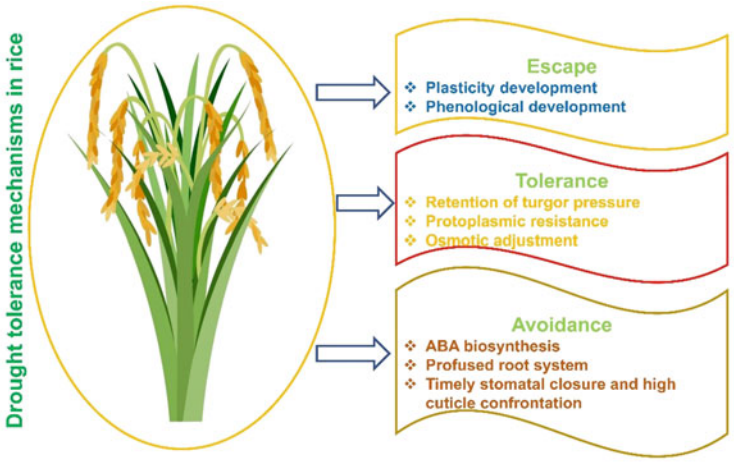

Fig. 3.1 Various mechanisms of rice to cope with drought stress

crop has a coping mechanism under drought stress by closing stomata, leaf rolling, and abscisic acid (ABA) production (Price et al. 2002). Rice plants respond to drought stress in either three of the following ways (Fig. 3.1).

3.2.1 Drought Escape

Here, rice plants escape the severe moisture stress condition by completing their life span before the onset of drought (Kumar et al. 2017). The escape is caused by two mechanisms: quick phonological development (flowering early and quick maturity) and plasticity development. During abundant rainfall, plants produce more vegetative growth, flowering, and seed set (Kumar et al. 2008).

3.2.2 Drought Avoidance

Rice plants having the capacity to retain more tissue water potential under a water-deficit condition will have an avoidance mechanism (Kumar et al. 2017). The drought avoidance capacity depends on plants having a coarse deep root system with more branching and penetrance capability in the soil, larger root and shoot ratio, timely stomatal closure, and higher cuticle confrontation (Wang et al. 2006).

3.2.3 Drought Tolerance

The tendency of the plants to survive the low moisture level without hampering the yielding ability of the plants is called drought tolerance (Zhang et al. 2019).

Drought-tolerant mechanism also involved turgor pressure retention via osmotic regulation, improved cell elasticity, reduced cell size, and protoplasmic resistance. Here, we will further learn about the morphological, physiological, biochemical, and molecular responses of rice plants during drought stress.

3.2.4 Morphological Responses of Drought Stress in Rice

Morphological parameters are used to study the various aspects of plant responses to drought stress (Zaher-Ara et al. 2016). These morphological parameters for drought response are reduction in leaf size, several stomatal reductions, leaf surface cutinization, and thickened leaf cell wall. In a drought-tolerant cultivar Nagina 22, it was observed that drought stress leads to total leaf area reduction significantly (Kadam et al. 2017). In addition to these, drought stress also alters the plant height, leaf area index, plant biomass, and leaf senescence (Kadam et al. 2017). However, drought stress has a distinct impact on rice plants: a decrease in root depth, distribution, number and length of primary roots, low root and shoot length, leaf rolling, curling, leaf area reduction, and wilting. It also hampers the timely flowering and grain filling, which directly impacts the yield.

3.2.5 Physiological Responses of Drought Stress in Rice

Physiological processes in the rice plant are adversely affected by drought stress which affects the growth and productivity of the crop. Relative water content (RWC), leaf water potential, stomatal resistance, rate of transpiration, and leaf temperature are the physiological traits that influence plant water relations. When drought stressed, plants have a lower RWC and the temperature increases due to decreased leaf water potential and transpiration rate (Fahad et al. 2017). Nitrogen metabolism in plants is also affected by drought stress. Nitrogen metabolism and increase in nitrogen provide rice plants to adapt to photosynthesis and water stress by mitigating stomata, higher Rubisco activity maintenance by further increases in nitrate and ammonium assimilation (Zhong et al. 2017). Drought stress altered the photosynthetic activity of the plants by limiting CO_2 availability impairing ATP synthesis and decreasing phosphorylation (Fahad et al. 2017). In acclimation to abiotic stress, mineral nutrition is necessary to regulate cellular ionic homeostasis. Many important minerals such as nitrogen, silicon, magnesium, calcium, and other essential minerals are impacted due to drought stress. In addition, drought induces ROS toxicity which is decreased by macronutrients like N, K, and Ca and micronutrients like Si, Zn, and Mg and will further increase the level of an antioxidant such as superoxide dismutase (SOD) (Waraich et al. 2011).

Various physiological responses of rice plants during drought stress are root signal recognition, loss of turgidity, and osmotic adjustment. It also affects the photochemical activity, loses leaf water potential, and reduces stomatal conductance. In addition, the reduction in plant growth rate also reduces the pollen-pistil

interaction, resulting in low spikelet fertility and ultimately affecting the crop yield (Upadhyaya and Panda 2019).

3.2.6 Biochemical Responses of Drought Stress in Rice

The biochemical process, such as redox reaction, or the transfer of electrons, is a natural cellular metabolism during energy transduction in the inner mitochondrial and thylakoid membranes (Upadhyaya and Panda 2019). Drought stress induces over-accumulation of pro-oxidants, referred to as oxidative stress, resulting in loss of redox homeostasis. Plant metabolism and development are adjusted by redox regulation during abiotic stress. In rice, ROS is generated during drought stress which results in oxidative stress by damaging the carbohydrates, proteins, lipids, and DNA (Gill and Tuteja 2010). Now, the ROS produced will increase the antioxidant level comprising enzymes (SOD, CAT, APX, GR, MDHAR, DHAR, GPX, and GST) or non-enzymatic molecules (ASA, GSH, phenolic compounds, alkaloids, nonprotein amino acids, and α-tocopherols). In return, an observed decrease in proline accumulation directly correlates with ROS accumulation, which causes oxidative damage by making the plant sensitive to drought and salinity stress (Miller et al. 2010). Changes in carbon and energy metabolism have also been reported in mitochondria and chloroplasts during drought stress.

3.2.7 Molecular Responses of Drought Stress in Rice

In rice during drought stress, molecular studies have led to the identification of several changes in the gene expression, which further helps to design a plant type having better survival and adaptation in the extreme environment (Upadhyaya and Panda 2019). During drought stress, ABA treatment leads to a better response of the drought inducible genes. There are two regulatory systems to control drought escape: ABA-independent and ABA-dependent in rice (Fu et al. 2017; Du et al. 2018). And the signal transduction cascade in rice has four different pathways, two ABA dependent (I and II) and two ABA independent (III and IV). The drought/dehydration-responsive elements (DRE) regulate drought, salt, and cold stress, which is regulated by ABA-independent pathways (IV). In the root of the upland rice, as reported by Rabello et al. (2008), many drought-responsive genes lead to signal transduction, such as Ca-dependent protein kinase, ethylene-responsive factors, genes for CO_2 metabolism, oxidative injury reduction, and osmoregulatory and ionic balance. The drought-responsive genes are regulated by several transcription factors (TFs) such as MYB, MYC, CBF/DREB (C-repeat-binding factor/drought-responsive cis-element binding protein), ABF/AREB, NAC, and WRKY TFs (Dey et al. 2017; Nahar et al. 2016: Zhang et al. 2016). The action of SnRK2, an ABA receptor complex, indicates the pivotal role in regulating the and responsiveness of plants to drought stress (Umezawa et al. 2010). The SnRK2 regulates the rapid, adaptive response of plants to drought. DREB and AREB are

the transcription activators of genes expressed in the different tissues. Additionally, a clear understanding of the plant responsiveness toward drought can be achieved by determining the molecular mechanism and the various signaling pathways.

3.3 Breeding Strategies for Drought Tolerance

3.3.1 Breeding Technologies in Pre-genomic Era

During the 1950s, increasing the genetic yield was the main motive of the plant breeders, where dwarf varieties Guang-Chang-Ai and Taichung (Native)-1 were successfully developed using spontaneously produced dwarf mutant *Ai-zi*-zhan (Huang 2001) and spontaneous dwarf mutant *Dee-Gee-Woo-Gen*, respectively. After the breakthrough of these varieties, in the 1960s, the International Rice Research Institute (IRRI) developed a miracle yielder rice named *IR8*, using the tropical japonica variety Peta, to hybridize with the dwarfing gene *Dee-Gee-Woo-Ge*. Hence, the era of the green revolution begins. The IR8 plant type was extensively bred worldwide because of its short-statured, having desirable physiological traits such as high leaf area index (*LA1*), photoperiod insensitive, high harvest index, and high fertilizer efficiency. Over the past five decades, more than 90% of the high-yielding varieties were developed using the DGWG dwarfing gene (*sd1*). Although these could be satisfied for quite some time, the huge demand for food due to the escalating population recapitulated the breeders to search for alternative strategies as the yield growth was flattened. Hence, the hybrid rice technology was introduced by the Chinese breeders using cytoplasmic male sterility (CMS) from more than 20 different sources, such as wild abortive (WA) in *indica* rice, and *Boro Tai (BT)* in *japonica* rice (Li and Yuan 2000; Fujii and Toriyama 2009) to exploit the heterosis in rice during the late 1970s. However, these could still not work out due to stagnant in the yield plateau, and the changing weather added another challenge. Therefore, with the advancement in technologies and strategies, there becomes a need to upgrade using the high-throughput techniques and molecular approach.

3.3.2 Population Development and Improvement

The drought-tolerant cultivar should outstand in terms of better yielding capacity over the present available popular cultivar under water-limiting situations, across various locations, environmental conditions, and seasons, having higher yield even in irrigated conditions (Rizza et al. 2004; Ober et al. 2004; Pidgeon et al. 2006). Baring on grain yield, the drought-tolerant cultivar should possess good characteristics like cooking quality, nutritional value, and the ability to withstand various biotic stresses. The need to introgress a drought-tolerant gene in the improved high yield, medium height, desirable grain type having lodging and biotic resistance becomes important because of their inability or poor yielding during the drought condition. The drought trait can be found in the traditional landraces with

undesirable agronomic traits such as lodging susceptible, tall height, poor cooking, and low yield. The new gene combination for an improved population is generated when this is transferred to the background of improved cultivars (Kumar et al. 2014). Thus, the cultivated population is exploited with genetic diversity, which makes it tolerant to extreme stress conditions. A drought-tolerant population can be developed by crossing with multiple parents having the contrasting trait of interest, making recombinant inbred lines (RILs), shelfing it, and then advancing it by single-seed descent (SSD) method. Various other populations such as near homozygous lines (NILs), backcross inbred lines (BILs) (Kumar et al. 2014; Mishra et al. 2013; Sandhu et al. 2014), near-isogenic line (NIL) populations, and double haploid (DH) populations with early generation homozygosity (Xu et al. 2010) serve the purpose for developing a drought-tolerant variety in rice.

3.3.3 Selection Criteria: Variability, Choice of Parents, and Suitability

Any breeding program is deemed successful if genetic variability adheres to the selection, the selection criteria, and the suitable parents for breeding. The most crucial job is to find out suitable parents based on the requirement of the target breeding program (Liang et al. 2013). Combining the donor low-yielding parent with the high-yielding drought susceptible can buffer the gene complexity to produce a drought-tolerant line. There is an advantage in choosing a donor parent who can combat multiple stresses other than drought. Some of the drought-tolerant donors are *N22*, *Dagad Deshi*, *Moroberekan*, *Aus 276*, *Vandana*, *Apo*, and *IR55419-04* and introgressing them in the background of popular high-yielding varieties from different countries such as *IR64*, *Swarna*, *TDK1*, *MTU1010*, *Samba Mahsuri*, and *Sabitri*, conducting phenotypic screening under both drought stress and normal conditions for grain yield. Apart from this, the selection of parents should be based on the targeted environment. Rice is a dynamic crop grown in very distinct conditions from each other such as upland, lowland, flooded, submerged conditions, etc. The requirements for flooded conditions are high tiller, medium to dwarf height plants, and drought-tolerant capacity. And high yielding with tolerance to lodging is the criteria under normal conditions. Most lowlands established varieties such as Swarna, IR64, and TDK1 which have the ideal characteristics but lack drought-tolerant mechanisms. The requirement for the upland condition is semi-dwarf to semi-tall, early-to-medium-duration lines. Keeping into consideration of the growing condition, suitable parents should be selected. Nevertheless, the ultimate goal is to develop a high-yielding variety under water-deficit conditions (Dixit et al. 2014a, b).

3.3.4 Conventional Breeding

Breeding rice via conventional breeding methods is cumbersome and labor-intensive and most of the breeding methods take around 8–10 years. In traditional methods, selection plays a key role in varietal development. However, continuous breeding exploits the plants' existing genetic variation, narrowing down the genetic pipeline (Haroon et al. 2020). Due to its low heritability and high G × E interaction, grain yield becomes an ideal selection criterion under drought stress. However, the traditional methods have its limitation; therefore, the focus has shifted to selection based on physiological characteristics (Monneveux et al. 2006). A modified plant breeding approach is applied for screening large populations of rice under both normal and irrigated conditions. The sequential selection and screening are suitable for grain quality, biotic stress, and yield parameters. Under drought-stress conditions, the high-yielding popular variety can still positively impact the yield parameters irrespective of the water condition (Dixit et al. 2014a, b). Before the change in the weather extremity, conventional breeding methods were used for germplasm conservation and wide hybridization between contrasting parents and to create novel genetic traits. As time goes by, the International Rice Research Institute (IRRI) started developing extraordinary rice varieties that resisted biotic and abiotic stress using conventional breeding methods (Khush 1984). Conventionally, pedigree selection, recurrent selection, backcross method, and mutation breeding develop drought tolerance for self-pollinated rice crops. Let us have a brief view of all the following.

3.3.4.1 Pedigree Method

Pedigree selection is the oldest and best method for handling the segregating generation in rice. Many prominent rice varieties like Jaya, Ratna, Bala, Kaveri, etc. have been developed using this method. The success of this method is when many major genes governing biotic and abiotic stress are combined to develop a condition (Posadas et al. 2014). The major drawback is maintaining the pedigree record, which is time-consuming; and discarding and evaluating every line and generation becomes tedious. This method requires the utmost dedication and skills of the plant breeders, and the trait under study should have high G × E interaction. The diallel mating design becomes handy when the trait is controlled by many genes (Khush 1984). The most discouraging the breeder has to face is the absence of one particular suitable method to breed for a particular trait of interest. In rice breeding, including most self-fertilizing crops, the pedigree method is outpowered by recurrent selection (Miah et al. 2013). Figure 3.2 depicts the procedures of selection done for developing drought-tolerant lines.

3.3.4.2 Recurrent Selection

Recurrent selection is widely preferred because of its short breeding cycles, genetic improvement involving multiple crosses, improved quantitative trait levels, and development of diverse breeding lines. Although it has been popularly used in maize breeding (Bolaños and Edmeades 1993) and wheat (Rebetzke et al. 2002),

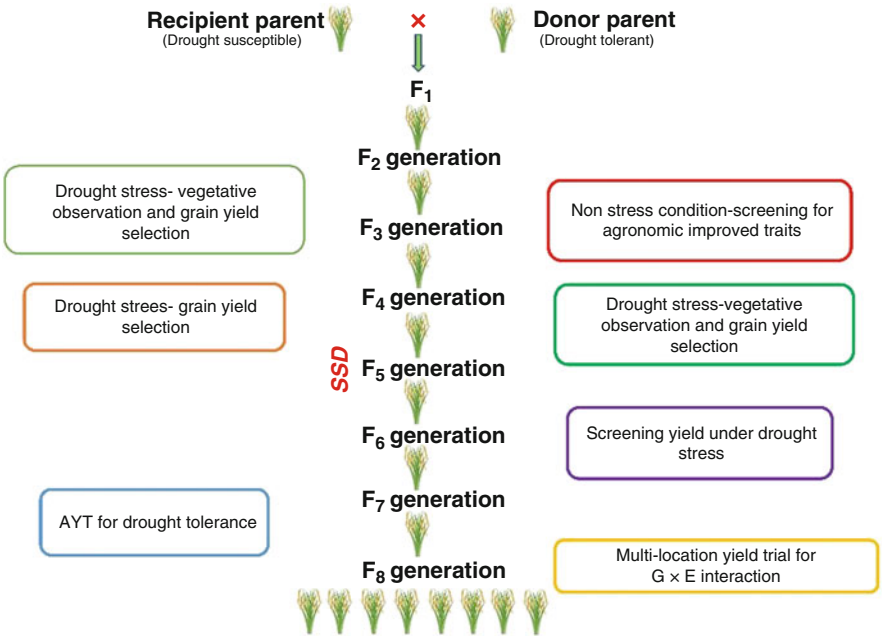

Fig. 3.2 Procedure for developing drought-tolerant lines

and other crops, however in rice, the approach started to be applicable only after the availability of "Jiabuyu," a male sterile line which is controlled by a single dominant gene (Pang et al. 2017). The DMS (dominant male sterile) line "Jiabuyu" (as outcrossing facilitator) was used to develop two different types of recurrent selection populations. In the study by Pang et al. (2017), 12 drought-tolerant (DT) lines were screened for grain yield. Reny et al. (2017) conducted the study at the seedling stage using 180 lines developed by recurrent selection for the agronomic trait in rice. On selecting 53 drought-tolerant lines, the concluded study favored using the recurrent selection method for DT improvement. In cereals like wheat, DT lines were also evaluated using the recurrent selection method (Singh et al. 2016). In recent years, merging the conventional recurrent selection method and molecular technologies modified as the marker-assisted recurrent selection has been extensively used in rice to develop and identify QTLs for drought tolerance (Sandhu et al. 2018).

3.3.4.3 Backcross Breeding

In conventional breeding methods, backcrossing is done involving two-parent donor and the other recipient. The gene of interest controlling the trait of interest is introgressed from the donor parent to the recipient parent. This method has been used to develop tolerant cultivars for various stress conditions. However, the major drawback in developing conventionally is the transfer of the unwanted genes in the high-yielding recipient parent due to linkage drag. Lately, the drought-tolerant rice

variety is developed by modified marker-assisted backcross breeding, which has become an ideal technique. Backcross methods have been prolific in the development of drought-tolerant varieties in rice.

3.3.4.4 Mutation Breeding

Traditionally, the major activity of plant breeders was the introduction, selection, and hybridization for crop improvement. However, with time and with the narrowing genetic bases, mutation breeding becomes the sole breeding strategy for generating genetic variation for crop improvement. In recent years, 800 varieties were released directly or by crossing with other suitable varieties by inducing mutation in rice. There are two categories of mutation-causing agents: physical mutagens and chemical mutagens (Mba et al. 2010; Acquaah et al. 2012). Physical mutagens alter the genetic makeup of the species, whereas chemical mutagens induce the point mutation. Using mutagens proved to develop agronomic traits like grain yield, disease, pest resistance, and drought-tolerant variety in rice. The induced mutation has the major advantage of creating genetic alleles that are not found in nature. India stands second in using mutagens for creating genetic variability in crops (Kharkwal et al. 2004). To date, around 1594 cereals and 3346 other crop varieties have been developed. MK-D-2 and MK-D-3 were two drought-tolerant lines selected as a result of irradiating the Manawthukha rice variety with a dose of 300 Gy of gamma rays (Soe et al. 2016). MR219-9 and MR219-4 are two superior drought-tolerant lines developed in Malaysia from a well-known rice variety, MR219 (Abdul et al. 2012). In breeding for drought-tolerant rice cultivars, various traits have been studied, but very less evidence has been found for their contribution to improving yield under drought stress (Lafitte et al. 2006).

3.3.5 Pre-breeding for Drought Tolerance

A narrow genetic base in the released varieties has made plateaus in the crop yield. The limited genetic resource use in breeding crops didn't help either. Widening the genetic base becomes a necessity to overcome the yield barrier. Lately, hybridization and pre-breeding have been carried out to broaden the genetic bases of the plants. In pre-breeding, the first activity is to identify the desirable traits in the wild or unadapted materials that are not compatible with the cultivated population and introgressed them to the intermediated plant material, where further the plant breeders can use them to improve the cultivated varieties. These pre-breeding pipelines help in having a minimum linkage drag and capturing the desirable genetic diversity from the natural resources. Pre-breeding has successfully contributed to several crops like rice, tomato, soybean, cotton, maize, wheat, barley, groundnut, chickpea, pigeon pea, sorghum, and pearl millet (Iqbal et al. 2001; Sebolt et al. 2000; Seetharam 2007). With the dramatic change in the climatic condition due to global warming, the plant's adaptation mechanism is depleting every year. In addition, drought tolerance being a complex trait is not helping either. So, it became necessary

to remove the left-behind genetic variation and re-introduce it into breeding programs.

3.3.6 Genomic Era (High-Throughput Genotyping Using NGS Platform)

Maintaining sustainable food production is a great challenge with a constantly growing population. The concept of genomic-assisted breeding (GAB) was introduced 15 years ago in response to advances in the genomic area. The availability of good-quality genome sequences of different crops and a wide range of advanced genomic approaches have created a new paradigm for smart crop breeding in the genomic age. It is by the emerging genomic technologies when the multi-parent synthetic population-based genetic blueprint is used to detect traits that have benefits for both linkage analysis and association mappings, such as QTL detection, better mapping accuracy, and higher genetic variability (Sharma et al. 2017; Kover et al. 2009). The ongoing advancement of next-generation sequencing (NGS) approaches improved access to whole-genome sequence information of various large-scale crops as well as high-efficiency genotyping and thus has contributed to filling gaps in the genome and phenome map. So far, approximately 10,000 rice accessions have been sequenced due to the availability of good-quality genome-wide reference sequences of *Oryza sativa, Japonica*, and *Nipponbare* (Wing et al. 2018). Thus, GAB developed around 130 cultivars over the past few years, accelerating the conventional breeding timeline for many different crop species (Vogel 2014). Most crops developed through GAB have shown resistance to both abiotic and biotic factors. For instance, rice cultivars developed through GAB are highly resistant to blast and bacterial blight. Furthermore, breakthroughs in the genomic era have been achieved by developing crops that can withstand major abiotic stress, including submergence, water stress, and osmotic stress. This happens due to (1) high-quality whole genome reference sequence and RNA-seq data availability, (2) automated, highly efficient genotyping principles and strategies such as genotyping by sequencing (GBS), (3) QTL quantification, and (4) the availability of genome-wide selection platforms such as genomic selection. By leveraging all these aspects, the breeders could select a large number of genotypes within a short period.

3.3.6.1 Marker-Assisted Breeding: A Promising Breeding Approach in the Genomic Era

In the era of genomics, marker-assisted breeding (MAB) is considered as ameliorating approach to conventional breeding that aims to elucidate the genetic basis of some complex traits, including tolerance to abiotic stress, disease resistance, quality, or productivity. Marker-assisted breeding takes advantage of a molecular marker to select the plants whose genome sequence is responsible for expressing a particular trait of interest. The exploitation of molecular markers in crop breeding programs is based on the premise that the presence of specific markers in the genome is correlated with the presence of specific traits. In this case, the big data of genomics

plays a significant role in studying the linkage between the marker and traits. In the genomic era, with the advancement and accessibility of a wide range of genetic markers and high-resolution genetic maps in agricultural plants, MAB can now be used to study traits influenced by key genes and QTLs. The more advances in the area of genomics, the more will become easier to apply MAB for polygenic traits that cannot be resolved by traditional breeding. This type of genomic-based marker-assisted breeding is termed genomic-assisted breeding (GAB) or smart breeding. Thus, molecular marker-based smart breeding enabled the identification of genes, genetic markers, and QTLs linked to major abiotic constraints such as water stress, temperature, salinity, and flood in rice. Drought is the major constraint among all the abiotic stress that most rigorously jeopardizes the global productivity of major crops. Drought stress has become more prevalent and severe as a result of climate change, resulting in a parched world with a substantial yield decline in drought-prone areas in recent years. Because the drought resistance trait is polygenic, conventional breeding for drought resistance rice varieties is challenging. However, in the genomic era, a substantial amount of progress has been achieved in identifying suitable parents or donors and developing suitable screening criteria for traits associated with drought tolerance (Guan et al. 2010; Kumar et al. 2008; Venuprasad et al. 2007). The International Rice Research Station (IRRI) has used marker-assisted breeding to identify multiple QTLs associated with yield parameters under water stress (Kumar et al. 2014) and developed several improved drought-tolerant varieties by introgressing the identified QTLs into high-yielding drought-sensitive varieties.

3.3.6.2 Marker-Assisted Breeding: Identification, Introgression, and QTL Pyramiding

In the pre-genomic era, many morphological traits are used as a benchmark for the identification of genes affecting complex characters by the plant breeders. However, the number of morphological markers available is insufficient to serve as an index of every region of DNA in the entire genome that can be manipulated, particularly the quantitative features. In this context, variation at the DNA level provides a unique marker known as a molecular marker to serve as an indicator of the genetic basis of the entire genome. Plant breeders define molecular markers as the DNA sequence traced to a specific location on the chromosome and associated with a particular trait. The advancement in the molecular tools led to the availability of different types of molecular markers, including restriction fragment length polymorphisms (RFLP), randomly amplified polymorphic DNA (RAPD), sequence characterized amplified region markers (SCAR), and simple sequence repeats (SSRs). Using map-based cloning to know the exact position of the genes, particularly the loci driving quantitative trait locus (QTL), is fundamental and of preeminent importance. The location of specific genes, DNA markers, and QTLs associated with specific traits is achieved through molecular marker-based QTL mapping. In the era of genomics, efforts have been put into the identification of QTLs linked with a particular trait, primarily grain yield under drought conditions in rice (Sandhu and Kumar 2017; Kumar et al. 2014). In general, the identification of QTLs governing specific traits under drought stress entails several steps: first, the development of a mapping

Table 3.1 QTLs related to yield-related traits under drought stress

Sl. no.	QTL	Associated trait	References
1.	*qDTY1.1*	Grain yield	Vikram et al. (2011a, b); Ghimire et al. (2012)
2.	*qDTY1.2*	Grain yield	Sandhu et al. (2014)
3.	*qDTY1.3*	Grain yield	Sandhu et al. (2014)
4.	*qDTY2.1*	Grain yield	Venuprasad et al. (2009)
5.	*qDTY2.2*	Grain yield	Swamy and Kumar (2013)
6.	*qDTY2.3*	Grain yield	Palanog et al. (2014)
7.	*qDTHI2.3*	Grain yield	Mishra et al. (2013)
8.	*qDTY3.1*	Grain yield	Dixit et al. (2014a, b)
9	*qDTY3.2*	Grain yield	Vikram et al. (2011a, b)
11.	*qDTY4.1*	Grain yield	Swamy and Kumar (2013)
12.	*qDTY6.1*	Grain yield	Dixit et al. (2014a, b)
13.	*qDTY6.2*	Grain yield	Dixit et al. (2014a, b)
14.	*qDTY9.1*	Grain yield	Swamy and Kumar (2013)
15.	*qDTY9.1A*	Grain yield	Dixit et al. (2012)
16	*qDTY10.1*	Grain yield	Vikram et al. (2011a, b)
17.	*qDTY10.2*	Grain yield	Swamy and Kumar (2013)
18.	*qDTY12.1*	Grain yield	Bernier et al. (2007)

Source: Panda et al. (2021) (modified)

population by crossing wild drought-tolerant genotypes and improved high-yielding genotypes as parents, accurate phenotyping using morphological markers in different conditions such as irrigated and drought stress conditions, genotyping of the population using suitable molecular markers, generation of chromosome map, and finally, QTL mapping based on available phenotypic and genotypic data. In the past few years, a large scale of major QTLs associated with important morphological traits (i.e., grain yield, root length) and physiological traits (i.e., osmotic adjustment, photosynthetic activity) showing response to drought stress have been detected and are being used broadly to select superior rice varieties (Vikram et al. 2011a, b, 2016; Venuprasad et al. 2012; Dixit et al. 2017; Vinod et al. 2019; Ramchander et al. 2016). Considering yield as a selection criterion, breeders all over the world are focusing more on mapping QTLs linked with grain filling under drought conditions and the introgression of identified QTLs in a suitable background to develop high-yielding drought-tolerant rice varieties. So far, QTLs explaining a wide range of phenotypic diversity for yield attributes have been detected, such as *qDTY1.1* (Ghimire et al. 2012; Vikram et al. 2011a, b; Sandhu et al. 2014), *qDTY2.1* (Venuprasad et al. 2009; Sandhu et al. 2014), and *qDTY3.1* (Venuprasad et al. 2009; Dixit et al. 2014a, b) (Table 3.1)

The genotyping strategies such as bulk segregate analysis (BSA) (Mishra et al. 2013; Vikram et al. 2011a, b; Ghimire et al. 2012), selective genotyping (SG), genotyping by sequencing, whole-genome genotyping (WGG), and genome-wide association studies (GWAS) (Begum et al. 2015) have been used for the detection of

QTLs associated with drought tolerance and introgression in the different genetic backgrounds using marker-assisted recurrent selection (MARC) (Xu and Crouch 2008); marker-assisted backcrossing (MAB) (Mishra et al. 2013; Venuprasad et al. 2009; Sandhu et al. 2014) and marker-assisted QTL pyramiding have been reported. The cost-effective genotyping and phenotyping approaches lead to the mapping of 12 key and stable QTLs (*qDTY1.1*, *qDTY2.1*, *qDTY2.2*, *qDTY2.3*, *qDTY3.1*, *qDTY3.2*, *qDTY4.1*, *qDTY6.1*, *qDTY6.2*, *qDTY9.1*, *qDTY10.1*, and *qDTY12.1*) (Table 3.1) in the background of some common broadly cultivated drought-sensitive rice varieties having high productivity including Swarna, IR64, Sabitri, MTU1010, and TDK1 and one drought-tolerant variety Vandana (Venuprasad et al. 2012; Mishra et al. 2013; Swamy and Kumar 2013; Dixit et al. 2014a, b; Vikram et al. 2011a, b; Ghimire et al. 2012; Bernier et al. 2007) through marker-assisted QTL pyramiding. The consistency of identified QTLs in multilocation, different seasons, genetic backgrounds, and ecosystems was reported in many studies. The major seven loci *qDTY1.1* (Venuprasad et al. 2009; Ghimire et al. 2012; Vikram et al. 2011a, b), *qDTY2.2* (Swamy and Kumar 2013; Sandhu et al. 2014), *qDTY3.1* (Venuprasad et al. 2009; Vikram et al. 2011a, b), *qDTY3.2* (Ghimire et al. 2012), *qDTY4.1* (Swamy and Kumar 2013), *qDTY6.1* (Vikram et al. 2011a, b), and *qDTY12.1* (Bernier et al. 2007) have shown steady effect across multilocation, multiseason, multi-environment, and genetic backgrounds in repeated years. In addition, the QTLs *qDTY1.1*, *qDTY2.2*, *qDTY6.1*, and *qDTY12.1* have also shown enormous effects across different cultivable environments like aerobic environments and direct-seeded (Sandhu et al. 2014; Bernier et al. 2007). Thus, efficient molecular marker-based breeding procedures based on a meticulous assessment of population size and structure resulted in the release of several drought-tolerant rice cultivars with high productivity.

3.3.6.3 Haplotype-Based Breeding

In the genomic era, the advanced platform of next-generation sequencing (NGS) technology has inspired the fast growth and accessibility of DNA sequencing in large-scale germplasm efforts. This NGS platform has brought up the intriguing option of mining single-nucleotide polymorphism (SNP) to use as a marker for crop breeding purposes. However, because SNPs are biallelic, the SNP marker has some limitations over multiallelic markers, providing less information and low resolution. In this regard, an efficient way to address the biallelic limitation of SNPs is to use haplotypes for genetic and genomic studies in modern plant breeding. A haplotype is a unique group of alleles or set of allelic variations or polymorphisms such as insertion/deletion or SNPs present in the same chromosome, which tends to inherit together with less probability of contemporary recombination (Garg et al. 2021). A haplotype is a collection of closely located genetic and structural variations such as two or more SNP alleles, with high linkage disequilibrium (LD) among them (Bernardo 2008). Nevertheless, generating haplotypes in terms of available marker data is crucial in a genomic-assisted breeding program. Generally, there are three methods to define or assign haplotype: (a) by taking haplotype diversity of a given stretch of the chromosome, (b) by pairwise LD between markers that are inherited

together, showing less or no evidence of historical recombination in the same chromosomal blocks, as usually measured by r^2 (Maldonado et al. 2019; Pritchard and Przeworski 2001), and (c) by combining polymorphic SNP via sliding windows of fixed or different length(s) (Huang et al. 2007). It is reported that the linkage disequilibrium-based method is more effective for assigning haplotypes in a particular chromosome segment (Qian 2017; Maldonado et al. 2019). This is due to the following: (a) the LD-based technique directly focused on identifying historical recombination in a specific population via haplotype identification, (b) it is also easily applicable to diploid data with unknown haplotype phase, and (c) detection of coefficient of LD is easy. Many factors influence LD in a particular population, including pollination mode, rate of mutation, size and structure of the population, genetic drift, type of selection, and frequency of recombination on particular chromosome segments (Gupta et al. 2005). During the evolutionary process, the directional selection of alleles or genes governing favorable traits of interest has played a substantial role in forming the selection signature of all significant crop species, including maize, rice, sorghum, rapeseed, and cassava (Qian 2017). The signature of selection, also termed as conserved haplotype block or selective sweep, comprises multiple genes, and the expression of these genes is jointly governed by more than one regulatory gene. The correlations among various characters expressed by multiple genes of signature of selection are likely to be caused either by the presence of linked genes or by the pleiotropic effect of the linked genes (Qian et al. 2016). Therefore, plant breeders should target these genomic areas or selection hotspots to unravel their consequences on desirable traits. The genomic-driven crossing approach where genomic big data is used to determine recombinants formed by crossing two different parents will highly simplify the elucidation of complex quantitative traits. Thus, this approach could be used to identify novel alleles and donors linked with traits of interest and enhance the development of climate-smart crops (Varshney et al. 2018). Because of the advancement and accessibility of large-scale sequence information for major crops, assigning haplotypes became easy in the genomic era. Thus, available large-scale genome-wide resequencing datasets along with haplo-pheno analysis paved the way to identify important haplotypes for breeding in rice (Lenaerts et al. 2019) and pigeon pea (Sinha et al. 2020) in the coming decades. The available genome-wide sequence information of diverse rice landraces in the rice gene bank is a suitable source for identifying allelic or haplotype diversity of key genes governing important traits. For example, a study on haplotype diversity of 93 aromatic rice germplasm and *Indica* germplasm reported four new SNPs haplotypes in the *GW2* locus associated with grain characteristics (Dixit et al. 2013). Also, a total of 9 SNP haplotypes (three major and six minor) of *GS3* locus associated with grain size were detected in a collection of 160 wild rice cultivars (Singh et al. 2017). The haplotype diversity analysis of 129 major genes governing grain productivity and quality identified certain superior haplotypes in rice across the 3K RG panel (Abbai et al. 2019; Li et al. 2014). All of these superior haplotypes associated with grain characteristics could be used in haplotype-based breeding, paving the way to breed high-yielding rice varieties under drought conditions.

In the genomic age, although marker-assisted selection (MAS) can be applied efficiently for traits with mono- or oligogenic inheritance, it is crucial to select highly complex quantitative traits with low heritability due to substantial environmental influence. However, the MAS approach has strong limitations due to the complex genetic structure and statistical overestimation markers linked to QTL for most important agronomic traits (Bernardo 2008). In this context, genomic selection (GS) methods have developed as a promising approach for addressing polygenic characters, such as developing drought-tolerant improved varieties. So far, haplotype-based genomic selection has been proposed to be a strong complementary tool to overcome the inaccuracy and inefficiency of classical genomic selection (Qian 2017). This is because the haplotype map allows the mapping of the QTLs and genetic segments associated with a particular trait of interest at greater resolution in populations with substantial linkage disequilibrium (LD) structures (Varshney et al. 2005). Thus, haplotype-based breeding is considered one of the efficient approaches for developing high-yielding drought-tolerant rice cultivars (Roy et al. 2021).

3.3.6.4 Speed Breeding

Conventional breeding of crops entails a significant time, land, input for phenotypic selection, and consequent crossing of suitable crops for many generations. Therefore, the time required for the breeding cycle is considered a major constraint in advancing crop breeding programs. Thus, speed breeding, which relies mostly on three factors, namely, temperature control, the extension of photoperiod, and early harvesting of seeds, accelerates the rate of crop breeding program to deliver improved high-yielding crop varieties (Ghosh et al. 2018). However, speed breeding is not a new concept; conventional methods like single-seed descent and shuttle breeding have been used to modify the duration of the crop breeding cycle since 1940. Further, plant breeders have widely adapted numerous techniques under the notion of speed breeding to adjust the controlled-environment growth conditions, resulting in a shorter breeding cycle. The techniques include rapid generation cycling (RGC: molecular marker-based selection increased the number of breeding cycles per year), single-seed descent (SSD: the fast generation of homozygous lines) method, rapid generation turnover (RGT: early seed harvesting and extension of photoperiod increase the number of breeding cycles per year), and fast generation cycling (FGC: more breeding cycles per year achieved through in vitro culture of immature embryos). As the name indicates, speed breeding regulates the photoperiod by using artificial light sources and reduces the duration of crop breeding cycles (Sysoeva et al. 2010). For the first time, speed breeding was done in wheat (*Triticum aestivum*) and studied the trait related to seed dormancy under controlled environment conditions (Hickey et al. 2019). Afterward, the National Aeronautics and Space Administration (NASA), USA, in collaboration with Utah State University, affirmed and approved the notion of speed breeding (Wheeler et al. 1996; Ghosh et al. 2018). A group of breeders from the University of Queensland (Australia) designed a new protocol of speed breeding to overcome the negative impact of steady light on the germination of immature seeds, crop growth, and harvesting.

Currently, new speed breeding protocols have been established for several major crops that allow plant breeders to grow four to six generations in 1-year crops such as wheat, barley, chickpea, rice, pea, and canola (Watson et al. 2018; Ghosh et al. 2018). Shuttle breeding or off-season nursery and embryo/in vitro culture were used to minimize the duration of the seed-to-seed cycle in several crops (Bhatta et al. 2021). In 1 year, the embryo rescue technique accelerates the number of generations to eight in the case of wheat and nine generations in the case of barley when applied in a controlled environment of light, water regimes, and temperature (Zheng et al. 2013). To this end, plant breeders in the era of genomic have used smart breeding to accelerate the breeding cycle of rice. Rice breeding programs aimed at improving tolerance to abiotic stress involves a long-duration labored process of growing diverse genotypes in a homogenous amount of land and water resource. Although extending the photoperiod is feasible for long-duration crops, it fails to be viable for a short-duration crop like rice, as an extended photoperiod delays flowering. In this context, a rigorous method of tweaking the photoperiod has been established as a suitable method for developing new rice varieties within a short time duration. A speed breeding protocol was reported where, after germination, seeds were allowed to receive light for 14 h and darkness for 10 h for enhancing vegetative growth and then again allowed to receive light for 10 h and darkness for 14 h to induce reproductive growth, which finally increases the number of generation (4–5) per year in rice (Collard et al. 2017; Rana et al. 2019). Similarly, biotron, a simplified speed breeding protocol, has been applied to minimize the seed-to-seed cycle's duration in rice by growing in a controlled growth condition (Ohnishi et al. 2011).

Furthermore, the concept of rapid generation advance (RGA) has been applied to truncate the breeding cycle of rice and develop high-yielding varieties of rice in a short time under different abnormal conditions such as drought (Collard et al. 2017). Thus, molecular breeding approaches in combination with a speed breeding system enable the breeders to develop new drought-tolerant rice varieties. Recently, an effort has also been put to integrate speed breeding with other smart crop breeding strategies such as highly efficient genotyping tools, genomic selection, and different genome editing tools to enhance the crop breeding program.

3.3.6.5 Rapid Generation Advance (RGA)

Rapid generation advance (RGA) is the most recent approach to speed breeding. The RGA reduces the generation cycle of a crop and enables early harvesting of seed in F_2 to F_6 generation in modified controlled conditions (Collard et al. 2013). Thus, as the name implies, RGA enables several generations to be completed within less time and increases genetic gain. The RGA has various advantages over other breeding methods such as the need for a small field area, technological simplicity, and fewer labor resources, making RGA one of the less time-consuming and cost-effective alternatives (Stoskopf et al. 1993; Poehlman and Sleper 1995). Although RGA was first reported in 1939, it was not widely used until the 1960s, and after the 1970s, plant breeders started to use RGA or single-seed descent (SSD) in barley, oats, and soybean (Grafius 1965; Kaufmann 1971; Brim 1966). So far, RGA has been broadly used in the field of crop breeding to develop mapping populations (Recombinant

inbred line, RIL) for the detection of QTLs linked to a particular trait (McCouch and Doerge 1995). These mapping population produced using SSD or RGA are suitable for QTL mapping as well as breeding studies because they are genetically homozygous, and seeds can be replicated in enormous quantities, allowing for the phenotyping of many traits over many years (Collard et al. 2005). Thus, SSD integrated with rapid generation advance (RGA) has been adapted in many crop breeding programs to truncate the duration of crop breeding under controlled growth conditions. In temperate countries like Japan and Korea, where rice is cultivated only once a year due to cold weather, the rapid generation advancement (RGA) method could have saved a great deal of time by requiring off-season cultivation. In 1977, the RGA method was applied to breed 24 popular Japanese rice cultivars, occupying more than 40% of the overall rice-growing area of Japan. The plant breeders in Japan adapted this RGA strategy to improve rice breeding for cold tolerance in rice, which resulted in the release of widely cultivated rice cultivars *Nipponbare*. In the past few years, rice breeders have used SSD or RGA to develop several high-yielding rice varieties that show tolerance to different abiotic stress (Janwan et al. 2013). Using RGA, researchers at the International Rice Research Institute (IRRI) developed drought-tolerant breeding lines such as IR74371-54-1-1 and IR74371-70-1-1 in 2009 (Raman et al. 2012). Thus, RGA-mediated introgression of suitable alleles may be a viable option to accelerate rice productivity under severe drought conditions.

3.3.6.6 Genomic Selection

The primary goal of rice breeding is to produce climate-resilient high-yielding rice varieties that are resistant to abiotic stress, pest, and disease. However, the conventional breeding approach, which is based on crossing contrasting parents and continuous phenotypic screening of offspring through multiple generations, takes more time to produce an improved variety. It takes 9–10 years to develop a novel rice variety. From 1990 onward, marker-assisted breeding (MAB) has used genetic markers to select desired traits indirectly. MAB is suitable for only those traits that are influenced by less number of QTLs having a large effect on the expression of the trait. For the polygenic quantitative traits which are controlled by more than one QTL having a minor effect, the selection based on the molecular marker is not suitable. In this context, genomic selection emerged as a promising tool to overcome the drawback of the marker-assisted selection approach. Genomic selection (GS) is a viable strategy that estimates the genetic basis of an individual using genome-wide marker information rather than a few markers used in MAS. Thus, at first, genomic selection approach on the basis of available genotypic and phenotypic information of the training population creates a prediction model. The designed model is utilized to generate genomic estimated breeding values (GEBVs) from the genomic profiles of all individuals in the breeding population (BP) (Meuwissen et al. 2001). The GEBVs help us to predict suitable individuals either as parents in hybridization or for advanced generation crop breeding programs because the genome-wide genetic marker information of those individuals is the same as that of the other training populations that have been estimated to perform well in a certain environment. The

estimated GEBV also aids in selecting a new breeding population, resulting in a shorter breeding cycle so that it no longer requires to wait for late filial (F_6) generation for phenotyping complex traits such as yield, abiotic and biotic stresses, and so on. However, in the era of genomics where advanced, cost-effective sequencing (NGS) has dramatically led to the development of cost and time-effective high-throughput, genome-wide, and flexible single-nucleotide polymorphism (SNP) genotyping platform, particularly the emergence of genotype sequencing (GBS), which has made deployment of SNPs ideal and reliable for genomic selection in almost all crop species (Poland et al. 2012). Plant breeders have been attempting to determine how NGS approaches can help them realize the true advantage of GS in the era of highly available genomic resources for the rapid enhancement of crop breeding programs to develop climate-smart crops species showing tolerance to complex quantitative traits, including biotic and abiotic stress, grain quality, grain productivity, etc. (Burgueño et al. 2012; De los campos et al. 2009; Jannink et al. 2010; Crossa et al. 2010).

3.3.6.7 Role of NGS or Genomic Resources in GAB

In the genomic age, advances in sequencing technologies have enabled the successful sequencing of the entire genome of large-scale agricultural species, providing a chance to investigate the relationship between phenotype and genotype with higher accuracy at the genome level. Thus, combined with precise phenotyping methods, NGS-based platforms are an efficient way to identify a complex quantitative trait's genetic basis and estimate the breeding value of individuals within a plant breeding population. There are some approaches to QTLs and gene identification where the use of NGS enhances the accuracy and efficiency of the mapping process.

3.3.6.7.1 Genome-Wide Association Study (GWAS)

Genome-wide association study (GWAS) is a promising method to determine the complex QTLs using a spontaneously occurring genetic variation. GWAS provides better mapping accuracy than biparental mapping and studies the association between genotypes and phenotypes based on linkage disequilibrium mapping or association mapping. GWAS has been utilized successfully in some important crops, including rice (Huang et al. 2010, 2012a, b; Zhao et al. 2011), maize (Kump et al. 2011; Tian et al. 2011; Li et al. 2013), wheat (Kollers et al. 2013), sorghum (Morris et al. 2013), soybean (Hwang et al. 2014), and foxtail millet (Jia et al. 2013). In combination with NGS, GWAS has drastically enhanced the accuracy of the mapping process by genotyping a large population of plants with a higher density of markers. Nested association mapping (NAM), a specialized mapping population, has also been developed inconsistent with the development of NGS technologies which significantly accelerate the efficiency and resolution of GWAS. Nested association mapping (NAM) takes advantage of association and linkage mapping and deletes the disadvantage of both. NAM was initially developed for the maize population by leveraging recent and historical recombination events, thus providing an opportunity for high-resolution mapping. NAM decreases the number of markers utilized in GWAS while taking advantage of more mapping resolution, high allele richness, and high statistical power of association mapping. Plant breeders have

utilized NAM and GWAS to determine key QTLs linked to various traits in diverse crop species (Huang et al. 2009, 2012a, b; Li et al. 2011; Bandillo et al. 2013).

3.3.6.7.2 Bulk Segregate Analysis: High-Resolution QTL Mapping

The NGS-based strategies enable sequence-based mapping (SBM), which, together with bulked segregate analysis (BSA), facilitates the detection of QTLs (James et al. 2013). Bulk segregate analysis (BSA) detects the molecular marker linked with the desired trait by genotyping DNA isolated from plants at the extremes of the phenotypic distribution for a particular trait, and then bulked samples from diverse plants at each of the extremes are pooled together and utilized to map genomic regions or QTLs governing the trait (Michelmore et al. 1991). Thus, advanced sequencing approaches that facilitate whole-genome sequencing can enhance the accuracy of BSA and open a way to develop climate-smart plant species (Abe et al. 2012; Austin et al. 2014; Cuperus et al. 2010; Fekih et al. 2013). So far, bulk segregated analyses have been applied to uncover QTLs having a major effect on grain yield under drought conditions (Venuprasad et al. 2009).

3.3.6.8 Tilling and Eco-tilling: Identification of Novel Mutants in the Genomic Era

TILLING (targeted-induced local lesions in genomes) is a reverse genetic tool for quickly identifying and mapping the induced causal mutation responsible for target traits. On the contrary, ECOTILLING is one of the types of TILLING techniques used to detect natural mutation in individuals (Wang et al. 2012). TILLING populations have been generated for diverse crop plants, including wheat (Uauy et al. 2009; Chen et al. 2012), rice (Till et al. 2007; Rakshit et al. 2010), brassica (Stephenson et al. 2010), etc. A novel approach called "TILLING by sequencing" has been developed by a group of researchers in which specific genes were amplified from a pooled population representing a total of 768 individuals per experiment and then amplified genes were sequenced using NGS technology, finally leading to the identification of rare novel mutants (Tsai et al. 2011). ECO-TILLING has also been used to identify a novel drought-responsive transcription factor in rice (Yu et al. 2012). Thus, in the coming decades, TILLING or ECO-TILLING approaches will pave the way to identify useful genetic variants that have rarely been utilized to develop improved crop varieties.

3.3.7 Postgenomic Era

3.3.7.1 Application of Transgenic Approaches for Developing Drought-Tolerant Rice

In the post-genomic era, genetic engineering of drought-responsive genes has become a common strategy for dealing drought stress (Mathur et al. 2008; Hervé and Serraj 2009). Throughout the past decades, highly efficient *Agrobacterium*-mediated gene transformation method and gene gun method have been used successfully to develop transgenic drought-tolerant rice lines by

introducing drought-responsive genes in the suitable host plant. These transformation methods have been widely used to transfer more than one drought-responsive gene involved in various key processes such as posttranslational modification, signaling, and secondary metabolite production into the host plant to induce drought resistance (Yang et al. 2010). In addition to employing native rice genes, transgenic procedures allow for the exploitation of genes from diverse sources, which is impossible with traditional or marker-assisted breeding methods (Cattivelli et al. 2008). Another merit of transgenic approaches is the ability to regulate the expression of genes in specific organs or tissues at different stages of development under stress conditions by employing suitable promoters and transcription factors. The reported drought-responsive genes include *Late embryogenesis abundant* (*LEA*) proteins, *MAP kinase* (Agrawal et al. 2003), heat shock proteins, ABA, proline, organic osmolytes (Xiao et al. 2007; Sato and Yokoya 2008), *DREB* (*DREB/CBF, DREB2*), *AREB* (Dubouzet et al. 2003), *NAC* genes (Hu et al. 2006; Leung 2008), calcium-dependent protein kinase (Saijo et al. 2000), and trehalose (Garg et al. 2002) which show different levels of expression under drought stress. Many studies have mentioned that selecting a better combination of insert genes and promoters is efficient for greater expression of the transferred genes. Transgenic rice varieties developed by inserting specific drought-responsive genes enhanced drought resistance in rice; nevertheless, genes introduced with a specialized inducible drought promoter showed better performance than constitutive promoters. The *DREB1A* gene performed better with inducible promoter *rd29A* instead of constitutive promoter CaMV 35S (Kasuga et al. 2004).

Similarly, *AtDREB1A* genes inserted with drought inducible promoter *OsHVA22P* overexpressed under drought stress and influenced better drought tolerance in rice. It has been possible to discover and comprehensively characterize different types of drought-responsive genes and regulatory factors in rice through transgenic approaches. However, their steady expression and consistent phenotypic traits under different stress conditions remain a major challenge. Furthermore, before being released for cultivation, transgenic rice plants must undergo rigorous testing and biosafety laws, which may cause the process of commercialization to be delayed. These major drawbacks need to be addressed immediately to make transgenic methods a promising approach for developing drought-tolerant rice (Yang et al. 2010; Bhatnagar-Mathur et al. 2008).

3.3.7.2 Genome Editing Methods

In the post-genomic era, genome editing methods have emerged as a promising tool, expanding the potential for crop improvement. Recently, CRISPR (clustered regularly interspaced short palindromic repeats) with CRISPR-associated protein Cas9 (CRISPR-Cas9) has developed as a novel genome editing tool. This genome editing technique is simple and easy in comparison to other genome editing tools such as TALEN (transcriptional activator-like effector nuclease) and ZFN (zinc finger nuclease) (Miao et al. 2013; Cong et al. 2013; Ma et al. 2015). CRISPR-Cas9 is cost-effective and highly accurate for multiplex genome editing that allows the manipulation of multiple genes at several genomic regions (Wang et al. 2017).

CRISPR-Cas9 is one site-specific nuclease (SSN) type that cleaves double-stranded bonds (DSB) at a specific site. This DSB is then repaired by natural repair machinery either through nonhomologous end joining (NHEJ) or homologous recombination, thus resulting in changes in the genomic regions, gene insertion, deletion, gene replacement, and gene knockout of targeted genes. CRISPR-Cas9 system has been successfully employed in rice breeding programs because of its simplicity and adaptability (Xu et al. 2016). A study reported that overexpression of transcription factor *OsNAC14* made the rice tolerant to drought stress during the early growth stage. Similarly, field trials revealed a high grain filling rate and more number of panicles in transgenic rice lines with overexpressed *OsNAC14* in comparison to non-transgenic ones under drought stress. Later, it was demonstrated that CRISPR-Cas9 induced overexpression of *OsNAC14*, which specifically regulates the expression of *OsRAD51A1* and controls the expression of other downstream target genes for defense-related DNA repair strigolactone biosynthesis and stress response, which together improve drought tolerance in rice.

3.3.7.3 Epigenomics for Drought Tolerance

Drought is one of the major abiotic stresses encountered by crops and other plants, resulting in significant yield loss. Plants that have been constantly subjected to drought stress can save themselves by altering their physiological and developmental process through changes in the whole genome expression. In this regard, epigenetics plays a key role in altering genome-wide expression via switching on/off machinery in a specific tissue or the growth stage of plants undergoing drought stress. Epigenetics deals with the study of epigenomes which could be defined as the combination of all the biochemical changes that occur in DNA, polypeptides, and small noncoding RNA of a cell. The branch of the genomic platform that addressed all these epigenetic alterations that happen in a cell in response to different internal and external environmental stresses is outlined as epigenomics. Until now, researchers have made tremendous progress in understanding the different metabolic and signaling pathways that occur in stressed plants at the molecular level (Kumar et al. 2019; Ku et al. 2018). Activation of the signaling pathway leads to transcriptional reprogramming and regulates the expression of dominant stress-responsive genes (Kim et al. 2019; Shahid 2019). The transcriptional adjustment and regulation of stress-responsive genes are primarily based on several epigenetic changes, including DNA methylation, histone modification, and long noncoding RNA-based regulation (Kim et al. 2017). Recently, it has been reported that different types of histone modification, including *H3K4me3*, *H3K27me3*, *H3K9me2*, *H3K9 acetylation*, *H3K23 acetylation*, *H3K27ac*, and *H4 acetylation*, together with DNA methylation act as regulators of stress-responsive gene expression in response diverse abiotic stress such as drought, salinity, and cold and heat stress (Luo et al. 2012). The different patterns of DNA methylation in response to drought condition are studied in drought-susceptible lowland and drought-tolerant upland rice varieties. The drought-susceptible rice variety "IR20" showed hypomethylation under drought stress, whereas the drought-tolerant varieties "Paiyur" and "PMK3" showed hypermethylation. These different DNA

methylation patterns were considered the key reason behind different expression levels of drought-responsive genes. In the post-genomic era, different techniques, including Chip-sequencing (Chip-seq), chromatin immunoprecipitation (Chip), and shotgun bisulfite sequencing, have revealed chromatin modification primarily at histone protein modification which leads to alteration in the expression of drought-responsive genes. Thus, it is imperative to focus on the epigenome profile such as DNA methylation, histone modification, long noncoding RNAs, and the three-dimensional genomic structure of rice to develop drought-tolerant rice cultivars (Liu and He 2020).

3.4 Present Status of Breeding Rice for Drought Tolerance

Breeding for drought tolerance in rice has always been one of the superior objectives among the rice breeders dealing with the water scarcity problem. In the past decades, molecular breeding approaches for developing drought-tolerant rice cultivars were carried out at the International Rice Research Institute (IRRI) (Sandhu and Kumar 2017; Kumar et al. 2017). The primary goal of the Rainfed Rice Breeding (RRB) program at the International Rice Research Institute (IRRI) is to develop drought-tolerant high-yielding lines with improved quality and then release them for cultivation among the farmers. Under this RRB program, many drought-tolerant high-yielding varieties have been developed from IRRI through direct selection for grain yield (Kumar et al. 2014; Bhandari et al. 2020; Sandhu and Kumar 2017; Dar et al. 2020). However, grain yield's polygenic nature has always been a significant challenge for developing improved drought-tolerant varieties. Despite these challenges, IRRI has consistently worked toward developing drought-tolerant rice varieties and disseminating them to farmers for cultivation in Africa and Asia-Pacific regions. One of the most successful research programs, the STRASA (Stress-Tolerant Rice for Africa and South Asia) project (2005–2019) launched at IRRI, has developed and released around 30 high-yielding drought-tolerant varieties in African and Asian countries for farming. Under the STRASA project, rice breeders have successfully introgressed major drought-tolerant QTLs in the background of high-yielding popular rice varieties such as IR64, TDK1, and Swarna (Venuprasad et al. 2009; Mishra et al. 2013; Sandhu et al. 2014, 2019, 2021; Henry et al. 2015, 2019; Bhandari et al. 2020; Yadav et al. 2021; Bernier et al. 2007; Vikram et al. 2011a, b; Majumder et al. 2021). The Indian Institute of Rice Research (IIRR) has developed several drought-tolerant rice cultivars, including DRR Dhan 42, DRR Dhan 43, and DRR Dhan 44 in India and released them for field trials. The multilocation field trials revealed that the average productivity of these released drought-tolerant varieties is 1.0–1.5 tons per hectare more than drought-susceptible varieties. The following are the list of drought-tolerant varieties developed so far (Table 3.2).

Table 3.2 List of drought-tolerant varieties released in different countries using molecular approaches

Sl. no.	Name of variety	Ecosystem	Released country	Released Year
1.	Sahod Ulan 1	Rainfed lowland	Philippines	2009
2.	Sahbhagi Dhan	Rainfed lowland	India	2010
3.	BRRI Dhan 56	Rainfed lowland	Bangladesh	2011
4.	Sookha Dhan 1	Rainfed lowland	Nepal	2011
5.	Sookha Dhan 2	Rainfed lowland	Nepal	2011
6.	Sookha Dhan 3	Rainfed lowland	Nepal	2011
7.	Katihan 1	Upland	Philippines	2011
8	Tarharra 1	Rainfed lowland	Nepal	2009
9	Sahod Ulan 3	Rainfed lowland	Philippines	2011
10	Sahod Ulan 5	Rainfed lowland	Philippines	2011
11	Sahod Ulan 6	Rainfed lowland	Philippines	2011
12.	Inpago Lipi GO1	Upland	Indonesia	2011
13.	Inpago Lipi GO1	Upland	Indonesia	2011
14	CR Dhan 201 (IET 21924)	Aerobic	India	2013
15.	CR Dhan 202 (IET 21917)	Aerobic	India	2013
16.	CR Dhan 203 (IET 21920)	Aerobic	India	2013
17.	CR Dhan 204 (IET 21922)	Aerobic	India	2013
18.	DRR Dhan 43 (IET 22080)	Irrigated	India	2013
19.	CR Dhan 40	Upland	India	2012
20.	Sahod Ulan 12	Rainfed lowland	Philippines	2013
21.	M'ZIVA	Rainfed lowland	Mozambique	2013
22.	DRR Dhan 44	Rainfed lowland	India	2014
23.	Katihan 2	Upland	Philippines	2014
24.	BRRI Dhan 71	Rainfed lowland	Bangladesh	2015
25.	Swarna Shreya	Rainfed lowland	India	2015
26.	Sahod Ulan 15	Rainfed lowland	Philippines	2015
27.	Sahod Ulan 20	Rainfed lowland	Philippines	2015
28.	MPTSA	Rainfed lowland, irrigated	Malawi	2015
29.	ATETE	Rainfed lowland, irrigated	Malawi	2015
30.	CAR14	Rainfed lowland, irrigated	Cambodia	2015
31.	Identified	Rainfed lowland	Philippines	2016
32.	CR Dhan 801	Rainfed lowland	India	2017
33.	Baghuguri Dhan 1	Rainfed lowland	Nepal	2017
34.	Baghuguri Dhan 2	Rainfed lowland	Nepal	2017
35.	Rajendra Neelam		India, Bihar	2017

Source Sandhu et al. (2019) (modified)

3.5 Conclusion and Future Perspective

Breeding for drought tolerance is one of the provocative tasks that require a comprehensive understanding of various physiological, morphological, biochemical, and molecular characteristics. While considerable progress has been made in marker-assisted breeding, there are still many challenges for drought-tolerant molecular rice breeding. Maintenance of yield in rice under drought stress is not an easy task owing to its complexity. In this regard, different approaches of the post-genomic era, including genetic engineering and genome editing (CRISPR-Cas9, ZFN, and TALEN), play a superior role in enhancing rice yield and other secondary characteristics. These modern approaches would be effective ways to accelerate breeding programs to develop high-yielding drought-tolerant rice varieties. So far, several genes related to drought tolerance have been characterized under laboratory conditions. Thus, it is also urgent to know the effect of these genes under drought in field conditions. Thus, conventional breeding, genomic-assisted breeding, different bioinformatics tools, and transgenic approaches are now providing a comprehensive approach to improving drought tolerance in rice. Combining all these strategies may pave the way to resolve the future need of farmers in drought-prone areas (Fig. 3.3).

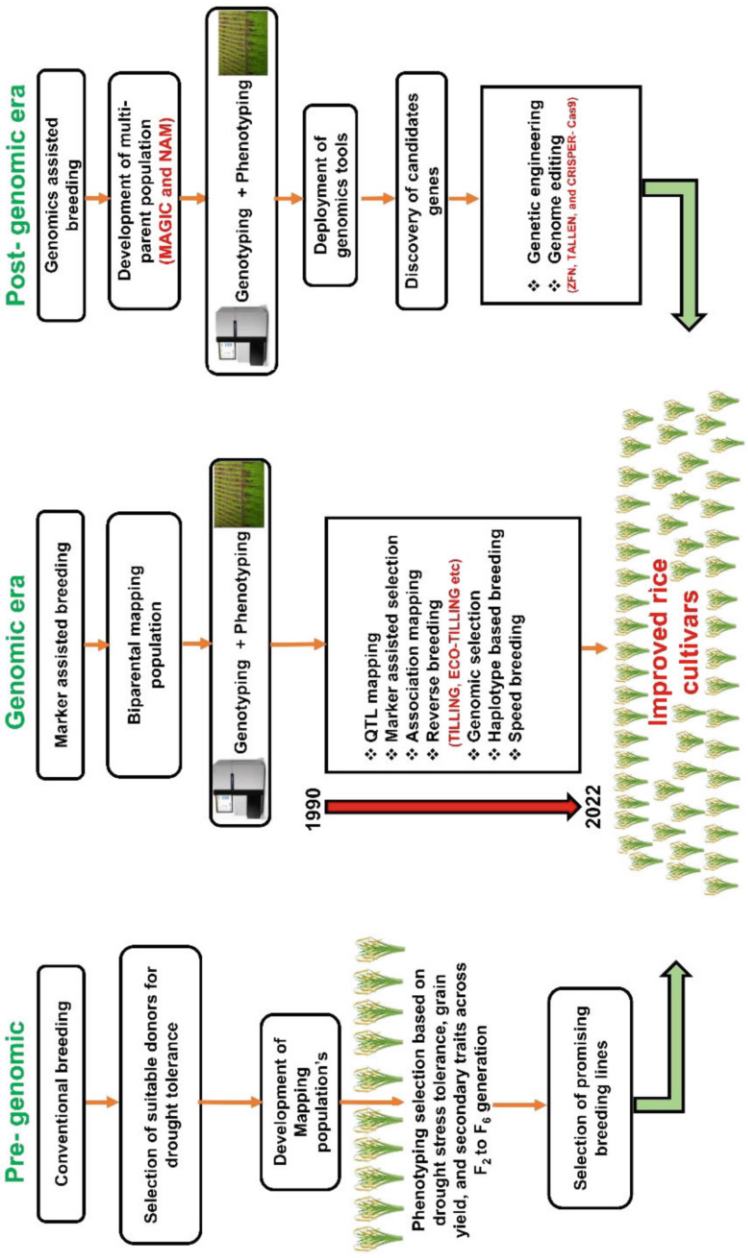

Fig. 3.3 Different breeding techniques from pre-genomic, genomic, and post-genomic eras used for crop improvement

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Augmenting Salinity Tolerance in Rice Through Genetic Enhancement in the *Post-genomic Era*

4

Sanchika Snehi, Santosh Kumar, Sanket R. Rathi, and Nitish Ranjan Prakash

Abstract

Rice is a prime dietary cereal of almost 90% of Asian population and is grown in more than 110 countries. Soil salinity is a major challenge in rice cultivation across the world. More and more land is becoming saline in coastal and inland areas due to irrigation with saline ground water, inherent salt in the parent material of soil, excessive use of fertilizers and chemicals, sea water intrusion, and erratic rainfall. Therefore, a crop with enhanced tolerance to salinity can withstand the situation of high salinity and is a promised approach to manage crop cultivation in such areas. Genetic enhancement of rice to such increased salt content at both seedling and reproductive stage can be sourced from several landraces, wild relatives, and germplasms. Novel genetic approaches such as genome-wide association studies (GWAS), QTL mapping, allele mining, candidate gene prediction, and marker-assisted gene tagging have been applied to identify, isolate, validate, and characterize genomic loci governing salinity tolerance in rice. Next-generation breeding strategies, including marker-assisted selection (MAS), have been deployed to transfer salt-tolerant QTL (*Saltol*) into susceptible cultivars. In the present chapter, we have critically described the physiological, biochemical, and genetic basis of salinity tolerance in rice. The breeding approaches utilizing several methodologies for evaluating genotypes

S. Snehi · S. R. Rathi
Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

S. Kumar
ICAR-Indian Agricultural Research Institute, Hazaribagh, Jharkhand, India

N. R. Prakash (✉)
ICAR-Central Soil Salinity Research Institute, Regional Research Station, Canning Town, South 24 Parganas, West Bengal, India

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under salinity for practicing selection have also been described. The recent success of genomic-assisted breeding and future proposed use of advanced breeding methods such as genomic selection and haplotype selection has been mentioned in detail.

Keywords

Salinity tolerance · Rice breeding · Coastal salinity · Genomic-assisted breeding

4.1 Introduction

Globally, rice is one of the most commonly consumed grains; hence, it plays a pivotal role in supplying the daily dietary requirement for more than half of the world's population and nearly 90% of the Southeast Asian population. Rice is grown mainly in South and Southeast Asia, but its area is now increasing in others parts of the world where it was not grown prior. The rice is exposed to several abiotic factors, including salinity, submergence, drought, etc., severely reducing its production and productivity. Salinity is a devastating abiotic factor that significantly reduces plant germination, growth, and productivity (Safdar et al. 2019). The rice plant exhibits a variable degree of sensitivity toward salinity. The most sensitive stage is the early seedling stage and late reproductive stage, while the germination stage shows some degree of tolerance toward salinity (Zeng et al. 2001; Roy et al. 2019). Saline soil is the one that contains a higher concentration of soluble salts of sodium chloride, sodium sulfate, and calcium chloride with pH less than 8.2, electrical conductivity of 4 dSm^{-1} or more, and exchangeable sodium content lower than 15% (Ghassemi et al. 1995; Chinnusamy et al. 2005). Soil salinity occurs due to both natural and anthropogenic processes (Hussain et al. 2017). It may result from weathering of rocks possessing a luxuriant proportion of harmful salts, disproportionate irrigation, continuous application of saline groundwater, silting of sea salt via airstream and rain near the coastal regions or flooding of coastal areas by tidal water, deforestation, shifting cultivation, and irrational use of agrochemicals (Shrivastava and Kumar 2015; Upadhyay et al. 2020).

Globally, it is estimated that around 4.4% of topsoil (0–30 cm) and more than 8.7% of subsoil (30–100 cm) of the total land area of 118 countries is salt-affected. A total of 424 Mha of topsoil is salt-affected, 85% of which are saline soil, 10% belongs to sodic soil, and the remaining 5% comes under both saline and sodic soils. In addition, 833 Mha of subsoil are salt-affected, 62% of which are saline, 24% are sodic, and 14% comes under both saline and sodic soils (Dasgupta et al. 2015; FAO 2021). Further, it is estimated that more than 50% of the arable land will be converted into saline land by the year 2050 if proper ameliorative measures are not taken to control it (Jamil et al. 2011; Khan et al. 2020). Rice is grown in around 120 countries, but China and India account for more than 50% of the global rice production. Furthermore, nine of the top ten rice-cultivating nations globally are in Southeast Asia, where the salinity problem is widespread in around 20% of the area

Table 4.1 Area distribution of salt-affected soils in different states along with the number of districts in each state of India

S. no.	State	No. of salt-affected district	Saline soil (Mha)	Coastal saline soil (Mha)	Alkali soil (Mha)	Total (Mha)
1.	Gujarat	15	71.2	37.1	14.3	32.9
2.	Uttar Pradesh	40	1.3	–	35.6	20.3
3.	Maharashtra	18	10.4	0.6	11.2	9.0
4.	West Bengal	4	–	35.4	–	6.5
5.	Rajasthan	29	11.4	–	4.7	5.6
6.	Tamil Nadu	12	–	1.1	9.4	5.5
7.	Andhra Pradesh	16	–	6.2	5.2	4.1
8.	Haryana	17	2.9	–	4.8	3.4
9.	Bihar	26	2.8	–	2.8	2.3
10.	Punjab	16	–	–	4.0	2.2
11.	Karnataka	9	0.1	–	3.9	2.2
12.	Orrisa	7	–	11.8	–	2.2
13.	Madhya Pradesh	25	–	–	3.7	2.1
14.	A & N islands	3	–	6.2	–	1.1
15.	Kerala	6	–	1.6	–	0.3
16.	Jammu and Kashmir	NA	–	–	0.5	0.3
	Total	**100 (243)**	**100 (1.71)**	**100 (1.25)**	**100 (3.78)**	**100 (6.74)**

The figure in parenthesis shows a total area in million ha. (Source: Compiled from Mandal et al. 2018)

accounting for up to 52 Mha (Vinod et al. 2013; Mandal et al. 2018). In India alone, 2.96 Mha of land is affected by saline and coastal saline soils, while 6.74 Mha is salt-affected soil, which accounts for around 2.1% of the total geographic area (Arora and Sharma 2017) (Table 4.1).

Salinity has a remarkable influence on agriculture, ecology, and the environment. Salinity leads to degradation of agricultural lands, conversion of highly fertile and prolific land into uncultivable and wasteland, leading to lesser agricultural productivity, contraction of cultivable agricultural lands, and ravaging soil flora and fauna (Kumar and Sharma 2020). Salinity impairs plant growth and alters several biochemical and physiological processes within the plants (Roy et al. 2019; Goyal et al. 2021). Surface salt accumulation negatively affects rice cultivation in two ways: First, salinity reduces the plant's ability to absorb water and nutrients from the soil because of stunted root growth, referred to as osmotic stress, which in turn hinders ion transport to other parts of plants such as leaves, shoots and affects metabolic

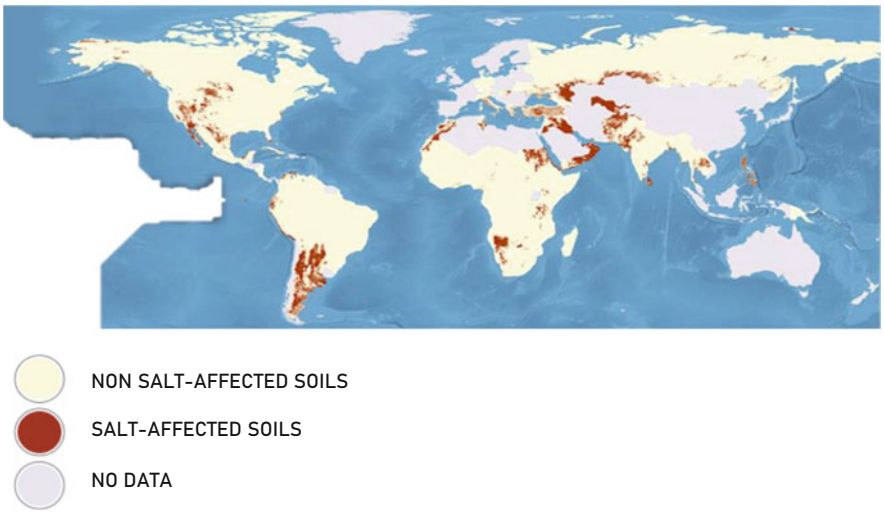

Fig. 4.1 Distribution of salt-affected topsoil across the globe (source: FAO report on Global map of salt-affected soils, 2021)

changes in plants (Munns 2005; Roy et al. 2019). Second, if there is excessive deposition of ions in the transpiration stream, particularly Na^+ ions, it may lead to a reduction of nutrient uptake of potassium and calcium, absorption of CO_2, and other metabolic changes such as accumulation of excess reactive oxygen species and damage to the cell membrane, referred to as ion toxicity (Sahi et al. 2006; Kumar and Sharma 2020) (Fig. 4.1).

This severely affects the rice plant yield by reducing several yield components such as the number of effective tillers/plants, panicle length, fertile spikelet, and seed set percentage, along with delayed flowering and reduced chlorophyll content and leaf area for photosynthesis (Vinod et al. 2013; Kumar and Sharma 2020). This, in turn, leads to a yield reduction of nearly 27–50% in rice cultivation, but in the coming days, the world will require more and more rice to fulfill the daily dietary requirement of the ever-increasing world population. There are two major ways to mitigate the detrimental effect of salinity stress: reclaiming the salt-affected soil by using bio-fertilizers and chemicals and developing and cultivating salt-tolerant varieties. The first option is practically impossible because of the large area of salt-affected soil and limited resources available to reclaim such soil. Still, the second option seems more practically possible because of the availability of a considerable amount of genetic diversity for salt tolerance among the rice germplasm. The breeder can effectively use these genetic variations to develop salt-tolerant rice varieties using several breeding methods starting from selection, hybridization, to modern-day breeding methods such as tissue culture, mutations, genetic engineering, distant hybridization, and the use of plant growth-promoting endophytic bacteria.

4.2 Germplasm for Salinity Tolerance

A large amount of genetic variability and diversity, accounting for more than 130,000 rice accessions belonging to different categories such as wild relatives, cultivated species, and related genera species, is being available and preserved at the IRRI's International Rice Gene bank (Chen et al. 2021). These rice accessions can serve as potential tools for screening rice germplasms for salinity tolerance at both the seedling and reproductive stages. The green revolution led to the development and introduction of semi-dwarf high-yielding rice varieties that replaced traditional rice landraces possessing genes for tolerance against several abiotic stresses. The screening at the reproductive stage is essential as it provides insight into the physiological mechanism underlying salinity tolerance. Still, minimal information is available for tolerance at the reproductive phase because of its intricate nature, limited availability of precise techniques for accurate phenotyping, high cost, and labor unavailability. Landraces such as Pokkali, Nona Bokra, and Horkuch are well known for their ability for salt tolerance. Still, more landraces possessing a higher degree of tolerance against salinity, particularly during the reproductive stage, need to be validated to develop salinity-tolerant varieties.

Rice is a staple crop of coastal agro-ecosystem and thus has various stress-responsive and salinity tolerant forms in these regions. Several salt-tolerant landraces are rooted from these regions with different mechanisms (Rasel et al. 2013; Hairmansis et al. 2017). India is home to many world-famous salt-tolerant landraces such as Pokkali, Cherivirruppu, Nona Bokra, Damodar, Dasal, Getu, etc. (Manohara et al. 2021). The Sundarbans region of West Bengal is home to several salt-tolerant landraces such as Talmugur, Odasal, Marisal, Darsal, Kalonunia, Dadsal, Matla, etc. (Pani et al. 2013). Similarly, Bangladesh is home to salt-tolerant rice landraces such as Horkuch, Capsule, Sona Toly, Nakraji, Komolbhog, Ghunsi, Holdegotal, Hogla, Kanchan, Vusieri, etc. (Rasel et al. 2013; Tahjib-Ul-Arif et al. 2018). In South India, Kerala is home to many salt-tolerant landraces in coastal marshy lands suitable for paddy-cum-fish farming. It includes world famous *saltol* QTL donor Pokkali along with Ayyampillypokkali, Anakodan, Cheriya Orpandy, Cherayipokkali, Elamkulampokkali, Karunagapallipokkali, Chootupokkali, Kulapandi, Kadamakudipokkali, Kozhippillipokali, Khuzhippallypokkali, Kuzhippulipokkali, Nedungodupokkali, Kuthirunellu, Oorpandy, Odachan, Orkyma, Vellapokkali, Vettakkalchettivirippu, Orumundakan (black), Vadanakkudipokkali, Pallipurampokkali, etc. (Latha et al. 2013). In Tamil Nadu, landraces such as Sornamugi Kuzhiadichan, Kallundai, Poonkar, etc., are salt tolerant (Mohanavel et al. 2021). Salt-tolerant rice landraces Kagga, Korgut, Shidde, etc., are native to Goa (Manohara et al. 2021). Coastal Karnataka is home to the landraces Kasanella, Bilithopu vadlu, etc. (Bhambure and Kerkar 2016). Maharashtra's Sahyadri coast also has many salt-tolerant landraces: Manjarvel, Malkudai, Harkhel, Vailechi, Ratal, Kilanz, Morchuka, Kalarata, Bhadas, Bhurarata, etc. (Bhambure and Kerkar 2016). Such germplasms are locally adapted and can be effectively used in dissecting novel genomic regions imparting salinity tolerance in rice. Similarly, other countries of the world have such salt-tolerant donor landraces.

It includes Moroberekan, Sadri (Iran), Pakhal, Fakhr-e-Malakand (Pakistan), Siputeh, Serendah, and Lahatan Jambu (Indonesia) as notable ones (Sabouri et al. 2008; Sakina et al. 2016; Hairmansis et al. 2017).

Apart from this, several salt-tolerant wild species related to rice are known (*Oryza nivara*, *O. australiensis*, *O. coarctata*) (Nguyen et al. 2021). Rice accessions from *Oryza sativa* and *O. glaberrima* were found to have a diverse range of genetic differences in regard to their capacity to tolerate salt in a study by Platten et al. (2013). Salinity tolerance was reported to be moderate in *O. rufipogon* and *O. nivara* (Mishra et al. 2016a). Changmaogu and Sea Rice 86 were two landraces discovered in China's coastal region that are adapted to seawater. Pokkali, a salt-tolerant rice variety, showed less tolerance to salinity during the germination stage than Changmaogu (Sun et al. 2019; Chen et al. 2017). The KKLL genome in the *Oryza coarctata* species was found to be more promising in terms of salt tolerance (Prusty et al. 2018).

4.3 Mechanism Governing Salinity Tolerance in Rice

Salinity build-up of at least 3 dS m^{-1} is detrimental to rice crop and affects severely during seedling and reproductive stages (Lutts et al. 1995). Plants show complex behavior for osmotic balancing, production of osmotic solutes, managing photosynthesis under stress, ion exchange, and stomatal regulation while exposed to a saline environment (Chen et al. 2021). Rice genotypic differences have been observed in tolerance to varying levels of salinity at different growth and developmental stages (Prakash et al. 2022). In general, plants have some mechanism to sustain growth under saline environment (glycophytes—salt haters) or mechanisms to outperform others under salinity (halophytes—salt lovers) (Munns and Tester 2008). Salt response initiates with sensing the salt accumulation, salt uptake, and salt movements within a plant and then modifying physiology to manage growth and development under these conditions.

4.3.1 Molecular and Genetic Mechanisms

Molecular players such as transmembrane proteins, intracellular signaling proteins, small RNAs, etc. sense salt stress and communicate via signaling pathways, resulting in a change in the expression of salt-responsive genes and overall cascade (Hernández 2019).

4.3.1.1 Sensing of Ions

Salt (NaCl and others) uptake results in ionic imbalances, for which cells increase Ca^{+2} levels, which act in switching the CBL-interacting kinase (CIPK)/calcineurin B-like (CBL) pathway and salt overly sensitive (SOS) signaling pathway (Martínez-Atienza et al. 2007; Qiu et al. 2002). Several rice genes (*OsSOS1*, *OsSOS2/OsCIPK24*, and *OsSOS3/OsCBL4*) are well characterized in these pathways. These

genes have a role in root K^+ uptake and Na^+ sequestration in vacuole Na^+/K^+ homeostasis (Li et al. 2014). Change of calcium concentration (Ca^{+2} level), as induced by salt uptake, is also known to regulate calcium-dependent protein kinases (CDPKs), which in turn activate downstream genes and calcium signaling pathways and thus create response to salt stress (Saijo et al. 2000). *OsCDPK7* is known to positively regulate salt response in rice. Apart from these, *OsCPK21* is reported to enhance the expression of ABA and salt-responsive genes such as *OsLEA3*, *OsNAC6*, *OsNHX1*, and *OsSOS1* (Asano et al. 2011).

4.3.1.2 Reactive Oxygen Species (ROS) Regulation

Many genes regulate hydrogen peroxide content in the cytoplasm and manage the ROS detoxification system, turgor pressure, and other metabolisms (Liu et al. 2022). Salinity-induced reduction in the rate of photosynthesis is induced by enhanced reactive oxygen species (ROS) in a cell. ROS signaling is also related to calcium-dependent signaling to maintain potassium homeostasis (Fetoni et al. 2019). Reactive oxygen species (ROS) are toxic to the cellular environment. They are responsible for the salinity-induced degradation of several cellular proteins and lipids, thus hampering the enzymatic and structural phenomenon in the cell. Thus, to maintain growth and development, plants must scavenge, degrade, or sequestrate ROS. This will lead to the maintenance of enzymatic functions, ionic homeostasis, and cellular structure and metabolism. Several genes from the ascorbate peroxidase (APX) gene family have been identified in rice which are found to have a role in peroxide (H_2O_2) scavenging, ABA accumulation, and Na^+/K^+ homeostasis (Zhang et al. 2013). These ABA-related genes are involved in regulating the expression of glutathione reductase (OsGR1, OsGR2, and OsGR3), also known for ROS scavenging (Wu et al. 2015). Similarly, mitogen-activated protein (MAP) kinase genes can sense salt stress and regulate cellular levels of toxic ROS and ethylene (Na et al. 2019). In rice, genes such as *OsMPK3* and *OsMPK6* are activated by the lectin receptor-like kinase (SIT1) gene, which is reported to mediate sensing of salt stress, ethylene accumulation, and ROS degradation (Li et al. 2014).

4.3.1.3 Regulation by Specific Transcription Factors

Transcription factors such as ABA-responsive element (ABRE)-binding factor (AREB/ABF), dehydration-responsive element (DRE) binding protein (DREB), and NAC (NAM, ATAF1/2, CUC2) family protein are known to have a role in salt response (Chen et al. 2021). DREB family transcription factors are well known for ABA-dependent regulation of salt-responsive genes, activation of ROS scavenging cellular machinery, and regulation of genes from the MAP kinase family (Wang et al. 2008). Several NAC transcription factors such as *OsNAC5*, *OsNAC106*, *OsNAC045*, and *OsNAC022* are known to regulate many genes (*OsDREB2A*, *OsbZIP23*, *OsSAPK1*, and *OsLEA3*) under salt stress having a role in ROS scavenging and ionic homeostasis (Jiang et al. 2019).

4.3.1.4 Regulation of Functional Salt-Responsive Genes

As a response to salt stress, stomatal opening and closing are affected in ABA-independent and ABA-dependent manner. Drought and salt tolerance (*DST*) is a key transcription factor in the regulation of stomatal closure in rice through regulation of peroxide (H_2O_2) homeostasis (Huang et al. 2009). This gene (*DST*) regulates several peroxidase-related genes such as *Leaf panicle 2* (*LP2*), *SIMILAR TO RCD ONE* (*OsSRO1c*), and *Prx24* through the binding sequence in their promoter regions. All these three genes are reported to regulate H_2O_2 homeostasis and stomatal closure in rice (Cui et al. 2015). Water-deficit tolerance in rice is performed via stomatal closure and is regulated by increased expression of salt and drought-inducible ring finger protein (*OsSDIR1*) (Gao et al. 2011). Reduction in stomatal density is also beneficial for salinity tolerance in glycophytic plants such as rice (Mohammed et al. 2019). Apart from this, proteins such as Aquaporins play a very different role in osmotic adjustment by regulating water transport across cell membranes. Therefore, enhanced expression of *OsPIP1;1* (a plasma membrane intrinsic protein) has a great role in imparting salt tolerance (Abdelkader et al. 2012). Osmotic adjustment is also provided by the accumulation of cell-compatible solutes, e.g., glycine betaine, proline, polyols, trehalose, etc., which are encoded and regulated by genes such as *OsTPS1*, *OsTPS8*, *OsCMO*, *OsTPP1*, *SAPK9*, *OsBADH1*, etc. (Chen et al. 2021). Ionic toxicity in plants is balanced by ionic homeostasis under salinity-induced stress, characterized by Na^+ efflux, K^+ retention, Na^+ sequestration, and Na^+ loading in the xylem. Many genes belonging to root absorption and Na^+ uptake (high-affinity K^+ transporters, HKT) and nonselective cation channels (NSCCs) are identified in rice and other plants. Many genes of the *HKT* gene family in rice (*OsHKT1*, *OsHKT2*), vacuolar Na^+ sequestration genes (OsNHX family genes), and plasma membrane Na^+/H^+ antiporter gene (*OsSOS1*) are involved in ionic homeostasis.

4.3.2 Physiological Mechanism

Although salinity tolerance is a very complex physiological phenomenon, many studies have been done so far to dissect the component trait, the interrelationship between traits, and their final contribution to the overall performance of the rice plants. Rice responds to salt stress by regulating ionic homeostasis, stomatal opening and closing, osmotic adjustments, and enhanced tissue tolerance (Reddy et al. 2017). Salinity in the root zone induces physiological drought by creating low soil-water potential and thereby decreasing stomatal conductance. The stomatal opening and closing are managed by internal ABA level and super-oxide generation (Van Zelm et al. 2020), which is responsible for a series of gene signaling. Ionic balancing of the Na^+/K^+ ratio is done through efflux/influx and transport regulation, exclusion, sequestration, and compartmentalization. These are important in balancing physiological drought, leaf expansion, stomatal function, and plant growth (Rajendran et al. 2009; Roy et al. 2014). Tissue tolerance can be enhanced through ionic sequestration, solute deposition, and enzyme detoxification (Chakraborty et al. 2020).

4.3.2.1 Plant Vigor

Vigorous crop growth will avoid the toxic effects of salts by accumulating and compartmentalizing them. Rice with faster growth and higher vigor is tolerant to salinity as higher biomass will have higher tissue volume to accumulate salts (Kumar et al. 2013). The plants at later stages can tolerate more salinity than those at the seedling stage. However, the reproduction stage in rice is very much susceptible to salinity stress as it induces pollen sterility and reproductive inviability.

4.3.2.2 Restricted Salt Entry into Plants

The salt entry into plants and absorption through root hairs are the first physiological phenomenon that happens during salt toxicity development. Plant physiological events such as ionic exclusion, the release of excess organic acids into the root zone, reduced ion exchange at root hairs, and fixation of metallic ions in the root zone are important in preventing entry of salts (Krishnamurthy et al. 2009; Kumar et al. 2013). These functions are supported by the root Na^+ exclusion mechanism in rice through enhanced growth of root cap cells. Generally, larger root cap cells are present in salt-tolerant genotypes (Ferdose et al. 2009). Rapid and faster growth and development are also important mechanisms that can dilute the impact of salinity in the root zone (Horie et al. 2012).

4.3.2.3 Intracellular Compartmentalization

In order to have uninterrupted cellular functions, plant cells must be devoid of toxic metabolites, superoxide radicals, excessive ions, etc. Therefore, the out exchange of excessive ions and active transport of ions play an important role in maintaining cellular structure and function. Excessive salts are stored in leaf sheaths and older leaves of rice plants which are less photo-synthetically active and metabolically less important. Young meristematic regions are kept out of stress by such compartmentalization (Reddy et al. 2017). Therefore, to have positive growth, rice plants must have a better rate of compartmentalization and meristematic activities in younger leaves than that of root uptake of toxic salt ions (Chakraborty et al. 2020). Vacuolar size is an important trait in governing such a mechanism as most of the toxic metabolites and ions are generally compartmentalized or inactivated in it (Kanawapee et al. 2012; Chakraborty et al. 2019). Intracellular compartmentalization in rice is also maintained by stress signaling (ethylene response, ABA response, and calcium-mediated signaling), ionic homeostasis (anti-porter/symporter/carrier proteins) (Chen et al. 2021).

4.3.2.4 Antioxidants

Salts in plant cellular environments facilitate the formation of reactive oxygen species (ROS). These ROS molecules (peroxide (H_2O_2), superoxide (O_2^-, O_2^+), hydroxy (OH^-), and singlet (oxygen) may cause toxic effects, including tissue damage, reduced photosynthesis, and metabolism enzymatic dysfunction and degradation (Kibria et al. 2017). These ROS create signaling pathways regulating ROS scavenging. Enzymes such as catalase, peroxidase, super-oxide dismutase (SOD), etc. are produced to manage ROS toxicity in the plant cell (Çelik et al. 2019).

4.3.2.5 Osmoprotectants

Osmoprotectants are soluble organic chemicals produced by plant cells in response to salt stress to manage turgor pressure, maintain ionic exchanges, and keep metabolic activity functional (Garcia et al. 1997). Several osmoprotectant genes are reported, producing molecules such as trehalose, proline, glycine betaine, mannitol, etc. Proline is the most celebrated osmoprotectant that can regulate cytosolic pH and facilitate the removal of singlet oxygen radicals (Omari Alzahrani 2021). Trehalose is an important signaling sugar that helps in enhancing metabolic and enzymatic function and maintaining the sugar content of the cell (Li et al. 2011).

4.4 Screening for Salt Tolerance

Salinity tolerance in rice is a physiologically complex phenomenon and hence requires trait dissection and accurate phenotyping for germplasm evaluation and plant breeding (Prakash et al. 2020).

4.4.1 Screening for Seedling Stage Salinity Tolerance

Visual scoring of plant physiological status, measurement of leaf photosynthetic efficiency under stress, and ionic accumulation in leaf, root, and shoots are important criteria for evaluating seedling stage salinity tolerance in rice (Prakash et al. 2022). IRRI has devised a robust protocol for assessing rice seedlings' salinity tolerance under hydroponics. The rice seedlings grown with under-supplemented nutrients and added salts are compared with unadded (control) and evaluated based on visual scoring, chlorophyll content, and ionic accumulation (Gregoria et al. 1997). The seeds are pretreated with heat to break dormancy, and treatment with salts may be given after 12–14 days of growth under unstressed conditions (Prakash et al. 2022). On every alternate day, nutrients must be changed and aeration must be maintained in the nutrient solution to maintain near natural conditions. Evaluation of genotypes must be done in reference to already known susceptible (e.g., Pusa 44) and tolerant genotypes (e.g., FL478, Pokkali, Nona Bokra, etc.). The scoring (Standard Evaluation Scoring, i.e., SES) based on visual observation can be recorded as standards given in the IRRI manual on a scale of 1–9 based on physiological and morphological observations shown in Table 4.2 (Gregoria et al. 1997). Root and shoot ionic concentration (Na^+, K^+, Ca^{+2}, Cl^-) along with root and shoot length are measured and compared between control and salinity-treated seedlings.

4.4.2 Screening for Reproductive Stage Salinity Tolerance in Rice

Salinity is able to disturb ionic exchange cell metabolic and enzymatic activities and cell turgor maintenance; hence, in the reproductive stage, where the plants are highly sensitive, floral development and photosynthetic assimilation are hampered (Ali

Table 4.2 Scoring criteria for assigning standard evaluation score (SES) for seedling stage salinity tolerance in rice (adapted from Gregoria et al. 1997)

SES score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or a few leaves are whitish and rolled	Tolerant
5	Growth is severely retarded; most leaves are rolled, only a few are elongating	Moderately tolerant
7	Complete cessation of growth, most leaves are dry, some plants are drying	Susceptible
9	Almost all plants are dead or dying	Highly susceptible

et al. 2014). The reproductive stage is more sensitive to salinity than the vegetative stage in rice. Field evaluation of rice is not very much satisfactory as effective salinity varies in patches across the field and hence may give inconsistent results. Therefore, because of the heterogeneous nature of environmental influence, it is better to evaluate them either under microplot conditions (with rain-out shelter) or under the earthen pot with maintained irrigation (as given in the IRRI manual) (Gregoria et al. 1997; Prakash et al. 2022). Twenty-five-day-old unstressed seedlings can be transferred into a saline microplot with salinization increased gradually and kept at 8 dSm^{-1} at the reproductive stage (Ahmadizadeh et al. 2016; Pundir et al. 2021). Soil and hydroponic systems can also be integrated to evaluate genotypes for both seedlings and reproductive stages (Gregoria et al. 1997). Traits such as yield, biomass, yield-contributing traits (number of tillers, panicle weight, panicle length, number of grains per panicle), spikelet fertility, pollen abortion, pollen fertility, leaf and grain sodium and potassium concentration, etc. can be observed. Standard visual observation score can also be derived. Some genotypes may show a nonsynchronous reproductive stage and hence impose difficulty in providing stress properly, making it challenging to do phenotyping (Ahmadizadeh et al. 2016). It can be overcome by evaluating the different sets of genotypes by grouping them based on duration and plant height (Ahmadizadeh et al. 2016).

4.5 Breeding for Salinity Tolerance in Rice

Rice diversity in coastal areas plays a major role in shaping the breeding for salinity tolerance as germplasms native to these areas have salinity tolerance and adaptability to saline environment along with submergence, high acid sulfate, low zinc, and marshy lands (Prakash et al. 2020). Therefore, breeding rice for salinity tolerance was attempted using classical approaches of selection, hybridization, and backcrossing. Modern-day breeding approaches, including genomic approaches such as marker-assisted selection (MAS), genomic selection (GS), and transgenic approaches have played a great role in the past decade. In today's time, post-genomic

approaches utilizing big data analysis, artificial intelligence, high-throughput genotyping, phenotyping, selection based on predictive models, and genome editing tools are set to play an important role (Snehi et al. 2022).

4.5.1 Pre-genomic Era

Breeding rice varieties for salinity tolerance has started with domestication of rice along with selection and introgressive hybridization in coastal regions of the world (Snehi et al. 2022). Almost all the landraces belonging to coastal areas of India and the world are salt tolerant and rich sources of diversity in developing salt-tolerant rice cultivars. Many of these salinity-tolerant landraces have been used in breeding programs in India and the world, such as Pokkali, Nona Bokra, Horkuch, Cherivirruppu, Odichan, Korgut, Ambemohar, etc.

4.5.1.1 Classical Breeding

As salinity is one of the most significant threats to crop productivity, the development of salt-tolerant varieties has been viewed as a crucial step toward feeding the millions of people who live in such hostile environments. Rice breeding for salinity tolerance requires dependable screening methods that have been previously described (Gregoria et al. 1997). Since ancient times, plant breeding has produced salt-tolerant productive lines. However, its use is restricted due to the multigenic nature of salt tolerance and the low genetic diversity of the most important crops. Success in conventional breeding hinges on correctly identifying a tolerant gene donor. Landraces and neglected crops display a wide range of genetic diversity and survival strategies, as well as a wide range of responses to stress (Reynolds et al. 2005). At the seedling stage, various parameters have been used to assess a multitude of rice genotypes under different salinity levels (Mishra et al. 2021). Plant breeders have utilized intraspecific, interspecific, and intergeneric genetic variation in crops to produce salt-tolerant lines. Breeding has produced numerous salt-tolerant crop cultivars and lines, such as the salt-tolerant CSR10, CSR13, and CSR27 rice cultivars developed at the Central Soil Salinity Research Institute in Karnal. More than 30 salinity-resistant rice varieties have been developed by the International Rice Research Institute (IRRI) since the 1970s through sexual hybridization. Traditional breeding methods result in rice varieties that contain unwanted DNA. Conventional breeding relies on the proper mapping/search of genes for new salt tolerant cultivars to achieve success. Finding the molecular and physiological mechanisms of enhanced ability to withstand salt may help breeders to incorporate certain desirable traits (Rajakani et al. 2019). Several breeders have bred salt-tolerant rice using hybridization, pedigree selection, recurrent selection, backcrossing, and induced mutations (Reynolds et al. 2007).

Landraces and local cultivars were primarily used to develop salt-tolerant rice cultivars through selection from the population during the 1950s and 1970s in India. Initially, varieties like CSR1, CSR2, CSR3, etc., were developed as a selection from salinity-tolerant popular landraces from coastal regions (Prakash et al. 2022).

Similarly, a bold grain high-yielding popular variety called Canning 7 is also developed through selection from local cultivars. Pedigree breeding, hybridization, and selection have led to the development of popular rice varieties such as Sabita, Lal minikit, Sada minikit, Gosaba-5, Gosaba-6, Panvel-1, Bhutnath, CST7-1, TRY1, TRY2, TRY3, etc. from various institutes in India. These genotypes are tolerant to salinity at the seedling and reproductive stages. Nowadays, people are preferring for better grain quality in rice. Therefore, in West Bengal, local varieties like Dudheshwar are popular with mild salt tolerance and excellent cooking quality preferred by local consumers (Snehi et al. 2022; Kumar et al. 2022). A list of rice varieties in India is given in Table 4.3.

4.5.1.2 Pre-breeding

Many accessions from *Oryza glaberrima* have salt tolerance. Therefore, *O. sativa* lines had been crossed with them to develop salinity stress-tolerant New Rice for Africa (NERICA) lines (Mondal et al. 2018). Similarly, efforts were also taken to use photosensitive landraces such as Pokkali and Nona Bokra in the breeding program and develop salt-tolerant pho-insensitive lines to be used in plant breeding (Prakash et al. 2020). Many *O. coarctata* lines are being utilized to attempt to cross with *O. sativa*. Although enough success has not been achieved, significant progress has been made (Latha et al. 2004; Solis et al. 2020).

4.5.1.3 Mutation Breeding

Saline-tolerant mutants have been successfully screened and confirmed through mutation breeding (Forster and Shu 2012). In wild rice germplasm, identifying the source of salt tolerance and the involvement of a candidate gene is a good example of targeting a specific mutation (Mishra et al. 2016a,b; Flowers et al. 1990). Mutation breeding can create genetic variability, and mutagenesis can support functional genomic research and lead to the development of new genotypes (Fraiture et al. 2016; Moin et al. 2017). Rice's small genome makes mutagenesis advantageous due to the need for a smaller population to provide a broader allelic series sequence (Serrat et al. 2014). After screening >270,000 EMS-mutagenized Zhonghua 11 rice seedlings of the M2 stage, the *dst* mutant was found. An important gene, *DST*, encodes a zinc-finger transcription factor that directly influences the modulation of genes involved in regulating stomatal aperture by H_2O_2 homeostasis in plant guard cells. Hitomebore having *hst1* gene responsible for salt tolerance was selected from 6000 EMS mutant lines of the local elite cultivar (Takagi et al. 2015). Rice has been mutated in several different ways through the use of random mutations to develop mutant varieties in many other countries, such as Zhefu 802 and 26 Zhaizao rice mutants in China (Wu et al. 2005); PNR-381 in India (Wu et al. 2005); Iratom-24, Binasail and Binadhan-6 mutants in Bangladesh; Amaroo in Australia (Parry et al. 2009); Basmati 370 in Pakistan (Wu et al. 2005); VND-99-1, VND 95-20 and VN121 in Vietnam; and Calrose 76 rice in the United States (Kharkwal and Shu 2009). According to a report published by the IAEA in 2003, eight salt-tolerant rice mutants have been identified. These mutants have a higher salinity tolerance than their parents. The parent Bicol has resulted in the production of six mutants, and the

Table 4.3 Varieties for salt-tolerant regions of India (Snehi et al. 2022)

S. no.	Institute	Varieties
1	SARC, Arundhuti Nagar, Tripura	AR-11
2	RARS, Bankura	Puspa, Dhiren, Sampriti, Dhruba
3	ZARS, UAHS, Brahmavar, Karnataka	KCP-1, Champaka, Phalguna
4	RARS, Chinsurah	Panke, Bhupen, Jamini, Khanika, Kiron, Puspa, Jogen, Bipasa, Sashi, Giri, Kaushalya, Kanak, Dhiren, Sujala, Sabita, Purnendu, Amulya, Sudhir, Nalini, Biraj, Suresh, Mandira, Matangini, Golak, Saraswati, Bhagirathi, Bhudeb, Hanseshwari, Ambika, Mahananda, Sunil, Jaladhi-1, Jaladhi-2, Jalaprabha, Neeraja, Jitendra, Dinesh
5	Dept. of Rice, TNAU, Coimbatore	CO43, ADT37, ADT39, TRY1, TRY2, TRY3 etc.
6	ARS, UAS (Raichur) Gangavati	Gangavati sona
7	Khar Land Research Station, Panvel	Panvel-1, Panvel-2, Panvel-3, etc.
8	RARS, Maruteru	MTU-1010, MTU 1001, MTU 1061, MTU 1075, MTU 1064, etc.
9	RRS, Moncompu, KAU, Kerala	Kallada Champavu, Kochathikkira, Karishma, Krishnanjana, Bhadra, Karthika, Makom, Uma, Revathy, Pavizham, Aruna, Remya, Kanakom, Renjini, Pavithra, Panchami, Pratheeksha, Jyothi, etc.
10	Main Rice Research Station, AAU, Nawagam, Gujrat	Dandi
11	RRS, Pattambi, Kerala	Thekkancheera, Rashmi, Mangala Mahsusri, Karuna, etc.
12	Dept. of Rice, PJTSAU, Hyderabad	Taramati
13	NRRI, Cuttack	Sarala, Luna Sankhi, Luna Swarna, Lunishree, Luna Sampad, CSR89-IR8, etc.
14	ICAR-CSSRI, Karnal	BR4-10, CSR43, CSR30, CSR27, etc.
15	RRS, KAU, Vytilla	Vytilla-3, Vytilla-4, Vytilla-5, Vytilla-7, Vytilla-8, Vytilla-9, Ezhome-1, Ezhome-3, Amritha, Jyotsana, etc.
16	ICAR-CCARI, Goa	Goa Dhan-1, Goa Dhan-2, etc.
17	Bangladesh varieties	BINA Dhan 8, BINA Dhan 10, BRRI Dhan 40, BRRI Dhan 53, etc.
18	ICAR-CSSRI, RRS, Canning	Bhutnath, CST7-1, Amalmana, SR26B, Canning7 etc.

salt-sensitive parent IR29 has produced two mutants (Hayashi et al. 2007). Irradiating the callus of the Korean rice variety Dongjinbyeo with gamma rays led to the development of salt-tolerant and salt-sensitive mutants, as described by Lee et al. (2003). In vitro anther cultures and double haploids were also used to create salt-tolerant mutants (Nakhoda et al. 2012). *Salt-hypersensitive 1* (*shs1*) mutant was

created using sodium azide, which plays a critical role in Na^+ homeostasis and antioxidant metabolism (Sathish et al. 1997). Several salt-tolerant rice varieties have been developed using gamma rays, including Emai No.9, Basmati370, A-20, Fuxuan No. 1, Changwei19, Atomita2, Shua92, Nipponbare, Mohan (CSR4). They have been released in many countries worldwide (Song et al. 2012). However, the use of mutagenesis breeding is restricted due to the randomness of mutation and problems with plant regeneration (Jaiswal et al. 2019).

4.5.2 Genomic Era

Salinity-tolerant rice cultivars can be developed through modern-day genomic tools. Several successful examples include the use of DNA-based molecular markers (SSR or SNPs) in marker-assisted selection (MAS) and genomic selection (GS). Salinity in rice has complex physiological and genetic behaviors and is hence governed by many loci contributing concomitantly. Therefore, major quantitative trait loci (QTLs) can only be selected via marker-assisted backcross breeding (Krishnamurthy et al. 2020).

4.5.2.1 Marker-Assisted Backcross Breeding

Marker-assisted selection-based improvement of several rice varieties has been made mostly using *saltol* locus (Kumar et al. 2022). *Saltol* is a major quantitative trait locus (QTL) on chromosome 1 of rice identified in salinity tolerant variety FL478 (a selection from landrace Pokkali) (Bonilla et al. 2002). This QTL explains an extraordinarily 43% of phenotypic variance for seedling stage salinity tolerance and became a major candidate for marker-assisted backcross programs for varietal improvement (Prakash et al. 2020; Krishnamurthy et al. 2020). With the fine mapping of this QTL, Ren et al. (2005) identified a major gene *SKC1* governing K^+ homeostasis in FL478 and imparting salinity tolerance. This gene works as Na^+ exporter and helps in maintaining K^+/Na^+ homeostasis in the cell in a saline environment. In coastal regions and regions where groundwater is saline, seedling stage salinity tolerance is very important in determining crop establishment and crop yield (Pundir et al. 2021). Several popular mega-rice varieties that had been improved for seedling and reproductive stage salinity tolerance are listed in Table 4.4. Some of these popular rice varieties are Pusa Basmati-1, Sarjoo52, Pusa Basmati 1121, Pusa 1509, etc. (Babu et al. 2017; Singh et al. 2018; Krishnamurthy et al. 2020). These near isogenic line (NIL) yields are on par with original varieties under stress and better under unstressed conditions. Another gene called *hitomebore salt tolerant-1* (*hst1*) had been identified in an EMS mutant line of popular japonica rice variety Hitomebore and had been used in marker-assisted backcross breeding (MABB) program to improve seedling and reproductive stage salinity tolerance (Rana et al. 2019). This EMS mutant line called "Kaijin" was used to improve the salt tolerance of "Yukinkomai" through MABB. *Hst1* (*OsRR22*) encodes a B-type response regulator (*Os06g0183100*), and a mutation in the third exon of this gene imparts salinity tolerance. Similarly, spikelet fertility under salt stress is governed by

Table 4.4 List of salinity tolerant improved cultivars in rice using genomic assisted breeding

S. no.	QTLs	Donor	Recipient	Trait	Reference
1	*Saltol*	FL478	ASS996	SSST	Huyen et al. (2012)
2	*Saltol*	FL478	BT7	SSST	Linh et al. (2012)
3	*Saltol*	FL478	Binadhan-5	SSST	Moniruzzaman et al. (2012)
4	*Saltol*	FL478	Q5DB	SSST	Huyen et al. (2012)
5	*Saltol*	FL478	BRRI dhan49	SSST	Hoque et al. (2015)
6	*Saltol*	FL478	Rassi	SSST	Bimpong et al. (2016)
7	*Saltol*	FL478	IR64	SSST	Ho et al. (2016)
8	*Saltol*	FL530	KDML105	SSST	Punyawaew et al. (2016)
9	*Saltol*	FL478	PB1121	SSST	Babu et al. (2017)
10	*Saltol*	FL478	Pusa Basmati-1	SSST	Singh et al. (2018)
11	*hst1*	Kaijin	Yukinko-mai	SSST and RSST	Rana et al. (2019)
12	*Saltol*	FL478	Improved WP	SSST	Valarmathi et al. (2019)
13	*Saltol*	FL478	Pusa44	SSST	Krishnamurthy et al. (2020)
14	*Saltol*	FL478	Sarjoo52	SSST	Krishnamurthy et al. (2020)
15	*Saltol*	Pokkali	RD6	SSST	Thanasilungura et al. (2020)
16	*Saltol*	FL478	PB 1509	SSST	Yadav et al. (2020)
17	*Saltol*	FL478	Aiswarya	SSST	Nair and Shylaraj (2021)

SSST seedling stage salinity tolerance, *RSST* reproductive stage salinity tolerance

a major QTL called *qSSISFH-8.1* in variety CSR27, and this QTL has been used nowadays in the MABB breeding program. However, no rice varieties have been developed using this QTL (Pandit et al. 2010).

4.5.2.2 Marker-Assisted Recurrent Selection

Marker-assisted recurrent selection (MARS) is a powerful approach to amalgamating the trait found in diverse germplasm and is important in co-augmenting multiple loci contributing to the trait of interest. In rice, MARS has been used in developing drought- and salt-tolerant lines of IR58025B. This line is a B-line (maintainer line) of three-line breeding system for hybrid rice and is very popular in the Indian hybrid rice breeding program (Suryendra et al. 2020). Under this scheme, salinity-tolerant QTLs from FL478 were introgressed into IR58025B to develop seedling stage salinity tolerance.

4.5.2.3 Genomic Selection

Genomic selection offers an enormous opportunity to dissect the genetics of a complex trait like salinity tolerance, assess the genetic worth of a large set of genotypes in real time, identify component traits, and develop superior breeding cultivars (Ahmadi et al. 2020). Genomic selection is the development of a prediction model based on extensive genotyping and phenotyping of individuals of the training population (a diverse population used to train the predictive model) and estimating the genetic worth of alleles. Based on this, allelic worth the phenotypic performance

of the genotypes is predicted using a prediction model based on genomic estimated breeding value (GEBV). The genetic worth of any germplasm, individuals from segregating generations, advanced breeding lines, or genetic stock, can be estimated using this (Ahmadi et al. 2020). Genomic selection is being assisted by the availability of enormous genomic resources in the public database, which helps in precisely selecting markers and identifying genes and allelic forms (Choudhary et al. 2019). Genomic selection for salinity tolerance in rice can be applied using two approaches: (1) targeted haplotype-based approach (local GS) and (2) whole genome-based approach (global GS). Whole genome-based genomic selection utilizes whole genome genotypic data to predict the genomic selection model and predict the germplasm's genetic worth. Under the targeted approach, the identified major QTLs are mined for various alleles and haplotypes present in the training population, and their effects are predicted in the genomic selection model. Such an approach is also called haplotype-based genomic selection (Haplo-GS). It can be chartered to customize rice variety with suitable alleles at all the targeted loci with salinity tolerance at seedling and reproductive stage (Sinha et al. 2020). The availability of publicly available genome sequence and variant data of the 3k-rice genome project has helped breeders worldwide design genomic selection-based breeding strategies for salinity tolerance in rice. However, a fruitful outcome in terms of variety is yet to come, but the approach is found to be promising in improving other traits in rice (Kumar et al. 2022). Nowadays, it is proposed to integrate speed breeding with the genomic selection-based strategy to handle a larger set of segregating generations and rapid generation advancement to develop rice varieties with precision and targeted breeding strategy (Snehi et al. 2022).

4.5.2.4 Genomic-Assisted Population Improvement

Wild relatives of rice, such as several accessions of *Oryza nivara*, *Oryza brachyantha*, *Oryza coarctata*, etc., are known to confer salt tolerance (Flowers et al. 1990). Several studies have been carried out to identify the genetics of salt tolerance in these species and find ways to introgress these genes/QTLs into domesticated rice (Prusty et al. 2018; Yichie et al. 2018). Mondal et al. (2018) reported the whole genome sequence of *Oryza coarctata*, a halophyte relative to rice and can be a potential donor for salt tolerance in rice varieties. Attempts are being made to introgress salinity-tolerant genes from this species to *Oryza sativa* using conventional biotechnological approaches. Presently, genomic approaches are also being used to develop the multiparent advance generation intercross (MAGIC) population to simultaneously map and utilize major QTLs for breeding programs (Ganie et al. 2021).

4.5.3 Post-genomic Era

Understanding salinity tolerance at molecular and genetic levels in diverse organisms has given many handy tools and techniques to precisely play with DNA and protein and thus is helping the breeding for salinity tolerance in rice. Transgenic

methods have come a long way to utilize any gene conferring salinity tolerance from any organism to be transferred to rice varieties (Ganie et al. 2021). Similarly, genome editing tools, epigenomic profiling (epigenetic behaviors and epigenetic QTLs), and precision phenotyping are also helping breeders and will pave the way for futuristic plant breeding in rice.

4.5.3.1 Genetic Engineering

Modern plant breeding has benefited dramatically from genetic engineering. A gene of interest can be introduced into elite cultivars without sacrificing desirable features. An agrobacterium-mediated transformation technique has significantly contributed to rice genetic improvement (Liao et al. 2016). Genetic modification for salinity tolerance focuses on genes that encode transcription and signal transduction factors, heat-shock proteins, compatible organic solutes, programmed cell death, ROS detoxification, and ion transport (Liao et al. 2016). In addition, all known techniques for coping with salinity have been used in genetic modification to improve rice salinity tolerance. Salt tolerance in rice is enhanced by the expression of salt-responsive genes, such as *phosphatase 1a* (*OsPP1a*). In transgenic lines, *OsPP1-2*, *OsPP1-3*, and *OsPP1a-6*, upregulation of the *nRK1A*, *OsNAC5*, and *OsNAC6* genes has also been observed (Amin et al. 2016). The accumulation of Na^+/H^+ in shoots and roots of transgenic rice exemplifies salinity tolerance. Landrace Pokkali derived *OsNHX1* genes, which are overexpressed in transgenic rice to increase the grain's tolerance to salt (Chen et al. 2007).

Increased *OsNHX1* gene expression increased the biomass production of shoots and roots and improved germination (Wang et al. 2016). Transgenic rice with elevated salinity, drought, and cold tolerance was found to have trehalose-6-phosphate synthase overexpression associated with the *OsTPS1* genes. Reduced wilting and maintenance of photosynthetic activities in transgenic rice also increase the accumulation of compatible solutes (Lan et al. 2019). The *PtCYP714A3* gene promotes active tillering in transgenic rice, which results in smaller seeds and semi-dwarfed phenotypes. *PtCYP714A3* plays an important role in rice shoot salinity responses, and these findings demonstrate the importance of molecular foundation in transgenic rice research (Li et al. 2016). Overexpression of *SIDP361* gene has been shown to increase rice tolerance to salinity at both the seedling and reproductive stages in rice (Sahoo et al. 2014). Using wild rice (*Oryza coarctata*), a plant native to Bangladesh, India, and Myanmar, researchers have created salt-tolerant transgenic rice. The transgenic approach has resulted in numerous salinity-tolerant rice cultivars, but none of them have been released to farmers for commercial cultivation. Transgenic rice production procedures make it difficult to expand these rice varieties but may be released for commercial cultivation in the future.

4.5.3.2 Genome Editing

Plant genomics has been revolutionized by targeted genome editing to improve the plant's resistance to biotic and abiotic stresses (Huang et al. 2020). Genome editing can be used to create new rice varieties that are more resistant to abiotic and biotic stressors, resulting in increased yield and quality. It's been widely used in rice,

showing great promise in producing desired changes in response to biotic and abiotic stress (Mishra et al. 2018). Several rice genes have been successfully edited using the CRISPR-Cas9 method, including the *MYB* family genes, editing of the *OsSPP* gene for early seedling leaf chlorosis, *OsMYB1-OsMYB5*, *OsMSH1*, and the photoperiod sensitive male sterility-responsive gene, *OsPMS3*. A Cas9-OsRR22-gRNA expressing vector was engineered to edit the targeted gene *OsRR22*, resulting in improved salt-tolerant rice (Shao et al. 2017). CRISPR/Cas9 and other genome editing methods have been used to modify several genes in rice to increase their tolerance to salt (Das et al. 2015). *OsPIN5b*, *GS3*, and *OsMYB30* genes were simultaneously edited using the CRISPR/Cas9 system, and the resulting rice mutants showed excellent cold tolerance and high grain yield (Zhang et al. 2019). Drought and salinity tolerance in rice variety MTU1010 was also enhanced by editing the *drought and salt tolerance* (*DST*) gene and creating a 366 bp deletion mutant which enhanced chlorophyll retention and physiological activities during salt stress (Santosh Kumar et al. 2020). It is clear that genome editing techniques like CRISPR-Cas9 have a huge potential as an accurate, promising, and effective technique for improving more traits based on these successful applications of CRISPR-Cas9 techniques (Zeng et al. 2020). However, salt-resistant rice must be improved by editing genes for salinity tolerance. Genome editing can take advantage of functionally relevant SNPs found in GWAS studies (Shan et al. 2013).

4.6 Smart Breeding Strategies for Salinity Tolerance in Rice

Advancements in genomics must be utilized along with excellent modern phenotyping methods using artificial intelligence, machine learning, and big data analytics (Prakash et al. 2020). Modern-day salinity breeding may utilize hyperspectral imaging based on plant phenotypic scoring in real time and determine the correlation of such image-based data with photosynthetic efficiency, plant vigor, plant growth, ionic content, and other physiological conditions (Pabuayon et al. 2021). Salinity creates a multitude of stress on the plant, impacting many physiological phenomena that generally coincide with other stresses and creating confusing effects. Modern-day phenotyping can help decipher novel component trait for salinity (such as NDVI for drought) and utilize them to predict overall performance under stress (Prakash et al. 2020). Genomic selection will play a vital role in days to come as genotyping has become cheaper day by day and is now easily accessible to all breeders, along with robust advancements in phenotyping. The present advancement in genomics and phenomics can significantly improve breeding rice cultivars with salinity tolerance (Fig. 4.2).

- Precision phenotyping: This can be achieved with better control of experimentation and increasing the multitude of experimentation. A larger area can be handled for experimentation and managed effectively under homogenous conditions using modern agronomic tools.
- Big data analysis: Image-based phenotyping and NGS-based genotyping generated a huge volume of data which must be handled with robust

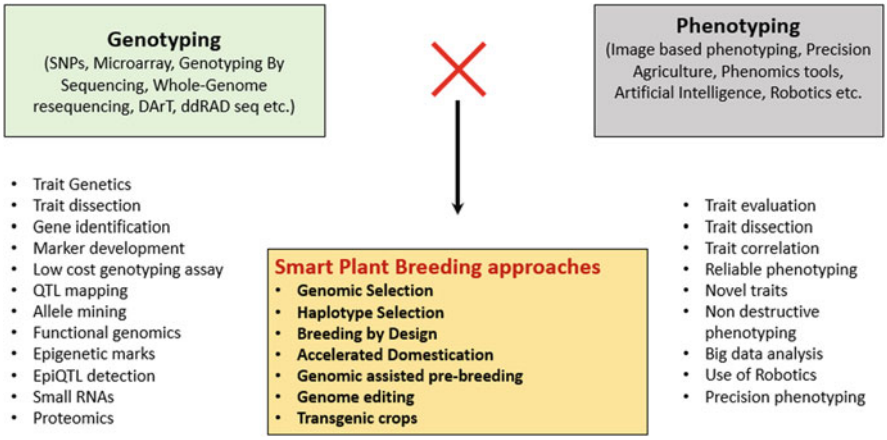

Fig. 4.2 Graphical display of smart breeding activity for salinity tolerance in the post-genomic era

computational tools and techniques. This will help breeding salt-tolerant rice cultivars through trait evaluation and mapping genes and QTLs.

- Next-generation sequencing: Modern sequencing methods are very robust and accurate, leading to accurate dense genotypic data of individuals, thus making genetic mapping, GWAS, genomic selection, and gene editing an easy task.

4.7 Challenges in Breeding Salt-Tolerant Rice

Breeding in the present-day post-genomic era has challenges in completing the following desired objectives:

- Identification of the novel source of tolerance in rice
- Understanding the molecular interplay of different physiological activities during salt stress
- Functional validation of identified genes under a stressed environment
- Administrative issues pertaining to the release of transgenic varieties
- Climatic abnormalities and problems in imitating field conditions
- The interplay between various stresses
- Understanding the long-term interplay between different stresses
- High cost of precision phenotyping

4.8 Conclusion

Salinity tolerance is one of the important abiotic stresses and affects rice crop significantly as rice is a staple crop in coastal areas. Rice in river basins is affected mainly by inland salinity from salty irrigation water. The development of salinity

tolerant rice varieties is the best promising and economical solution to manage salt stress. Concerted breeding efforts in the past 100 years in India started with classical breeding to present-day genomic-assisted breeding has yielded many salt-tolerant varieties, which were very popular. Proper genetic and physiological understanding of salinity tolerance in rice has been planned and resulted in understanding the molecular mechanism of sodium exclusion in Pokkali. It has helped in breeding using *saltol* QTL. Marker-assisted breeding (MAB) has come up in a very big way to develop salinity-tolerant NILs of popular rice varieties such as PB1, PB1121, Sarjoo52, etc. Modern-day genomic selection and haplotype-assisted breeding have been planned and are yet to give any proper variety. Although posed with various challenges, breeding salinity-tolerant rice in the post-genomic era is blessed with a better understanding of component traits, easy genotyping, and robust phenotyping.

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5 Understanding Heat Stress-Induced Morpho-Phenological, Physiological and Molecular Modulations in Wheat for Improving Heat Stress Tolerance

Surinder Paul, Ratan Tiwari, Joginder Singh Duhan, and Poonam Kumari

Abstract

Wheat is one of the most important cereal crops cultivated and consumed worldwide. In the current changing climate scenario, ever-increasing environment temperature is one of the major abiotic factors affecting worldwide wheat production. Severe reduction in the produce quality occurs when wheat faces elevated temperature conditions. Thus, it is important to elucidate the mechanisms of heat stress response at morphophysiological and molecular levels. As wheat possesses one of the most complex and largest genomes among plant kingdom, the molecular studies using advanced next-generation sequencing (NGS) technology-based omics studies, including genomics, transcriptomics, proteomics, metabolomics and micromics, have proven to be a reliable, accurate

S. Paul (✉)
Chaudhary Devi Lal University (CDLU), Sirsa, Haryana, India

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

ICAR-National Bureau of Agriculturally Important Microorganism (NBAIM), Maunath Bhanjan, Uttar Pradesh, India

ICAR-Indian Grassland and Fodder Research Institute (IGFRI), Himachal Pasturelands, Palampur, Himachal Pradesh, India
e-mail: surinder.paul@icar.gov.in

R. Tiwari
ICAR-Indian Institute of Wheat and Barley Research (IIWBR), Karnal, Haryana, India

J. S. Duhan
Chaudhary Devi Lal University (CDLU), Sirsa, Haryana, India

P. Kumari
CSIR-Institute of Himalayan Bioresource Technology (IHBT), Palampur, Himachal Pradesh, India
e-mail: poonam@ihbt.res.in

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and rapid way to study and better understand the complex mechanisms of heat stress response in wheat. The molecular markers, QTLs, heat-responsive genes and their regulation can further be utilized in designing breeding strategies for developing heat-tolerant wheat varieties.

Keywords

Heat stress · Morphophysiological responses · Genomics · QTLs · Transcriptomics · miRNA

5.1 Introduction

Wheat, the world's second most important staple cereal, is adaptable to a diverse range of global eco-climatic conditions (Riaz et al. 2021). Approximately 36% of the world's population, around 4.5 billion people, rely solely on wheat to meet their calorie needs, most of which live in developing nations (Braun et al. 2010). It meets 20% of their caloric and 20% of their protein needs. In 2019, wheat was grown on 215.9 million hectares worldwide with a total production of 765.80 million tonnes (MT) and a mean yield of 3547 kg/ha (FAO 2020). But this wheat quantity would not be enough to feed the ever-growing global population, necessitating an additional 198.0 million tonnes (MT) of wheat by 2050. In order to reach this goal, wheat grain yields in developing nations must increase by 77% (Sharma et al. 2015). This goal could be accomplished by selecting wheat varieties/genotypes that are better yielding and have climate-smart and various biotic and abiotic stress resistance/tolerance attributes which can perform at their best actual field conditions (Riaz et al. 2021).

Food security is being jeopardized by rising temperatures and the occurrence of droughts (Lobell et al. 2012). Wheat production is severely affected by heat stress globally (Paul et al. 2022). Between 1880 and 2012, worldwide mean sea and land temperature increased by 0.85 °C, with another 1.5–2.0 °C rise anticipated by the century ending (Pachauri et al. 2014). Wheat production in Russia between 1980 and 2008 declined by 15% (Lobell et al. 2012). A short duration of HS (heat stress) above 35 °C resulted in compromised wheat productivity and quality (Mason et al. 2010). During its grain-filling stage, the Australian wheat belt wheat crop is exposed to HS above 34 °C temperature every season on an average speed up leaf senescence resulting in 5% yield loss per day (Asseng et al. 2011). In India, the North-western Plain Zone (NWPZ), which includes Punjab, Haryana, and western Uttar Pradesh, and the North-eastern Plain Zone (NEPZ), which includes eastern Uttar Pradesh, Bihar, Jharkhand and West Bengal, are mega wheat-Growing zones which face a variety of abiotic and biotic challenges including HS (Pathak et al. 2003). These are the most prolific and fertile terrain of the Indo-Gangetic Plains, which produce 15% of global wheat output. Still, climate change is expected to categorize this area as heat-stressed by 2050, with around 51% of this area designated as such (Ortiz et al. 2008).

Wheat grain production falls by 3.0–4.0% for every 1 °C increase in mean temperature above 15 °C during grain filling in moderate temperature stress (25–32 °C) in both controlled and field circumstances (Wardlaw et al. 1989). This issue impacts wheat production in around 9.0 million hectares of land in tropical and subtropical countries, where temperatures often exceed 17 °C in the coldest month of the crop season (Ortiz et al. 2008). Upcoming environments will also be marked by more temperature unpredictability and a higher frequency of summertime (Pittock et al. 2003). As a result, the long-term sustainability of wheat farming systems under future changing climate circumstances is a serious worry (Rodriguez et al. 2014). The growing season temperatures in major wheat-producing regions are on the rise (Alexander et al. 2006). With an aim to develop wheat genotypes/varieties as a well-adapted crop to future harsh climates, researchers must first learn how plants respond to high temperatures and how heat tolerance may be increased (Halford 2009).

5.2 HS Impact on Wheat Morphology and Phenology

Abiotic stresses are the leading cause of crop losses worldwide, lowering crop yields by more than half in some cases, including wheat (Buttar et al. 2020; Lal et al. 2021). Increased global temperature poses a serious hurdle to agriculture globally, as it has a detrimental impact on wheat growth and development, resulting in lower yields and productivity. By the end of the century, the average global temperature is expected to rise from 1.3 to 3.7 °C. The reproductive stage is one of the critical developmental stages influenced by HS (Rezaei et al. 2018). Therefore, breeding heat-tolerant cultivars is a major limitation (Haque et al. 2014). HS impacts wheat productivity in the arid, semiarid, tropical and subtropical wheat-growing regions worldwide (Stocker et al. 2013; Stocker et al. 2014). Elevated temperatures during day and night are harmful to the plant, especially during the reproductive phase. Over the last few decades, there has been a diurnal asymmetry in the temperature rise, resulting in a more rapid increase in the night temperature. Heat stress affects the morphology and phenology of wheat (Fig. 5.1). HS significantly affects various growth processes, including germination, the emergence of root/shoot, tillering, floret development, anthesis and fertilization in wheat, which ultimately impacts the overall yield and quality of the produce (Rezaei et al. 2018). The two important determinants for measuring the severity of HS on various growth and developmental stages in wheat are exposure duration and heat-shock intensity (Buttar et al. 2020).

High temperatures have a negative impact on seed germination, seedling emergence and seedling establishment (Hossain et al. 2012; Zhang et al. 2016). HS exposure to about 45 °C negatively impacts the wheat seed embryonic cells and reduces the germination rate and emergence, so poor crop stand results (Essemine et al. 2010). Kosova et al. (2011) observed that HS primarily impacts plant meristematic tissue, limiting its development, accelerating leaf senescence and abscission in leaf tissue and drastically decreasing the photosynthesis rate. Inhibition of photosynthesis results in decreased carbon assimilates, resulting in drastically reduced leaf surface area, biomass and yield induced by HS (Buttar et al. 2020). Prolonged heat

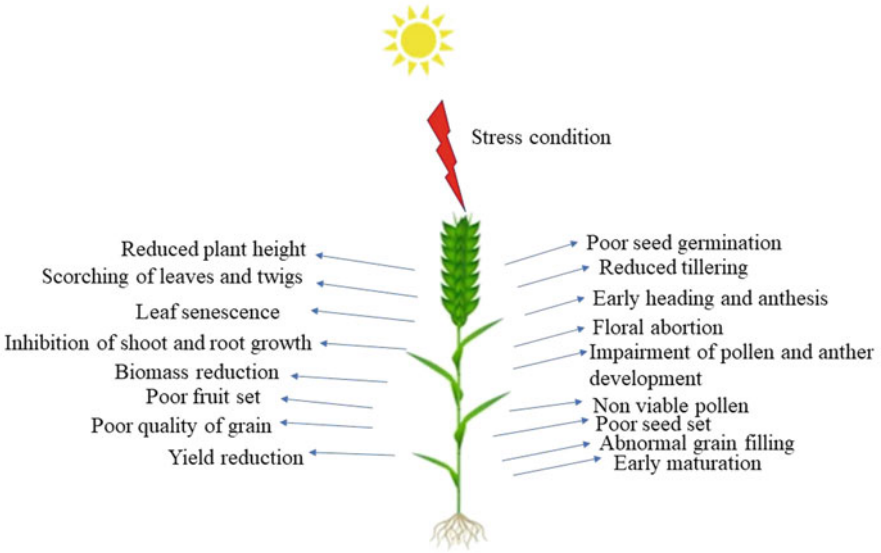

Fig. 5.1 Effect of heat stress on morphology and phenology of wheat

exposure causes organ damage and death, as well as leaf shedding and floral abortion (Kumar et al. 2019a). Several studies have been carried out to study the impacts of HS on wheat at various growth stages, and it was concluded that exposure to elevated temperature (45 °C) during germination causes compromised root/shoot development, reduced biomass, total chlorophyll and cell membrane stability index (Gupta et al. 2013). In HS conditions, wheat flag leaves undergo morphological modification, viz. leaf rolling and various other modifications at the physiological level to reduce water loss and improve water-use efficiency (WUE) in wheat crop (Hasanuzzaman et al. 2013). Also, elevated temperature conditions during day (>30 °C) and night (>25 °C) hampered leaf growth and the number of productive tillers in wheat, resulting in a reduction in grain yield (Din et al. 2010).

The life cycle of wheat is shortened in HS compared to normal temperatures (Alam et al. 2014). HS also affects the root system development, ultimately reducing overall yield (Mishra et al. 2011). In wheat, the reproductive phase is the most vulnerable for HS (Nawaz et al. 2013), and for the post-anthesis stage, the maximum threshold temperature is 26 °C (Stone et al. 1994). A rise in temperature above the threshold temperature is extremely detrimental to various developmental processes during the reproductive stage of wheat (Dubey et al. 2020). The ideal temperature for wheat anthesis (flowering) and grain filling ranges between 12 and 22 °C. When HS occurs during meiosis, it affects the early stages of gametogenesis (Ji et al. 2010), and HS has a deleterious impact on microspore and pollen cell development. HS severely affects the wheat grain quality by impairing many important physiological processes which are critical for quality seed formation (Balla et al. 2012) and influence the length of grain filling (Lobell et al. 2012; Yu et al. 2014). Seed weight

is reduced by a 1–2 °C increase in temperature due to a reduction in grain-filling period (Nahar et al. 2010).

5.3 Impact of Heat Stress on Physiology of Wheat

It is predicted that a 1 °C increase in temperature results in a 6% decline in global wheat yield (Asseng et al. 2011). Heat stress causes morphophysiological changes in wheat plants that impede growth and ultimately result in significant yield loss. Figure 5.2 describes various physiochemical changes that occur in wheat in response to wheat stress. Plants sense temperature changes and accordingly modify their metabolic processes, protein structures, cytoskeletal assembly and membrane composition (Rangan et al. 2020). Wheat genotypes with better heat tolerance attributes had significantly increased leaf length and width, leaf surface area, weight and area (Sing 2009). Various physiochemical traits affected by HS are described below.

5.3.1 Water Relations

Under changeable climate patterns and high-temperature conditions, the plant's water status is critical. Water intake and transpiration regulate the temperature of plant tissue, resulting in stable water content in the tissue. Water loss accompanied by high-temperature shock is fatal for the plant (Fahad et al. 2019). Among all the physiological impacts of HS, water relation is one of the important physiological processes disturbed in wheat. HS causes excessive cellular water loss, causing dehydration, which negatively impairs normal growth and development processes (Akter and Rafiqul Islam 2017). As a result of the increased temperature of leaf

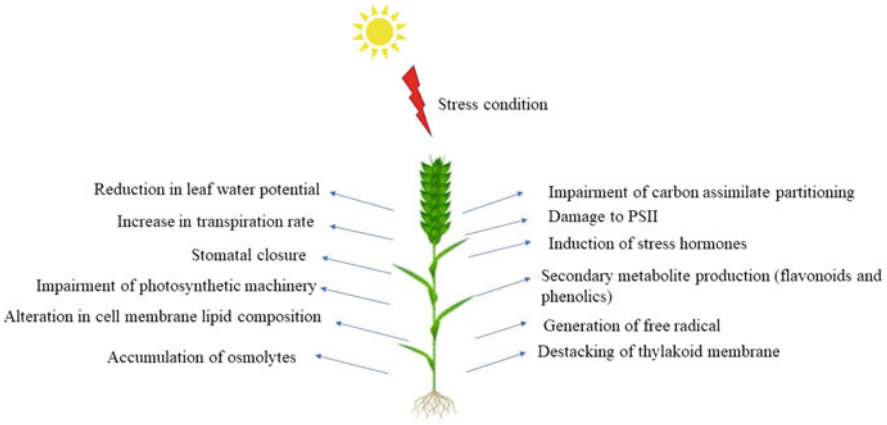

Fig. 5.2 Physiological and biochemical changes in wheat in response to heat stress

tissue during exposure to HS, the water potential of the leaf tissue cells and their relative cellular water content are reduced due to which photosynthesis capability gets diminished, leading to lower biomass accumulation (Farooq et al. 2011). HS (35/25 °C) exposure during tillering resulted in a considerable reduction in water potential, particularly in HS-sensitive wheat genotypes (Almeselmani et al. 2009). A rise in leaf surface and canopy temperature (CT) significantly affects the leaf's relative water content, transpiration rate, leaf water potential and stomatal conductance during HS (Farooq et al. 2011). Due to the high temperature, the soil moisture content is depleted, resulting in a drop in leaf water potential (LWP) and leaf relative water content (LRWC). According to Sairam et al. (2000), during HS, when there is a temperature rise, the LRWC is significantly lowered, which affects the reproductive and grain-filling stages. The transpiration mechanism in wheat aids the plant in energy dissipation which helps in HS avoidance. In this case, the plant with the highest transpiration rate has better survival under HS. HS did not affect parameters, viz. leaf RWC and water potential, when the soil water content was close to field capacity; however, day/night temperatures of 40/35 °C had a minor effect on water content. Enhanced activity of aquaporin altered the membrane fluidity and permeability and enhanced the reduction in cellular water viscosity and tissue hydraulic conductivity (Cochard et al. 2007). A higher transpiration rate also helps alleviate overheating due to the cooling effect on the leaf and canopy.

5.3.2 Impact on Photosynthesis

Photosynthetic activity is directly related to crop productivity and, according to Wahid (2007), is the most temperature-sensitive process. HS has a deleterious impact on plant growth and development and photosynthetic efficiency in wheat (Arshad et al. 2017). HS promotes thylakoid membrane expansion and leakiness (Djanaguiraman et al. 2018), causing detachment of the PSII complex and the chlorophyll light-harvesting complex II (LHC II) (Pastenes and Horton 1999). The activities of important photosynthetic enzymes ribulose1, 5-bisphosphate carboxylase/oxygenase (RuBisCo) and RuBisCo activase (RCA) are drastically reduced under elevated temperature conditions. RuBisCo is the most widely distributed enzyme in the world, and it is a key enzyme in the carboxylation of CO_2 in plants. It is extremely temperature sensitive, and as RuBisCo activity is reduced, the rate of photosynthesis drops. It is also established that the reduced activity of RuBisCo under HS is also attributed to the high-temperature sensitivity of RuBisCo activase (RCA), which modulates RuBisCo activity (Salvucci and Crafts-Brandner 2004). RuBisCo activase (RCA), a novel chaperone enzyme, restores the catalytic activity of RuBisCo by eliminating sugar-phosphate derivatives, which act as inhibitors from catalytic sites of the enzyme (Wachter and Henderson 2015). RCA acts as a catalytic molecular chaperon and ATPase involved in many cellular processes that remodel RuBisCo active site using energy from ATP hydrolysis (Neuwald et al. 1999). It is heat-labile in nature, so RCA is thought to be responsible for the decline in photosynthetic function of plant parts during HS for plants experiencing HS

(Perdomo et al. 2017). RuBisCo inactivation is produced at least in part due to activase inactivation under HS and is primarily responsible for inhibiting normal photosynthetic processes during mild thermal elevations (Crafts-Brandner and Salvucci 2000). It is reported that a single amino acid alteration (M159I) in RCA in bread wheat significantly changes its thermal and regulatory properties (*Triticum aestivum* L.) (Degen et al. 2021). As a result, boosting Rca's thermostability is a very important characteristic for identifying and developing wheat with improved photosynthetic ability aiming for higher yield under HS (Parry et al. 2011). Moreover, the relative abundance of wheat RCA isoforms and specific amino acid residues associated with their activity can further be linked to HS tolerance in changing environmental conditions (Degen et al. 2021).

5.3.3 Impact on Reactive Oxygen Species (ROS) Production and Antioxidant System

HS causes the generation of reactive oxygen species (ROS), which functionally cause oxidative stress to the plant, impeding normal growth and development of the plant (Caverzan et al. 2016). Under oxidative stress, the formation of reactive oxygen species (ROS), including superoxide anion (O2•), hydroxyl radical (•OH), and such as hydrogen peroxide (H_2O_2) and singlet oxygen get enhanced (Asada 2006). HS in wheat has been shown to cause multiple modifications of normal physiological characteristics, viz. proline (osmolyte accumulation), lipid peroxidation and mitochondrial and chloroplast membrane deterioration, and H_2O_2, a secondary metabolite involved in stress signalling (Gupta et al. 2013; Kumar et al. 2019b). Kumar et al. (2019b) also reported that in wheat HS, including superoxides, hydroxyl radicals and H_2O_2, caused the formation and build-up of reactive oxygen species (ROS). The process of generating reactive oxygen species (ROS) and antioxidant-mediated neutralization is critical for shielding the plant from the negative effects of HS (Roy et al. 2017). Reactive oxygen species (ROS) production rises during HS, causing lipid peroxidation and increased membrane damage (Djanaguiraman et al. 2018). A very high proline content (1.0 mole/g FW) was observed during temperature rise >25 °C during the vegetative growth stage in wheat (Kumar et al. 2012).

When ROS generation exceeds cellular scavenging capacity, redox equilibrium becomes unbalanced, resulting in increased membrane damage; as a result, more electrolytes leak from the damaged membrane, compromising the cell's function and causing oxidative stress (Sharma et al. 2012). The wheat plant uses various tactics to counteract ROS effects, including scavenging the ROS molecule and protecting the membrane and other organelles from damage (Lal et al. 2021). Wheat is very prone to oxidative stress during its reproductive stage. Thus, a scavenging system to neutralize the ROS comprises peroxidases (ascorbate peroxidase), dismutases (superoxide dismutase), superoxidases and catalase and protects against the adverse effects of accumulated ROS (Kumar et al. 2012).

5.3.4 Impact on Cellular Respiration

Respiration is a crucial mechanism that determines a plant's development and survival. Mitochondrial respiration is a key factor influencing production and productivity in wheat plants under HS. The gross photosynthesis rate is slowed or inhibited as the temperature rises over the optimal temperature, whereas both respiration and photorespiration rates accelerate (Lal et al. 2021). For temperature ranging from 0 to 35 °C, respiration rate increases exponentially, plateauing at 40–50 °C, and declines at temperatures higher than 50 °C, as the respiratory mechanism and proteins are destroyed (Yadav et al. 2022). During HS, respiration rate accelerated, further reducing and transporting photo-assimilate partitioning and mobilization from leaves to grain, resulting in poor yield and development (Asthir et al. 2012). HS has a stronger negative impact on chloroplast, resulting in growth disruption and reduced maintenance of the normal respiration process (Wang et al. 2018). It is reported that during high-temperature stress, the rate of leaf respiration increases (Almeselmani et al. 2012). The influx of photo-assimilates is counterbalanced by respiratory losses in the wheat grain during HS, resulting in a drop in yield and production (Akter and Rafiqul Islam 2017). According to the research shown above, increased respiration efficiency and resilience to HS benefit wheat crop growth and output. Plants adjust their metabolism to preserve equilibrium in their respiration rate.

5.3.5 Impact on Nutrient Relation

There is very little evidence of the impact of high-temperature stress on crop nutritional status (Rennenberg et al. 2006). Under high temperatures, nitrogen fixation enzyme activity was reduced (Klimenko et al. 2006). It is reported that sulphur can improve resistance to high temperatures as a nutrient. Sulphur metabolites maintain the cell's redox state and protect the cell membrane, thylakoid membrane and cytoplasm from damage during HS, resulting in an increase in photosynthetic activity (Alghabari et al. 2019).

5.4 HS Impact on Wheat Reproductive Biology

5.4.1 Impact on Pre-anthesis

HS has a significant impact on flower initiation and development. It was observed that about 90% of florets flowers during the early morning or evening when the temperature is comparatively low (Aiqing et al. 2018). HS has crucial effects on the viability of male and female reproductive parts, particularly at the anthesis stage (Prasad et al. 2011), with negative consequences for microspores and pollen cell development (Kaur and Behl 2010). HS in wheat causes flower initiation to be delayed and reproductive development to be harmed. HS during gamete formation in

wheat results in irreversible structural abnormalities in stigmas, styles, pollen and ovaries, as well as adverse effects on subsequent physiological functions such as pollen tube growth, fertilization performance and pollen vitality (Prasad and Djanaguiraman 2014). Heat shock causes a defective meiosis process in pollen mother cells characterized by micronuclei formation, absences of metaphase plate, aberrant tetrad, pyknosis, etc. (Omidi et al. 2014). HS causes abnormalities in microsporogenesis and ultrastructural alterations in pollens. HS induces pollen infertility (Jager et al. 2008). Pollen vitality, proliferation and fertilization were greatly harmed by HS, resulting in the formation of pseudo-seeds (Kumar et al. 2013). A rise in temperature (>30 °C) during anthesis in wheat was reported for triggered floral abortion in wheat (Wardlaw and Wrigley 1994). HS induced damaging of tapetal cells and pollen formation in wheat induced collapsed and shriveled pollen grains with uneven surface structures (Bokshi et al. 2021; Prasad and Djanaguiraman 2014). During anthesis, HS reduces floret viability by causing alterations in male and female reproductive parts' (pollen and pistil) structure and functioning (Prasad and Djanaguiraman 2014; Bokshi et al. 2021). High-temperature conditions (up to 36/26 °C) for 24 h before 10 days post-anthesis (dpa) or 4 days post-anthesis stage negatively impacted floret vitality, with elevated severity occurring 8 days pre-anthesis and 0–2 days post-anthesis (Prasad and Djanaguiraman 2014). HS (>30 °C) during flower development triggered sterility in wheat (Kaur and Behl 2010). Wheat yields were lowered by 24 and 16% when air and ear temperatures exceeded 31 °C at anthesis (Rezaei et al. 2018). HS has a deleterious impact on the flowering onset, floral establishment and pollen vitality resulting in compromised fertilization and reduced seed counts (Rieu et al. 2017).

5.4.2 Impact on Post-anthesis or Grain-Filling Stage

During the post-anthesis stage, elevated temperature (above 35 °C) resulted in decreased grain-filling time. It restricted the mobilization of photo-assimilates to developing wheat grains, lowering wheat productivity by 6–51% in controlled environment cultivation and 2–27% in cultivation under field conditions (Bergkamp et al. 2018). In wheat, grain-filling duration and grain-filling rate are significantly both affected by elevated temperature conditions (Farooq et al. 2011; Sharma et al. 2018; Arjona et al. 2020). Early stage is more susceptible to HS than the later stages. The influence of HS on grain filling and development is determined by the duration and severity of the stress. HS caused a seriously compromised grain development process by negatively affecting photo-assimilate synthesis in vegetative organs and its delivery during grain development in wheat. Pre-anthesis photo-assimilate delivery and the quantity of assimilating deposit stored in vegetative organs are critical in heat-stressed wheat because floret onset and subsequent seed development got seriously compromised (Girousse et al. 2021).

5.4.3 Impact on Grain Filling, i.e. Assimilation and Translocation of Photosynthetic Reserves

Grain-filling rates determine the final grain mass (Dias and Lidon 2009). In wheat, temperature exposure above 20 °C during spike formation and anthesis speeds up spike growth but diminishes grain number and yield potential (Lukac et al. 2011). The duration of wheat grain filling >25 °C was shortened by 12 days (Yin et al. 2009), drastically reducing the final yield because of the decreased leaf and spike photosynthetic activity along with reduced carbon assimilation and nutrient remobilization (Akter and Rafiqul Islam 2017). In heat-stressed conditions (32/22 °C), Song et al. (2015) found a substantial decreased rate of grain filling in wheat. HS cause reduced grain filling and hence reduced grain size, quality and thousand-grain weight (TGW). Under raised temperature (>30 °C), carbon assimilate translocation from flag leaf to developing seed inhibited via the apoplastic and symplastic pathways substantially and thus formed shriveled seed formation with reduced thousand grain.

5.4.4 Impact on Starch and Protein Biosynthesis in Wheat Grains

The wheat endosperm mainly comprises carbohydrates and proteins, and starch contributes about 65% of kernel dry weight (Barnabás et al. 2008). Seed composition is regulated by wheat's duration and rate of grain filling. Starch biosynthesis in wheat is mediated by three enzymes: sucrose synthase (SS) enzyme, soluble starch synthase (SSS) enzyme and granule-bound starch synthase (GBS) enzyme (Hawker and Jenner 1993). Starch biosynthesis is severely affected by HS in comparison to protein synthesis in wheat grain due to the hypersensitivity of soluble starch synthase (SSS) enzyme (Zahra et al. 2021). During extreme temperatures (40 °C), wheat starch synthase enzyme efficiency decreases drastically (around 97%) in wheat, reducing starch biosynthesis and aggregation substantially. HS also alters the grain quality due to the reduction of amylopectin to amylose ratio (Liu et al. 2011). Protein content in wheat seed increased from temperatures ranging from 15 to 25 °C, while it reduced drastically by 32% at 35 °C (Viswanathan and Khanna-Chopra 2001) and is crucial for dough quality in wheat. Alpha-gliadin was found to be upregulated in the present study. Have reported that the expression of many storage proteins is under heat or water deficit, viz. α-gliadin, γ-gliadin, low molecular weight glutenin and globulins in the seeds altered.

Further, the abundance of gliadins was found to increase under heat stress from anthesis up to 10 DPA. DuPont and Altenbach (2003) also studied that amount of α-gliadin increases in response to elevated temperature conditions during endosperm development in wheat. HS also alter the accumulation of two major wheat seed proteins, i.e. increasing gliadins and decreasing glutenins, thus reducing dough quality (Zahra et al. 2021). Because the gliadin gene contains 5′ heat-shock elements, gliadin synthesis rises at high temperatures (30–35 °C). However, poor

dough quality is caused by decreased glutenin production and disulphide cross-linking of glutenin subunits (Blumenthal et al. 1995).

5.5 HS Tolerance Trait Assessment and Mechanisms in Wheat

The plant is usually exposed to HS or elevated temperature conditions above the optimal threshold. HS cause severe loss in term of survival, yield and quality of the plant. In terms of plant reaction and tolerance to HS, all plant species can be divided into heat-sensitive, relatively heat-resistant and heat-tolerant species based on their thermotolerance (Larcher 1995). Global wheat production faces a great threat of HS or elevated temperature conditions due to global warming (Melloy et al. 2014; Liu et al. 2017). Wheat crop is exposed to an elevated temperature between the heading and maturity stages of its life cycle, termed terminal HS. Terminal HS is a temperature increase between the crop's heading and maturity stages (El Hassouni et al. 2019). HS during the wheat reproductive phase impacts anthesis and the grain-filling process, resulting in a significant productivity drop (Hays et al. 2007). The ideal temperature for anthesis and grain filling in wheat is between 12 and 22 °C (Kumudini et al. 2014). It is reported that if wheat is exposed to a temperature of >35 °C for even for a short duration, significant grain yield loss might occur (Sarkar et al. 2021).

Wheat plant physiological responses to higher temperatures are categorized into avoidance and tolerance (Adams et al. 2001). Plants gain heat tolerance through morphological, physiological, biochemical and molecular changes and adaptive strategies in response to HS.

5.5.1 Avoidance

Wheat morphological adaptations to HS include improved germination capacity, improved plant development, rolling/folding of leaves, suppression of early senescence in leaves, higher biomass accumulation and so on (Sarkar et al. 2021). Early maturation with a lower reduction in yield could potentially be linked to an HS avoidance mechanism. Improved and well-developed roots, improved stomatal exchanges, the altered orientation of leaves, thickening of leaves and lowering of temperature due to higher transpiration are some of the HS avoidance mechanisms that assist plants in sustaining under HS conditions when water is not a limiting factor (Fahad et al. 2019). Early maturing wheat cultivars can avoid terminal HS and thus minimize the detrimental effects of and thus minimize the HS-induced yield loss (Menshawy 2007).

5.5.2 'Stay Green' Trait

In wheat, 'Stay-Green', a character that refers to the preservation of photosynthetic capacity and leaf chlorophyll during HS for a longer duration, is a marker for heat tolerance of a particular genotype (Sakuraba et al. 2014). For several years, visual evaluation has used the 'Stay-Green' trait in breeding line screening (Thomas and Ougham 2014). Stay-Green cultivars have the ability to photosynthesize for a longer duration during HS and are thus able to maintain the normal grain filling. Therefore, the 'Stay-Green' trait has been found very effective for reducing yield loss induced by HS in wheat (Pinto et al. 2016). The direct impact of HS includes denaturation, inactivation and aggregation of functional proteins, while indirectly, it inhibits the normal translation of cellular proteins, mitochondrial and chloroplast enzyme inactivation and cellular membrane disintegration protein (Howarth 2005). Several transitions happened at the molecular level, viz. regulation of gene expression and the accumulation of transcripts controlling various stress-induced protein biosynthesis and stress-tolerance mechanisms being operated (Iba 2002). Even under HS, the ability to sustain normal productivity is critical during wheat improvement programmes (Aziz et al. 2018). Wheat cultivars that possess and maintain high yield serve donor parents in the heat tolerance wheat breeding programme (Al-Otayk 2010).

5.5.3 Physiological Trait Assessment for HS Tolerance in Wheat

5.5.3.1 Canopy Temperature Depression

Canopy temperature depression (CTD) refers to the temperature difference between the canopy and the ambient temperature (Deva et al. 2020). CTD is a good predictor of a genotype's ability to cope with HS (Urban et al. 2018; Sharma et al. 2021). Wheat genotypes that can maintain the lower canopy temperature in HS during grain-filling stages are a bit better heat tolerant (Munjal and Dhanda 2016). Because CTD is linked to various adaptive physiological properties for HS tolerance, it has allowed breeders to investigate wheat yield stability (Saxena et al. 2014). It is found that CTD is positively correlated with root traits, leaf area index, stomatal conductance, water-use efficiency, transpiration rate and grain yield in different varieties with comparatively cooler canopies (Gautam et al. 2015). Total leaf chlorophyll content and canopy temperature depression (CTD) were found to be useful in identifying wheat varieties with better heat tolerance attributes (Saxena et al. 2014).

5.5.3.2 Photosynthesis

Under HS, the photosynthetic machinery is shown to be impaired in heat-sensitive wheat cultivars than in heat-tolerant due to high levels of reactive oxygen species (ROS) and malondialdehyde (MDA) build-up (Zou et al. 2017). To protect themselves from ROS's harmful effects, heat-tolerant plants synthesize diverse ROS scavenging and detoxifying systems (Apel and Hirt 2004). Thermotolerance can be generated by enhancing antioxidant capacity while preserving improved cell

membrane temperature stability and reducing ROS generation (Chakraborty and Pradhan 2011; Hameed et al. 2012). Plants use a variety of strategies to preserve their photosystems, including cyclic electron flow, alternate oxidase (AOX) pathways, oxidative electron transport and photorespiration processes (Sunil et al. 2019). Among such strategies, the activities of CEF, AOX and photorespiration are critical (Hodges et al. 2016).

5.5.3.3 Chlorophyll Content and Fluorescence

By being linked to transpiration efficiency, chlorophyll concentration may play a role in the mechanism of heat tolerance (Reynolds and Trethowan 2007). In heat-resistant genotypes, a strong positive association between leaf chlorophyll concentration and transpiration efficiency has been discovered (Sheshshayee et al. 2006). During the grain-filling period, yield is linked to photosynthesis rate and leaf chlorophyll content (Reynolds and Trethowan 2007). Under HS, chlorophyll in leaves is rapidly broken down, resulting in chlorophyll loss (Jespersen et al. 2016). In-depth research into the start of protein modifications in the nucleus along with signalling cascades in the chloroplast could aid in understanding chloroplast nuclear signalling in response to environmental cues (Schmidt et al. 2020). Several gene products are activated and regulated to aid and safeguard chloroplasts in their regular functioning and to improve plant heat tolerance (Hu et al. 2020). Improving stem resources' mobilization is an efficient heat tolerance strategy in wheat (Bala and Sikder 2017). In wheat stems, water-soluble carbohydrate (WSC) stores are depleted, and remobilization of these carbs improves grain yield in extreme heat (Gupta et al. 2011).

5.5.3.4 Membrane Thermostability

Membrane thermostability (MTS) is an important strategy on a physiological level for heat tolerance in plants, allowing them to adjust to hot conditions (Barma et al. 2010) as HS deteriorates the 3° and 4° structures of membrane proteins. Increased solute leakage has been suggested as a sign of compromised cell membrane thermostability, which may be utilized as an alternative indicator of wheat HS tolerance (Bala and Sikder 2017). Plant tolerance to high temperatures is aided by membrane systems that stay functional under HS (Blum 2018). As a result, the plant's ability to retain membrane integrity and function determines its tolerance to HS (Almeselmani et al. 2011).

In wheat cellular membrane stability in HS serves as an excellent measure of heat tolerance and serves as a reliable relationship with plant performance under HS, suggesting that it might be used as a key selection criterion for heat tolerance (Sarkar et al. 2021).

Soluble starch synthase is most susceptible to HS and regulates starch production (Keeling et al. 1993). HS reduces enzyme activity in wheat, decreasing total grain weight and starch content. New findings established that the heat tolerance capability of soluble starch synthase enzyme might be a useful indication for improving heat tolerance and better seed development in wheat under HS directly linked to this enzyme's catalytic efficiency (Tian et al. 2018).

5.5.3.5 Antioxidant Production

Plants activate their antioxidant defence mechanism to prevent cell damage caused by these reactions (Suzuki et al. 2014). Plants under HS accumulate a variety of antioxidants from several mechanisms (Bokszczanin and Fragkostefanakis 2013). Two types of antioxidant defence systems are identified in wheat: enzymatic and non-enzymatic (Sattar et al. 2020). The enzymatic antioxidant system includes catalase (CAT), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione *S*-transferase (GST), superoxide dismutase (SOD) and guaiacol peroxidase (GPX) (Noctor and Foyer 1998). SOD is a key antioxidant that aids in converting superoxide to H_2O_2. APX, GPX and CAT, on the other hand, regulate ROS detoxification (Buttar et al. 2020). To remove H_2O_2, APX needs AsA and glutathione (GSH) in reduced form, which are created through the AsA-glutathione cycle, for the conversion of H_2O_2 into H_2O via AsA oxidation to monodehydroascorbate (MDHA), which then dismutates to dehydroascorbate (DHA) through the process (Asthir 2015).

At 50 °C, catalase (CAT), ascorbate peroxidase (APX) and superoxide dismutase (SOD) functioning is reduced, but their activities are initially raised (Chakraborty and Pradhan 2011), while peroxidase (POX) and glutathione reductase catalytic functions lowered at temperatures ranging between 20 and 50 °C. Total antioxidant activity was maximum in tolerant wheat types at 35–40 °C, while maximal antioxidant activity was reported in susceptible wheat varieties at moderately high temperatures (Chakraborty and Pradhan 2011). The enzymatic efficiency of these enzymes varies depending on the wheat growth stage and the season it is grown (Chakraborty and Pradhan 2011). When heat-tolerant wheat genotypes were treated with HS, enzyme antioxidants were significantly elevated during the reproductive phase (Balla et al. 2009). In wheat, catalase and superoxide dismutase activities are capable of achieving thermotolerance and show a substantial link with HS throughout the reproductive phase (Almeselmani et al. 2009; Zhao et al. 2007).

5.6 Molecular Biology of HS Tolerance in Wheat

Plants have defence mechanisms in response to HS, including overexpressing certain genes that are specifically induced by stress conditions. Such genes include heat-shock proteins (HSPs), which act like chaperons, and stress-induced proteins (SIPs) (Lindquist and Craig 1988).

Heat-shock proteins (HSPs) are induced when wheat coleoptiles are exposed to HS (Blumenthal et al. 1990). Because about 65% of chloroplast HSPs are transported to thylakoid membranes under HS, membrane affiliations must be investigated to understand better the role of HSPs in HS adaption (Bernfur et al. 2017). HSPs function as molecular chaperones in plants, regulating protein accumulation, folding, localization and elimination (Gupta et al. 2010).

Under HS, proteins in the ER (endoplasmic reticulum) and cytoplasm of wheat were discovered to become unfolded through reactive oxygen species (ROS) regulation pathways (Kataoka et al. 2017). Heat-shock proteins (HSPs) with molecular

chaperones' functions prevent cellular proteins' aggregation by assisting in regaining their native structures (Morrow and Tanguay 2012), preventing apoptosis (Altenbach et al. 2003). In wheat, high temperature-induced genes got overexpressed in the grain-filling process under HS, resulting in more heat-shock proteins in developing wheat seeds (Blumenthal et al. 1991; Zhang et al. 2018).

According to numerous studies, overexpression of HSPs leads plants to gain thermotolerance (Grover et al. 2013). Different forms of HSPs are produced in various plant tissues of the wheat plant depending on the timing and encountering stage of HS (Xu et al. 2011). Swindell et al. (2007) have categorized HSPs into five different categories, viz. HSP100, HSP90, HSP70, HSP60 and HSP20, based on their molecular weight. The development of HSPs is accompanied by an increase in the wheat embryo's ABA (abscisic acid) level during grain filling and maturation (Xue et al. 2014). During the acclimatization process in HS, heat-shock factors (HSFs) regulate heat-inducible genes (Yabuta 2016).

Elongation factor 1 α- is a multifunctional protein. Transcript elongation factors (EFs) play an essential role in mediating critical cellular processes related to cellular growth, proliferation and cell differentiation by interacting with other cellular proteins (Zheng et al. 2014). Its high expression during heat stress conditions in animals and plants has been reported and thus suggests its essential role in survival under stress conditions (Shamovsky et al. 2006). They further suggested its role in wheat stress as accumulation was high in cultivars with better heat tolerance. Zheng et al. (2014) characterized a transcript elongation factor gene in wheat through expression and association analysis, near-isogenic line comparison and then overexpressing in *Arabidopsis* after reporting its role in the regulation of yield-related traits associated with growth and development. Heat tolerance in wheat may be improved by the EF-Tu (elongation factor thermo-unstable) chloroplast protein synthesis elongation factor, which acts as a molecular chaperone and protects chloroplast protein against thermal aggregation (Ristic et al. 2007).

According to Djukic et al. (2019), a winter wheat cultivar named Zvezdana had a 25% overexpression of chloroplast-associated EF-Tu under HS (38 °C) than normal temperature (23 °C), due to which this genotype has shown reduced protein denaturation under HS than other heat-susceptible cultivars. Plants with high EF-Tu expression are found to be better adapted to HS, suggesting the importance of EF-Tu in plant HS adaption (Ristic et al. 2008).

5.7 HS Tolerance Mechanism Elucidation Using Omics

The omics (genomics, transcriptomics, proteomics and metabolomics) are important tools for understanding plant growth survival's molecular pathways under various abiotic and biotic stresses (Tiwari et al. 2020). Wheat genetic improvement for improving wheat productivity can be achieved by integrating advanced genomic technologies (Sheoran et al. 2019). Several HS-responsive genes and QTLs have been reported in utilizing genomics identified and characterized in wheat (Deshmukh et al. 2014) (Fig. 5.3).

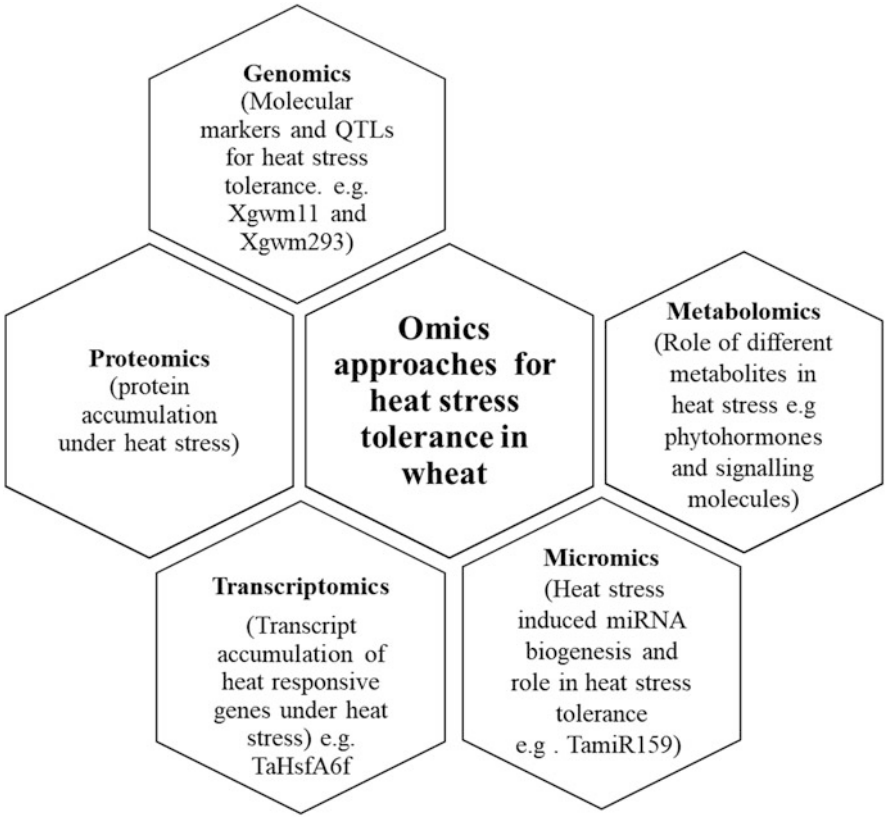

Fig. 5.3 Omics approaches for heat stress response study in wheat

Multiple genes govern HS tolerance (Abou-Elwafa and Shehzad 2021), posing a barrier to wheat breeding tolerance-related traits. Advances in genomics, bioinformatics tools and high-throughput phenotyping, on the other hand, have aided in dissecting the genetic areas linked to numerous agronomic and physiological variables in wheat under HS. Large numbers of wheat genomic regions have been identified using interval mapping (IM) and linkage mapping/genome-wide association study (GWAS) mapping for HS tolerance traits, viz. days to heading (DH), thousand-grain weight (TGW), yield and grain-filling duration, canopy temperature depression, 'Stay-Green' and senescence-related traits (Jamil et al. 2019; Abou-Elwafa and Shehzad 2021). Approximately 300 QTL/MTAs have been identified in wheat for various agronomic and physiological parameters (Gupta et al. 2020; Sharma et al. 2020). The stable significant QTLs could further be used in wheat molecular breeding programmes for marker-assisted selection (MAS) to improve HS tolerance. Web-based methodology/approaches also play a vital role in the varietal identification of wheat using throughput SNP data (Singh et al. 2019). Some of the QTLs reported in wheat are summarized in Table 5.1.

Table 5.1 Some QTLs for heat stress tolerance in wheat

QTL	Trait associated	Chromosomal location	Reference
QGNP-HS-R1	Grain number per main spike	1A	Li et al. (2019)
QGYP-HS-R1	Grain yield per plant	1A	Li et al. (2019)
QHkwm.tam-1B	Kernel weight of main spike	1B	Mason et al. (2011)
QTKW-HS-R1	Thousand kernel weight	1D	Li et al. (2019)
QSpn.agt-SG.1D	Spikelet number per spike	1D	Telfer et al. (2021)
QHtscc.ksu-1B	Chlorophyll content	1B	Talukder et al. (2014)
QHttmd.ksu-1D	Thylakoid membrane damage	1D	Talukder et al. (2014)
TaHST1	Chlorophyll fluorescence (Fv/Fm)	4A	Zhai et al. (2021)
QLCCHR.nri-4A	Leaf chlorophyll content	4A	Maulana et al. (2018)
Qndvi.ccshau-5A	NDVI	5A	Sangwan et al. (2019)
QPro-5B	Proline content	5B	Hassan et al. (2018)
QWSC-4A	Water-soluble carbohydrates	4A	Hassan et al. (2018)

Transcriptomics has been utilized to investigate the up- and downregulation of key genes in response to several crops, including barley, wheat, rice and maize (Mangelsen et al. 2011; Frey et al. 2015; González-Schain et al. 2016; Wei et al. 2017). Many transcriptome studies exist for elucidating molecular mechanisms involving overlapping and distinct regulatory transcriptional mechanism of abiotic stress response in model plants and in some crops at a specific plant development (Li et al. 2019; Kang et al. 2020; Rangan et al. 2020).

Wheat, a hexaploid with a large and complex genome, requires modern NGS-based approaches to elucidate tissue and growth stage-specific heat-responsive gene expressions. Wheat transcriptome profiling can reveal the differential gene expression, genome annotations, regulatory factors, molecular markers and expression quantitative trait loci (eQTLs) and their sequence variants, controlling the traits of importance (Lal et al. 2021). HS tolerance mechanisms in model plant systems have been well studied, but understanding HS-induced genes and tolerance-associated proteins regulating carbon assimilation and starch biosynthesis, particularly in wheat, is still in progress (Kumar et al. 2013).

In wheat, RNA-seq has been adopted mainly to identify new and conserved transcripts associated with abiotic, biotic stress and nutrient-responsive genes (Rangan et al. 2020). It is accurate, rapid and comparatively cheaper and can be applied to non-model plant systems to extract novel genetic information (Unamba et al. 2015). De novo transcriptome assembly may be utilized to study the temporal and spatial gene expression of non-model organisms, which is an otherwise difficult

task without complete genome sequence information (Grabherr et al. 2011). HS-responsive transcriptome investigation using wheat genome arrays found changes in expression of *hsf*, *hsp* OF biosynthesis and signalling phytohormone genes, carbohydrate and calcium signalling pathways, ribosomes and RNA metabolic processes and metabolic genes for biosynthesis and regulation of primary and secondary metabolism. HS-induced gene expression of key genes, including transcription factors, heat-shock proteins (HSPs) and ROS scavenging enzyme expression, contributes to wheat's survival under HS (Comastri et al. 2018).

MicroRNAs and micromics research aid in revealing the complex regulation HS tolerance in wheat (Chinnusamy et al. 2007). Proteomics can also be utilized for structural and functional annotation of HS-related proteins and enzymes in wheat. Using quantitative proteomic analysis, the novel stress-associated active proteins (SAAP) have been reported to be crucial for HS tolerance. HSP17, RuBisCo, RuBisCo activase (RCA), superoxide dismutase (SOD), catalase (CAT), oxygen-evolving extrinsic protein (OEEP) and calcium-dependent protein kinase (CDPK) were among the 4272 SAAPs identified in wheat (Kumar et al. 2019a). Protein posttranscriptional modifications (PTM) are also playing an important role in HS regulatory mechanism in wheat (Chen et al. 2011), and proteomics technique can be utilized detecting and functioning modifications of various protein modifications. Metabolomics investigations can reveal changes in plant metabolites due to HS (Roessner and Bowne 2009). Wheat grain yields under HS are influenced by the mobilization rate of stem reserves to developing wheat grains (Hutsch et al. 2019). HS-induced metabolic reconfiguration in wheat plants has also been discovered to preserve homeostasis and necessary metabolism (Thomason et al. 2018). The metabolites anthranilate, dimethyl maleate, drummondol, guanine, galactoglycerol and glycerone that showed the greatest decline under HS were identified in the study. Advanced 'omics'-based techniques have provided a great deal of insight into the mechanism of HS responses and elucidated key regulators/mechanisms regulating cellular machinery for wheat survival under extreme temperature conditions.

5.8 Epigenetic Responses in Wheat to HS

The genetic basis of HS tolerance, including HS-induced genes and QTLs in wheat, along with mechanisms and regulation, has been extensively studied (Niu and Xiang 2018; Janni et al. 2020; Haider et al. 2021). But the epigenetic basis of HS tolerance in wheat, including DNA methylation, modifications of histone proteins, chromatin remodelling and the role of smRNA and short RNAs in HS-responsive gene regulation, is yet unknown (Gahlaut et al. 2020; Kong et al. 2020). HS significantly affected gene expression in a genome-wide examination of DNA methylation in wheat, but only minor alterations in methylation patterns were found (Lal et al. 2021). However, methylation has been linked to minor alterations in the expression of key genes in response to HS (Gardiner et al. 2015). Gahlaut et al. (2020) recently

discovered 52 cytosine-5 DNA methyltransferases (C5-MTases) and investigated their expression under HS and drought.

Interestingly, most of them are induced by both HS and water stress. It is discovered that TaDRM10-5A, TaDRM10-5B and TaDRM10-5D showed increased expression response to 6 h of HS (Gahlaut et al. 2020). Histone modification through acetylation in heat-shock factor A3 (HSFA3) at H3K9 as well as H3K14 and UV-hypersensitive 6 (UVH6) in *Arabidopsis* regulated by the General Control of Nonrepressed Protein 5 (GCN5) gene which encode a histone acetyltransferase confers HS tolerance in plants (Hu et al. 2015). Hu et al. (2015) also reported that the TaGCN5 gene was discovered to be upregulated in wheat in response to HS, suggesting that GCN5-regulated HS tolerance is conserved in both wheat and *Arabidopsis*. In wheat, the function of miRNAs are essential epigenetic key players which regulate HS-related signalling pathways in addition to DNA methylation and histone modifications (Xin et al. 2010; Gahlaut et al. 2018; Ravichandran et al. 2019). Xin et al. (2010), for example, discovered many HS-responsive miRNAs and further revealed upregulation of taemiR156 and consequently downregulation of putative target genes, SQUAMOSA, the promoter-binding (SBP) protein-like proteins (SPLs) in wheat under HS.

Additionally, Kumar et al. (2015) discovered six new miRNAs in response to HS in wheat. HS-regulated miRNAs along with their putative target genes in wheat were recently identified and verified using small RNAs and degradome sequence analysis (Ravichandran et al. 2019). They found 202 miRNAs in all, 36 of which were differentially expressed in response to HS. They also discovered that several of these miRNAs target HS response genes. MiR156 targets SPLs protein, MYB transcription factor is targeted by miR159, and superoxide dismutase is regulated by miR398 (Ravichandran et al. 2019). All these findings could be utilized for further understanding HS response and its regulation as well as could be utilized by the researcher to improve HS tolerance attributes in wheat.

5.9 Conclusion

Climatic change has affected the yield and quality of major food crops and thus poses a significant threat to global food security. Wheat is one of the most important cereal crops cultivated and consumed all over the world. In the current changing climate scenario, ever-increasing environment temperature is one of the major abiotic factors affecting worldwide wheat production. Increased global temperature poses a severe hurdle to agriculture globally, as it has a detrimental impact on wheat growth and development, resulting in lower yields and productivity. Exposure to elevated temperature conditions severely impacts all the aspects of wheat biology, including morphology, phenology, physiology and molecular biology. These alterations in wheat in response to heat stress can be better understood using modern biological tools, including genomics, transcriptomics, proteomics and epigenetics. The knowledge can be further applied to elucidate the complex mechanism of heat stress tolerance in wheat and other important cereal crops. This knowledge can be

further utilized in the identification, characterization and breeding strategies to develop heat stress-tolerant wheat varieties.

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Doubled-Haploid Technology in Maize (*Zea mays* L.) and Its Practical Implications in Modern Agriculture

6

Indu, Vijay Kamal Meena, Ranjit Saroj, Manoj Kumar Patel, Devender Sharma, Subhash Chand, Rajat Chaudhary, Rajesh Kumar Singhal, Reena Rani, and Amit Dadheech

Abstract

Maize (*Zea mays* L.) is the most important cereal crop in the world, consumed directly and indirectly. Doubled-haploid (DH) technology in maize has emerged as a promising tool for accelerating the development of completely homozygous lines in a much shorter time than conventional breeding methods. The breeding cycle is shortened and genetic gain is enhanced using the rapid doubled-haploid line generation method. Haploids are created mainly using traditional techniques, such as in vitro and in planta processes, and are then transformed into doubled haploids either naturally or through chemical means. The recent developments in

Indu · R. K. Singhal
Crop Improvement Division, ICAR-Indian Grassland and Fodder Research Institute, Jhansi, India

V. K. Meena
Agriculture Research Sub-Station (Sumerpur), Agriculture University, Jodhpur, India

R. Saroj · M. K. Patel · R. Chaudhary
Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi, India

D. Sharma
Crop Improvement Division, ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, Uttarakhand, India

S. Chand (✉)
Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi, India

AICRP on Forage Crops and Utilization, ICAR-Indian Grassland and Fodder Research Institute, Jhansi, India

R. Rani
Division of Plant Improvement and Pest Management, ICAR-Central Arid Zone Research Institute, Jodhpur, India

A. Dadheech
Department of Plant Breeding and Genetics, RCA, MPUA & T, Udaipur, India

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understanding the genetic and molecular mechanisms of doubled haploidy have opened new avenues for precise genetic improvement in a shorter time. Marker-assisted breeding can be combined with doubled haploidy to fix favorable alleles for a variety of traits in a single DH line. Additionally, the method can be employed for reverse breeding, CMS line development, and uncovering the genetic diversity found in untapped germplasm and landraces. The future of DH breeding is bright since reliable DH production techniques are available and marker-assisted technologies are being more closely incorporated.

Keywords

Doubled haploid · Haploid inducer · Maize · Genetic gain · Induction rate

6.1 Introduction

Maize (*Zea mays* L.) is the third most important cereal crop after wheat and rice which is grown for its versatile use for food, feed, and industrial products. It fulfills the primary calorie requirement in many developing and developed nations. Demand for maize worldwide would be increased by over 50% from 558 million tons in 1995 to 837 million tons by 2020 (Pingali and Pandey 2001). The demand for maize in developing countries alone was projected to rise from 282 million tons in 1995 to 504 million tons by 2020 (IFPRI 2000).

It can grow successfully in tropical, subtropical, and temperate agroclimatic situations anywhere in the world due to its greater genetic variability. Development of fully homozygous inbred lines is the prerequisite for maize hybrid breeding programs. It was established that the primary goal of the maize breeder is to identify the best hybrid combination of parents in a maize population to grow seed corn that results in a good harvest (Shull 1908). In modern agriculture, farmers can grow two types of maize varieties: open-pollinated varieties (OPV) and hybrids. Since the development and popularization of single cross hybrids, the maize improvement programs are mainly focused on developing high-yielding single cross maize hybrids. Single cross maize hybrids are derived from two genetically diverse homozygous inbred lines. The inbred lines generally developed through conventional breeding techniques require more time and resources. This results in nearly homozygous inbred lines after six to ten generations of selfing (Odiyo et al. 2014). The availability of haploid inducers (maternal/paternal) made it possible to generate a completely homozygous (100%) inbred line in just two seasons. This saves time and resources for developing the maize inbred lines. The maternal haploid inducers and paternal haploid inducers have different induction rates.

It all began with identifying and developing naturally existing haploid lines in maize (Chase 1969). The study of Coe (1959), who used the haploid inducer "Stock 6" to produce haploids in maize, represents the main advancement in haploid breeding of maize. Maternal and paternal inducers are the two main categories of

haploid inducers. Paternal haploids employ haploid inducers as female parents, while maternal haploids use haploid inducers as pollen parents. In earlier investigations, the gene *ig1* (indeterminate gametophyte 1) was discovered to be a trigger for paternal haploid induction (Kermicle 1969; Evans 2007). However, the paternal haploid induction method is the least recommended by the researchers for maize breeding programs due to the low frequency of haploid induction (Kermicle 1994) and inheritance of cytoplasm from inducer line in haploids (Kermicle 1973). Maternal haploids, in contrast, inherit their cytoplasm and nucleus from the same female parent, making this approach superior to paternal haploid induction. With time, this approach has been improved because of the discovery of temperate inducers (WS14, MHI, PHI, CAUHOI, and RWS) with a higher haploid induction rate than Stock 6 (Wu et al. 2014), which have been extensively employed in maize breeding programs. Tropically adapted haploid inducer lines (TAILs) with high induction rates have also been developed for tropical regions where temperate inducers produce inferior outcomes. Various breeding efforts have been made in the last two decades to develop the haploid inducer lines with a greater induction rate. CIMMYT has developed the second-generation haploid inducer lines (CIM2GTAILS) with a higher haploid induction rate.

In the first season of the doubled-haploid technology, haploidy is induced in diploid maize plants, which results in the progeny's chromosomal pairs being reduced to single chromosomes. The haploid chromosome set is duplicated in the second season using a specific chromosomal doubling process (mainly colchicine), which entails making copies of each chromosome to produce pairs of identical chromosomes. The outcome is a diploid maize plant, often known as a "doubled-haploid (DH)" plant, because in each pair of chromosomes, one chromosome is a copy of the other chromosome and the plant has homozygosity levels of up to 100% (Fig. 6.1). Guha and Maheshwari (1964) presented anther culture method to produce haploids in a lab environment for the first time. Niizeki and Oono both created rice haploids in 1968. Since over 250 species have used DH technologies, creating DH lines from heterozygous material is not very time-consuming.

DH lines display the complete genetic variability at the beginning of the selection program, simplifying the screening of outstanding genotypes. As we know, greater genetic variance leads to high heritability of genotypes per se; testcross evaluations improve this accuracy; therefore, purely 100% homozygosity suggests that no remaining heterozygosity is hiding the genotype performance, thus assuring that line selection can be achieved earlier. DHs have more per se performance for morpho-agronomical characters because more selection is enforced during the haploidy level. When recessive alleles come in a homozygous state, it's pretty easy to chuck out recessive deleterious alleles effectively from germplasm pools because haploids cannot counteract their unfavorable impacts.

Resources that may be supplied for testcross evaluation are unavailable due to testing of multiple later generations. In short, DH technology allows breeders to examine more hybrid combinations in a shorter time, realizing maximum genetic gain per cycle, reducing developmental cost, and enhancing the efficiency of the

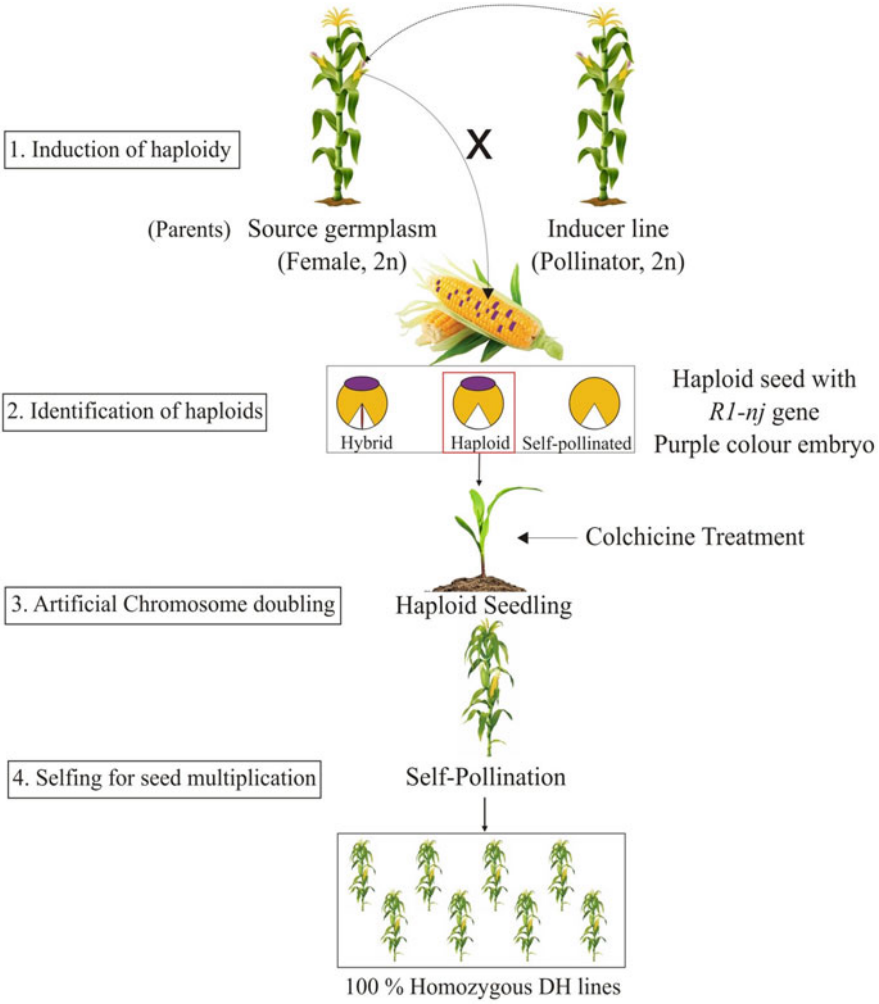

Fig. 6.1 An overview of steps involved in doubled-haploid technology

breeding program. The doubled-haploid approach may be regarded as the third most significant technology in a maize breeding program, after hybrid technology and off-season nurseries.

6.2 Haploid Generation

Production of haploids is a crucial stage in creating DH lines because it allows for the induction of haploidy both in vitro and in vivo. In vitro production of haploids requires aseptic conditions for the cultivation of anthers or microspores or female

(ovules) gametophytes to induce embryogenesis leading to the development of haploid plants. Generating in vitro haploids has not become a useful strategy in maize breeding because in vitro culture responsiveness in maize is entirely genotype dependent (Buter 1997; Tang et al. 2006).

Haploids can be generated in vivo by the following steps:

- Interspecific crossing in barley (*Hordeum vulgare*; by pollination with *H. bulbosum*) and wheat (by pollination with maize)
- Pollinating with the pollen that has been irradiated with heat or chemicals
- By crossing with a specific haploid inducer genotype (Chase 1952)

6.2.1 In Vivo-/Inducer-Based Approach

The proportion of haploids produced to the total induced cross progeny refers to the haploid induction rate (HIR) of the inducer employed. It is used to develop doubled haploids in maize. Haploid seed induction rate of inducer lines is 8–12%. Using the inducer as female will yield paternal haploids, while employing the inducer as male will produce maternal haploids. Table 6.1 mentions various maternal haploid inducer lines developed at different international institutions.

The DH technology adoption process in tropical countries is not as much as in temperate countries due to lacking of inducer lines having high HIR and wider adaptability. Molecular markers are being recently used for development of inducer

Table 6.1 List of maternal haploid inducer lines developed at various international institutions

Inducer line	Characteristics	HIR (%)	Researchers
Stock-6	Population of self-pollinated progeny	~3.2	Coe 1959
Wisconsin-23	Parental haploid inbred	3	Kermicle 1969
ZMK-1	Inducer population	6–8	Zabirova et al. (1996)
ZMK1U	Direct selection from ZMK1	11–13	Shatskaya (2010)
WS-14	W23gi × Stock-6	3–5	Lashermes and Beckert (1988)
RWS	WS14 × KEMS	7–9	Röber et al. (2005)
MHI	Carries *A1*, *B1*, *C1*, and *Rj-n1* alleles	7–9	Chalyk (1999)
PHIs	Four inducer lines (1–4)	10–15	Rotarenco et al. (2010)
CAU0I	High oil content	~3	Chen and Song (2003)
CAU5 and CAU079	High oil content	6–8	Xu et al. (2013)
UH600 and UH601	High oil content	~10	Melchinger et al. (2013, 2014)
TAIL	Tropical inducer line	~5–15	Prigge et al. (2012a, b)
CIM2GTAILS	Tropical inducer line	~5–15	Chaikam et al. (2018)

Table 6.2 A list of factors that affects the haploid induction rate

Factors	Particulars	HIR rate	References
Season	Winter	Higher	Kebede et al. (2011)
	Warmer	Higher	De La Fuente et al. (2018)
Crop	Barley (summer)	Higher	Pickering (1984), Pickering and Morgan (1985)
Silk age	Older	Higher	Chase (1969), Seaney (1954), Tyrnov (1997), Chase (1974)
Mode of haploid production	Hand pollination	Higher	Rotarenco (2002)
Donor genetic background	Flint Dent Flint × dent	Lower	Eder and Chalyk (2002)
	Hybrid derived from inbreds	Higher	De La Fuente et al. (2018)

lines (Dong et al. 2014). It was recently reported that genes encoded for pollen-specific phospholipase are necessary for producing seeds having haploid embryos. Prigge et al. (2012a, b) reported a gene *GRMZM2G471240* named *ZmPLA1*, located at locus QTL *qhir1*, showed 66% genetic variance in three populations obtained from a cross between inducer and normal germplasm line, and their results showed that HIR have epistatic genetic control. In haploid inducer development, single fertilization happens. A sperm cell fertilizes only the egg or central nuclei cell and forms a haploid embryo (Sarkar and Coe 1966). Maize impend double fertilization by starting the formation of a second pollen tube, which is fused with a second synergid cell. This mechanism is known as heterofertilization (Uliana Trentin et al. 2020). Sprague first reported the heterofertilization in maize in 1929, who stated that it occurs at an average of <2.0% (Sprague 1932). Inducer development is also affected by certain factors that are enlisted in Table 6.2.

The introduction of CRISPR-Cas9 construct into maize haploid inducer line having a transgene *CENTROMERIC HISTONE3* (CENH3) induces maternal and parental haploids (Ravi and Chan 2010). Kelliher et al. (2017) showed evidence that CRISPR-Cas9 can be combined with a different method of haploid induction efficiently and effectively into cultivars. Inbreds, hybrids and synthetics are developed in maize and could also be exploited for development of inducer lines.

6.3 Types of Inducer Parents

Most widely inbred lines are used as inducer parents to produce doubled-haploid progeny in plant breeding programs. However, hybrid inducers such as tropical climate-adapted hybrids developed by CIMMYT (Prasanna et al. 2012), RWS/RWK 76-a German hybrid (Flint-Garcia et al. 2003), and inducer population such as ZMK

1 (Shatskaya 2010) are also used. Each type of inducer parent has some advantages and disadvantages over each other.

6.3.1 Inbred as a Inducer

Globally inbred inducers are used as maternal haploid inducer parents due to their breeding true to type and uniformity, easy maintenance and multiplication, and rouging advantage of off types over all other inducers by only visual observations. Alleles for specific traits will be in homozygous state and would also be easy to incorporate them into new inducer inbred lines. If haploid sorting is done based on OC (oil content) value, then an inbred inducer will be best because there will be no classification error as if hybrid and composite inducer lines are used. The major limitations are that inbreeding depression tends to reduce hybrid vigor, changing plant morphological behavior, increases the susceptibility to major and minor diseases and insects pests, low seed setting, and fertility of pollens for a long spell. They show weaker performance in the isolation field to produce many haploids. However, they show better ergonomics when hand pollination is used in induction nurseries for limited seed production.

6.3.2 Hybrid as a Inducer

Hybrids used as inducers, being heterotic in nature, tend to produce larger tassels and have abundant fertile pollen and tolerance to diseases and insects. However, due to its gametophytic nature, HIR does not show hybrid vigor (Prasanna et al. 2012). The major challenges are that trait of interest must be in a homozygous state and to achieve it, both the parents must be in a homozygous state for the desired trait; otherwise, heterozygosity will tend to generate variability in the haploid progeny which makes them unsuitable for accurate screening and identification. Hybrid inducer lines need to create and maintain a separate genetic pool and spatial and temporal isolation for inbred maintenance and hybrid seed production. Hybrid inducer lines are much taller than inbred and synthetics, making them lodging susceptible, which is one of the key challenges, and unfit for areas where high wind speed prevails. Qualitative trait such as *R1-nj* is easy to incorporate, while quantitative trait such as OC is difficult, challenging, and time-consuming to incorporate in hybrid inducer at homozygous condition.

6.3.3 Synthetic as a Inducer

Synthetic inducers contain the desirable traits of both inbreds and hybrids. Synthetic inducer lines also show some extent of hybrid vigor over inbreds, but less than hybrid inducers, and the extent of vigor depends on the genetic dissimilarity between crossable genotypes. They are easier to develop and maintain if inbreds are used as parents. They produce fertile pollen for a long time spell due to more genetic

variability than inbreds and hybrids. These lines are not as heterotic as hybrids, thus producing less amount of pollens and comparatively more susceptible to diseases and insects. These lines must be reproduced at a periodic and regular time interval to maintain their vigor and desired trait level, which was jeopardized due to natural contamination and drift. A major challenge is the fixation of desirable marker traits in the developed population when more parents are involved in genesis.

6.4 Development of New Maternal Inducer Inbred Lines

Inducer inbred lines were extensively developed for temperate climatic conditions. But these inducer lines were not well eco-adapted to tropical conditions and showed poor agronomic performance under appropriate management practices. Thus, there is a separate need to develop new well-adapted inducer lines with good agronomic performance in tropical environments by using a robust tropical breeding program

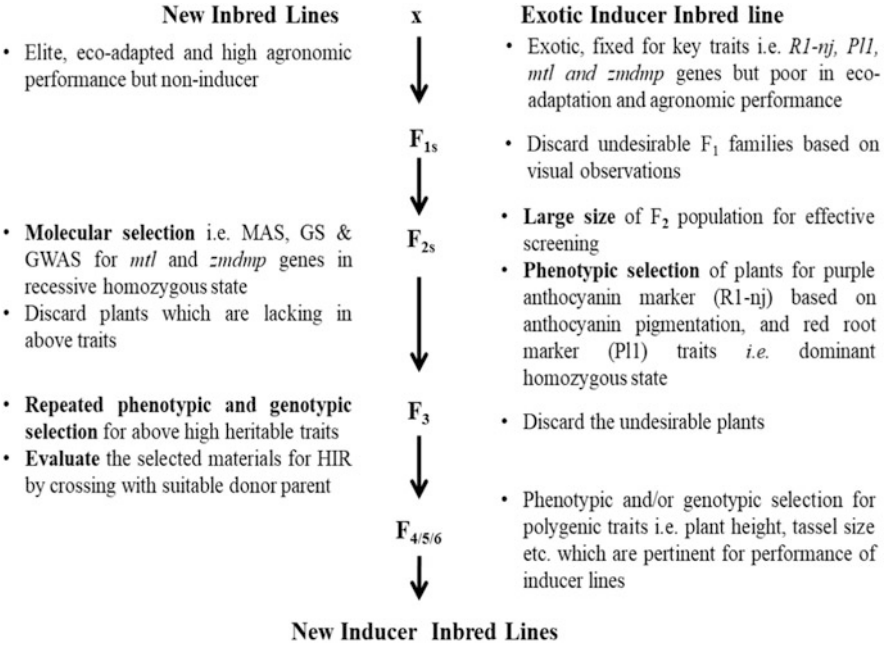

Fig. 6.2 Development of new tropical maternal haploid inbred lines by using exotic-cum-non-adapted and poor agronomic performance of temperate inducer inbred lines. Crossing between elite-non-inducer inbreds and exotic inducer inbred tend to produce F_1 families. Selection for moderate and high heritable traits such as purple embryo pigmentation (PEP), red root traits, and *mtl*, i.e., disrupts maternal haploid induction at an early generation of selfing (F_2 and F_3), while for low heritable traits such as resistance to some disease and yield, the selection is desirable in F_4 generation onward. The combination of phenotypic selection (PS) with genomic selection (GS) and genome-wide association studies (GWAS) accelerates the breeding progress, reduces the breeding cycle, and, ultimately, increases the genetic gain of desirable traits

(Fig. 6.2). Exotic inducer lines are fixed, i.e., homozygous state, with wide differential morphological marker genes such as *R1-nj* (purple embryo marker), *Pl1* (red root marker), *mtl* (maternal haploid induction), and *zmdmp* (increases the haploid induction rate of inducers). These exotic inducer lines are used as pollen sources, while elite or advanced well adapted and good in agronomic performance inbred lines are used as seed parents under the crossing program. A large number of F_1 families must be produced to avoid the loss of good genotypic material in the subsequent generations. Discard F_1 families which are undesirable for inducer lines. Selected F_1 progenies are self-pollinated to produce a large number of F_2 populations to obtain a desirable number of genotypes (~0.4%) with fixation of the above-mentioned genes (Uliana Trentin et al. 2020). Phenotypic selection (PS) can be made for the *R1-nj* trait, while marker aided selection (MAS) for *Pl1*, *mtl*, and *zmdmp* for their fixation in F_3 generation. In the subsequent generations, i.e., F_4 and onward, selection must be made for polygenic traits such as plant height, tassel length, pollen production and duration, haploid induction rate, lodging tolerance, and seed set.

Genomic selection (GS) has been used to improve traits essential for inducers. Nowadays, the DH breeding program combines GS to achieve maximum genetic gain. Through the integration of GS at a haploid level during haploid inducer line development, we can select only superior haploids through individual haploid genotyping for self-pollination, reducing the time and size of the population to be selfed. GS is based on prediction accuracies, and analysis is done by using genotypic and phenotypic data. Higher prediction accuracy tends to create a more accurate and precise selection for trait of interest. The most challenging is evaluating HIR for inducer lines which are complex, time-consuming, and labor-intensive. There must be a high seed production to evaluate accurate HIR. Human error is greater while separating the haploid seed from a mixture of selfed diploid and crossed diploid seeds. Haploid seeds are selected based on the expression of *R1-nj* gene (purple embryo pigmentation) in the embryo. Its expression also depends on factors such as environmental conditions (Prigge et al. 2011), seed morphology, and inhibitor gene from the donor parents (Paz-Ares et al. 1990). Thus, analyzing the HIR rate large sample size and the number of people involved might be time-consuming but will be most effective. Newly developed inducer lines can be effectively used for haploid seed production in tropical areas of the world.

6.5 Steps Involved in Doubled-Haploid Production Technology

6.5.1 Step 1: Detection of Putative Maize Haploid Seeds

The cross between normal germplasm and inducer inbred line generally produces three types of seeds: hybrid, haploid, and self/outcross. We can visually distinguish these seeds through an effective phenotypic marker system. Inducers carry a dominant gene, *R1-nj*, which can be used as an embryo- or endosperm-specific marker

gene, which induces purple coloration of the scutellum and the aleurone of seeds. The endosperm and embryo of normal maize plant are triploid and diploid, respectively, because they are aroused from fusion of two female polar nuclei with one male sperm cell and the fusion of the egg cell with the remaining sperm cell. Therefore, as the purple *R1-nj*-encoded coloration is dominantly inherited, only seeds of the haploid embryo will have a nonpigmented scutellum, while seeds with diploid embryos have purple-colored scutellum. In line with the before, scutellum pigmentation helps differentiate haploid and diploid seeds, whereas aleurone pigmentation helps to categorize haploid and diploid seeds from the outcrosses (without pigmentation) (Khulbe et al. 2022). Another phenotypic marker involves the Pl1 gene in which hybrid plant roots show red coloration, whereas haploid plant roots remain white. The mutant carrying recessive morphological traits such as liguleless or glossy appearances on leaves is the most authenticate method of identification of haploids. Tester for liguleless and glossy traits has been widely used to examine HIR during genetics-cytological studies, development of inducer, and maintenance activities. At the molecular level, through marker-assisted selection (MAS), we can identify the haploids by fixing the genes like *R1-nj* (purple colored embryo), *Pl1* (red root marker), *mtl*, and *zmdmp* in the adopted inducer inbred lines. Recently authors also stated that oil content of seeds can also be used in haploid seed selection. We have summarized some trait-specific genes important to haploid inducers and are helpful in distinguishing haploid seeds from diploids with their mode of gene action in Table 6.3.

6.5.2 Step 2. From Haploids to Doubled Haploids via Duplication of Chromosomes

In vivo production of maize doubled haploids involves artificial chromosome doubling as most haploid plants are sterile due to disrupted gamete formation. Therefore, doubling the haploid chromosomes is required for the seed set and maintenance of the genotype, so self-pollination can occur in doubled-haploid plant. In maize, the most common integral part of doubled-haploid standard protocol is colchicine, an alkaloid extracted from meadow saffron (*Colchicum autumnale* L.) that inhibits spindle fiber formation during mitotic division (Prigge et al. 2012a, b). Chromosome doubling through colchicine is the most promising and economic method as it has the most success rate; on the other hand, it is hazardous also. Trained persons are required for its handling, personal care, storage, and proper disposal after its use. The steps for chromosome doubling make the doubled-haploid technology expensive for its extensive use in developing countries.

Altogether, these constraints underline the necessity of replacing the colchicines with other alternative methods to spontaneously enhance chromosomal doubling. The treatment of haploids with nitrous oxide also observed anti-microtubule effects (Kato 2006). Cycloalkane is also reported as chromosomal doubling agent but it has not been adopted on large scale and limited information on its success rate is available (Cori Cui et al. 2013). To further have an alternative approach for

Table 6.3 List of genes/QTLs important for haploid induction and discrimination between haploid and diploid seeds

Genes/ QTLs	Trait	Genetic control	Gene action	Desirability	References
R1-nj	Purple embryo marker	Monogenic	Dominant	At seed stage haploid selection	Chase and Nanda (1965)
Pl1	Red root marker	Monogenic	Dominant	At seedling stage haploid selection	Emerson (1921)
B1 and Pl1	Purple sheath, husk, and culm	Digenic	Dominant	Before flowering haploid selection	Chandler et al. (1989)
mtl/nld/ zmpla 1, zmdmp	Haploid induction in maternal inducer	Monogenic	Recessive	Required for haploid embryo formation	Kelliher et al. (2017), Liu et al. (2017), Gilles et al. (2017), Zhong et al. (2019)
qhir 2–7, zmdmp	HIR of maternal inducers	Polygenic	Additive, dominant, and recessive	Efficiency determination in which haploid seeds are formed	Prigge et al. (2012a, b), Liu et al. (2015), Zhong et al. (2019), Chase (1947), Melchinger et al. (2014)
ig1	HIR of paternal inducers	Monogenic	Recessive	Efficiency determination in which haploid seeds are formed	Kermicle (1969), Kindiger and Hamann (1993), Lashermes and Beckert (1988)
lec1, DGAT1-2, OBAP1, WRI1	Oil content	Polygenic	Mainly additive	Oil content can be used to differentiate between haploid and diploid seeds	Moreno-Gonzalez et al. (1975), Berke and Rocheford (1995), Laurie et al. (2004), Zhang et al. (2008), Moose et al. (2004), Shen et al. (2010), Cook et al. (2012), López-ribera et al. (2014)

chromosomal doubling, Melchinger et al. (2015) used two phytohormones [amiprophos-methyl (APM) and pronamid] in their experiment in different ratios to treat the maize haploid seedling. They reached almost the same result as colchicine without risk of toxicity and suggested that pronamid at optimum dose is as good as colchicine for chromosomal doubling. A recent review suggests that detecting quantitative trait loci (QTLs) inducing spontaneous haploid genome doubling (SHGD) can be introgressed into the genome of the source germplasm by crossing

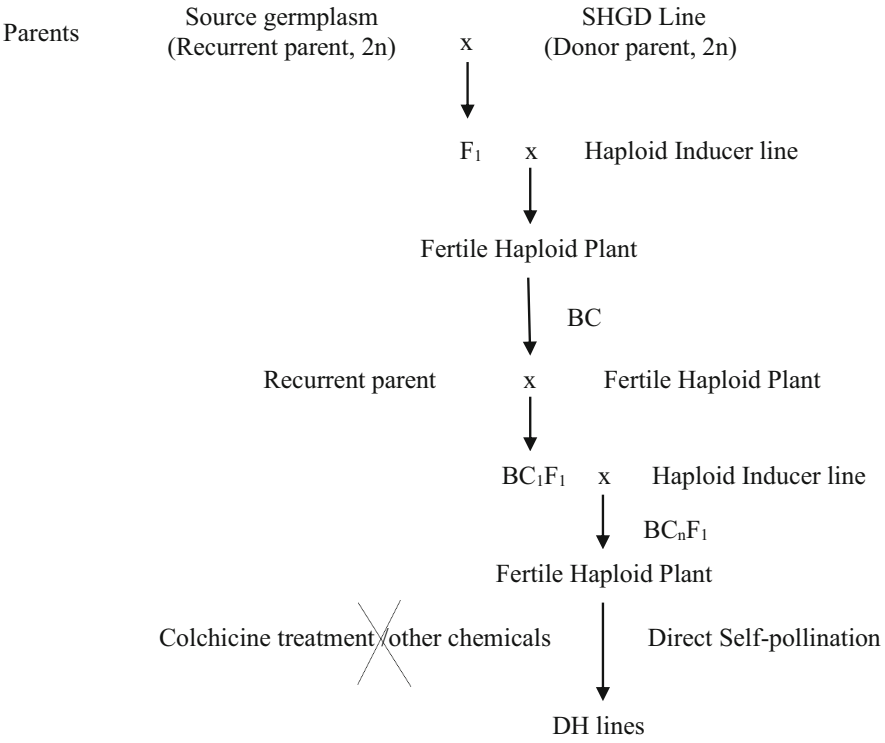

Fig. 6.3 Schematic representation of a breeding procedure for introgression of SHGD into non-SHGD source germplasm line and showing the haploid plants can directly be selfed without undergoing any chemical chromosomal doubling treatment

it with the donor SHGD line and their F_1 crossed with a haploid inducer line. These haploid seeds are repeatedly backcrossed to recurrent parents up to the desired number of times. The end progeny will have induced SHGD in its genome, and no chemical treatment is necessary for chromosomal doubling. These backcrossed introgressed SHGD-induced progenies can directly be selfed to produce DH lines (Boerman et al. 2020). It has been observed that SHGD chromosomal doubling increased from 5 to 50% and explained that epistatic gene interactions were present for SHGD, which could be exploited instead of artificial chromosomal doubling that ranges from 10 to 30% (Molenaar et al. 2019) (Fig. 6.3).

6.5.3 Step 3. Self-Pollination and Genetic Nature of D_1 DH Population

Plants treated with colchicine are called as D_o. Selfing of D_o plants will produce D_1 seeds. The D_1 consists of newly developed completely homozygous DH inbred

lines. Many D_o plants produce a limited number of seeds, as low as one. Just 3–5% of all haploid plants of a genotype will develop into DH lines. It has been reported that the genetic variance of a DH population is greater compared to segregating F_n populations obtained from the same parental cross (Seitz 2005). The more homozygous and homogenous nature of doubled haploid enhances the heritability compared to F_n segregating families. The genetic gain that increased through the use of the DH line can be calculated by using the following equation:

$$G_C = \frac{ih^2\sigma p}{t}$$

where i is the selection differential, h^2 is the narrow sense heritability of the selected trait (s), σp is the phenotypic standard deviation, and t is the time taken per breeding cycle (Boerman et al. 2020). DH population exhibits only additive genetic variance because of homozygosity at all loci and reflects higher covariance than any other population. The use of the DH population increases the genetic gain due to only additive genetic variance, which parallelly increases the response to selection, positively increases the heritability, and ultimately allows greater repeatability, through which environmental variation can be reduced by increasing replications.

6.6 Utilization of Doubled Haploids in Various Maize Breeding Programs

1. Geiger and Gordillo (2009) conducted an experiment by using maize doubled-haploid technology and suggested that the use of doubled haploids (DH) can be routinely used in maize (*Zea mays* L.). If off-season nurseries are available, two testcross generation evaluations can take place in only 4 years through developing one cycle DH line. When three breeding steps, including recombination, haploid induction, and DH plant development, are completed in a single year, then the duration of the cycle can be reduced to 3 years. Genome-wide marker-assisted selection can be incorporated effectively into DH line-based breeding technologies.
2. Smith et al. (2008) have suggested that DH progeny inherit a major portion from parental chromosomes. Third-generation DH progeny were selected that were more than 90% similar to one of the parents. They suggested that DH technology allows taking up the genome of a commercial hybrid already present in the domain. The study showed that the DH population has the largest area because it extends utmost toward extremes of parents' values. The study also conveyed that the DH population is more effective and efficient than the RIL and F_2 population in accessing the parental genotype to the utmost level.
3. Wu et al. (2014) used the inducer line CAU5 to pollinate a mapping population made up of 186 $F_{2:3}$ family lines developed from spanning Zheng58 and Chang7-2 and then choose the haploid kernels using *R1-nj* kernel markers to address the maternal genetic contribution to haploid formation. To find QTLs relating to

haploid inducibility, they created an $F_{2:3}$ population. On chromosomes 1 and 3, two quantitative trait loci (QTLs), *qmhir1* and *qmhir2*, were found which are involved in the maternal genetics of haploid induction.

4. Odiyo et al. (2014) experimented with 160 DH testcross hybrids and five checks. The material was evaluated under two locations; one was well watered and the other was at a drought location. Their combined analysis showed that the best 20 hybrids expressed better performance for grain yield and other agronomical characters of maize than the checks. The top ten DH testcross hybrids yielded 16% higher than the best check. While under drought location, the top ten DH yielded 62% higher than the best check. According to these findings, maize hybrids developed using DH lines had comparable grain yields and acceptable agronomic features to commercial hybrids produced using traditional pedigree techniques.
5. In 2006, Mayor and Bernardo (2009) examined 430 DH testcross lines in many environments, and marker-trait connections for grain yield, moisture, plant integrity, and staying green were found. The best DH lines in the initial mapping population were then intercrossed after three rounds of marker-assisted recurrent selection (MARS), performed from the F_2 of the original cross. They also chose the top DH lines for 2006 (Phen-1) and 2007 based on testcross phenotypic scores (Phen-2). In this study, Phen-1 came from screening the DH testcrosses in just 1 year at eight different sites, whereas Phen-2 came from screening a better selection of DH test crosses in 2 years at 17 different locations. Researchers have hypothesized that the additional screening conditions employed in Phen-2 compared to Phen-1 would allow more accurate identification of better DH lines.
6. Mahuku et al. (2011) studied temperate inducers UH400 and RWS for induction of tropical source germplasm that includes landraces, OPVs, and single cross hybrids. The identification of haploid seed was done using a seed purple color marker controlled by the *R1-nj* (R-Navajo) gene. Crosses were made between CIMMYT advanced lines as females and inducer hybrids RWS × UH400 and RWS × RWK as pollinators, as well as backcrosses to both parents. HIR for the two temperate inducers was generally high and similar with results obtained in the temperate zone, indicating that they can be directly used in the tropical environment. The source germplasm showed a significant difference in HIR. That indicates that source germplasm is an important factor that contributes to different HIR in addition to the inducer. Therefore, the number of plants to be induced to obtain the desired number of DH lines differs for different source germplasm. Furthermore, the winter season had higher HIR, which shows that the environment plays one important factor in determining HIR; thus, the winter season was more suitable than the summer season for induction at Agua Fria, Mexico. This confers that DH technology can be initiated directly with the temperate inducers by pollinating a sufficient number of plants of source germplasm under suitable environmental conditions.
7. Georgeta and Cristea (2016) used Procera Haploid Inducers (PHI), which are highly suited to temperate temperature circumstances due to their high inducer

rate (HIR) and ample and high-quality pollen and excellent phenotype. To produce haploids and doubled-haploid parent lines through PHI, three synthetic populations (SP) from the most significant heterotic groupings were crossed. Twenty DH parent lines plus the four original parental lines that served as the study's controls made comprised the 24 lines in each trial. There were three experiments, one for every set of DH parental lines that were a part of the three synthetic populations. As shown, DH parent lines outperform parental line components in synthetic populations for all attributes studied. The traits associated with atmospheric heat tolerance, like anthesis-silking interval and prolificacy, showed the best results. From their research, it can be inferred that haploid technologies are characterized by complete homozygosity of doubled-haploid lines, phenotypic and genotypic uniformity of doubled-haploid and hybrids, and increased anthesis-silking interval. These traits reduce time and costs in maize breeding and significantly increase the efficiency of selection procedures.

8. Ryu et al. (2016) settled this technology in Korea to identify haploid-inducing factors and to develop temperate inbred lines for hybrid breeding. Haploid induction was done by using eight populations crossed with inducer line (TAILs) and through treatment with colchicine (0.04%), and 12-h chromosome doubling was done. The 11 inbred lines' doubled-haploid lines were selected. The average haploid induction rate was 4.1% when the inducer was crossed with three maize populations. They may significantly shorten the time required for line development and improve Korea's maize breeding research technique.

6.7 Application of Doubled Haploidy

6.7.1 Rapid Development of Homozygous Lines

The development of homozygous lines such as inbreds in any cross-pollinated crops is an important breeding objective. Conventional breeding techniques such as pedigree, bulk, SSD, and backcross methods require much more time to develop inbreds. Even off-season nurseries and shuttle breeding require several rounds of inbreeding to select a homozygous line (Tadesse et al. 2012). However, due to residual heterozygosity in cross-pollinated crops, complete homozygosity cannot be attained (Baenziger and Peterson 1992; Baenziger and DePauw 2009). Hence, to save the valuable time of breeders, doubled-haploid technique can be adopted to obtain a complete homozygous line in one or few generations. Doubled-haploid (DH) technique aids in rapid crop improvement by reducing several cycles of inbreeding to obtain a homozygous line (Tadesse et al. 2012). After obtaining a homozygous line, It can be utilized further in several ways, such as a new variety (in self-pollinated crops), as parent in a hybridization program, or as a mapping population in a gene/QTL mapping program.

6.7.2 Cytogenetic Studies

Doubled-haploid technique is useful in cytogenetic studies such as chromosomal pairing and production of aberrant chromosomal complements like monosomics, nullisomics, etc. Being univalent, haploids provide special opportunities to study pairing relationships among chromosomes. Using a modern biotechnological technique like plant tissue culture, the production of homozygous lines became easy by exploiting the haplo-diploidization system (Baenziger and DePauw 2009; Wu et al. 2012). In some crops, the DH technique has also developed chromosome substitution and chromosome addition lines.

6.7.3 Selection Breeding

The DH technique results in a complete homozygous line; consequently, it favors additive genetic variance that eventually increases selection efficiency. DHs also had a role in the recurrent selection; the superior DH of the first cycle can be used as a parent for hybridization in the next cycle; however, slow genetic improvement is expected using this technique due to frequent crossing, DH production, and selection (Tadesse et al. 2012). Using the DH technique, rapid crop improvement was observed in maize and barley (Seguí-Simarro 2015). DH technique is the third most important milestone in maize breeding after hybrid and off-season nurseries (Seitz 2005). It has also been used in crops like *Brassica*, wheat, barley, and rice (Dwivedi et al. 2015). Haploids having a single copy of the genome express deleterious recessive alleles and can eliminate them in early generations.

So, this technique permits a more effective assessment of the genetic diversity of landraces and open-pollinated varieties that could be hampered by heterogeneity and deleterious effect (Melchinger et al. 2018). Homozygous lines obtained from the DH technique could be grown in different environments as these lines have wider adaptability due to a broad genetic base.

6.7.4 Mutation Breeding

Mutation breeding is an important application of the DH technique (Zhu et al. 1993). In *Brassica* species, in vitro screening of herbicide-resistant mutants can be achieved through the DH technique (Beversdorf and Kott 1987). Further, recessive mutants can easily be recognized by DH techniques as compared to conventional breeding methods. In DH lines, the selection of mutants for quantitative traits became easy due to the fixation of mutation and desired recombinant (DePauw et al. 2011; Wu et al. 2012).

6.7.5 Production of Male or Female Plant

DHs could have applicability in producing male or female plant from dioecious crop species like asparagus, hemp etc., as haploids can be produced from both male and female gametes.

6.7.6 Mapping Quantitative Trait Loci (QTL)

DH lines have been used as mapping populations in molecular mapping program (Chauhan and Khurana 2011). These lines are non-segregating and hence can be used as perpetual mapping populations. These lines are free from residual heterozygosity; consequently, they are equally effective in self- and cross-pollinated crops. In barley, doubled-haploid lines are used in marker-assisted backcrossing program to select strip-resistant lines (Chen et al. 1994). DH technique produces a mapping population in a few generations, resulting in rapid gene identification compared to other mapping populations. Further, using this technique, landraces and biparental populations can be applied for genomic selection and association studies (Melchinger et al. 2018).

6.7.7 Stability of Agronomic Traits

Haploids of wheat/maize crosses are used for genetic studies and crop improvements (Amin et al. 2010). DHs being homozygous lines are genetically stable; therefore, introduced variance could be identifiable at any stage of the breeding program (Suenaga and Nakajima 1993). Rapid production of fixed lines using the DH technique helps in improving the stability of various agronomic traits.

6.7.8 Bulked Segregant Analysis (BSA)

BSA uses two extreme bulks to identify putatively linked makers. Selecting extreme types for a particular trait is difficult in segregating mapping populations like F_2 as it may involve both heterozygotes and homozygotes in bulks of the dominant allele. In contrast, perpetual mapping populations like DHs involve only homozygotes in bulk, which excludes the possibility of ambiguity in the experiment. The DH lines remove the heterozygosity and confirm the disease reaction and its testing can be repeated several times (Knox et al. 1998). The use of DHs in BSA has wider applicability in crops like rapeseed and barley.

6.7.9 Exchanging Cytoplasmic and Nuclear Genome

Haploids could be easily applicable in rapid development of different cytoplasmic and nuclear genome combinations by transferring nuclear genome into a heterologous cytoplasm. Alloplasmic lines are the best-suited example, which can be developed using haploid inducer lines. Further, cytoplasmic male sterility can be transferred in two generations using this approach.

6.7.10 Reverse Breeding

DH technique has an important application in reverse breeding. Reverse breeding inhibits the meiotic crossing over in F_1 generation and results in nonrecombinant parental gametes; further, using the DH technique, these parental gametes can be developed into doubled-haploid plants. Original hybrids can be obtained by crossing complementing DH lines assigned to different heterotic pools based on genetic diversity.

6.7.11 Application in Crop Improvement

Doubled-haploid technology can be utilized in crop improvement. The best instance of crop improvement using DH technology is maize, which was used to develop inbreds within a short period of time. According to the breeders' equation, the genetic gain is inversely proportional to the time required. Therefore, the genetic gain can be maximized by reducing the time needed for inbred development, which could be achieved by adopting DH technology. In maize, inbreds and hybrids have been produced in a short period (Prasanna et al. 2012). Doubled-haploid populations contain more desirable agronomic traits of interest. Smaller population size is required to obtain homozygous targeted genes in doubled-haploid populations compared to traditional F_2 populations. In DH populations, an increase in the target genes helps identify favorable genotypes that carry all or maximum desirable alleles of genes under consideration. Marker-assisted gene stacking in combination with DH populations could be the best alternative to target gene fixation (Que et al. 2010). Apart from maize, DH technology could also be used for genetic improvement of other economic crops where haploid production is easy.

6.7.12 Genetic Studies in Crops

DH lines have been successfully utilized in understanding the genetics of any crop species. Doubled haploids carry duplicated haploid genomes through a chromosomal doubling mechanism; as a result, recessive genes can be expressed in early generations. Hence, phenotypic evaluation of recessive traits can be easily performed using such populations. DHs are also helpful in identifying random

recessive mutants in the population. Further, using DHs, gene action of any quantitative trait can be estimated by the sample mean of genotypic variance (Choo 1981) or by developing different segregation generations involving selected DH lines as parents.

6.8 Limitation of Doubled Haploids

Haploids and doubled haploids have been technologically advanced, employing several approaches such as genotypic selection, alterations in the composition of growth media and its conditions, and modifications to the plant growth environments (Maluszynski et al. 1996, 2003). However, the transition phase of the gametophytic to the sporophytic system, its genesis, and morphogenesis are still blurred. In the past, countless efforts have been made to decode the genetic and molecular basis of doubled-haploid developments in plants (Kyo et al. 2003). For example, anther culture technique has been widely used to develop doubled-haploid plants, particularly species belonging to Brassicaceae, Poaceae, and Solanaceae; however, this technique has a very low success rate in the species, particularly *Glycine max* belonging to Fabaceae (Hu et al. 1996; Rodrigues et al. 2004).

In forest tree breeding, haploid production is difficult due to uncontrolled pollen donor sources. These tree species have a robust structure that might be crucial in other species for DH production (Palmer and Keller 1999). Therefore, for the production of DH in these species, the main focus should be on the isolation of flower buds or inflorescences and their pretreatments. Two major challenges have been reported with DH production in tree species: successive rate and efficacy of embryo formation and enlargement (Bueno and Manzanera 2003; Bueno et al. 2003) and missing callus formation during the direct embryogenesis phase from microspores that is needed for reducing the gametoclonal dissimilarities and provides stability for the embryo at the genetic level (Deutsch et al. 2004). But these types of variation might be beneficial for the isolation of different and unique genotypes. There are several missing links to vividly understand the process of initiation and development of embryogenic tissue from microspores.

In addition, DH production using microspores faces major challenges due to recalcitrant type of nature and genotypic variability at the species level (Zheng et al. 2003). Male sterility does not permit the production of DH using microspores in the species belonging to Cucurbitaceae, Liliaceae, and Chenopodiaceae families; however, gynogenesis might be the best option. The development of DH from gynogenesis also has a lot of limitations, such as genotype specificity, a very less rate of haploid production, a high level of restriction during tempted chromosome doubling, and reduced fertility (Alan et al. 2003). The chromosome elimination method has also been used for DH production, especially when both androgenesis and gynogenesis could not be exploited (Mujeeb-Kazi and Riera-Lizaraza 1996); however, this technique could be used only in monocots. In addition, there are a few challenges while using this technique. For example, embryo development is regulated by pollen-contributing genotype, and the exact mode of chromosome elimination is

also unknown. Therefore, robust in vitro culture techniques such as embryo rescue and efficient chromosome doubling approaches are required for speeding up the DH production in crop species.

6.9 Conclusion

In experiments, the hybrids developed in maize by exploiting DH lines can give high corn yield and acceptable agro-morphological traits that are as good as hybrids developed by conventional breeding approaches. Hence, the elite DH lines could be used in hybrid maize breeding programs for high corn yield and tolerance to different biotic and abiotic stresses, particularly for drought and heat. Further, DH technology shortens the breeding cycle and increases genetic gain. The amalgamation of molecular or morphological markers with DH technology in breeding programs has different challenges in following the IPR issues under Plant Variety Protection regimes.

6.10 Future Prospectus

As previously mentioned, DH technology has many advantages over conventional breeding methods. In maize, it has modernized the breeding programs as the cost of investment in producing completely homozygous lines is less and these lines could be used for hybrid development and deployment for other trait improvements. However, sophisticated technology coupled with high technical skills is needed for producing DH lines and their effective implementation in breeding programs. Haploid production and chromosome doubling techniques are the main pillars required for DH technology. Although several decades of research have extensively been used for DH production, its genetic mechanism, in maize, for producing maternal haploids is still unclear. Conventional approaches for haploid genome duplication are toxic, labor-extensive, and cumbersome and use expensive reagents leading to restrictions for DH line development. However, haploid genome doubling technologies such as combining haploids and minichromosome approach could be of immediate use for accelerating DH production. In addition, we must search for novel markers that can easily detect the haploids with a very low false-positive rate.

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Finger Millet Improvement in *Post-genomic Era*: Hundred Years of Breeding and Moving Forward

7

Priyanka Joshi, S. K. Gupta, Henry Ojulong, Rajan Sharma, M. Vetriventhan, Himabindu Kudapa, Sunita Choudhary, D. Naresh, Jana Kholova, and Sobhan Sajja

Abstract

Finger millet, grown on about 5 Mha globally under semi-arid environments of East Africa and South Asia, serves as an important dual-purpose crop to address food, forage, and nutritional needs in these marginal regions. Despite the tremendous yield potential, the area cultivated for small millets, including finger millet, decreased by 25.7% globally between 1961 and 2018. Finger millet improvement program began in 1913 in India; however, concentrated efforts to realize genetic gains in this climate-resilient crop are yet to be deployed compared to the efforts invested in improving other major cereals. This has resulted in lower productivity of finger millet in farmer's fields than its potential yield even after more than 100 years of breeding. However, significant genetic variability is available for traits of importance. The breeding programs in Asia and Africa have refined the hybridization techniques and breeding objectives as per local needs. ICRISAT, an international center with finger millet as one of its mandate crops, is engaged with partners to generate new germplasm to enhance the productivity of this crop in marginal regions. This program, based in India and Kenya, has developed and distributed germplasm and breeding lines globally in the last few decades. Many promising and widely adapted cultivars have been released and adopted in many countries. Hybridization between the Indian and African gene pools of finger millet in the 1990s brought a paradigm shift in finger millet production in India. Now, breeding pipelines have been strengthened with the identification of newly

P. Joshi · S. K. Gupta (✉) · R. Sharma · M. Vetriventhan · H. Kudapa · S. Choudhary · D. Naresh · J. Kholova · S. Sajja
International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Telangana, India
e-mail: s.gupta@cgiar.org

H. Ojulong
International Crops Research Institute for the Semi-Arid Tropics, Nairobi, Kenya

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identified germplasm for traits of importance, especially for blast resistance. Recently, finger millet genome sequencing was accomplished, and with the availability of advanced phenotyping protocols for various traits of importance, it has opened new opportunities to enhance genetic gains in this crop. This chapter informs about historical breeding efforts and discusses the prospects and challenges of finger millet breeding to enhance breeding efficiency and genetic gains in finger millet. International collaborative efforts toward improving agronomic traits, value addition, and the trade value of finger millet would help marginal farmers of southeast Asia and Africa but will also help enhance the commercial value of this underutilized millet.

Keywords

Crossing · Speed breeding · High-throughput phenotyping · Trait discovery and mapping · Genomics

7.1 Introduction

Finger millet (*Eleusine coracana*) is an important component of low input agriculture prevalent in semi-arid tropics of South Asia (India, Nepal, and Sri Lanka) and the drylands of Africa (Uganda, Kenya, Zimbabwe, Zambia, Malawi, Tanzania, Rwanda, Zaire, Democratic Republic of the Congo, and South Africa). In terms of area and production, finger millet is the third-most important millet worldwide, after sorghum and pearl millet (Meena et al. 2021). Currently, the crop is cultivated across 25 countries, semi-arid regions, and tropical regions, up to an altitude of 2300 m. The major producing countries of finger millet are Uganda, India, Nepal, and China (Onyango 2016). In Africa, finger millet is mainly cultivated in minimal-scale cereal farming systems, mostly in the upland areas of Eastern Africa (Uganda, Ethiopia, Tanzania, and Kenya). It is cultivated on around 3–4 million ha in several Eastern and Southern African (ESA) countries, while on about 1.2 million ha with a production of 1.82 mt in India, Karnataka, Andhra Pradesh, Tamil Nadu, Maharashtra, Odisha, Jharkhand, etc. being the main cultivating states (ICAR-AICRP on small millets report, 2018; Reddy et al. 2008). Archaeological evidence suggests that it originated approximately 5000 years ago in the highlands of Ethiopia and western Uganda, whereas in India, records of its cultivation trace back to 3000–4000 BC in the western Ghats (Hilu and Dewet 1976; Hilu et al. 1979). Among all the known species of genus *Eleusine*, *E. coracana* spp. *africana* and *E. coracana* spp. *coracana* are the only two cultivated subspecies (Rawat et al. 2022).

Recognizing its immense health benefits, the consumption of finger millet in various functional food formulations, such as pasta, cookies, bread, cake, and noodles can be observed in Africa, Asia, Europe, and the USA (Deshpande et al. 2021). Traditionally, tribal people consume finger millet in the form of porridge, malt, and beverages, while straw is fed to the farming animals. The nutritional

superiority over major cereals (i.e., wheat and rice) in terms of the gluten-free nature of the protein, exceptionally high calcium content, low glycemic index, and bioactive secondary metabolites of diverse therapeutic uses makes it a highly valuable crop. Furthermore, its wide adaptation to drought-prone environments, low input dryland agriculture, and marginal and fragile hilly agroecosystems make it a crop of the future. However, despite its high agricultural value, the global area of finger millet production and cultivation has declined. The largest reduction was observed in Asia, whereas the smallest was observed in Africa (Meena et al. 2021). The productivity of finger millet doubled from 0.7 t ha^{-1} (1950–1951) to 1.6 t ha^{-1} (1976–1980) in India owing to the cultivation of high-yielding blast-tolerant varieties (http://www.aicrpsm.res.in/). However, after that, the crop productivity stagnated at 1.6 t ha^{-1} in India with minor improvement despite the crop's high nutritive properties and excellent sustainability in semi-arid systems (http://data.icrisat.org/dld/). Padulosi et al. (2015) reported the potential productivity of finger millet as 10 t ha^{-1}. However, the actual productivity of finger millet is very low in Uganda (0.4–0.8 t ha^{-1}; Tenywa et al. 1999), India (1.6 t ha^{-1}; ICAR-AICRP on small millets report, 2018), Nepal (1.1 t ha^{-1}; Khadka et al. 2016), and Asia (1.3 t ha^{-1}; Onyango 2016). Kurosaki and Wada (2015) presented spatial patterns of long-term changes in finger millet cultivation in India from 1965 to 2007. The study also reported the declined area of finger millet in Tamil Nadu, Karnataka, Andhra Pradesh, and Odisha as opposed to the fine cereals (rice, wheat, and maize). This downfall may be due to the lack of focused research and policy support compared to major cereals.

Large germplasm collections have been maintained in international and national genebanks, and adequate genetic variation has been reported for various agronomic traits (Upadhyaya et al. 2006). However, appreciable genetic gains through harnessing the available genetic variation for agronomic and nutraceutical traits have not been achieved due to inherent problems such as cumbersome floral biology, small seed size, seed shattering, and unsynchronized maturity (Sood et al. 2019). The latest trends in cutting-edge biotechnological and omics tools, particularly the availability of reference genome sequences (Hittalmani et al. 2017; Hatakeyama et al. 2018) and their integration with conventional breeding, hold immense potential to overcome these limitations. In fact, acquiring high-density genomic data coupled with high-dimensional phenotypic records will certainly improve our understanding of genetic control of complex traits of agronomic and nutraceutical importance.

In this chapter, we summarize the importance of finger millet in diversifying the future cropping systems as well as its origin, phylogeny, genetic resources, production constraints, breeding achievements, and genomic advancements. We also provide perspectives and a roadmap on utilizing emerging genomic tools like gene editing and next-generation genotyping to make finger millet a viable and competitive crop in contemporary agroecosystems.

7.2 Taxonomy, Biology, and Genetic Resources

7.2.1 Taxonomy

The cultivated finger millet (*Eleusine coracana* (L.) Gaertn.) is an allotetraploid belonging to the family Poaceae, subfamily Chloridoideae, and tribe Chloride (Vetriventhan et al. 2020). *E. coracana* subsp. *africana* is considered an assumed progenitor to the cultivated finger millet, and it is completely cross-compatible with the cultivated finger millet and produces fertile hybrids (Mehra 1963; Hiremath and Salimath 1992). The genus *Eleusine* comprises about ten species, including annuals and perennials, with three basic chromosome numbers 8, 9, and 10. The cultivated species *E. coracana* can be classified into races and subraces (Prasada Rao et al. 1993). The species *E. coracana* contains of two subspecies, *africana* (wild type) and *coracana* (cultivated type). The subsp. *africana is* again divided into two wild races, *africana* and *spontanea*.

7.2.2 Biology

Finger millet is a robust, tufted annual-growing crop from about 30–150 cm tall and takes 3–6 months to complete the seed cycle. The stems are erect, slender, compressed, glabrous, and capable of producing many tillers and nodal branches. At maturity, the stems are somewhat laterally flattened. The inflorescence is an arrangement of many spikelets, which are known as fingers. The inflorescence consists of a variable number of spikes ranging from 3 to 20 arranged in a bird's foot style. It resembles fingers on a hand, hence its common name "finger millet." Spikes are straight or slightly incurved and up to 11 cm in length. Each spike contains serially arranged four to ten florets on the finger. Two large barren leaves cover the florets, each enclosed between a pair of scales known as palea. The flowerets are in the axil of the lower flowering glumes, known as a lemma; near the base of the ovary, two little scaly lodicules are present (Gupta et al. 2011; Dodake and Dhonukshe 1998). The three stamens are 0.5–0.8 mm long, not penicillate (Nanda and Agarwal 2008). The gynoecium is bicarpellary and unilocular, with a larger ovary having two styles with plumose stigma (Seetharam et al. 2003). The androecium mostly surrounds the stigma. Anthers are bigger than filaments (Gupta et al. 2010); spikelets are usually 5–8 mm long and 3–4 mm wide. Spikelets are arranged alternately on the rachis, and each spikelet contains about four to seven seeds. The seeds vary in diameter from 1 to 2 mm (Reddy et al. 2008). Except for the terminal ones, which can occasionally be sterile, all florets are excellent flowers. The caryopsis is globose and smooth; the color can be white, light brown, reddish-brown, ragi brown, and dark brown. The seed pericarp is easily removed from the seed coat because it is independent of the kernel. The shape of the grain varies from oval and round to oblong. In cultivate, seed shattering at maturity is not that common, while in wild species, it is common (Sood et al. 2019; De Wet et al. 1984) (Fig. 7.1).

Fig. 7.1 Finger millet plant, leaf, root, panicle, and seed

7.2.3 Genetic Resources

A large number of finger millet germplasm accessions are available for the scientific society. Globally, >37,000 germplasm accessions of finger millet have been conserved in various genebanks (Vetriventhan et al. 2016; Dwivedi et al. 2012). The major collections of finger millet accessions are conserved in India, Kenya, Ethiopia, Uganda, and Zambia. The National Bureau of Plant Genetic Resources, New Delhi, India, has the largest germplasm collection, which maintains >10,500 accessions under long-term conservation. Most of them are indigenous in nature. The ICRISAT genebank in Patancheru, India, comprises a total of 7519 germplasm accessions from 26 countries, of which 205 are wild species, 7121 traditional cultivars/landraces, 143 advanced/improved cultivars, and 50 breeding/research material. The concept of core and mini-core collections has been proposed for better utilization of diversity in crop improvement programs. Following this approach, the ICRISAT has developed core and mini-core collections in finger millet. The finger millet core collection contains 622 accessions (~10% of the total collection), and the mini-core collection contains 80 accessions (10% of core collection or 1% of the total collection). In addition, a composite collection of germplasm consisting of 1000 accessions has been developed under the Generation Challenge Program (Upadhyaya et al. 2006). The core and/or mini-core collections established at the ICRISAT genebank have been evaluated for agronomic, grain nutrients (Upadhyaya et al. 2011), salinity (Krishnamurthy et al. 2014), drought (Krishnamurthy et al. 2016), and fodder quality traits (Backiyalakshmi et al. 2021a, b) and identified promising trait-specific sources for use in crop improvement.

7.3 Target Traits and Their Relationships

Despite finger millet's enormous potential attempts to enhance its genetics lag well behind those of other main crops. Breeding targets for finger millet improvement may be classified as must-have and long-term traits. It is possible to increase yield by improving its components like plant height, days to flowering, synchronous maturity, inflorescence length and number of productive tillers, grain size, and threshability while taking the must-have traits into account (Sood et al. 2019). Besides these basic traits, breeding for blast resistance is the most important objective of finger millet genetic improvement programs across the globe (Kumar et al. 2021a). The blast caused by the fungus *Pyricularia grisea* is the most important biotic constraint which severely affects the production of finger millet worldwide. It affects the finger millet plant at all the growth stages and expresses symptoms in the form of leaf blast (LB), neck blast (NB), and finger blast (FB), with neck blasts causing the greatest yield losses. In endemic and hotspot areas, 70–80% yield loss has been reported (Mbinda and Masaki 2021). Like blast, the infestation by a parasitic weed *Striga* is a serious biotic constraint that severely affects finger millet production in sub-Saharan Africa (Teka 2014).

Drought is the main abiotic stress of finger millet, especially in the low rainfall, low altitude areas of sub-Saharan Africa. Finger millet is mainly affected by terminal drought after flowering at the grain filling stage. Producing short-duration varieties that escape terminal drought is the main measure for drought. Therefore, breeding for stable blast and striga resistance and drought escape/tolerance is one of the primary breeding objectives and must-have traits of all the finger millet breeding programs in Asia and Africa. Breeding for snapping varieties for the ease of harvesting, medium height (≤90 cm), good plant aspect and strong stem to prevent lodging, compact heads as an indicator for high yield, and three to four productive heads are the traits to be considered for popularization and commercialization of finger millet. With the improvement of yield, usually leading to increased head size, plant lodging is becoming an inherent problem, with two main negative effects: (1) finger millet grain usually germinates the moment it gets in contact with soil leading to great yield losses and (2) lodging complicates machine harvesting. Efforts to breed for stronger or stiff stalks are addressing this problem. Enhancing fodder yield by selecting and including genotypes with high basal tiller numbers in hybridization programs is another important target of finger millet breeding programs. Emphasis on breeding for high fodder nutrient digestibility and high threshability is required for sustainable food and food security in semi-arid areas of Asia and Africa.

Besides these basic traits, enhancing the seed size coupled with synchronized and early maturity of the tillers is a major long-term trait of the finger millet genetic improvement program. Very small seed size and unsynchronized maturity of most of the available finger millet cultivars are causing difficulties in mechanical planting and harvesting of the crop (Meena et al. 2021). Some inherent problems like high seed shattering also need to be addressed in the long-term breeding goals of finger millet. The poor initial vigor of finger millet leads to a heavy infestation of weeds

bringing in more competition for light and nutrients, leading to a poor crop stand and significant yield losses. Seedling vigor is highly correlated to drought tolerance and has been used as an early selection criterion. It is also highly correlated to high yield and higher 1000 grain weight. Further, manual weeding increases quality seed production costs without an effective pre- and post-emerging herbicide. Therefore, breeding for herbicide-tolerant finger millet through modern approaches like transgenic development and genome editing is an important long-term target for finger millet genetic improvement (Joshi et al. 2018).

Quality and market-driven traits of finger millet include grain color (red and white), high puffing percentage, and taste. Due to the high quantities of tannins and phenolics, finger millet grains are typically dark brown, making the product's appearance unappealing. Sometimes the plentiful tannins and phenolics give a bitter taste to the improved products, thereby reducing their consumer acceptability. Therefore, breeding for white-seeded finger millet is an effective approach for adding its value and enhancing market demand for the products (Joshi et al. 2021a). Traits for consideration for yield are normally correlated and can be assessed together with yield, or a number of them can be assessed as yield indicators, especially in early generations where yield per se is not assessed or in the early vegetative period of the crop. Correlation analysis on phenotypic characterization data of trials conducted at Kiboko, Kenya, showed yield to be highly correlated ($P < 0.01$) to all agronomic and yield-related traits, viz., grain yield, agronomic aspects, days to 50% flowering, days to maturity, plant height, productive tillers, ear weight, ears harvested, 1000-grain weight, blast resistance, lodging estimate, and seedling vigor evaluated (Table 7.1), implying that the traits can be used to indirectly select for yield. Ear weight, days to flowering, and plant height were highly correlated to all or most of the traits implying they are good traits for selection per se.

There's immense potential for enrichment in finger millet. The exploitation of yield parameters might lead to weakening impacts for numerous nutritionally valuable components within the seeds, which are available in different cereals. Therefore, such dilution effects need to be considered while breeding for higher yield. However, improving the major nutrient contents in the finger millet grain has been shown not to affect yield significantly. Correlation of yield with grain nutrient content from 480 accessions evaluated in Kiboko showed nonsignificant associations with yield, implying that breeding for high nutrient content will not have any significant effect on yield and vice versa. Similarly, Gupta et al., (2009) also reported no penalty on grain yield and seed size while breeding for grains rich in these micronutrient. Calcium (Ca), the main nutrient in finger millet, was highly correlated to the other important nutrients in finger millet such as iron (Fe), zinc (Zn), and nitrogen (protein), while Fe, Zn, and nitrogen (protein) were highly correlated ($P < 0.001$) to all nutrients in the grain, implying that it is possible to improve the different mineral contents in the grain simultaneously. Studies suggest that grain yield and Ca have a low correlation or negative correlation—the same in grain yield and Fe, Zn, and protein (Ojulong et al. 2021). Previous studies on finger millet have also suggested a low or negative correlation between grain yield and grain nutrient traits (Upadhyaya et al. 2010; Kumar et al. 2010; Ng'uni et al. 2011), and iron and

Table 7.1 Correlation of agronomic and yield traits assessed during characterization of figure millet germplasm at Kiboko, Kenya, in 2010

Correlations	Grain yield	Agronomic aspect	Blast resistance	Days to 50% flowering	Days to maturity	Ear weight	Ears harvested	1000 grain weight	Lodging estimate	Plant height	Productive tillers	Seedling vigor
Grain yield (t/ha)	–											
Agronomic aspect (1–5)	−0.51***	–										
Blast resistance (1–9)	−0.45***	0.56***	–									
Days to 50% flowering	−0.39***	0.13NS	−0.04NS	–								
Days to maturity	−0.26**	0.19*	−0.11NS	0.64***	–							
Ear weight (g)	0.91***	−0.54***	−0.43***	−0.30**	−0.28**	–						
Ears harvested	0.67***	−0.38***	−0.25**	−0.30**	−0.18NS	0.70***	–					
1000 grain weight (g)	0.27**	0.03NS	−0.08NS	−0.28**	−0.13NS	0.15NS	0.05NS	–				
Lodging estimate (1–5)	0.26**	0.15NS	0.02NS	−0.44***	−0.31**	0.17NS	−0.05NS	0.36***	–			
Plant height (cm)	0.53***	−0.24*	−0.20*	−0.32***	−0.34***	0.48***	0.21*	0.28**	0.58***	–		
Productive tillers	0.43***	−0.14NS	−0.09NS	−0.25**	−0.07NS	0.44***	0.78***	−0.06NS	−0.14NS	−0.01NS	–	
Seedling vigor (1–5)	−0.25**	0.17NS	0.13NS	0.13NS	0.10NS	−0.24**	−0.19NS	−0.03NS	−0.04NS	−0.29**	−0.05NS	–
Thresh %	0.39***	−0.10NS	−0.21NS	−0.33***	−0.04NS	0.00NS	0.08NS	0.33***	0.24**	0.25**	0.04NS	−0.00NS

Degree of significance, * => 0.05, ** => 0.01, *** => 0.001, NS = Nonsignificant

zinc have a low negative correlation with yield in sorghum. Ojulong et al. (2021) suggest a highly significant ($P < 0.001$) correlation among the yield and calcium, copper, iron, potassium, magnesium, manganese, potassium, sulfur, zinc, and protein.

Although finger millet has a lot of room for development, higher yield parameters might dilute some of the nutritionally important components of seeds, as shown in several cereals. Therefore, such dilution effects need to be considered while breeding for higher yield. Knowing the genetic architecture of crucial breeding targets like flowering, early duration, yield, resistance to disease/pest, and nutritional quality is a must to execute suitable breeding strategies for enhancing genetic gain. However, very limited studies have been conducted on finger millet to understand the genetics of these important breeding targets. Therefore, genetic mapping studies need to be implemented to learn more about the underlying genes for the traits of economic importance.

7.4 Target Product Profile and Market Segments for Africa and Asia

For the success of breeding programs, it is very important to work closely toward the trait-specific requirements of its stakeholders. The breeding programs in Africa and Asia are well-aligned with the farmer and consumer needs in the finger millet-growing countries. For instance, ICRISAT's East African breeding program has identified five different market segments, while the Indian program has identified two segments. Product profiles have been developed considering the trait-specific requirements for each segment. The type of cultivar requirement, area, target regions, and regions of different product profiles (segments) are shown in Table 7.2 as an example of these two breeding programs. Must-have traits are the ones that can be addressed with the available trait variability and tools. They are immediately needed in the current-day cultivars, while long-term traits are the ones which are visioned for the future, and efforts are required to strengthen them in the breeding pipeline (Table 7.2).

7.5 Genetic Variability for Traits of Importance

The global germplasm of finger millet conserved at the ICRISAT genebank shows a large variability for morpho-agronomic, grain and fodder quality and stress tolerance traits (Vetriventhan et al. 2016). For example, a huge variability trait is for important agronomic traits such as days to 50% flowering that varied from 40 to 120 days, plant height from 30 to 240 cm, number of basal tillers from 1 to 70 (wild species accessions produce a large number of tillers), and inflorescence length from 40 to 320 mm (http://genebank.icrisat.org/), and germplasm diversity representative subset called core collection (Upadhyaya et al. 2006) and mini-core collection (Upadhyaya et al. 2010) were established to enhance the use of diverse germplasm

Table 7.2 Market segments for Asia and Africa

Market segment	Market segment description	Estimated area (m ha)	% area/ effort	Target and spillover agroecologies	Product development goals
Market segments for Asia					
MS1	Medium- to long-duration varieties for semi-arid areas	0.6	60	**Target:** Semi-arid/arid to humid southern peninsula of Indian subcontinent; major states: KN, OD, MH, TN **Spillover:** sub-humid areas of Ethiopia, Uganda, Tanzania, Kenya, Zimbabwe, Malawi, and Zambia	**Must-have traits:** 1. >5% more yield than check 2. Resistance to blast (leaf blast: <=3 on 1–9 scale; neck blast: <=2 on a 1–5 scale; finger blast: <=10% infection) 3. Maturity (80–100 days) for MS2, and 105–130 days for MS1 4. Seed color: brown 5. Synchrony of tillers 6. Good threshability **Long-term traits:** 1. Lodging resistance 2. High Fe, Zn, Ca 3. Drought tolerance 4. Heat tolerance 5. Easy snap trait 6. Fodder yield and quality
MS2	Short-duration varieties for sub-humid areas	0.4	40	**Target:** Sub-humid areas of India high altitude, arid; Himalayan Range, UK, Sikkim **Spillover**: semi-arid areas of Ethiopia, Uganda, Tanzania, Kenya, Zimbabwe, Malawi, and Zambia	

Market segments for Africa					
MS1	Short-duration brown-colored varieties for food and malting	0.34	30	Dry lowland low-rainfall agroecologies of ESA: Ethiopia, Kenya, Tanzania, Uganda, Malawi, Zimbabwe **Spillover:** WCA, Asia	**Must-have traits:** 1. Yield >5% above commercial check 2. Maturity: early <100 days for MS1 & 2; medium 101–120 days for MS 3 and 4); and long 121–140 days for MS 5 3. Resistance to blast: <4 on a 1–9 scale 4. Drought tolerance: <2.0 on a 1–5 scale 5. Striga tolerance: >10% above commercial grain yield or equal to the best trait check 6. Resistance to lodging <10% 7. Synchrony in tillering 1 on 0/1 scale 8. Good threshability <3 on a 1–5 scale 9. Grain color: red/brown for traditional food, white for value-added products: confectionaries, dark brown for malting 10. Grain quality for porridge, ugali, malting quality <3 **Long term:** 1. Snapping trait 2. High Ca, Fe, Zn, protein 3. Stemborer resistance: <40% dead hearts 4. Fresh biomass yield: >15% above the check 5. Response to inputs: 5% above the commercial check 6. Tillering attitude (for intercropping): 3 for sole and 5 for intercrop (3 = decumbent, 5 = erect, 7 = prostrate)
MS2	Short-duration light-colored varieties for confectionaries and other value-added products (industry)	0.1	10		
MS3	Medium-duration varieties for food and malting (local)	0.64	40	Medium-duration, medium-rainfall areas of ESA: Ethiopia, Kenya, Tanzania, Uganda, S. Sudan, Malawi, Zimbabwe **Spillover:** WCA, Asia	
MS4	Short-duration light-colored varieties for confectionaries and other values	0.1	10		
MS5	Low temperature-adapted varieties for food and malting	0.1	10	High altitude >1500 masl, high-rainfall low-temperature agroecologies of Ethiopia and Uganda Spillover: Rwanda and Burundi	

in crop improvement. Evaluation for grain nutrient content of the finger millet core collection revealed a substantial variability for Fe (21.71 mg/kg), Zn (16.58–25.33 mg/kg), Ca (1.84–4.89 g/kg), and protein (6.0–11.09%) and also reported a weaker and nonsignificant correlations of grain yield with Fe, Zn, Ca, and protein indicating better prospects for combining higher grain nutrients with higher yield background (Upadhyaya et al. 2011). Finger millet is primarily grown as a food crop in Asia and Africa, but its stover serves as an important source of fodder, producing excellent hay and green forage for cattle, sheep, and goats (Sampath 1986; Gupta et al. 2017). The finger millet diversity panel conserved at the ICRISAT genebank was assessed for fodder quality traits, and the study showed a substantial variability for fodder quality traits (2.8) to 10.7 t/ha of dry fodder yield, 6.47–8.15% of crude protein, >90% of dry matter content, and 45.21–49.09% of in vitro organic matter digestibility (IVOMD) and identified promising accessions for developing dual-purpose cultivars (Backiyalakshmi et al. 2021a, b). Similarity, a large variability for salinity (Krishnamurthy et al. 2014) and drought (Krishnamurthy et al. 2016) were reported in the international collection (core/mini-core) of finger millet, and promising sources were identified for use in crop improvement.

7.5.1 Genetic Variability

Significant genetic variability for different traits has been reported in finger millet crop. For instance, a very high variation was observed among the agronomic and yield-related traits in a study conducted on 480 accessions constituted from collections and farmers and improved varieties from Eastern and Southern African countries and the finger millet mini core (Table 7.3). Days to 50% flowering ranged from 46 to 92, indicating that sources for short-, medium-, and long-duration varieties were available. Very high variation in productive tillers (2–21) highlighted the great chances of improving this important trait for yield, and so was the number of heads harvested (2–21). Numbers of fingers and other important traits contributing to yield had high variability (4–12). Grain spikes (3–8) were variable too. Grain yield, the main trait for improvement, was highly variable (0.7–4.6 t/ha), and so was thresh percentage (47.2–94.8%). All these show high prospects of improving from the available germplasm using conventional means.

Nutrient profiling showed high diversity in the materials evaluated. Calcium values ranged from 115.5 to 540 mg/100 g, Fe from 1.4 to 24.5 mg/100 g, and Zn from 0.1 to 10.1 mg/100 g, again showing the promising aspects of improving the nutrient content from the germplasm. An ICRISAT genebank trial in 2018 and 2019 quantified for Ca, Fe, Zn, and Aluminium (Al) showed large genetic variation micronutrients, which could be further exploitable in nutrition-inclusive breeding programs (Fig. 7.2). Ojulong et al. (2021) also established high variability among the different nutrient traits in the region. Finger millet cluster analysis studies suggested two main clusters. The first cluster contains varieties from countries of finger millet origin, Uganda and Ethiopia (Hilu and DeWet 1976; Dida et al. 2008), and the major

Table 7.3 Summary statistics of different agronomic and yield parameters assessed during characterization of 480 finger millet accessions at Kiboko, Kenya

Statistical parameters	Plant aspect (1–9)	Days to 50% flowering	Plant height (cm)	Stem diameter	No. of leaves	Leaf length	Leaf width	Peduncle length (cm)	Prod. Tillers	Number of fingers	finger width (cm)	Glume cover	Grain spikes	Heads per plant	Panicle size	Grain yield (t/ha)	Thresh %
Mean	4.3	69.5	74.6	8.4	10.8	42.0	1.2	24.1	6.6	7.1	0.9	2.4	5.2	6.2	2.1	2.5	75.9
Minimum	2.0	46	24.1	4.1	4.0	22.2	0.7	10.1	1.8	3.8	0.6	1.9	2.9	1.8	1.0	0.7	47.2
Maximum	7.0	92	123.9	12.5	17.0	64.0	2.4	34.8	20.7	11.8	1.2	3.0	7.6	20.5	3.0	4.6	94.5
SE	0.9	6.0	17.4	1.3	1.6	7.6	0.2	4.1	3.3	1.3	0.1	0.4	0.7	3.1	0.6	0.8	7.0
Variance	0.8	36.2	301.1	1.8	2.5	58.1	0.0	16.5	10.8	1.7	0.0	0.2	0.6	9.5	0.3	0.6	48.5
CV	21.0	8.7	23.3	15.9	14.7	18.2	13.6	16.9	49.6	18.5	9.7	16.4	14.2	49.4	27.4	29.8	9.2

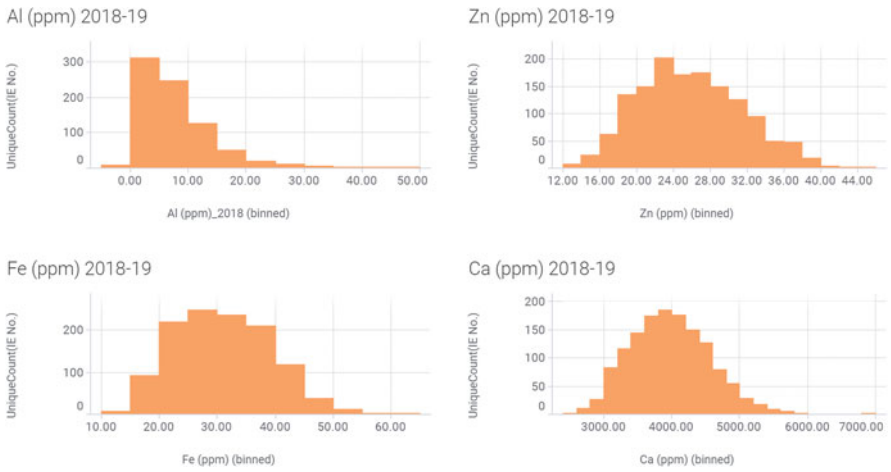

Fig. 7.2 Large genetic variation in micronutrients

finger millet-growing countries in the East and Southern Africa region, Tanzania, Kenya, Malawi, and Zimbabwe. The second cluster contains germplasm from countries in the diverse region that are among the largest finger millet producers: India and Nepal (Hilu and DeWet 1976; Dida et al. 2008). Cluster analysis studies suggested that the highest diversity for the different nutrient traits for the enrichment of finger millet exists in centers of origin. Earlier studies also observed that the domesticated varieties were low in maximum nutrient content, most likely a result of farmer selection by the farmers, who preferred brown grain, which is relatively lower in nutrient content compared to dark brown. Studies on finger millet found it to be rich in protein (8–10%) which is associated with seed color (Vadivoo et al. 1998), and lower in fat (2.5–4%) which makes it a healthy option for the modern diet.

Manyasa et al. (2014) conducted genetic diversity studies on 340 finger millet germplasm from Kenya, Tanzania, and Uganda, and 15 mini-core accession using single-sequence repeat markers and qualitative traits found explained the diversity by variability within the countries and subregions than that among the countries and subregions. The low variability among the countries explained the shared gene pool, as the crop originated from the East African region. Studies suggest that farmer's selection for adaptation and end-use could have contributed to the high diversity within the countries. The genetic diversity studies explained that finger millet was domesticated in Africa and later introduced to India (Dida et al. 2008). It is observed that Asian accessions are earlier in maturity with short plant height and small flag leaf length when compared to African germplasm, which has high plant height and longer and wider flag leaves with higher intraspecific diversity (Dida et al. 2008; Bharathi 2011; Babu et al. 2014d). Further, as compared to the African gene pool, it is reported that the Asian gene pool was created from limited founder populations lacking unique genes. Heritability of the different traits is high in finger millet.

7.5.2 Breeding Methods

Hybridization in finger millet started around the early 2000s in many African countries, with the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in Nairobi, Kenya; the Agricultural and Livestock Research Organization (KALRO) in Kenya; and the National Semi-Arid Resources Research Institute of the National Agricultural Research Organization (NaSARRI-NARO) in Uganda taking the lead. Pedigree breeding was the most used method in both African and Asian countries. The progenies advanced based on the combination of several highly heritable traits as per the needs of different segments, like selecting for seedling vigor, plant aspect, plant height, number of productive tillers, tillering type, days to flowering, days to maturity, head shape and size, finger number and size, thresh percent and ease of threshing, grain yield, 1000 grain weight, and grain color and shape. In India, finger millet improvement work started in 1913, but hybridization started in 1951.

7.5.3 Historical Breeding Efforts in India

Crop improvement efforts for finger millet were initiated in India at Zonal Agricultural Research Station, V.C. Farm, Mandya, Karnataka state, by Dr. Leslie C. Coleman in 1913. Productivity was very low at that time due to a lack of high-yielding varieties, improved crop management practices, soil health issues, and new technological interventions. According to the available literature, efforts for finger millet improvement in India can be divided into five stages.

7.5.3.1 Stage I (1913–1938): *Pure Line Selections—Indigenous Varieties*

During this period, the varietal improvement work was conducted at different research centers in Karnataka states of India. Pure line selections were made from indigenous varieties. During this period, Dr. Leslie noted that the complete emergence of inflorescence required about 10 days, and flowering takes 7–8 days while working on floral biology, anthesis, and pollination in finger millet. He also observed that cross-pollination is very rare because the period of anthesis is very short.

7.5.3.2 Stage II (1938–1963): *Initiation of Recombination Breeding*

During this period, pure line selection work was continued from indigenous germplasm lines and landraces. To enhance the genetic base of the crop, hybridization was initiated through the contact method in 1951. In the contact method, panicles from the plant of the recipient parent and from the plant of the desired/donor parent were chosen as they were both about to start the anthesis process. To prevent unintended cross-pollination, both panicles were joined together and covered with butter paper bags. The varieties released using this method exhibited a yield advantage of approximately 50% over the existing pure line varieties with wide agroecological adaptation capable of fulfilling the need of different growing seasons of finger millet.

7.5.3.3 Stage III (1964–1988): *Widening the Genetic Base by Combining Divergent Gene Pools*

This period is regarded as the most significant period in finger millet improvement resulting in a quantum jump in area and crop production. Hybridization was initiated between the two divergent gene pools of finger millet by Dr. C.H. Lakshmanaiah in 1964. The locally adapted, early maturing but low-yielding Indian genotypes were crossed with late maturing, high-yielding, and stress-tolerant African genotypes (Sood et al. 2022). This effort resulted in the developing of 16 Indo-African varieties designated as "INDAF" varieties.

7.5.3.4 Stage IV (1988–2013)

During this period, emphasis was given to the development of dual-purpose varieties (high straw and grain yield) along with resistance to blast adaptability to rainfed and irrigated conditions.

7.5.3.5 Stage V (2013 to Date) *Genomic Interventions*

During this period, the finger millet's draft genome was sequenced by Hittalmani et al. in 2017 and Hatakeyama et al. in 2018, creating opportunities to use genome-level information to accelerate the improvement of finger millet. Forward breeding or marker-assisted selections are being used to fast track the varietal development. A set of SNPs (49) was developed to refine the crossing technique and identify true hybrids. A set of SNPs has also been developed for forward breeding of blast resistance (Table 7.4).

7.5.4 Breeding for Traits of Importance

7.5.4.1 Climate Adaptation

Finger millet production is limited majorly by two critical constraints: high-temperature stress and terminal drought. This is linked to the low and erratic rainfall (150–800 mm) of production environments where finger millet is traditionally grown in eastern South Africa and India.

Heat Stress High-temperature stress has been reported as another most important cause of change in physiology by arresting the cell expansion, which causes a reduction in plant growth and development, leading to loss of productivity (Sato et al. 2002; Abdelmageed et al. 2003). The optimum temperature for the growth of finger millet is 28–32 °C and can be well sustained up to 36 °C (Yogeesh et al. 2016). It has been evident from the literature (Sato et al. 2002; Abdelmageed et al. 2003) that finger millet deviates from its normal morpho-physiology when temperatures cross the cardinal thresholds (day = 36 °C; night = 26 °C), which affects the stable physiological functions resulting in yield reduction. When seedlings are exposed for 5 h to temperatures between 38 and 54 °C, shoot and root growth is affected (Venkatesh Babu et al. 2013). It has been reported that yield and yield-contributing traits like flowering, maturity, ear head length, finger length,

Table 7.4 A brief account of historical breeding efforts in finger millet

Research area	Year	Research description	Reference
Pure line selection	1918	H-22 variety released from indigenous varieties	NA
Mutation breeding	1941	H-1 variety released from a mutant variety in Gidda Aryam	NA
Recombination breeding	1959	High-yielding variety (Udaya) released	NA
	1964	Combination of Indo-African gene pool	NA
Genetic analysis	1976	Racial evolution in finger millet	Hilu and DeWet (1976)
	1979	Archaeobotanical studies	Hilu et al. (1979)
	1985	Callus initiation and plant regeneration	Mohanty et al. (1985)
Blast-resistant variety	1989	Bred finger millet varieties (Pant Mandu 3 and PES 110) tolerant to blast	Tyagi and Rawat (1989)
	1991	Influence of head blast infection on seed germination and yield components	Ekwamu (1991)
Development of international core and mini-core collections	2006	Core subset of finger millet (622 accessions) is developed from the global (26 countries) collection of 5940 accessions	Upadhyaya et al. (2006)
	2010	Mini-core collection of finger millet is developed (80 accessions) using accessions from 14 different countries	Upadhyaya et al. (2010)
Diversity	2007	Finger millet germplasm imported from Southern and Eastern Africa exhibits morphological diversity	Upadhyaya et al. (2007)
Production enhancement in Africa	2010–2015	Under the Hope Project yield increased in Ethiopia (2–3 t/ha), Uganda (1.8–2.3), Kenya (0.8–1 t/ha)	NA
Popular variety of Africa	–	Popular variety U15 release in Uganda in 2002, Kenya in 2013, and Tanzania in 2014	NA
Nutrient-rich variety released	2016	Nutrient-rich finger millet varieties released for the first time in Kenya	NA
Release of varieties	2017	Finger millet varieties released based on social/cultural trait (easy harvest) based on the snapping trait in Kenya	NA
Nutrient-rich variety released	2018	Nutrient-rich finger millet varieties released for the first time in Uganda	NA

(continued)

Table 7.4 (continued)

Research area	Year	Research description	Reference
Molecular markers and genetic mapping	2007	The genetic map of the tetraploid of finger millet	Dida et al. (2007)
	2017	Genome and transcriptome sequence of finger millet provides insights into drought tolerance and nutraceutical properties	Hittalmani et al. (2017)
	2018	Multiple hybrid de novo genome assembly of finger millet	Hatakeyama et al. (2018)
	2020	Genome-wide association study for nutritional traits (iron (Fe), zinc (Zn), calcium (Ca), magnesium (Mg), potassium (K), and sodium)	Swati et al. (2020)
	2020	Through association mapping, QTLs identified for grain calcium content using SSR markers	Yadav et al. (2020)
	2020	By GBS technology, QTL identified for grain yield, days to maturity, and seed protein content and using 2977 SNPs	Tiwari et al. (2020)
Genetic diversity analysis	2021	Genome-wide analysis of population structure and genetic diversity in the global finger millet germplasm panel	Backiyalakshmi et al. (2021a, b)
Other related research	2021	VL *Mandua* 382: the first early maturing, white-seeded finger millet cultivar suitable for rainfed organic agroecology of the Himalayan region	Joshi et al. (2021a)
	2021	Characterization for mineral content in finger millet germplasm	Ojulong et al. (2021)

number of branches, and grain size are severely affected when the crop is exposed to temperature stress (42–44 °C) (Yogeesh et al. 2016). A suitable crop management strategy for finger millet would be to avoid heat stress during the most vulnerable reproductive stages by selecting the right genotypes (phenology and duration) and planting dates.

7.5.4.2 Drought Stress

Drought is known to affect finger millet in many ways and depends on the crop's stage. The soil moisture stress during flowering and grain filling stages is a very frequent form of drought in finger millet, contributing to a significant yield loss (Maqsood and Ali 2007). This is also referred to as terminal drought stress. It is mainly caused by cessation of rain toward the end of the rainy season in semi-arid tropics where the cropping period is limited. Breeders define a drought-adapted variety as having the ability to give a high or reasonable yield under drought

conditions. The variety can achieve this through drought escape-short duration varieties or being tolerant to drought. Screening the germplasm and farmer-preferred varieties resulted in the identification of such varieties. In the African region, short duration varieties U15, Ekama, and Gulu E from Uganda and KNE 741 from Kenya have been used to reduce the days to flowering of a number of lines, and currently ICRISAT-Nairobi has a pipeline of short duration lines, a number at advanced stages for release in Africa. A number of lines have also stayed green characteristics and remain green under drought conditions giving reasonable yields. We now have kits of materials that flower before 60 days and give a high yield.

7.5.4.3 Biotic Stress Resistance

Finger millet is affected by numerous diseases caused by fungal, bacterial, and viral pathogens, including blast, seedling blight, wilt or foot rot, Cercospora leaf spot, downy mildew, smut, bacterial blight, ragi mottle streak, and ragi severe mosaic. Most of these diseases are region-specific and of minor importance. However, blast, caused by an ascomycete fungus *Magnaporthe oryzae* (anamorph: *Pyricularia oryzae*), is the most destructive and widespread disease that affects the yield, utilization, and trade of finger millet within East Africa and South Asia (Mgonja et al. 2007). The disease affects the crop at all growth stages leading to leaf, neck, and finger blasts; neck and finger blasts are the most destructive forms of the disease. The average yield losses due to finger millet blast have been reported to be around 30%; however, yield losses could be as high as 80–90% in the susceptible cultivars under favorable conditions of disease development (Vishwanath et al. 1986; Rao 1990; Nagaraja et al. 2007).

The pathogen is known to infect more than 50 graminaceous hosts, including food security crops such as rice, wheat, finger millet, pearl millet, and foxtail millet. Blast is largely managed through host plant resistance, the most economical, efficient, and ecologically sustainable method of disease management. For the development of durable disease-resistant varieties, information on diversity in the pathogen populations is essentially required. The pathogen causing a blast of finger millet is highly variable, which necessitates generating information on virulence diversity in the pathogen populations adapted to finger millet. This can help in developing finger millet varieties with durable resistance to blast disease. Though efforts have been made to study the genetic diversity and aggressiveness of the pathogen populations (Kiran Babu et al. 2013a; Takan et al. 2012), limited information is available on the virulence diversity in finger millet-infecting populations of the pathogen. Kiran Babu et al. (2015) developed the host differential set and reported the pathogenic variation in the isolates collected from finger millet grown in different states in India. This differential set is being used at ICRISAT to monitor variation in the pathogen population and select diverse pathotypes for greenhouse screening of a finger millet lines for blast resistance. Kiran Babu et al. (2013b) screened finger millet mini-core collection for blast resistance. Nine accessions (IE 1055, −2821, −2872, −4121, −4491, −4570, −5066, −5091, and −5537) with desirable agronomic traits, such as early flowering (<65 days), medium plant height (105–125 cm), and semi-compact to compact inflorescence were identified for use in a breeding program. The *africana*

type mini-core accession IE 4709, with a high level of resistance to blast, agronomically desirable characters, and high content of grain nutrients such as Fe, Ca, Zn, and protein, was identified as a promising source for use in finger millet breeding. An African cultivar IE 1012 has been extensively used in India as a source of blast resistance (Gowda et al. 1986).

Resistance sources have been identified through multilocation screening and are used in breeding programs. Multilocation evaluation of 29 genotypes at hot spots led to the identification of five promising genotypes (IE Nos. 2883, 2871, 6240, 2710, and GE3767) with stable resistance to both finger and neck blasts for further use in breeding programs (Das et al. 2021). Of the 81 finger millet germplasm accessions from East Africa evaluated for blast resistance, three accessions (G18, G43, and G67) were identified as resistant to all three stages: leaf, neck, and panicle blasts (Manyasa et al. 2019). Similarly, Dida et al. (2021) identified one improved variety (KACIMMI22), and four landraces (TZ1637, BKFM0031, ACC214988, ACC203544) in Kenya with high resistance to the blast isolate for use in breeding programs. Resistance to multiple pathogen isolates was observed in IE 2911, IE 2957, and GPU 28 in the greenhouse screening at ICRISAT, India (Kiran Babu et al. 2015). GPU 28, the cultivar, occupies a maximum area of about 80% of the total area cultivated in India and has shown resistance to blast over time in different states of India and exhibited lineage-wide resistance to *M. oryzae* populations as well (Nagaraja et al. 2008; Kiran Babu et al. 2015).

Of late, this cultivar has started showing susceptibility to blast. GPU 28 was released for cultivation in Karnataka in 1996, and after that GPU 26, GPU 45, and GPU 48 were released for cultivation on farmers' fields in India. Information on blast-resistant varieties identified and released for cultivation in different finger millet-growing areas in India has been compiled by Palanna et al. (2021). As virulence change in the pathogen populations has been cited as the main cause of the breakdown of resistance in the released cultivars to blast disease, monitoring virulence shift in the pathogen population, identifying new virulent pathotypes, screening breeding lines for resistance against the new virulent pathotypes under controlled conditions, further screening of promising lines at hotspots to identify stable sources of resistance, and strategically using these resistance sources in the breeding programs form the strategy of management of finger millet blast.

7.5.4.4 Nutrition-Inclusive Breeding

Grain quality A large proportion of the population in developing countries is deficient in essential nutrients like iron (Fe), zinc (Zn), and calcium (Ca) (Maharajan et al. 2021). Finger millet is especially rich in Ca (~350 mg per 100 g), which could be the potential crop to combat Ca deficiency. Apart from Ca, finger millet grains have a protein content of 6–13%, which is better balanced with sulfur-containing amino acids, such as methionine and cystine, as well as lysine, threonine, and valine, than other millets (Shobana et al. 2013; Saleh et al. 2013; Sharma et al. 2017; Rodríguez et al. 2020). Large variability exists for grain nutrient content in the core collection (Upadhyaya et al. 2010) and identified 15 promising accessions each for grain Fe, Zn, Ca, and protein and 24 accessions were identified which are

superior for two or more nutrients and provide an opportunity for breeding nutrient-dense cultivars. The ICRISAT product profiles included grain nutrient improvement, particularly Ca improvement as a target trait in the breeding program.

Fodder quality Finger millet is used as an important forage to some extent but not extensively used, like sorghum and pearl millet, due to a lack of scientific research on the quantity and quality of finger millet crop residues. The recent study on the fodder quality of finger millet germplasm conserved at the ICRISAT genebank indicated considerable variability. It provided evidence that finger millet crop residues have higher forage quality than rice and wheat, comparable with sorghum and pearl millet (Backiyalakshmi et al. 2021a, b). Thus, the promising lines identified could be used in the breeding program for breeding dual-purpose finger millet cultivars. With food security and nutrition-sensitive agriculture gaining momentum, this nutria-cereal is finding demand in urban food markets.

7.6 Novel Breeding Methods

7.6.1 Prebreeding: Widening the Gene Pool

Wild and weedy relatives of the genus *Eleusine* are the treasure troves for various economic traits, which are lacking in the primary gene pool of the finger millet. Introgression of novel traits like drought tolerance, blast and striga resistance, plant vigor, and superior nutritional quality from unadapted wild species to locally adapted popular cultivars of finger millet through a prebreeding approach will be an effective strategy for its genetic enhancement. Finger millet has two subspecies: *africana* and *coracana*. Subspecies *africana* is a diploid ($2n = 18$), while subspecies *coracana* is a tetraploid that evolved from the diploid subspecies (Paschapur et al. 2021). The diploid species *E. indica*, *E. floccifolia*, and *E. tristachya* form the secondary gene pool and *E. intermedia*, *E. gaegeri*, *E. kigeziensis*, *E. multiflora*, and *E. semisterlis* (*E. compressa*) from tertiary gene pool holds a great potential to address major production constraints of finger millet (Joshi et al. 2021b). However, incompatibility barriers must be investigated for developing interspecific hybrids between cultivated finger millet and its distant gene pool. Advancements in molecular breeding applications like advanced backcross and QTL analysis (Tanksley et al. 1996) enhance the possibility of utilizing a wild gene pool in the genetic improvement of finger millet.

Hybridization between Indian (*E. coracana* subspecies *coracana*) and African gene pool (*E. coracana* subspecies *africana*) of finger millet in the 1990s brought a paradigm shift in finger millet production in India, and the *Indaf* (Indian × African accessions) varieties replaced almost all the earlier released varieties. Apart from high grain yield, these varieties are known to possess unique traits like drought tolerance, lodging and enhanced protein quality acquired from the African gene pool. The ICRISAT breeding program is focused on widening the genetic base of the crop by combining the better stress tolerance traits of *E. africana* in Indian genotypes

to enhance the genetic gain and identify the best heterotic combinations through multilocation testing in collaboration with NARS partners across Asia.

7.6.2 Improving Crossing Efficiency

Variability plays a vital role in crop improvement, but inducing new variability is a daunting challenge in highly self-fertilized crops like finger millet with cleistogamous flowers. In general, there are about 280–1330 spikelets per panicle (4–7 seeds per spikelet), and a spike is reported to be 8–15 cm long and 1.3 cm broad, and it takes 5–7 days to complete anthesis in finger millet. Therefore, ensuring male sterility through hand emasculation in such small florets is a cumbersome and time-consuming task. Further, growing seeds for identifying a few hybrid plants in a traditional contact method (Sood et al. 2019) requires more resources, time, space, and labor. Therefore, ICRISAT, Patancheru, Hyderabad (17.3° N, 78.5° E), has done a good amount of work and recommended temperature and duration on a particular anthesis stage for effective emasculation in finger millet. Few seeds are set in the female panicle using this technique, and most are true hybrid plants. In addition, the hot water emasculation method was also studied by the ICRISAT breeding team (data unpublished). Compared to chemical treatment, hot water treatment is more efficient in emasculating female lines and enhancing the breeding process. After developing F_1s by hot water treatment method, we can quickly identify hybrid plants in F_1 generation using knowledge of identifiable morphological markers (e.g., pigmentation and panicle shape) in the case of male/donor line should have a dominant character. In the absence of a dominant pigmented marker on the nodes and panicles of the donor parent, the F_2 generation is raised and critically observed for the segregation of panicle or other plant traits. Recently, ICRISAT has performed whole-genome resequencing, and a set of 48 SNPs were identified for quality control and identification of putative F_1s.

Some programs are using the plastic bag technique. This method, adapted from the sorghum technique, involves covering the florescent with a plastic bag of the right gauge and leaving it overnight or until the stigmas open. Covering with a bag leads to the condensing of the water due to respiration, which will soak the anthers, making them not to disperse the pollen. The plastic bag is then removed, and the plant stalk is tapped gently to let the anthers fall. Pollen from the desirable donor is brought and dusted over the flower, the inflorescent which have not opened are removed, and the flower is covered. This technique has been very successful and is now universally used in many African breeding programs. As a result, thousands of lines have been developed by ICRISAT-Nairobi and shared with NARS partners in the region, west Africa, and with ICRISAT-Hyderabad.

7.6.3 Advanced Phenotyping Methods

The interaction of genotypes with the environment restrains genetic gain and insights into adaptation to different environmental constraints (abiotic stress). Therefore, it is important to characterize the environment in which the crop is grown (G×E) and design the phenotyping strategy relevant to the environment to empower the breeding programs for better selection.

ICRISAT has developed innovative methods and a high-throughput phenotyping platform (HTP) to facilitate precise characterization and screening for abiotic stress adaptation. It has helped NARS researchers from national programs to screen for several cereal and legume genetic materials (elite lines, national checks, advanced breeding lines, breeding populations) for crop improvement programs for changing climate adaptation (drought, heat, and salinity adaptation) using high-throughput phenotyping platform (LeasyScan, http://gems.icrisat.org/leasyscan/; Lysimeter facility, http://gems.icrisat.org/lysimetric-facility/). LeasyScan is "camera to plant"-based technology to characterize component traits of adaptation in just 4–6 weeks. A Lysimetric system with a rainout shelter facility is designed to impose various kinds of stress and evaluate the plant's performance. Efforts are underway to use AI technology for UAV-based field phenotyping to digitalize the field phenotyping of breeding trials and multilocation trials. There is also robust development in sensor-based technology for quick assessment of nutritional traits like macronutrient (Benchtop NIRS and mobile NIRS), micronutrient (XRF), and post-harvest traits (HarvestMaster, computer tomography) to support the nutrition inclusive breeding programs (Fig. 7.3).

Fig. 7.3 LeasyScan: high-throughput phenotyping platform

7.6.4 Speed Breeding

Over the last ten decades, plant breeders developed and released crop varieties through conventional approaches in many crops, but the conventional process is time-consuming because it involves crossing in between parental lines and generating progenies, followed by four to six generations of selfing or maintaining homogeneity to advance/fix the lines to evaluate productivity traits and agronomic performance. This is a time-consuming breeding approach for crop improvement that is often limited to only one to two generations per year, depending on the crops (Hickey et al. 2019). Speed breeding is a swift technique to enhance genetic gain and accelerate the breeding program/crop improvement in a shorter time with limited resources, manpower, and space compared to conventional breeding.

The generation period for finger millet cultivars in the field is around 4–5 months (Kumar et al. 2021b). However, under completely controlled conditions, the rapid generation advancement (RGA) technique may produce up to three to four generations of finger millet each year. The RGA protocol will accelerate the plant life cycle, and, on the other hand, it shortens the generation/breeding cycle time in light-, temperature-, and humidity-controlled conditions. In the case of short-day plants like finger millet, the protocol has already been developed based on light-emitting diode for some other short-day crops (soybean, rice, and amaranth) (Jähne et al. 2020), and efforts are underway to standardize speed breeding for finger millet. For rapid generation turnover, the rapid single-seed descent (rSSD) method applies to get near-homozygous lines in a year or two, depending on the crop species and duration. Five generations per year can be achieved in the case of soybean by using the protocol of Jähne et al. (2020). This is an economically and scientifically important and useful method compared to the conventional generation advancement method and shuttle breeding. Speed breeding allows and has significance in the development of populations, biparental populations (RILs and NILs), and mapping populations via robust phenotyping for trait specificity using X-ray fluorescence (XRF), near-infrared reflectance spectroscopy (NIRS), and computed tomography (CT) imaging, the marker-assisted selection (MAS), genomic selection (GS) models, and genome editing (Fig. 7.4).

7.7 Finger Millet Improvement Using Genomic Tools for Prospects of Accelerating Genetic Gain

7.7.1 Genomic Resources

Compared to major crops such as rice, wheat, maize, etc., few reports are available on genomic resources in small millets, including finger millet. Genomes of five small millets, namely, foxtail millet, finger millet, proso millet, teff, and Japanese barnyard millet, have been made available (Antony-Ceasar et al. 2018). Of these small millets, the genome of foxtail millet is the smallest (423–510 Mb), while finger millet has the largest one (1.5 Gb). Recently, the DArTseq approach was employed to assess finger

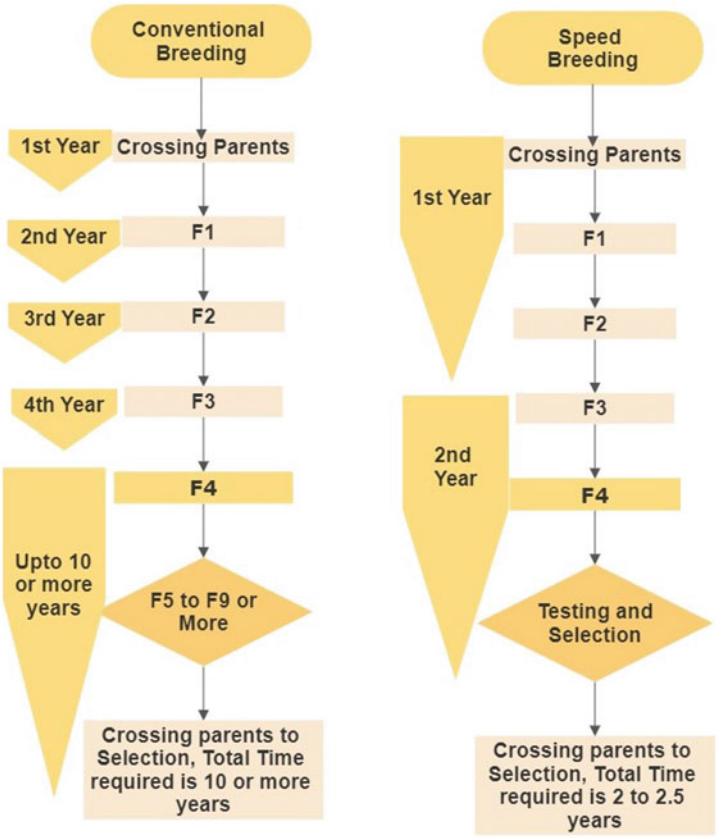

Fig. 7.4 Linking of conventional, novel breeding with post-genomic approaches

millet genetic diversity and population structure. Analysis of about 33,884 high-quality single-nucleotide polymorphism (SNP) markers on 318 accessions revealed considerable genetic diversity (Backiyalakshmi et al. 2021a, b). As limited genomic resources are available until recently in finger millet, comparative genomics has played an important role with high genomic co-linearity reported between finger millet and rice (Srinivasachary et al. 2007). The SSR markers were correlated to the genetic relatedness among the species with the cross-transferability of these markers to finger millet. For example, it has been reported that 71% of SSRs in rice (Babu et al. 2018) and 73–95% in foxtail millet SSRs (Pandey et al. 2013) were cross-transferable. The finger millet EST sequences showed homology with rice blast-resistant genes which suggested that genes responsible for rice blast resistance play an important role in finger millet blast resistance (Babu et al. 2014b, 2018). Further, as mentioned earlier in this chapter, under the subheading biotic stress, finger millet accessions from African countries are highly resistant to blast disease, whereas most

of the Indian subcontinent accessions are susceptible, as revealed by SSR markers (Babu et al. 2014a, b, c).

7.7.1.1 Reference Genome

The whole-genome sequence of finger millet genotype ML-365 (drought-tolerant and blast-resistant genotype) was sequenced on the platform Illumina and sequencing by oligonucleotide ligation and detection (SOLiD) technologies (Hittalmani et al. 2017). In the sequencing, about 45 Gb paired-end and 21 Gb mate-pair data were generated with a genome assembly consisting of 525,759 scaffolds (>200 bp) and N50 length of 23.73 Kb. In another study by Hatakeyama et al. (2018), genome assembly of the genotype PR 202 (IC: 479099) was reported using a novel polyploidy genome assembly workflow. Their analysis identified the genome size of finger millet as 1.5 Gb, and the genome assembled was 1189 Mb covering 78.2% of the genome. The whole genome consisted of 2387 scaffolds with the N50 value of 905.318 Kb with a maximum sequence length of 5 Mb and an overall gene number of 62,348, of which nearly 91% genes were functionally annotated and 96.5% were single-copy genes (Hatakeyama et al. 2018).

7.7.1.2 Trait Discovery and Mapping

Although next-generation sequencing technologies for genomic studies are now available, progress in identifying and tapping genes for important traits has been slow in finger millet until recently. The use of genetic markers to characterize functional traits diversity in finger millet has accelerated in recent years. The first genetic map using genomic SSRs, RFLP, AFLP, and EST markers was reported by Dida et al. (2007). Based on the genotype-phenotype association data, significant quantitative traits loci (QTLs) responsible for agronomic traits, as well as resistance for blast diseases, were identified, which showed strong associations with SSR primers designed from the blast genes (Babu et al. 2014b, 2018). Blast resistance gene homologs from rice and genes responsible for nutritional traits from other cereal crops have been developed and used invariably. Recently, the -omics approaches have efficiently been used in several studies to identify candidate genes responsible for nutritional variation as well as biotic/abiotic stress tolerance in finger millet (Rahman et al. 2014; Gupta et al. 2013). The identified markers are to be validated and fine mapped for use in marker-assisted breeding (MAB) programs of finger millet. In summary, the development of markers and comparative genomics paved the way for marker-assisted breeding. However, limited studies reported characterization of abiotic stress tolerance in finger millet using molecular markers.

7.7.2 Genomics-Assisted Breeding in Finger Millet

Biparental QTL mapping approach has been rarely initiated in finger millet due to the difficulty in crossing, variable synchronization in flowering, unavailability of stable contrasting parental lines, etc. for important quantitative traits. Further, fine mapping of the QTL region is unlikely due to high linkage disequilibrium (LD) in

populations (Sood et al. 2019). Likely, the first biparental mapping population developed is an interspecific mapping population of *E. coracana* subsp. *coracana* cv. Okhle 1 (a landrace from Nepal) and its wild progenitor *E. coracana* subsp. *africana* accession MD 20 to develop the first linkage map in finger millet (Dida et al. 2007). In finger millet, the availability of diverse germplasm resources has allowed the use of LD-based association mapping to detect marker-phenotype associations and identify linked markers associated with agronomic traits and disease resistance (Babu et al. 2014a,b; Bharathi 2011).

Recently, the application of NGS in finger millet has resulted in genome sequencing and identification of thousands of SNP markers for use in trait mapping and molecular breeding (Gimode et al. 2016). Significant and promising marker-trait associations for five important agronomic traits were identified using a genome-wide association study (GWAS) (Sharma et al. 2018). Identified SNPs through the whole-genome resequencing (WGRS) approach of global finger millet collections would provide useful genomic resources for identifying QTLs and linked molecular markers for important biotic/abiotic stresses and quality traits that can be used in early-generation selection. In this direction, the finger millet research team at ICRISAT endeavored WGRS in approx. 170 important germplasm lines (unpublished). On the other hand, genomic resources are being attempted to optimize genomic selection (GS) and genomics-enabled prediction in finger millet. The GS approach combines genotypic as well as phenotypic data of training populations to estimate the genomic-estimated breeding values (GEBV) of each individual of test populations (Crossa et al. 2017).

Further, molecular markers distributed throughout the genome would be used to predict individuals' GEBV, reducing the cost and time requirement of developing new crop varieties (Varshney et al. 2005). However, robust training populations and well-defined marker maps are the prerequisites for applying approaches such as GS in finger millet. Findings from these studies would facilitate rapid selection of superior genotypes overcoming the limitations of MAS (Fig. 7.5).

7.8 Summary and Outlook

Finger millet productivity in African and Asian countries is much below the real potential of this crop, even after 100 years of breeding. However, significant genetic variability is available for traits of importance. Germplasm exchange between Africa and Asia can be a game-changer. The challenge of crossing finger millet due to small-sized flowers can now be handled using recently devised new methods to increase crossing percentage, and a set of identified markers can be used to detect true crosses. With the availability of sequence data of the finger millet genome, important traits linked to productivity and biotic and abiotic stress tolerances have been mapped. With the improved understanding of the genetics of traits of importance, identification of donor lines for different traits, availability of improved methods of phenotyping, and the possibility of three to four crops in a year using

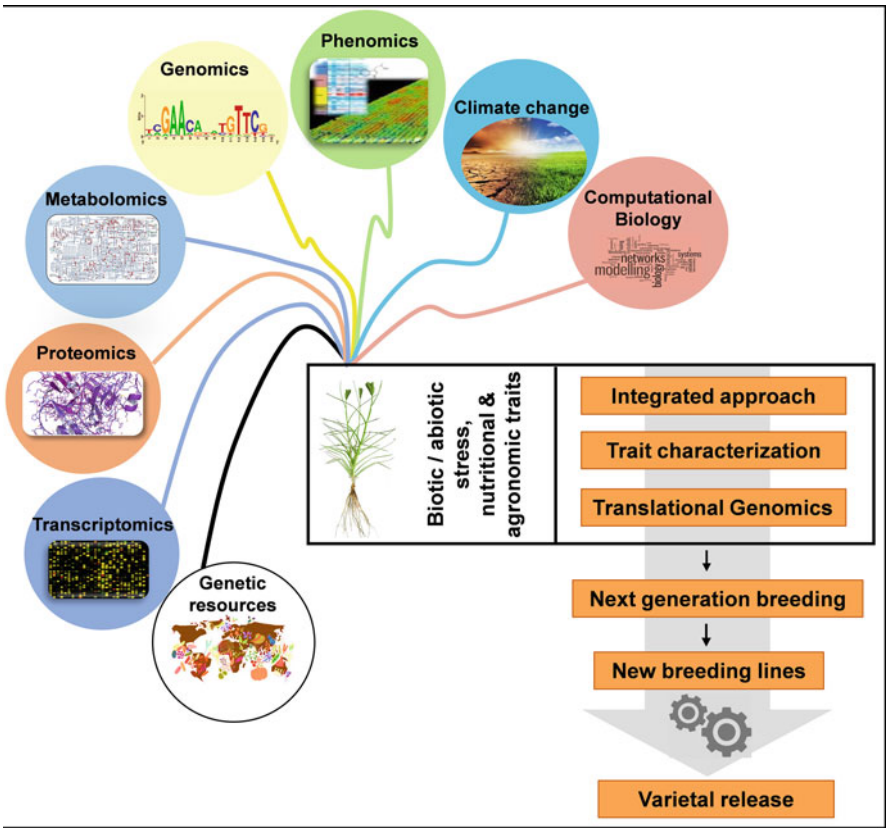

Fig. 7.5 Application of smart breeding in post-genomic era in finger millet breeding

speed breeding protocols, finger millet breeding programs across the world will have a major push to enhance genetic gains in this crop in the coming years.

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Barnyard Millet Improvement: From Pre-genomics to Post-genomics Era

8

Mahendar S. Bhinda, Nazarul Hasan, and D. C. Joshi

Abstract

Barnyard millet (*Echinochloa* species) is an eminent small millet that proficiently offers food, feed, and nutritional security to the ever-increasing population and adapts to climate change. Despite its numerous nutritional and agronomic remunerations, barnyard millet has received less research attention than the efforts devoted to major cereals over the past decades. In barnyard millet, the prerequisite genetic variation for enhancing the various agronomic and nutritional attributes is available in germplasm collections. However, utilization of these genetic resources for tangible improvement is frequently hampered by the poorly known genetic architecture of the traits. Furthermore, the genomic resources in this crop are less elaborate due to accompanying ploidy complexity and narrow genetic base. Therefore, more intensive research exertions are requisite to illustrate germplasm resources, recognize trait-specific donors, develop mapping populations, and discover QTL/gene(s). This chapter summarizes the brief introduction and significance of barnyard millet, the up-to-date state of the art in breeding, genetic, and genomics research.

Keywords

Barnyard millet · Nutritional security · Small millets · Genetics · Genomics

M. S. Bhinda · N. Hasan · D. C. Joshi (✉)
ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, Uttarakhand, India
e-mail: Dinesh.Joshi@icar.gov.in

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8.1 Introduction

Despite a significant increase in agricultural growth during the second half of the twentieth century, just around 12 crops contribute 75% of global food supplies, with 3 key cereals, rice, wheat, and maize, contributing about 50% of world foodstuff opportunities (Joshi et al. 2018). Such a limited agrarian portfolio poses severe concerns to agro-biodiversity. Dietary supplementation of major cereals with small millets featuring superior nutrient content and nutraceutical attributes might be a promising strategy to enhance dietary diversity and reduce the risk of adverse climatic circumstances (Joshi et al. 2019). Barnyard millet (*Echinochloa* spp.) is one of the oldest cultivated crop species among small millets. The *Echinochloa* comprises about 250 annual and perennial species extensively spread across the world's warmer and temperate regions (Bajwa et al. 2015). The Echinochloa encompassed mainly two cultivable species: *Echinochloa frumentacea*, which is acknowledged as Indian barnyard millet, and *Echinochloa esculenta*, acknowledged as Japanese barnyard millet (Sood et al. 2015b). Japanese species of the genus are grown in Japan, Korea, and the northeastern part of China, whereas the Indian species is the inhabitant of Pakistan, India, Nepal, and central Africa (Yabuno 1987; Wanous 1990).

Barnyard millet is principally self-pollinating (Potvin 1986) and self-compatible crop species. However, some outcrossing was also known to be assisted by wind pollination. It has a very short life cycle and can thrive in hostile environments with minimum inputs. However, cultivable species like *E. esculenta* and *E. frumentacea* are generally susceptible to many biotic hassles, largely pests and diseases at different phases during crop development (Jain et al. 1997; Jagadish et al. 2008). Consequently, the major breeding objective to conquer biotic limitations in barnyard millet is resistance against diseases, mainly grain smut, loose smut, and sheath blight, and among the insects are shoot fly, stem borer, and aphid.

Cultivable barnyard millet species is an annual, sturdy, and tall crop that may reach a height of 2–2.5 m. Barnyard millet is a dual-purpose crop that is grown mainly for human intake as well as cattle feed. It has recently gained prominence due to its superior nutritional profile, acknowledged health benefits, flexible environmental adaptation, feasibility under marginal land, and adaptability to organic and low-input agriculture. It is the fastest developing crop among the millets and is best suited to the fragile and undulating mountainous ecology (Gururani et al. 2021).

8.2 Present Status

It is extensively grown in Asian countries, mainly India, China, Japan, Korea, Malaysia, etc. Among the small millets, barnyard millet is the fourth most produced crop, offering food security to numerous underprivileged individuals worldwide (Meena et al. 2021).

India is the leading grower of barnyard millet, both in terms of area (0.146 million hectares) and production (0.147 million tons), with an average yield of 1034 kg/ha

over the last 3 years (IIMR 2018). Barnyard millet is mainly grown in India under two distinct agro-ecological: one in the northern part under the lower and mid hills of the Himalayan region and the other in the southern part, largely in the Deccan plateau region (Sood et al. 2015a). In India, it is primarily grown in states, viz., Orissa, Maharashtra, Madhya Pradesh, Tamil Nadu, Bihar, Punjab, Gujarat, and Uttarakhand (Kumar et al. 2000).

8.3 Barnyard Millet's Nutritional Composition and Nutraceutical Potential

Diets with medicinal properties that help maintain well-being, improve health, modulate immunity, and thus prevent specific diseases are known as "Nutraceuticals" (Kumar and Kumar 2015). The major nutraceutical constituents in millets are antioxidants, polyphenols, crude fibers, and minerals. Therefore, millets such as barnyard millet can be used in functional foods as a nutraceutical for the prevention and treatment of illness related to lifestyle owing to numerous listed health welfares. As a result, they are also acknowledged as "nutricereals."

Like rice grains, barnyard millet grains are dehusked, cooked, and consumed. In the Himalayan region, it is consumed as paleu or chencha, a savory boiled porridge made with buttermilk. In South India, however, the grain is parboiled and utilized in the preparation of idli, dosa, and chakli, among other dishes (Bhat et al. 2019). Aside from these, barnyard millet is also used to make biscuits, cakes, pasta, and other culinary items (Arora and Srivastava 2002).

Compared to major cereals, barnyard millet is a noble source of high-quality digestible protein with the least calorie bulk and gluten content. Barnyard millet is exceptionally nutritious, including high fat, fibers, essential amino acids, and mineral content, especially calcium and iron. The comparative nutritional profile of barnyard millet with major cereals based on per 100 g edible portion is provided in Fig. 8.1.

The low glycemic index of barnyard millet makes it an ideal diet for diabetic patients (Sharma et al. 2013). It is also known for its cholesterol-lowering properties (Rao and Bhaskarachary 2017). It is an ideal food for those patients suffering from gluten sensitivity and celiac illness. Problems related to digestion such as constipation, excess gas, bloating, and cramps can all be addressed due to the high fiber content (Rao and Bhaskarachary 2017). These characteristics make it a viable candidate for manufacturing industrially processed foods such as infant meals, snacks, and other dietary items (Vijayakumar et al. 2009).

8.4 Genetic Architecture of Barnyard Millet

Knowledge of flower biology and pollination behavior assists in the effective emasculation and crossing procedure. In barnyard millet, hot water treatment of inflorescence (3–4 days after emergence) at 48 °C for 4–5 min was perceived to induce male sterility (Gupta et al. 2011). In barnyard millet, emasculation and

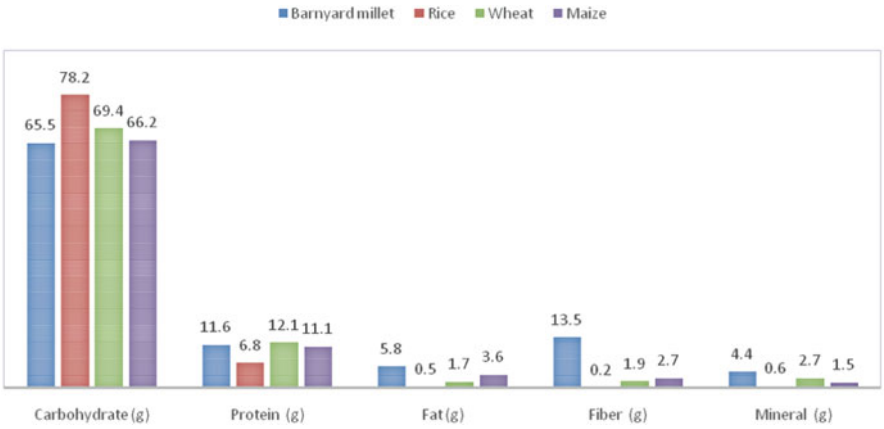

Fig. 8.1 Nutritional profile of barnyard millet (per 100 g) compared to major cereals (Sources: FAO 1995)

artificial hybridization are troublesome procedures due to the small size of the flower, the early hours of flowering, the short pollen viability period, the non-availability of pollen grain, and the short period of flowers opening (Nirmalakumari and Vetriventhan 2009). Therefore, in small millets, the realized fraction of making the effective crosses is normally very low, even for expert hands. Thus, like other small millets, the floral structure and anthesis behavior obstruct the opportunity of genetic studies and yield augmentation in barnyard millet.

In order to raise the efficacy of genetic improvement efforts, a better comprehension of the genetic context of objective traits is crucial. The genetic advances of barnyard millet are challenged by the fact that all members are polyploidy in nature. *E. colona* and *E. crus-galli* are classified as hexaploids, with chromosomal constitution $2n = 6x = 54$ (Prasada Rao et al. 1993; Upadhyaya et al. 2008). However, other numbers have also been described (Wanous 1990), probably suggesting that this genus is heterogeneous in nature. The flow cytometry studies revealed that the total genome size is around 1.4 gigabases (Bennett et al. 1998, 2000).

8.5 Available Germplasm Resources

The preliminary stage in classifying germplasms into diverse sets is the comprehensive collection and their characterization and further documentation based on different aspects. These activities are supported through gene banks, which perform a key role in preserving crop genetic variation in the face of continuing loss. They offer genetic diversity for breeding programs to adapt to fluctuating environmental factors and client requirements (Dwivedi et al. 2008).

Gene banks worldwide have vast collections of germplasm of different species of barnyard millet. However, because of the high G × E interactions for quantitative traits, it is practically tough to precisely and effectively assess these enormous

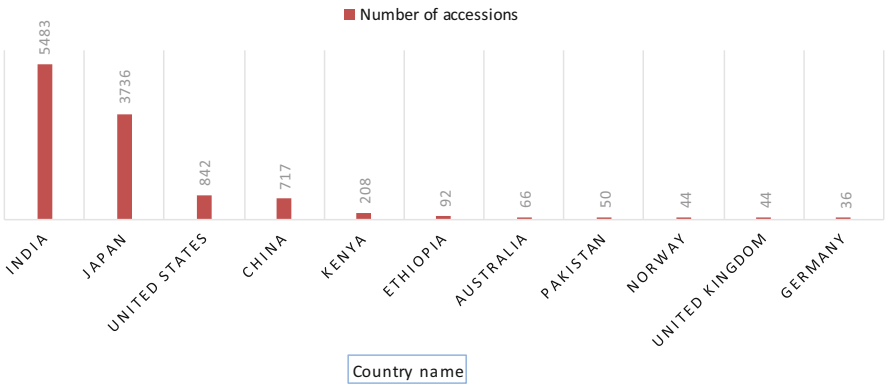

Fig. 8.2 Major countries around the globe have germplasm collection of the *Echinochloa* species (Source: Renganathan et al. 2020)

datasets for valuable morpho-agronomic variables. To address this issue, the formation of condensed subsets, for example, core or mini core collections, is one of the most practical approaches to evaluating them precisely and cost-effectively at multi-locations and replicated trials. These subsets could be used to recognize agronomically valuable accessions for further usage in breeding program.

At present more than 11,000 germplasm accessions of barnyard millet have been conserved at various gene banks across the globe (Fig. 8.2). The effective deployment of these genetic resources resulted in the release of more than 20 barnyard millet cultivars throughout India (Gomashe 2017).

8.6 Breeding in *Pre-genomics Era*

8.6.1 Classical Breeding

Little national and international consideration has been attained by the small millets, even though millets have been an integral part of the farming system from time immortal at the regional level. Initially, the genetic improvement projects started worldwide, focusing on collection, characterization, and conservation of small millets, including barnyard millet accessions. In India, the organized millet breeding work started at the national level in 1969 through the establishment of the All India Coordinated Millets Improvement Project (AICMIP). Conventional breeding approaches include pure line selection, pedigree selection, mass selection, and mutation, which are mainly relevant to self-pollinated crop species and are equally pertinent in barnyard millet. In barnyard millet, a majority of cultivars, about 18 in number in India, were released resulting from a selection from local landraces/cultivars, accompanied by pedigree selection (hybridization and selection) (AICSMIP 2014).

8.6.2 Pre-breeding/Inter- and Intraspecific Hybridization

Generally, the Japanese type (*E. esculenta*) holds more variation for agronomic traits compared to the Indian barnyard millet (*E. frumentacea*) (Sood et al. 2015a). To cater this diversity, efforts have been attempted for interspecific hybridization using *E. frumentacea* and *E. esculenta*. However, the interspecific hybrids amid distant gene pools have generally been unsuccessful due to strong compatibility barriers among the species (Hilu 1994). Consequently, intraspecific hybridization between the genetically divergent Indian types is one perspective to develop transgressive segregants for agronomical and nutritive attributes. To take advantage of their better adaptability to the Himalayan region and disease-resistant nature, both-way crosses were attempted between PRB 903 and PRJ 1 of *E. esculenta* at ICAR-VPKAS, Almora. The effort resulted in F_6 progenies with larger panicles (22.5–26 cm), more panicle branches (>15), medium plant height (120–148 cm), and smut resistance. In the genetic makeup of PRJ 1, the intraspecific hybridization effort led to the creation of stable awnless segregants that are more robust than the parental line (Joshi et al. 2021).

8.6.3 Mutation Breeding

Mutation breeding has been crucial for creating variability in self-pollinated crops, where the hybridization procedure is complex. Mutation breeding can be employed to create genetic polymorphism to improve yield and quality-related attributes. However, mutant phenotype induction in the case of polyploid species like *Echinochloa* is very problematic (Sood et al. 2019). Gamma irradiation exposure to barnyard millet enhanced genetic diversity for seed yield and yield attributes, including the number of tillers, plant height, and ear head length (Mehra et al. 1985). Induced mutagenesis through gamma radiation resulted in a full waxy stable mutant line of low amylase landrace “Nogehie” (Hoshino et al. 2010).

8.7 Genomics Era

Augmenting genetic gain by means of a comprehensive strategy that integrates conventional and genomic approaches is required to produce stress-tolerant cultivars with improved yield potential and dietary superiority (Varshney et al. 2010). The introduction of next-generation sequencing (NGS) techniques seems to have the potential to have an important influence on crop improvement practices. Advancements in recent years in next-generation sequencing (NGS) tools have offered excellent opportunities for producing genomic resources and revealing vital molecular mechanisms regulating particular biological paths in millets. These resources could facilitate gene discovery for economic traits, marker-assisted breeding, genome-wide association mapping, and genomic selection.

8.7.1 Gene/QTL Mapping

In barnyard millet, several SSR and SNP markers have been identified (Wallace et al. 2015; Chen et al. 2017; Manimekalai et al. 2018; Murukarthick et al. 2019) to aid in the establishment of linkage maps and QTL mapping. Although, no genetic linkage map has been available so far. Few mapping investigations in barnyard millet are available, one for waxy traits (Ishikawa et al. 2013). They used functional SNP markers and demonstrated that three loci, namely, EeWx1, EeWx1, and EeWx3, were accountable for regulating waxy traits in barnyard millet. Similarly, a bulk segregant analysis (BSA) with 51 EST-SSR markers was employed to investigate the genetics of anthocyanin pigmentation. This study revealed that anthocyanin pigments in barnyard millet are tightly linked to the SSR marker, BMESSR 39 (Renganathan et al. 2019). However, the genome mapping work in barnyard millet is still in its early stages. Consequently, advanced experimental research on mapping studies is required before implementing the marker-assisted selection strategy (Renganathan et al. 2020).

8.7.2 Genomic Resources

The whole-genome sequencing approach is required to understand a crop's genome configuration and gene sets and to detect critical genes and trails associated with economically imperative characteristics in crop plants (Joshi et al. 2021). The molecular characterization of the barnyard millet is hampered by a lack of genomic information, such as DNA markers, genetic/linkage maps, and genome sequences. However, the genome sequence information is available in other millets such as sorghum, pearl millet, foxtail millet, finger millet, and proso millet (Zhang et al. 2012; Mace et al. 2013; Hittalmani et al. 2017; Varshney et al. 2017; Zou et al. 2019). Due to the intricacy of the genome and the lack of research in barnyard millet, only a few attempts have been done to determine the genetic organization and associated advances. Further, the barnyard millet genetic resources could be supplemented by genomic resources from closely related species where genome sequences are already available.

The whole genome assembly of the wild progenitor (*Echinochloa crus-galli*), of *E. esculenta*, was recently completed (Guo et al. 2017). The genome size was measured to be 1.27 Gb, demonstrating 90.7% of the genome coverage with a scaffold N50 length of 1.8 Mb.

8.7.3 Genomic Selection (GS) for Barnyard Millet Improvement

Genomic selection (GS) is a useful approach with a lot of prospective for exploring and increasing genetic gain in the breeding scheme for selection per unit time frame (Spindel et al. 2015). It accelerates breeding efforts and increases the efficacy of crop improvement programs. Genome-wide dispersed DNA markers are employed in GS

to envisage genomic estimated breeding values (GEBV) for breeding materials (Varshney et al. 2013). Except for pearl millet, the GS data for other millet crops are lacking (Srivastava et al. 2020).

The GS is a viable method for selecting breeding materials. In the future, it may enhance the gene pool in millet germplasm. Plant breeders must employ GEBV to characterize millet germplasm (Bhat et al. 2016). Recently, many crop plants have been subjected to the rapid crop improvement system known as speed breeding (Wanga et al. 2021). Through short breeding cycles, this decreases the time needed for crop improvement. A combination of these potent breeding approaches with GS will hasten the genetic gains required for the speedy advancement of complex attributes to improve millet's yield potential. The discovery of QTL/genes accompanying millet bio-fortification attributes may support increasing micronutrient content in the seeds, thereby reducing micronutrient shortage globally (Krishna et al. 2022). However, there has yet to be a study by engaging the GS approach to recognize bio-fortification attributes in small millets, particularly barnyard millet, to alleviate micronutrient deficiency.

8.7.4 Comparative Genomics

The comparative genomics approach is vital because it uses synteny evidence between conserved regions among crop plants from the same family (Moore et al. 1995; Gale and Devos 1998; Pattanayak et al. 2019). The evidence for similar conserved genomic relationships in major cereals like rice (Zhao and Kochert 1993) and wheat (Roder et al. 1995) is already well established. Comparative genomic studies will be an effective means for genomic research in barnyard millet due to the inadequacy of genome sequence information.

The genomic microsatellite markers discovered by in silico mining for foxtail millet revealed a high level of cross-transferability in barnyard millet. According to Kumari et al. (2013), 90% of EST-based foxtail millet SSRs were transferable to barnyard millet. Similarly, Pandey et al. (2013) discovered that foxtail millet SSRs were 91% transferable to barnyard millet germplasm. Further, these microsatellites or simple sequence repeat (SSR) markers have been proven to be helpful in connecting phenotype-genotype variations to choose preferred genotypes through marker-assisted selection.

In addition, 100 intron-length polymorphic markers extracted from the foxtail millet genome revealed 94% cross-transferability with the Indian barnyard millet (Muthamilarasan and Prasad 2014). Similarly, greater than 70% cross-transferability of rice genic SSR was established gained from calcium transporters and calcium kinase primers to Indian barnyard millet (Yadav et al. 2014). Babu et al. (2018) employed genomic SSRs markers from rice and finger millet to assess cross-transferability in barnyard millet to identify polymorphic markers, syntenic regions, and analysis of genetic diversity as well as population structure. In the case of finger millet SSRs, they perceived 100% cross-transferability, of which 91% were

polymorphic, whereas for rice markers, 71% cross-transferability was recorded, out of which 48% were polymorphic.

These markers might be of enormous and meaningful worth for studies of genetic diversity, establishing linkage maps and recognizing significant agro-morphological traits associated with QTLs in barnyard millet. Furthermore, these identified QTLs will be efficiently introgressed via marker-assisted selection for high yield and stress regulation in barnyard millet genotypes that are locally adapted. Thus, till a huge number of molecular markers become readily accessible, comparative genomics offers a chance to generate orthologous molecular markers in barnyard millet by using sequence variants of key genes from major cereals and other millets. Further, the markers associated with target traits could be explored to screen a large set of germplasms.

8.7.5 Functional Genomics

8.7.5.1 Transcriptomics

Gene expression profiling focusing on transcriptome study is a potent functional genomics tool for identifying candidate genes responsible for an array of biological pathways (Kumar et al. 2016). Massive transcript profiles for the characters having role in numerous invasiveness and adaptation processes, viz., herbicide resistance, photosynthesis, and flooding, and other genes related to homeostasis have been constructed in the weedy *Echinochloa* species (Li et al. 2013a, b; Yang et al. 2013; Nah et al. 2015; Xu et al. 2015; Guo et al. 2017; Gao et al. 2018). It has also been used effectively in constructing linkage maps (Varshney et al. 2007), the germplasm diversity evaluation, and the exploration of molecular markers for MAS strategy (Kalia et al. 2011; Miah et al. 2013; Pandey et al. 2013). Several investigations have revealed that transcriptome analysis using NGS approach is the simplest way to recognize SSR loci (Obidiegwu et al. 2013; Gimode et al. 2016).

Jayakodi et al. (2019) identified 4159 protein-coding and 2258 long noncoding RNA (lncRNA) transcripts in Indian barnyard millet through comparative transcriptome analysis. These transcripts exhibited either up- or down-regulated expression when compared with *E. crus-galli*. Among these, 3489 protein-coding transcripts were found unique to Indian barnyard millet. Further, the investigation discovered that photosynthesis is most likely important in the Indian barnyard millet's drought adaptation mechanism. Moreover, transcriptome characterizations for economically important characters like resistance to diseases and nutritional quality have yet to be established in the Indian barnyard millet.

8.7.5.2 Proteomics

Proteomics is a functional genomics procedure that entails an in-depth exploration of many proteins in terms of their organization, expression, and functional characteristics. Proteomics studies frequently used 2D gel electrophoresis, mass spectrophotometry, and gel-free shotgun sequencing techniques (Matros et al. 2011). The technological advancement in proteomics through proteome mapping,

comparative proteomics, and the discovery of protein-protein interactions is consenting to new perceptions about plant genomes (Varshney et al. 2009). Proteomics studies with a focus on understanding seed quality characters may not have yet been applied in barnyard millet.

8.7.5.3 Metabolomics

Metabolomics is a new "omics" technology that identifies, characterizes, and quantifies biomolecules with low molecular weight in a biological environment (Kumar et al. 2016). The term "metabolomes" is used to describe these low-molecular-weight biomolecules. Several secondary metabolites, including polyphenols and flavonoids, have been discovered to perform a key role in the nutraceutical properties of the barnyard grain. But even so, metabolomics characterization of barnyard millet grain is not available. Therefore, an extensive metabolomics investigation is essential to determine the most suitable options for human food.

8.8 Post-genomics Era

8.8.1 Genetic Engineering

Using genetic engineering techniques, plants can also be modified to have desired features that don't express naturally. However, some health, environmental, and ethical issues are responsible for the large-scale cultivation of genetically engineered crops, despite their immense potential.

In the case of staple cereals, effective transformation procedures have been developed. But, till now, the transformation protocols in barnyard millet are not well standardized. Hence, this required further concerted focus on barnyard millet. Even though a 90-day regeneration procedure utilizing MS media was shaped to speed the in vitro plant regeneration development for barnyard millet using CO_2 cultivar, this has resulted in the establishment of a quick, effective, and reproducible regeneration strategy (Rajak et al. 2018).

There is only one report concerning genetic transformation studies in barnyard millet. This experiment was based on biolistic transformation to evaluate the effectiveness of various promoters in GUS expression (Gupta et al. 2001). Although, no attempts have been made so far to alter barnyard millet using an *Agrobacterium*-mediated transformation approach.

8.8.2 Genome-Editing Tools for Millet Improvement

Accessibility of WGS opened the door for genome editing tactics and the opportunity of introducing anticipated characters in millet crops (Ceasar 2021). The genome editing (GE) technique is a relatively new approach for genetic enhancement in crop plants. Genome editing through site-specific nucleases is considered the utmost

established means for accurate and efficient genome manipulation, and it has the prospects to transform applied research in crop improvement. The GE approach aids crop growth and yield in both biotic and abiotic stress environments. This technology entails inserting, removing, or replacing a DNA fragment at precise genome sites using designed nucleases that cause explicit double-strand breaks (DSBs) and activate cellular DNA repair processes.

Only a small attempt has been made in millet for genome editing by employing the CRISPR/Cas9 system. In millets, numerous genes are discovered that are responsible for tolerance to various abiotic stresses such as drought, salinity, heat, cold, oxidative, and nutrient deficiency (Gupta et al. 2013; Parvathi et al. 2013; Ceasar et al. 2014; Nagarjuna et al. 2016; Jadhav et al. 2018; Cao et al. 2019). Furthermore, researchers must explore the effect of biotic and abiotic stress-responsive genes available in millets via the genome editing system, which might aid in advancing anticipated traits (Krishna et al. 2022).

8.8.3 Conclusion

Currently, the agriculture production system is suffering from many challenges, especially from climate changes and over-dependency of world food supply from a few major crop species. Under such scenarios, using underutilized and potential crops like barnyard millet, which is climate resilient in nature and can offer diversification for food and genetic resources, is one of the prospective ways to combat these hitches. To overcome the yield barrier in barnyard millet, the male sterility system and heterosis can be exploited along with the improved crop management and mechanization practices. Barnyard millet will benefit from genomic-assisted breeding since it will make it easier to discover new alleles and genes with superior agronomic concert and resilience to biotic and abiotic challenges. As a result, a roadmap for supporting barnyard millet growth is urgently needed, including establishing unique cultivars for specific environments and exploring trait improvement through modern breeding and genomic methods. These strategies would support to fight against hunger and malnutrition while also helping farmers and other stakeholders tangle in barnyard millet cultivation in the context of climate change.

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Pigeonpea Crop Improvement: Genomics and Post-genomics

9

Raju Ghosh, Avijit Tarafdar, M. Kasi Rao, Srinivas Katravath, and Mamta Sharma

Abstract

Pigeonpea remains an excellent lucrative crop in the face of adding climatic adversities. In the past five decades, constant exploration has been directed toward yield enhancement in pigeonpea in the deployment of several commercially decent cultivars in India. Empowering crop enhancement strategies with genomic and post-genomic tools tackle is imperative to attain the design earnings in crop yield. The vacuity of the draft genome sequence with a large-scale marker resource acquainted the exploration toward particularity mapping for flowering time, determinacy, fertility restoration, yield-attributing traits, and print insensitivity. Defined core and mini-core collection still eased the pigeonpea breeding being accessible for being inheritable diversity and developing stress resistance. Ultra-modern genomic tools like coming-generation sequencing and genome-wide selection helping in the appraisal of selection effectiveness are leading toward coming-generation parentage, an awaited corner in pigeonpea inheritable improvement. This book chapter emphasizes the ongoing inheritable enhancement in pigeonpea with a blend of genomic and post-genomic exploration.

R. Ghosh · A. Tarafdar · M. Sharma (✉)
International Crops Research Institute for Semi-Arid Tropics, Patancheru, Telangana, India
e-mail: mamta.sharma@cgiar.org

M. Kasi Rao
International Crops Research Institute for Semi-Arid Tropics, Patancheru, Telangana, India

University of Agricultural Sciences, Raichur, Karnataka, India

S. Katravath
International Crops Research Institute for Semi-Arid Tropics, Patancheru, Telangana, India

Professor Jayashankar Telangana State Agricultural University, Hyderabad, Telangana, India

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Pigeonpea · Genomics · Post-genomics · Transcriptomics · NGS · Male sterility · Heterosis

9.1 Introduction

Pigeonpea is a potentially important pulse crop of rain-fed agriculture. The genus *Cajanus* includes 32 species; several are found in India (Bohra et al. 2011). Pigeonpea now shifted from an ignored crop to a genomic resource-rich crop (Varshney et al. 2010; Pazhamala et al. 2015). The primary pigeonpea expressed sequence tag (EST) dataset provides a transcriptomic database for genetic information and expansion of functional markers related to biotic stress resistance (Raju et al. 2010). The demand for the grain legumes is continuously rising, and it has been estimated that 32 million tons of pulses will be needed by the year 2030 and 50 million tons of pulses by the year 2050 (Vision 2050: Indian Institute of Pulses Research, 2013, www.iipr.res.in). Legume breeders have struggled to develop superior cultivars to provide more food and nutritional security to fulfill the requirements. We in ICRISAT are continually strengthening our pigeonpea breeding program through conventional breeding (Saxena 2008), and molecular breeding has a great potential to achieve crop production (Varshney et al. 2010). Limited genomic data information coupled with a narrow genetic diversity in the cultivated gene pool have been a serious concern to successful molecular breeding of pigeonpea (Varshney et al. 2009). Genomic data like molecular markers, genetic maps, transcriptomic, or genome sequence are the basics to go forward. Combining genomic resources with breeding can improvise our breeding strategy. The genomic science can efficiently select a heterotic hybrid of male and female parents to incorporate resistances and their stability. Recently, we have generated the application of genomic information, particularly DNA markers and draft genomes which represent major accomplishments in pigeonpea (Varshney et al. 2012).

9.2 Breeding for Future Resources

We are trying to develop pigeonpea genomic resources with food and nutrition security. Arhar (pigeonpea) is supposed to be an orphan crop, but it is surprising that in every meal, from breakfast to dinner, we consume pigeonpea as an idly, dosa, bara, etc. ICRISAT has developed many genetic resources to serve the whole nation and provide food and nutrition security Palit et al. 2020). These genetic resources (molecular markers and genetic maps) upsurge the option of finding the underlying important features of genes and QTLs, ensuing in genetic improvement of the crop. Furthermore, markers and candidate genes accountable for traits such as resistance to biotic stresses (Mir et al. 2013), yield, and phenology in pigeonpea have been identified using association mapping, marker-based QTL mapping, candidate

gene-based association mapping, transcriptomics, and whole-genome sequencing (Mir et al. 2017).

Additionally, the current genomic tools, such as next-generation sequencing (NGS), genome-wide genetic markers, and transcriptome/genome assemblies, have permitted the creation of various genomic resources to aid pigeon pea breeding program. The whole mitochondrial genome sequence has unlocked new roads for research into cytoplasmic male sterility systems and hybrid breeding in pigeonpea. Multiparent advanced-generation intercross (MAGIC) and nested association mapping (NAM) populations not only confirm the best utilization of high-throughput genotyping platforms but also offer numerous returns over traditional mapping populations in terms of better resolution and allelic richness, aiding in family-based QTL study and linkage disequilibrium analysis (Bohra et al. 2017a).

9.3 Achievements in Pigeonpea Genetics and Genomics

Even though conventional breeding and hybrid technology are still being used to advance pigeonpea (Saxena and Kumar 2003), molecular breeding should speed up the utilization of the extensive variability among pigeonpea landraces and germplasm lines for several traits. The genetic basis of the mainstream of important traits in pigeonpea is unknown, and no linkage map has been reported to date. This could be accredited to low levels of DNA polymorphism within the primary gene pool, as well as a lack of molecular markers (Burns et al. 2001; Yang et al. 2006; Odeny et al. 2007, 2009; Saxena et al. 2009a). Pigeonpea genomics initiative (PGI) has focused on generating a robust set of polymorphic markers, including microsatellites or simple sequence repeats (Gupta and Varshney 2000), single-nucleotide polymorphisms (SNP), and diversity array technology (DArT), to address the necessity for genomic tools in pigeon pea. Using these molecular markers in diverse mapping populations of pigeonpea will make it easier to generate a genetic map, mapping disease resistance genes and quantitative trait loci (QTL), and integrate phenomics data from several mapping populations. To enable map-based cloning and functional analysis of traits in pigeonpea, it is essential to concurrently develop mutant lines and a sizable DNA-insert library, such as the bacterial artificial chromosome (BAC).

Moreover, the accessibility of suitable genetic resources is a prerequisite for efficiently applying tools derived from genomics in any crop species (Varshney et al. 2005). As a result, the PGI consortium focused on generating an appropriate collection of genetic resources from the beginning. Over the past few years, substantial developments have been made in developing several populations, genetic mapping, and reverse genetic analysis of pigeonpea improvement. Even though some mapping populations already existed when the PGI started, numerous partner institutes made an effort to generate a reasonable set of mapping populations suitable for the molecular tagging of many biotic and abiotic stresses in pigeonpea. The key production constraints in pigeonpea are *Fusarium* wilt, SMD, *Phytophthora* blight, drought, and waterlogging, segregated when regionally adapted elite cultivars of

interest to PGI partners were evaluated with SSR markers to select a diverse set of parents (Saxena et al. 2009b). One interspecific [ICP 28 (*C. cajan*) × ICPW 94 (*C. scarabaeoides*)] and one intraspecific (Asha × UPAS120) mapping population have also been developed to generate high-density reference genetic maps. As previously described, ICRISAT has effectively generated hybrid pigeonpea plants in association with several NARS partners. ICRISAT is developing populations to map the fertility restorer (Rf) gene for A4 cytoplasm. Sustainable pigeonpea hybrid production critically depends on the identification of fertility restorer lines for a specific cytoplasm. In this context, ICRISAT has generated an additional eight mapping populations (BC1F1 and F2) for mapping the Rf gene. Breeders can use marker-assisted selection (MAS) to introduce fertility restorer loci into other elite cultivars using molecular markers closely linked to the Rf gene.

Functional genomics, a new arena for determining gene function, has grown up in prominence due to the rapid achievement of genomic sequence data. Based on TILLING, PGI has started creating a reverse genetic resource for pigeonpea (McCallum et al. 2000) to make it smooth to conduct functional genomic studies in the plant after its genome sequence has been available. TILLING is a reverse genetic tool that categorizes individuals carrying a variety of single nucleotide-induced variants in genes of interest from a library of saturation-mutagenized individuals, each with several hundred to low 1000 s of point mutations. For instance, in Banaras Hindu University (BHU), to generate the TILLING population, the reference genotype Asha (ICPL 87119) was mutagenized with the EMS mutagen. In the pilot trial, BHU treated 3000 seeds in each of the four EMS concentrations (0.01, 0.02, 0.03, and 0.04 M) between 2007 and 2008. As predicted, the germination (70%) and pollen fertility (87.9%) were higher with the 0.01 M treatment of EMS and declined with subsequent doses. So far, 505 M1 lines (single plant) have generated fertile M2 seeds. Several mutant lines with changed levels of chlorophyll and plant architecture (very dwarf, dwarf, fasciated, and tall) have been revealed in the M2 generation. These mutant populations are actively expanding to between 1000 and 10,000 plant lines. The lack of genetic variation made generating maps or molecular markers challenging. Three molecular maps have been generated from the interspecific procedure ICP 28 × ICPW 94 (Bohra et al. 2011; Yang et al. 2011). Six intraspecific molecular maps, including two stated earlier, were combined to generate intraspecific molecular maps with 120 and 467.97 cM distances (Gnanesh et al. 2011a). Also, a pigeonpea KASPar assay (PKAM) interspecific map developed from ICP 28 × ICPW 94 is 1.11 cM (Saxena et al. 2012). GoldenGate SNPs have been produced using 296 loci and a distance of 4.95 cM from a cross between Pusa Dwarf and HDMO41 (Kumawat et al. 2012).

To discover the genes that express resistance to biotic stresses like *Fusarium* wilt and SMD diseases, extensive research has been conducted in this domain. To find the gene loci that contribute to resistance to biotic stresses, numerous segregating mapping populations were developed. Considering these mapping populations, numerous polymorphic markers were reported (Bohra et al. 2011; Saxena et al. 2010a, b, c) by judiciously inspecting thousands of plants in wilt-sick plots throughout different areas. For *Fusarium* wilt resistance in pigeonpea, two RAPD markers

(Kotresh et al. 2006), four SCAR markers (Prasanthi et al. 2009), and six SSR markers (Singh et al. 2013) have been reported. In the case of SMD, in LG 7 and LG 9, six QTLs have been identified that account for 24.72% of the variation in phenotype (Gnanesh et al. 2011a). Employing transcriptome profiling on the leaves and roots of pigeonpea plants infected with *Fusarium* wilt and SMD, several genes of about 118 and 33 have been discovered (Raju et al. 2010; Dubey et al. 2011). Candidates for genes that confer drought tolerance should be explored to increase drought tolerance in legumes (Narina et al. 2014). Pigeonpea determinacy is a crucial adaptive trait and using DArT arrays, and the GoldenGate assay, 6 DArTs and 19 SNPs were discovered (Mir et al. 2013).

9.4 Modern Genomic Tools in Pigeonpea Improvement

Recent developments in pigeonpea genomics have enabled it to generate a range of modern genomic tools and technologies that are highly relevant to breeding and for use in pigeonpea crop improvement. In this section, large-scale genomic tools such as high-throughput DNA markers, saturated genome maps, comprehensive transcriptome assemblies, whole-genome assemblies, and, importantly, the DNA markers associated with the breeding traits were developed to support pigeonpea improvement, which were presented and summarized in Table 9.1.

9.4.1 Molecular Marker Technologies

In pigeonpea breeding studies, molecular markers were successfully deployed to increase genetic gain and accelerate the breeding process (Varshney et al. 2014a). They remain vital to genomic research and molecular breeding for crop genetic improvement. In pigeonpea, various marker assays were used for various functions, including genetic diversity assessment, linkage mapping, and QTL analyses. Initial assessments of genetic diversity and trait-specific molecular mapping in pigeonpea used a variety of first-generation molecular markers, including amplified fragment length polymorphism (AFLP) (Panguluri et al. 2005), random amplified polymorphic DNA (RAPD) (Ratnaparkhe et al. 1995), and restriction fragment length polymorphism (RFLP) (Nadimpalli et al. 1992). Later, pigeonpea molecular analyses were sparked by developing second-generation simple sequence repeat (SSR) markers. Creating SSR markers from genomic libraries and mining expressed sequence tags (ESTs) were initially labor-intensive and expensive procedures (Burns et al. 2001; Saxena et al. 2010a). To resolve this, a survey of BAC end sequences (BESs) led to the generation of the first substantial set of 3072 SSR massive DNA markers for pigeonpea genotyping (Bohra et al. 2011). Also, Bohra et al. (2011) successfully used more than 3000 SSRs they created from BAC-end sequences (BESs) in linkage analysis, hybridity testing, and other genetic analyses. The DArT and SNP markers, part of the new generation of markers, improved marker coverage to the genome level.

Table 9.1 List of various genomic resources utilized in pigeonpea crop improvement

Resource	Tool/technologies	Reference
High-density genotyping systems	Illumina BeadXpress	Roorkiwal et al. (2013)
	GoldenGate	Kassa et al. (2012), Kumawat et al. (2012)
	Veracode	Roorkiwal et al. (2013)
	KASP	Saxena et al. (2012, 2014)
	Restriction site-associated DNA (RAD) sequencing	Arora et al. (2017)
	Genotyping-by-sequencing (GBS)	Saxena et al. (2017)
DNA markers	Diversity array technology	Yang et al. (2006, 2011)
	Simple sequence repeats (SSR)	Burns et al. (2001), Odeny et al. (2007, 2009), Saxena et al. (2010a), Bohra et al. (2011, 2017b), Dutta et al. (2011)
	Single feature polymorphisms	Saxena et al. (2011)
	Single-nucleotide polymorphisms (SNP)	Kumar et al. (2016), Saxena et al. (2012)
	Large structural variations (CNV, PAV, InDels)	Kumar et al. (2016), Singh et al. (2017b), Varshney et al. (2017)
	Intron spanning region	Kudapa et al. (2012)
Large-scale genetic variants	Simple sequence repeats (SSR)	Bohra et al. (2011), Singh et al. (2011), Varshney et al. (2012)
	Single-nucleotide polymorphisms (SNP)	Dubey et al. (2011), Singh et al. (2011), Varshney et al. (2012), Saxena et al. (2012)
Modern experimental genetic populations	Biparental	Varshney et al. (2013)
	Multi-parental (MAGIC and NAM)	Huang et al. (2015), Pazhamala et al. (2015)
High-density genome mapping	910 loci (interspecific F2 population)	Saxena et al. (2012)
Transcriptomic resources	Transcriptome assemblies (4557 TACs, 43324 TACs, 48726 TACs, 21434 TACs)	Raju et al. (2010) Dutta et al. (2011) Dubey et al. (2011), Kudapa et al. (2012)
	Expressed sequence tags (ESTs)	Priyanka et al. (2010), Raju et al. (2010)
	Gene expression atlas	Pazhamala et al. (2017)
	Reference genes for expression studies	Sinha et al. (2015a, b)
DNA marker-trait associations (MTAs)	Sterility mosaic disease (SSR/SNP)	Gnanesh et al. (2011a, b), Singh et al. (2016, 2017b), Saxena et al. (2017)
	Fusarium wilt (SSR/SNP)	Singh et al. (2016, 2017a), Patil et al. (2017a, b), Saxena et al. (2017)
	CMS fertility restoration (SSR)	Bohra et al. (2012); Saxena et al. (2017)

(continued)

Table 9.1 (continued)

Resource	Tool/technologies	Reference
	Plant type and earliness (SSR/SNP)	Kumawat et al. (2012), Geddam et al. (2014)
	Flowering pattern/determinacy (SNP)	Mir et al. (2014)
	Seed traits (protein content/size) (SSR/SNP)	Obala et al. (2019); Yadav et al. (2019)
Whole-genome sequencing/ resequencing	Reference genome sequence (510.8 Mb and 605.7 Mb)	Singh et al. (2011), Varshney et al. (2012)
	WGRS	Kumar et al. (2016), Singh et al. (2016, 2017b), Varshney et al. (2017)
Cell-organellar genomic resources	Chloroplast genome sequence assemblies and SSRs	Kaila et al. (2016)
	Mitochondrial genome sequence assemblies	Tuteja et al. (2013)
	Mitochondrial DNA markers (SSRs and Indel)	Khera et al. (2015), Sinha et al. (2015c)
Genetic purity testing kits	SSR assay	Saxena et al. (2010a, b, c), Bohra et al. (2011, 2017b)
Molecular assays to assist CMS breeding	42 SSRs	Bohra et al. (2011)
	21 SSRs	Saxena et al. (2010a, b)
BAC-based resources	Two BAC libraries comprising 34,560 and 34,560 clones	Bohra et al. (2011)
	88,860 BAC-end sequences (BESs)	Bohra et al. (2011)
Genetic maps	Consensus	Bohra et al. (2012), Arora et al. (2017)
	Population specific	Gnanesh et al. (2011a, b), Saxena et al. (2012)

The DArT markers in pigeonpea permitted the evaluation of genetic variation and linkage mapping. SNP is gradually replacing SSRs as the preferred DNA marker among the various marker systems developing nowadays. Following that, a panel of 24 genotypes and a high-density linkage map were generated using a set of 1616 SNPs known as pigeonpea KASPar assay markers (PKAMs) (Saxena et al. 2012). Also, a subset of these PKAMs was chosen based on polymorphism between cultivar types, polymorphism information content (PIC) values, and assay design tool (ADT) scores, and 256 genotypes of the pigeonpea reference set were examined using 48-plex Veracode assay technology on the BeadXpress platform (Roorkiwal et al. 2013). This important study assessed genetic diversity and was more pertinent to the breeder community. A greater number of polymorphic DNA markers were discovered by screening 184 *Cajanus* accessions (77 cultivated and 107 wild relatives from secondary and tertiary gene pools) using 1616 SNPs (1226). Significantly, more

knowledge about the domestication of the pigeonpea was gained, confirming the widely held belief that *C. cajanifolius* is the closest progenitor and Madhya Pradesh is the region of origin (Saxena et al. 2014). In parallel, large-scale DNA markers were also discovered using whole transcriptome and genome assemblies. Exploring transcriptome assemblies revealed genetic variations in the form of expressed sequenced tag (EST) SSRs, intron spanning region (ISR) markers, and SNPs (Raju et al. 2010; Dutta et al. 2011; Dubey et al. 2011; Kudapa et al. 2012). Likewise, the entire pigeonpea genome sequence allowed for genome-wide SSRs and SNPs to recover.

SSR use in marker-trait association (MTA) studies was constrained in the case of cultivated pigeonpea due to low molecular (genetic) diversity. Because of this, the focus of pigeonpea researchers has shifted to high-throughput, automated, and affordable genome sequencing and will undoubtedly help the pigeonpea breeding program. In pigeonpea, the parallel development of genotyping platforms like GoldenGate assay (Kassa et al. 2012) and BeadXpress (Roorkiwal et al. 2013) allowed for medium- to high-throughput SNP genotyping. Saxena et al. (2011) used a low-cost KASP technology to genotype 1616 SNPs known as pigeonpea KASPar assay markers. A genome-wide SNP analysis of various pigeonpea accessions has aided in determining crop domestication and pigeonpea demographic history (Saxena et al. 2014).

This elucidates the evolution of genetic marker technology from gel or hybridization methods (RAPD, RFLP, DArT, SFPs) to sequence-based SSR and SNP markers. Genotyping methods such as genotyping-by-sequencing (GBS) have opened a promising way to concurrently discover and genotype thousands of SNPs due to their ease of library preparation and increased multiplexing capacity (Saxena et al. 2017). Other approaches, such as whole-genome resequencing (WGRS)/skim sequencing, have been greatly aided by the availability of the pigeonpea reference genome sequence. However, the inherent limitations of the GBS assay, such as a large number of missing data points and ascertainment bias, provoked researchers to develop array-based platforms for high-density genotyping in pigeonpea. Resequencing of pigeonpea diverse germplasm and advanced breeding lines helped in the development of the Axiom Cajanus SNP array, which has 56,512 unique and informative sequence variations tiled on the array.

Notably, adding 1554 SNPs and 385 InDel polymorphisms potentially associated with some key agronomic traits makes the array more appropriate for finding new haplotypes for associated traits. Based on WGRS data from 20 *Cajanus* accessions, the first-generation HapMap of pigeonpea revealed 5.5 million genome-wide variants, including 4.6 million SNPs and 0.7 million InDels, as well as large structural variations (SVs) such as copy number variation (CNV: 2598) and presence/absence variation (PAV: 970) (Kumar et al. 2016). Varshney et al. (2017) recently performed WGRS on 292 accessions, which included landraces, elite breeding lines, and wild accessions. The study revealed evidence of large SVs (1000 bp) in breeding lines (282 CNVs, 35 PAVs), landraces (228 CNVs, 37 PAVs), and wild species (173 CNV, 77 PAVs).

9.4.2 Next-Generation Trait Mapping Resources

Using traditional gene/QTL discovery technology, a variety of gene/QTL responsible for important agricultural traits in pigeonpea has been mapped (Varshney et al. 2013; Bohra et al. 2017a, 2019). To find important associations between the DNA markers and the trait(s) under deliberation, a moderate-sized genetic population segregating for the desired trait(s) is required. In pigeonpea, experimental populations generated from a cross of two contrasting genotypes have been developed to target a variety of traits, including resistance to important biotic/abiotic factors, fertility restoration, and growth habit/flowering patterns. Reverse genetic tools such as targeted induced local lesions in genomes (TILLING) populations derived from EMS-treated Asha were also reported in pigeonpea. The reference mapping population in pigeonpea was derived from an interspecific cross [ICP 28 (*C. cajan*) × ICPW 94 (*C. scarabaeoides*)], which advanced as a basis for the creation of reference linkage maps ranging from moderate (SSR based) to high density (SNP based) linkage maps. Multiparent advanced generation intercross (MAGIC) and nested association mapping (NAM) are popular mating designs incorporating multiple parents. Several crops, including maize (McMullen et al. 2009), wheat (Huang et al. 2012; Delhaize et al. 2015), rice (Bandillo et al. 2013), pea (Tayeh et al. 2015), and sorghum, have been generated through multiparental populations (Ongom et al. 2016). These new-generation mapping populations not only maximized the use of high-throughput genotyping/sequencing platforms but also have several advantages over traditional (biparental) mapping populations, such as higher resolution and allelic richness.

Next-generation trait mapping techniques, particularly sequencing-based bulked segregant analysis (Seq-BSA), have been used in pigeonpea for fast gene discovery in response to the development of NGS technologies and the availability of the reference genome sequence. Eight non-synonymous (ns) SNPs in seven genes were reported using the Seq-BSA and nonsynonymous (ns) SNP substitution method (Singh et al. 2016). Seq-BSA was used to extremely large extents and was obtained from the recombinant inbred (RIL) population (ICPL 20096 × ICPL 332). In contrast, the nsSNP substitution approach was based on the WGRS data of four pigeonpea genotypes (ICPL 20097, ICP 8863, ICPL 99050, and ICPB 2049). Four nsSNPs showed associations with FW, while the remaining four showed associations with SMD. Evidence for the causality of genes C. cajan_01839 and C. cajan_03203 for SMD and *Fusarium* wilt resistance, respectively, was provided by in silico protein analysis and gene expression study.

Sixteen InDels were found using a similar InDel Seq method to identify genomic regions linked to SMD and *Fusarium* wilt in pigeonpea; five of these InDels were further validated through the analysis of resequencing data (Singh et al. 2017b). Two InDels controlling SMD resistances were found on linkage groups (LGs) 2 and 10, while three InDels responsible for FW resistance were found on LGs 2, 7, and 8. Recently, common variant analysis was used to find candidate genes associated with the seed protein content (SPC) from WGRS data from high seed protein content

(SPC) lines (HPL 24, ICP 5529), a low SPC line (ICP 11605), and the draught genome of ICPL 87119 (low SPC) (Obala et al. 2019).

9.4.3 Transcriptome Resources and Significant EST Assemblies in Pigeonpea

Transcriptome analysis is one of the inexpensive but most powerful tools for improving the genetic resources of any crop. Pigeonpea transcriptome analysis explores the spatiotemporal expression of important key genes and their biological process and regulatory mechanisms. In addition, many functional or genic molecular markers were identified for use in pigeonpea breeding programs and in genetic research. Since 2014, a total of 25,577 ESTs have been available in NCBI database. Primarily, the transcriptome assembly contigs of pigeonpea (CcTAv1) were created through 127,454 tentative unique sequences (TUSs) and later updated with CcTav2 transcriptome assembly contigs using Illumina 454 platform. The data is available in the Legume Information System (LIS; http://cajca.comparativelegumes.org/). Several research groups used Sanger-derived EST resources to access the transcribed regions of the pigeonpea genome (Priyanka et al. 2010; Raju et al. 2010; Kumar et al. 2014). In 2010, the first set of EST markers was developed for pigeonpea *Fusarium* wilt and SMD (Raju et al. 2010). There are 9468 high-quality ESTs of four pigeonpea genotypes, two for *Fusarium* wilt (ICPL 20102 and ICP 2376) and two for SMD (ICP 7035 and TTB 7), which were characterized from 16 cDNA libraries. It was found that the expression of 19 and 20 genes differed between the *Fusarium* wilt- and SMD-responsive genotypes. Similarly, when abiotic stress-responsive genes were characterized and functionally verified with *Arabidopsis thaliana*, a total of 75 high-quality ESTs, 20 of which were pigeonpea specific, were obtained from cDNA libraries of drought-stressed pigeon pea. The specific genes in pigeon pea like CcCDR (*Cajanus cajan* cold and drought regulatory), CcCYP (*C. cajan* cyclophilin), and CcHyPRP (*C. cajan* hybrid-proline-rich protein) were overexpressed in *Arabidopsis*, demonstrating the plant's resistance to abiotic stress. Kumar et al. (2014) found that 105 high-quality ESTs were isolated from the root tissues of pigeon pea genotype GRG295, and the expression of the four genes, encoding methionine aminopeptidase, synthetase, phosphoglycerate kinase, serine carboxypeptidase, and *S*-adenosylmethionine synthetase, was validated using reverse transcriptase PCR.

Using 454 GS-FLX pyrosequencing, Dutta et al. (2011) yielded over 3000 genic SSR markers from a total of 43,324 transcript assembly contigs (TACs) of two pigeonpea genotypes, Asha and UPAS 120, analyzed. Another assembly was generated for Pusa Ageti (ICP 28) with 10,817 Sanger ESTs using 454-derived 494,353 short transcript reads (STRs) and the assembly consisted of 48,726 (38.1%) contigs and 79,028 singletons. Kudapa et al. (2012) generated a comprehensive assembly of 18,353 Sanger ESTs reads from 16 pigeonpea genotypes. They produced 128 ISR markers that scoreable amplicons are successfully used to screen eight pigeonpea genotypes. Although 116 markers were validated, 70 markers

showed one to three alleles, with an average of 0.16 polymorphism information content (PIC) value. A comparison of this assembly with the soybean genome sequence led to the discovery of 10,099 ISR markers.

A comprehensive understanding of gene expression may aid in bridging the knowledge gap between plant phenotypes and whole-genome sequence data. Additionally, homology-based gene assignment methods and de novo gene prediction programs are essential for determining the gene functions of genome assemblies. Pazhamala et al. (2017) created RNA Seq data covering the entire pigeonpea life cycle to complement this gene information. A set of 28,793 genes expressed at various developmental stages (from embryo to senescence) are cataloged, including a focus on genes involved in fertilization and seed formation, which explore the role of epi-transcriptomics, i.e., posttranscriptional modifications in pigeonpea seed and embryo development. Co-expressing network analysis was used to identify 28 genes and three hub genes for flower-related traits in pigeonpea. Thus, the transcriptomic tools serve as valuable community resources to provide transferable DNA markers for cross-genera studies and support comparative genomics of the pigeonpea genome.

9.4.4 Molecular Linkage Maps

The lack of genetic linkage information in pigeonpea until 2011 may be attributed mainly to the inadequacy of polymorphic DNA markers leading to the absence of mapping populations. A less genetic variation in pigeonpea made it challenging to construct linkage maps or develop molecular markers. Out of interspecific operation ICP 28 × ICPW 94, three molecular maps have been developed (Bohra et al. 2011). In the same year, Yang et al. (2011) developed molecular maps with the help of DArT markers where 172 DArT loci represented paternally and 122 DArT loci represented the maternal linkage and covering a distance of 270.0 cM and 451.6 cM. Intraspecific molecular maps were developed with 120 and 467.97 cM distances (Gnanesh et al. 2011a) by combining 6 molecular maps that are intraspecific in nature, including two molecular maps mentioned earlier. The following year, the distance of the interspecific map was developed by Saxena et al. (2012) from ICP 28 × ICPW 94 through pigeon pea KASPar assay. Kumawat et al. (2012) crossed between Pusa Dwarf × HDMO41 and 296 loci and a distance of 4.95 cM had been identified with the help of GoldenGate SNPs. For the first time, an interspecific cross of *C. cajan* × *C. scarabaeoides* was conducted to access contemporary marker technology like DArT. Bohra et al. (2011) reported an SSR-based genetic map for the first time with 239 loci using the same interspecific cross-spanning 930 cM of the pigeonpea genome. Like wild-type crosses, genetic linkage maps for cultivated crosses were also created, with 59–140 SSR loci mapped (Gnanesh et al. 2011a, b; Bohra et al. 2012). Kumawat et al. (2012) created a 296-loci (genic SNP and SSR) genetic map for the 1520 cM cultivated pigeonpea genome. In addition to these maps specific to each population, the first consensus genetic map with 339 loci was created by combining marker data from six different F2 populations (Bohra et al. 2012).

Extremely low DNA polymorphism revealed by SSRs or other previously used DNA marker systems necessitated a shift toward the use of high-throughput marker technologies such as genome-wide SNPs, and as a result of SNP markers assayed via the KASP platform, a saturated genetic map for an interspecific F2 population (*C. cajan* × *C. scarabaeoides*) was obtained. The map covered a 996 cM map distance with 910 (SNPs and SSRs) markers spaced at an average marker distance of 1.09 cM (Saxena et al. 2012). Gnanesh et al. (2011a) combined six molecular maps to create intraspecific molecular maps with distances of 120 and 467.97 cM.

9.4.5 QTL(s)/Candidate Genes Linked to Target Traits

Knowing the linked gene or linkage with the specific trait in a breeding program is very important. Biotic and abiotic stresses are the major constraints to pigeonpea crop improvement. India is the largest producer of pigeonpea and its production is significantly affected by *Fusarium* wilt and SMD (Sharma et al. 2012). So, it is necessary to identify the genomic region associated with resistance to those diseases for developing disease-resistant varieties. Various segregating mapping populations have been developed to identify the genomic regions associated with resistance to these biotic stresses. The traditional QTL mapping approach entails identifying parental polymorphisms and genotyping populations with polymorphic markers, which takes time and resources (Abe et al. 2012).

On the other hand, trait-associated markers can provide bulked segregant analysis (BSA) on extreme bulks and parents through marker screening. Thus, future NGS-based BSA approaches for rapid and accurate trait mapping are expected. Gnanesh et al. (2011a) identified six QTLs (qSMD1, qSMD2, qSMD3, qSMD4, qSMD5, and qSMD6) for sterility mosaic disease in LG 7 and LG 9 populations. Apart from that Saxena et al. (2017) discovered ten QTLs, including three major QTLs linked to SMD resistance in three different populations. Furthermore, the pigeon pea research community has identified 10,000 SNPs in pigeonpea (Varshney et al. 2013). These markers are extremely helpful in saturating genetic maps with a large number of molecular markers and labeling QTLs/candidate genes for critical traits such as disease resistance. The functional genomic approaches, such as homology searches, transcript profiling, and microarrays, aid in studying candidate genes that express resistance to various stresses. Until now, 118 and 33 gene transcripts were identified from leaves and roots of infected pigeonpea plants associated with *Fusarium* wilt and SMD, respectively (Raju et al. 2010; Dubey et al. 2011). This information on candidate genes would help genomics-assisted breeding in pigeonpea for developing multiple disease-resistant lines.

9.4.6 Genomics-Assisted Breeding (GAB): Designing Future Pigeonpea

Several markers for different traits in pigeonpea are available for varietal improvement and are used in the pigeonpea breeding program primarily aimed at pure line breeding or hybrid development. The marker can be used for various inherited traits like SMD and *Fusarium* wilt resistance in marker-assisted backcrossing (MABC) (Varshney et al. 2014b, c). Similarly, Varshney et al. (2014c) developed groundnut lines to improve rust resistance. Following the success stories of the pigeonpea breeder's community, the MABC program developed superior lines by pyramiding several desired alleles into cultivar and disease (SMD and FW) resistance cultivars by introgressing resistance genes in the susceptible cultivars. Furthermore, trait mapping populations like MAGIC and NAM generated through biparental and multiparental crosses are being conducted to identify additional loci for GAB in pigeonpea. Over the last two decades, seven cytoplasmic male sterile (CMS) systems have been identified in the pigeonpea hybrid breeding. The CMS lines were derived from wild *Cajanus* spp., viz., *C. platycarpus* (Mallikarjuna et al. 2006), *C. acutifolius* (Mallikarjuna and Saxena 2005), *C. cajanifolius* (Saxena et al. 2005), *C. lineatus* (Mallikarjuna and Saxena 2005), *C. scarabaeoides* (Saxena and Kumar 2003), *C. volubilis* (Wanjari et al. 1999), and *C. sericeus* (Ariyanayagam et al. 1995). Later, cytoplasmic-genetic male sterility (CGMS)-based hybrid system was developed using wild pigeonpea cytoplasm (Saxena et al. 2002; Saxena and Kumar 2003) and resulted in the development of hybrid varieties ICPH 2740, ICPH 2671, and ICPH 3762 which produce 30–48% higher yields than the widely used local varieties in multilocation field trials and have been released successfully for cultivation in central and southern parts of India (Saxena and Nadarajan 2010). Access to genomics-assisted breeding can solve the problem of frequently facing challenges in recognizing fertility restorers, determining the hybrid seed's purity, and preserving three lines (CMS and maintainer and restorer lines). Tuteja et al. (2013) discovered the mismatch in a genetic arrangement in mitochondrial genomes of a CMS line (ICPA 2039), its maintainer line (ICPB 2039), and wild species (*C. cajanifolius* ICPW 29) when sequenced using molecular markers. A total of 22 rearrangements between CMS and the maintainer line along with 34 genes coding for proteins in addition to presence and absence variations (PAVs) at 29 regions have been identified. These structural abnormalities and variations in the mitochondrial genome produce irregular proteins (Ma 2013). Documenting the genes responsible for such abnormalities can help better understand the molecular mechanisms underlying the development of CMS in pigeonpea. In addition, Saxena et al. (2010a) and Bohra et al. (2014) developed the kits based on SSR molecular markers for purity testing of pigeonpea hybrids (F1s derived from CMS and restorer line). Thus, marker-based genetic seed purity testing is developed for a hybrid breeding system of pigeonpea, which is a relatively quick and most efficient method than the normal grow-out test (GoT).

Additionally, the three-line hybrid breeding system makes the technology time-consuming and expensive. Therefore, efforts are being made to investigate an

alternative two-line hybrid breeding system, which requires a male sterile line that could precisely transfer to a fertile line and reverts under certain environmental factors. In this direction, a temperature-sensitive male sterile line has been identified based on the pigeonpea observations at the field level (Saxena 2014). The accurate characterization and assessment of such an environment-sensitive male sterile line are crucial in developing and utilizing a two-line hybrid breeding system. In addition, it is also vital to identify the parental combinations that would give higher yields and better resistance to diseases. In this regard, defining heterotic groups that cater to the needs of various locations and resistance to various stresses is the need of the hour. In this context, seven heterotic pools were defined based on the specific combining ability (Saxena and Sawargaokar 2014). Several approaches based on genome-wide markers for identifying favorable alleles in different parental genotypes would greatly aid in this aspect. In summary, the abovementioned possibilities and efforts would greatly help hasten the pigeonpea hybrid breeding program in Asia and other regions of the semi-arid tropics.

9.4.7 Reference Genome Sequence

Sequencing and resequencing technologies are important in improving legume crops through the construction of assembly for draft genomes (Bohra and Singh 2015). Pigeonpea is the first orphan crop and the second food legume after soybean to be sequenced by following a de novo sequencing technology. Two whole-genome sequence assemblies by two research groups have been documented in pigeonpea for the genotype Asha (ICPL 87119). With the help of Sanger-sequenced BESs and Illumina technology, the genome of pigeonpea was assembled to 605.78 Mb with a scaffold N50 of 516.06 kb (Varshney et al. 2012), representing more than 70% of the entire 833 Mb genome. A total of 48,680 genes were identified in pigeonpea genome assembly with an average transcript length of 2348.70 bp. Analysis of the genome assembly provided new insights into important traits such as drought response in the genetic landscape of pigeonpea. The sequence analysis revealed that 111 drought-responsive genes/candidate genes are present in pigeonpea, whereas 109 genes are reported in another legume soybean. The genome assembly delivered a large set of SNP (28,104) and SSR (23,410) markers. However, Singh et al. (2011) assembled another set of the whole-genome sequence of pigeonpea, which was 510 Mb (nearly 60%). The number of protein-coding genes in this assembly was similar to what was reported by Varshney et al. (2012); however, the average gene size was reported to be 1170 bp. The genome analysis revealed that it contains 47,004 genes. Of them, 1213 genes were disease/defense responsive, and 152 were predicted to regulate the plant's response to abiotic stress. Decoding the entire genome sequence of pigeonpea will greatly help breeders to develop a better variety or hybrid, especially for overcoming the biotic and abiotic constraints.

9.4.8 Potential Challenges for Implementing GAB in Pigeonpea

Besides the potential advantages of implementing GAB in pigeonpea, certain potential challenges are needed to be considered during the application of genomics in pigeonpea crop improvement. The major drawback of GAB in the pigeonpea crop improvement program is the long life cycle of pigeonpea, which allows pigeonpea to produce only one generation in field conditions during the cropping season. To overcome this limitation, ample resources are essential to growing large populations in controlled environmental conditions during the off-season. Another major challenge is the often cross-pollinating nature of pigeonpea, which produces a variable degree of heterozygosity. As a result, crossing programs in pigeonpea were slowing down, hence lesser development of mapping populations compared to other crop species. Besides, the cross-incompatibility barriers hamper the advancement of interspecific mapping populations. In addition, low heritable traits and levels of genetic polymorphism and photo-sensitivity pose other impending difficulties for pigeonpea GAB.

As a result, an identified marker associated with a particular trait from a population may not work for another population from other genetic backgrounds during marker-assisted selection. Multiparent mapping populations (MAGIC/NAM), which will make it easier to identify tightly linked markers for a variety of traits with high-throughput genotyping and phenotyping, are being developed to avoid such a situation. For any trait mapping experiments in pigeon pea, with high throughput and precision, phenotyping is crucial to be a significant bottleneck in the pigeonpea. Additionally, GS can be a promising futuristic strategy when breeding for complex traits with low heritability. Proper decision support tools need to be made available for applying GAB in pigeonpea to translate the information into knowledge which will ultimately be helpful to the pigeonpea breeders.

9.5 Future Prospects

Genomics permitted the pigeonpea crop improvement at its early stages. The development in the last 15 years has been satisfactory in creating important genomic tools in the pigeonpea crop. The present period is the developmental/training phase of molecular breeding, during which important marker-trait associations (MTAs) are established for downstream selection procedures or prediction models for genomic selection (GS) are trained (Nakaya and Isobe 2012; Bohra 2013). Once we enter the breeding phase, the true potential of genomics-assisted breeding will be revealed. Marker-assisted backcrossing (MABC) will be the most appropriate strategy for defect elimination for traits controlled by major effect QTL/gene, precisely improving an otherwise elite cultivar for the trait under consideration. At the same time, advanced backcross (AB)-QTL provides exciting avenues for trait detection and transfer. Advanced segregating generations derived from wide crosses involving *C. scarabaeoides* as the wild donor are one example (Varshney et al. 2013).

AB-QTL, by definition, seeks unexploited wild genes/alleles that are typically absent in the cultivated gene pool.

Furthermore, in light of NGS advances, genome-wide approaches such as GWAS and MAGIC/NAM are likely to expand the array of robust genomic segments associated with the trait while guiding the community in prioritizing candidate genes. Increasing schemes like GS will help reduce the cost and time spent on repeated phenotypic screening. Further, it is possible to make use of available variation using sequencing and resequencing techniques. Because there is very little diversity in pigeonpea, there is a need to introduce novel genetic variation through mutations or collecting wild relatives. Still, linkage drag may prevent favorable traits from being transferred from wild species to commercial cultivars. In this case, NGS, or draft genome sequencing, is used to investigate molecular-level variations in species and their relationship to phenotypic variation (Varshney et al. 2012). Resequencing aids in the study of existing variation and genes linked to phenotypes. It is possible to create new superior genotypes by utilizing the available genetic diversity (Varshney et al. 2017). Even though the QTL mapping approach is a time-consuming and resource-intensive process, it aids in identifying the best parents and determining their gene sequence using polymorphic markers (Abe et al. 2012). Bulked segregant analysis (BSA) aids in parent screening and provides trait-linked markers. Both of these would be useful in the future for accurate and rapid trait mapping in pigeonpea crop improvement programs. In pigeonpea, the current method for introducing resistant traits into elite and commercial cultivated varieties or marker-assisted purity test of hybrids, parents, and DNA-based fingerprinting is genomics-assisted breeding (Singh et al. 2017a, b). The pigeonpea whole-genome sequence is now available at ICRISAT (Varshney et al. 2012). In the future, combining traditional breeding with genetic approaches such as next-generation sequencing, high-throughput genotyping used for screening in an early generation, marker-assisted backcrossing, and marker-assisted selection would aid in the advancement of pigeonpea breeding.

9.6 Conclusion

In response to rapid changes in the global climate scenario resulting from the scarcity of land and water resources, the significance of drought-tolerant and nutrient-rich crops like pigeonpea has been appreciated. Pigeonpea can play a key role in ensuring food security and subsistence farming, especially in Asia and the semi-arid tropics. It can be cultivated in marginal environmental conditions with inadequate resources. Despite its limitations, pigeonpea productivity is severely affected by various biotic stresses such as pests and diseases, and the narrow genetic base of the crop has been a foremost constraint toward deploying GAB in pigeonpea. As a result, significant progress has been made in generating different genomic resources, including molecular markers, genetic maps, and transcriptome assembly. In contrast, specialized genetic stocks such as multiparent MAGIC and NAM populations focus on trait-linked marker studies. Therefore, several efforts are now concentrated on the

genomic marker-trait association, such as identification of candidate gene/QTLs and marker-assisted selection for resistance to biotic stresses (FW, PB, and SMD), tolerance to abiotic stresses (terminal drought, salinity, and water-logging), and agronomically important traits including plant type, determinacy, and earliness.

Genomics is also being made to determine the seed purity, identify candidate genes for CMS and fertility restoration, and define heterotic pools for identifying parental combinations for accelerating the hybrid breeding program in pigeonpea. With recent AB-QTL techniques, it is possible to introgress the useful genes/traits from the wild species into the commercially cultivated species. The accessibility of genome information of pigeonpea has allowed numerous NGS-based methods for allele mining, candidate gene identification, and high-resolution genetic mapping, which had enhanced the pace, accuracy, and effectiveness of trait mapping. At present, efforts should be made to focus on trait-associated markers. Cost-effective genotyping platforms and expertise are available for implementing GAB in pigeonpea. As a result, a paradigm shift from the progress of genomic resources to the implementation of GAB hastened genetic improvement programs in pigeonpea crops. However, there is a need to have low-cost, high-throughput, and efficient field-relevant phenotyping. We anticipate that in the upcoming years, MAS and GS will be widely deployed in combination or alone for enhancing productivity in pigeonpea.

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10 Innovative Approaches for Genetic Improvement of Safflower (*Carthamus tinctorius* L.): Current Status and Prospectus

H. D. Pushpa, H. H. Kumaraswamy, Helan B. Thomas, B. Ushakiran, Devender Sharma, K. Anjani, and M. Sujatha

Abstract

Safflower (*Carthamus tinctorius* L.) is a nutritionally and pharmaceutically important oleaginous crop cultivated for its seed oil. There is ever-increasing demand for edible oil in the country. However, the area under safflower cultivation globally has declined over the last decade. Low productivity is one of the major reasons for the decline in the area. The safflower faces several adaptation challenges, which leads to a low seed yield. Advances in biotechnology and genomics-assisted plant breeding benefited the genetic improvement of safflower in several ways. However, there is much scope for further deployment of innovative approaches for plant idiotype development, oil quality engineering, and crop adoption for changing climate scenarios and consumer needs. With this background, an attempt has been made in this chapter to comprehend the latest works of safflower researchers across the globe and present the information systematically and in a thematic pattern. Further, the future research direction is discussed, particularly highlighting the need for quality whole-genome reference sequencing, robust tissue culture and transformation protocols, genome editing, metabolomics, and transcriptomics. The information presented in this chapter is useful for evolving speed breeding strategies in safflower.

H. D. Pushpa (✉) · H. H. Kumaraswamy · H. B. Thomas · B. Ushakiran · K. Anjani · M. Sujatha
ICAR-Indian Institute of Oilseeds Research, Hyderabad, India
e-mail: Pushpa.hd@icar.gov.in

D. Sharma
Crop Improvement Division, ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, Uttarakhand, India

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Keywords

Safflower breeding · Biotechnology · Innovative approaches · Molecular markers · Quantitative trait loci (QTLs) · Transgenics

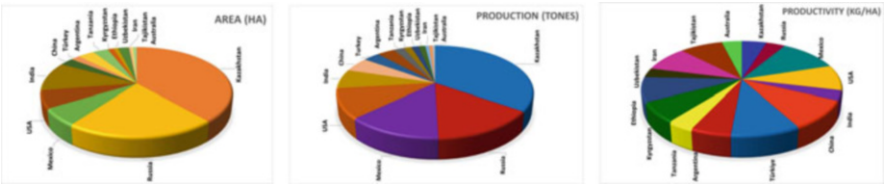
10.1 Introduction

Safflower (*Carthamus tinctorius* L.), an important cultivated oilseed, belongs to the tribe Cardueae (thistles), family Asteraceae (Compositae), and subtribe Centaureinae (Chavan 1961; Weiss 1971; Bérvillé et al. 2005). It is one of the most ancient crops and is cultivated for nutritionally and pharmaceutically important oleaginous seeds in 60 countries in the world, including major contributors such as Kazakhstan, the Russian Federation, India, the USA, Mexico, Argentina, China, Ethiopia, Australia, Kazakhstan, Uzbekistan, and Turkey (Singh and Nimbkar 2006). In 2020, the world harvested 0.65 million tons of safflower seeds from 0.82 million hectares, at a world average productivity level of 799.6 kg/ha (FAOSTAT 2022). Countries contributing to the world safflower economy are listed in Table 10.1.

Table 10.1 Major world economies contributing to safflower production

S. no.	Country	Area (ha)	Production (tones)	Productivity (kg/ha)
1	Kazakhstan	315,177	226,739	719.4
2	Russian Federation	174,974	96,636	552.3
3	Mexico	50,414	86,793	1721.6
4	United States of America	51,270	67,040	1307.6
5	India	85,475	44,000	514.8
6	China, mainland	22,724	33,404	1470
7	China	22,724	33,404	1470
8	Türkiye	15,114	21,325	1410.9
9	Argentina	27,349	22,565	825.1
10	United Republic of Tanzania	25,170	13,721	545.1
11	Kyrgyzstan	9836	9870	1003.5
12	Ethiopia	7442	9349	1256.2
13	Uzbekistan	18,324	8885	484.9
14	Iran (Islamic Republic of)	3568	4701	1317.5
15	Tajikistan	3438	4293	1248.7
16	Australia	6195	3602	581.4
	World	**816,699**	**653,030**	**799.6**

Source: FAO Stat, 2022



Source: FAO Stat, 2022

10.1.1 Safflower as a Crop

Safflower is an herbaceous annual plant 30–150 cm in height, heavily branched, mostly spiny, and cultivated during the winter/spring seasons (Chavan 1961) (depicted in Fig. 10.1).

Soon after the emergence, safflower seedlings enter the rosette stage, where many leaves are arranged close to each other, and this stage endures for 30 days, followed by stem elongation and branching. During the rosette stage, the crop exhibits weaker competition with weeds compared to the subsequent stages of its growth. During the vegetative stage, leaves and a substantial taproot system begin to develop (Smith 1993). There is a lot of variability in the floral petal colors of the safflower. Different petal colors of the safflower, such as yellow, orange, red, and white (cream), are illustrated in Fig. 10.2.

The size of leaves varies according to the type of the genotype and their positions on the plant. However, the leaves' average breadth and length range from 2.5 to 5 cm and 10 to 15 cm, respectively. While the pattern of leaf arrangement is invariably alternate-type, leaf shape varies: sessile, ovate, and lanceolate (Classen 1950; Ashri and Efron 1964; Teotia et al. 2017). Lower leaves on the stem typically lack spines, whereas upper leaves frequently produce stiff spines. Non-spiny varieties are preferred in non-traditional areas of safflower cultivation for the convenience of easy operation while harvesting. Generally, seed oil content is lower in non-spiny than the spiny cultivars of safflower (Belgin et al. 2007).

Fig. 10.1 Overview of safflower plants: (**a**) safflower field view; (**b**) spiny safflower plant; (**c**) non-spiny safflower plant

Fig. 10.2 Safflower floral morphology depicting color variability: (**a**) lemon yellow; (**b**) light yellow; (**c**) yellow; (**d**) orange; (**e**) red; (**f**) white

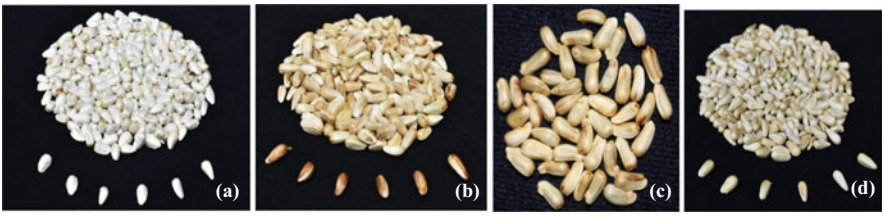


Fig. 10.3 Types of seed hulls: (**a**) normal thick hull; (**b**) partial hull; (**c**) striped hull; (**d**) thin hull

The flowering stage begins between 35 and 45 days and matures 45 days after the first floral initiation, resulting in a crop duration of 112–122 days. Safflower exhibits drought tolerance due to its deeper tap root system of up to 3 m. This enables the plant to draw moisture from the deeper soil layers (Henderson 1962; Heuzé et al. 2015).

Safflower fruit is botanically called "achene," where the embryos are surrounded by a tough fibrous hull that constitutes 32–65% of the total seed weight. The hull plays a critical role in protecting the seed kernel, comprised of two cotyledons attached to the embryo, constituting 40–67% of the remaining seed weight (Applewhite 1966). According to the sclerenchyma cell growth on the inner and outer surfaces of the hull, four hull types are found in safflower seeds: normal hull, partial hull, thin hull, and striped hull, as vividly presented in Fig. 10.3. Partial hull-type is genetically dominated by thin hull and striped hull traits (Urie 1986; Urie and Zimmer 1970). The seed dispersal mechanism in wild-type safflower is regulated by varying degrees of tufts of hairs attached to the proximal end of the seed, the feature called pappus, which is absent in the cultivated species (Kotecha and Zimmerman 1978a, b), possibly due to negative selection pressure, in the course of breeding cultivated species.

10.1.2 Uses of Safflower Plant and Its Parts

Since leaves are a rich source of vitamin A, they are used as green leafy vegetables. The whole plant is also used as hay for animal feeding (Dajue and Mündel 1996a, b). Due to their spiny nature, safflower plants were raised as border rows to protect wild animals (Chavan 1961).

Water-soluble yellow pigment called carthamidin and an alkali-soluble red pigment called carthamin, used as dyes in textile and food industries, are obtained from safflower petals. These are also used in preparing herbal tea rich in antioxidants (Weiss 1971; Dajue and Yunzhou 1993; Zohary and Hopf 2012). Safflower oil which is odorless and has no color is used for culinary purposes to produce margarine in the pharmaceutical and cosmetic industries. Due to its antipyretic, purgative, and analgesic properties, safflower seed oil is used for treating numerous human bodily ailments including joint pains, trauma, amenorrhea, dysmenorrhea, and postpartum abdominal pain (Jun et al. 2011; Kruawan and Kangsadalampai 2006). The major content of the safflower oil is polyunsaturated fatty acids (71–75%) called linoleic acid, followed by oleic acid (10–16%, monounsaturated), 1–2% stearic acid, and 7–8% palmitic acid (Knowles and Mutwakil 1963; Smith 1993). As it does not emit odor and smoke due to its high oleic acid content, it is an ideal vegetable oil for frying (Gyulai 1996). Further, due to its high stability during hydrogenation, safflower oil is better suited than soybean and canola oils for margarine manufacturing (Kleingarten 1993). The medicinal uses of different parts of safflower are listed in Table 10.2.

Table 10.2 Pharmaceutical importance of safflower

Plant part	Properties	References
Flower extract	Anticoagulant Antioxidant Suppress skin tumor	Yousefi and Rakhshandeh (2015) Choi et al. (2010) Yasukawa et al. (1996)
Carthamins yellow	Lowers blood pressure levels Lowers plasma renin activity and angiotensin level II Reduced the viscosity of blood and plasma, erythrocyte aggregation index	Liu et al. (1992); Li et al. (2009)
Water extracts of *Carthamus*	Inhibiting glutamate-induced C6 glia cell death, neuroprotective activity	Hiramatsu et al. (2009)
Flowers and seed oil	Purgative Rheumatism and paralysis	Weiss (1971) Razi and Fi (2000)
Fruit, leaves	Treatment of psoriasis, mouth ulcers, anti-poison, vitiligo, and black spots	Ibn Sina (2007)
Seeds	Laxative Semen improvement	Knowles (1965) Jorjani (2012)

10.2 Background

10.2.1 Genetic Resources

The genetic resources are the primary raw materials required to improve crops by creating new variability and increasing the value-added properties of the crop. Safflower's improvement benefits from the wide diversity of its genetic resources, which are protected and made available by gene banks (Dajue and Mündel 1996a, b). China, India, and the USA have significant national safflower collections, appraisals, and documentation. Many researchers have collected safflower genetic resources over the years but were significantly aided by Paulden F. Knowles, known as "the father of California safflower" (Mündel and Bergman 2009). The USDA World Collection of Safflower is a significant source of safflower germplasm resources worldwide. The USDA maintains more than 2300 accessions of safflower, including the material that Knowles gathered and developed during his expeditions. With the assistance of IBPGR since 1989, the Safflower Research Group of the Beijing Botanical Garden of the Chinese Academy of Sciences has documented a total of 2051 accessions from 49 countries and 465 specimens from within China (Zhaomu and Lin 1991; Zhaomu 1993). Zhang and Johnson (1999) created a germplasm directory for safflower that listed 18 distinct collections from 14 nations. The Western Regional Plant Introduction Station (WRPIS) in Pullman, Washington, has been keeping track of the US collection of 2383 accessions (Mukta and Reddy 2012). The National Crop Gene Bank gathered 1100 accessions at the Institute of Crop Germplasm Resources of the Chinese Academy of Sciences in Beijing (Eighth Five Year Plan, 1996–2001). India reported (Unpublished data Mukta 2020) the most significant collections of safflower genetic resources, with nearly 7637 accessions kept at the Project Coordinating Unit for Safflower and the National Bureau of Plant Genetic Resources in New Delhi.

10.2.2 Cytogenetics

The genus *Carthamus* L is known to have diploid, autopolyploid, and allopolyploid species, primarily found in the eastern region of the Mediterranean basin. The number of species in the genus *Carthamus* and its taxonomic boundaries are both subject to debate (Sheidai and Sotoode 2009). According to López González (1989), the newly circumscribed genus *Carthamus*, which includes only annual species and has members with 20, 22, 24, 44, and 64 chromosomes, includes several putative allopolyploid species. Several researchers have reported the polyploidy nature of the *Carthamus* species (Ashri and Knowles 1960; Harvey and Knowles 1965; Khidir and Knowles 1970a, b; Efron et al. 1973; Vilatersana and Susanna 2000; Vilatersana et al. 2005; Garnatj et al. 2006).

The taxonomic enigma of the genus has been solved using many methods, including morphology, cytology, experimental hybridizations, isozyme analysis, and molecular investigations. Using molecular phylogenies based on DNA

sequences, further molecular investigations have resolved the issue of the generic limitations of *Carthamus* and validating *Carthamus* genus as a natural group (Vilatersana et al. 2005). *C. oxyacantha* and *C. persicus* are thought to be the probable progenitors of the cultivated *C. tinctorius* (Ashri and Knowles 1960). As per genetic analysis and geographic evidence conducted by Pearl and Bowers (2014), it was concluded that *C. palaestinus*, the wild ancestor of safflower, originated in the Middle East and is cross-compatible with cultivated safflower. Safflower's center of origin, species classification, and reclassification and molecular classification of Carthamus have been well reviewed by Singh and Nimbkar (2006), Sujatha et al. (2008), and Dobrin et al. (2021).

10.2.3 Safflower Genetics

A good research has been carried out in safflower for understanding the genetics of various traits of agronomic importance. A summary of safflower genetic research, including the mode of gene action for different morphological traits and seed yield-related traits, is presented in Table 10.3.

Correlation measures the mutual relationship among various plant characters and helps determine the yield components on which indirect selection can improve seed yield. Several researchers have conducted research work to check the correlation between seed yield and other traits and concluded that there is a positive and significant correlation between plant height, number of branches per plant (Semahegn and Tesfaye 2016; Pavithra and Patil 2016; Salunkhe 2014; Priyanka et al. 2020), number of capitula per plant (Pattar 2014; Mohamed and Elmogtba 2018; Gujar 2018), number of seeds per capitulum (Pushpavalli et al. 2017; Mohamed and Elmogtba 2018; Priyanka et al. 2020), and 100-seed weight (Pushpavalli et al. 2017; Jadhav et al. 2018). For traits like days to 50% flowering, days to maturity, and oil content, there was a negative correlation with seed yield (Anjani 2005; Hoshang and Abas 2013; Salunkhe 2014; Pattar 2014; Priyanka et al. 2020).

Path analysis splits the correlation coefficient into the measures of direct and indirect effects and determines the direct and indirect contribution of various characters towards yield. In safflower, it is found to have a positive and immediate impact between seed yield,100-seed weight, and number of capitula per plant; for traits like plant height, number of seeds/capitula, and oil content, there was positive and indirect effect with number of capitula per plant and 100-seed weight (Karimi et al. 2014; Dambal and Patil 2016; Mohamed and Elmogtba 2018; Pattar and Patil 2020). There was a negative direct effect on seed yield with plant height, number of seeds/head, and oil content (Moghaddasi and Omidi 2010; Ahmadzadeh and Alizadeh 2012; Pattar and Patil 2020). The results in the variation of different traits must likely be combined to make valid conclusions about the heritability, mode of action, and potential for breeders to use genes associated with specific traits.

Table 10.3 Genetics of important agronomic traits in safflower

S. no.	Traits	Gene action	Reference
1	Germination	Non-additive variation	Kotecha and Zimmerman (1978a, b)
2	Plant height	Additive gene action	Kotecha (1979); Shahbazi and Saeidi (2007); Golkar et al. (2012)
3	Stem diameter	Additive	Kotecha (1979)
4	Leaf length	Non-additive gene action	Kotecha (1979)
	Days to budding and days to bolling	Additive	Golkar et al. (2011)
5	Days to flowering	Dominance gene	Golkar et al. (2011)
		Partial dominance	Gupta and Singh (1988a, b)
		Both additive and dominance gene actions	Singh and Kolekar (2008)
6	Earliness in safflower	Both additive and dominance effects	Golkar et al. (2011)
7	Number of branches per plant	Additive gene effects	Gupta and Singh (1988a, b)
		Epistasis effects	Narkhede and Patil (1987)
		Non-significant effect of epistasis	Golkar et al. (2012)
8	Node number on the main stem	Additive-dominance model	Abel and Driscoll (1976)
9	Internode distances	Epistatic effects	Abel and Driscoll (1976)
10	Flower color	Four dominant genes (Y, C, O, and R)	Claassen (1952); Narkhede and Deokar (1986)
		Epistatic effects	Joglekar and Deshmukh (1956)
		Two different models of epistatic gene action	Golkar and Arzani (2010)
11	Number of capitula/plant	Non-additive	Ashri (1971); Gupta and Singh (1988a, b)
		Dominance, duplicate epistasis	Narkhede and Patil (1987)
		Dominance gene effects	Pahlavani and Razavi (2007)
		Additive × additive and dominance × dominance epistasis	Shahbazi and Saeidi (2007)
		Additive-dominance model	Sahu and Tewari (1993)
12	Number of seeds/capsule	Additive gene effects	Mandal and Banerjee (1997); Singh and Pawar (2005)
13	Head diameter	Low broad-sense heritability	Çamaş and Esendal (2006)
		Dominance gene effects	Golkar et al. (2012)
14	Days to maturity	Additive gene action	Kotecha (1979); Shahbazi and Saeidi (2007)
			Gupta and Singh (1988a, b)

(continued)

Table 10.3 (continued)

S. no.	Traits	Gene action	Reference
		Overdominance of gene action	
15	Seed dormancy	Non-additive effects, heritability ranging between 33 and 55%	Kotecha and Zimmerman (1978a, b)
16	Spininess	Dominant over spinelessness with four genes (Sa, Sb, Sc, and Sd)	Narkhede and Deokar (1990)
		Monogenic and that the spiny trait was completely or partially dominant	Golkar and Arzani (2010)
		Spininess is affected by an unknown number of modifier genes	Claassen (1952)
17	Partial hull	Recessive to the white hull	Urie (1986)
18	Stripped hull	Recessive gene *th* monogenic control	Ebert and Knowles (1966)
19	Reduced pericarp/hull	Recessive gene *stp*	Ebert and Knowles (1966)
20	Seed weight	Digenic model (additive-dominance)	Shahbazi and Saeidi (2007)
		Additive gene effects	Golkar et al. (2012)
21	Pappus	Monogenic inheritance (pappus was dominant over non-pappus)	Claassen (1952); Efron et al. (1973)
		Digenic inheritance	Kotecha and Zimmerman (1978a, b)
		Dominance gene	Ashri and Efron (1964)
22	Seed dormancy	Non-additive effects	Kotecha and Zimmerman (1978a, b)
23	Seed yield	Additive gene effects	Golkar et al. (2012)
		Predominantly dominant gene action	Ragab and Fried (1992); Mandal and Banerjee (1997); Singh and Kolekar (2008)
24	Protein	Additive-dominance model	Pahlavani and Razavi (2007); Golkar et al. (2012).
25	Oil content	Epistatic effects	Yermanos and Hemstreet (1967); Ramachandram and Goud (1981); Pahlavani and Razavi (2007)
		Non-additive gene effects	Golkar et al. (2011)
26	Fatty acids and oil content	Both broad and narrow–sense heritabilities	Golkar et al. (2011)
	Linoleic acid	Additive gene effects	Hamdan et al. (2008)
	Linoleic acid and stearic acid content	Maternal effects	Golkar et al. (2011)
	Oleic acid	Additive gene effects	Hamdan et al. (2009)
		Additive gene effects	Hamdan et al. (2009)

(continued)

Table 10.3 (continued)

S. no.	Traits	Gene action	Reference
	Palmitic acid and stearic acid		
	High oleic acid content	Genetic control of recessive alleles	Fernandez-Martinez et al. (1993)
	Stearic acid inheritance	Monogenic	Ladd and Knowles (1971)
27	Genetic male sterility	Single recessive genes	Heaton and Knowles (1980); Singh (1996)
		Dominant gene-controlled	Joshi and Nerkar (1983)
	Cytoplasmic-genic male sterility in safflower	Single gene (1:1) segregation	Anjani and Mukta (2008)

Source: Golkar (2014)

10.3 Safflower Breeding Approaches in the Pre-genomics Era

Knowledge of genetic control and inheritance pattern of a given trait, mode of pollination and protocols of controlled pollination, and plant phenological and physiological attributes is the pre-requisite of safflower breeding. Major safflower breeding objectives include increased seed yield coupled with high oil content, improved protein content, hybrid development, non-spiny and early varieties for non-traditional areas, winter hardiness, and disease and insect resistance. Breeding methods in safflower are selected based on knowledge of genetics, heterosis, combining ability, gene action, and correlation of the traits. The selection method and heterosis breeding approach were followed to improve the traits governed by additive and dominant gene action, respectively. Trait enhancement is best accomplished by the selection method for those characteristics least influenced by the environment.

10.3.1 Introduction and Selection

The varietal introduction is one of the earliest breeding methods followed to introduce the new genotypes developed in different regions of the world. Since the plants of an introduced cultivar react differently to the altered environment, introduced varieties typically require a few cycles of adaptation, followed by selection and evaluation, before they are formally approved for commercial production. In safflower, there are no reports of directly introduced varieties to India, although enough exchange of safflower genetic material is there (Singh and Nimbkar 2006).

Selection is the most widespread breeding method used for cultivar development. Pure line selection is practiced extensively, and it is one of the oldest methods of crop improvement for safflower. The development of several germplasm lines with many desirable traits in safflower was the outcome of the pure line selection from local cultivars of the safflower (Mündel and Bergman 2009). In Montana (USA), researchers have employed mass selection to create cultivars with enhanced field resistance to several diseases, including bacterial blight caused by *Pseudomonas syringae* van Hall and leaf blight produced by *Alternaria carthami* (Bergman et al. 1985, 1987, 1989). Out of 36 released varieties in India, 17 are developed through selection in the existing genotypes (Anjani and Mukta 2008).

10.3.2 Hybridization

Safflower breeders often use hybridization to combine desirable traits and to create variability as the crop is autogamous in nature. Pollination in safflower happens when the style and stigma extend into the surrounding anther column; following elongation, the stigma is typically covered in pollen from the same floret (Classen 1950). About 40–50% extent of outcrossing is reported to be influenced by insect activity and other environmental factors (Classen 1950; Ramachandram and Ranga Rao 1984). Outcrossing rates vary based on various factors, including variety, size of the pollen source, and habitat. For crossing in safflower, the flowers should first be emasculated by having their anther tubes removed during the late budding stage. The emasculated florets are then fertilized with pollen from a different chosen bloom once the styles have lengthened (Knowles 1980). In addition to producing variance for numerous traits in F_2 and later generations, hybridization has proven useful in identifying the genetic basis of certain phenotypes (Singh and Nimbkar 2006). Hybridization has assisted in developing suitable approaches to produce the required improvement in various crops.

10.3.3 Pedigree Breeding

The pedigree method handles segregating generations, i.e., from F_2 onwards, for selecting good recombinants with desirable traits with high heritability. These recombinants are further selfed to fix the traits. The pedigree breeding approach is laborious but yields the most precise genetic data. The most exemplary traits from prominent parental lines are combined to generate new lines and cultivars. This approach is used to develop genotypes with high seed oil content coupled with high seed yields as the pedigree method allows to recombine of several desirable traits in one background.

10.3.4 Bulk Method

In the bulk method, F_2 and subsequent generations are harvested in mass or as bulk to advance the generation. From F_6 generation onwards, individual plants are selected to raise individual plant progenies, and the selected progenies are tested in preliminary yield trials for further evaluation of yield and other traits. Desired recombinants are more likely to evolve because of the high natural selection pressure. Bulk method is employed to develop genotypes with biotic stress resistance (Fusarium wilt and Alternaria resistance) in a natural infestation. Another benefit of this approach is that breeders can manage many bulk populations simultaneously.

10.3.5 Single-Seed Descent Selection (SSD)

SSD is suggested by Goulden (1941) as a modification of the bulk method. Using only one seed per plant and F_2-derived plants in each generation, homozygosity was achieved with the least selection. Once inbred lines have been developed, they can be chosen based on data from repetitive field trials for desirable traits, including agronomic performance, biotic and abiotic stress tolerance, and/or end-use quality testing. This technique is typically used when crossing elite safflower cultivars, many of which already have many of the beneficial alleles fixed. This method is commonly used in safflower to develop a structured population for mapping genes for a particular trait rather than to develop cultivated varieties.

10.3.6 Pre-breeding

Important sources of genetic variation for crop development can be found in wild relatives with increased levels of resistance to or tolerance of various stresses. However, linkage drags and cross-incompatibility barriers restrict their use for cultivar development. Safflower has quite a good number of germplasm collections. Pre-breeding is the current method employed in safflower breeding as pre-breeding offers a special chance to introduce desired genes from wild germplasm into genetic backgrounds that the breeders easily utilize with minimal linkage drag.

10.3.7 Back Cross Breeding

Backcrossing generally transfers highly inherited traits like disease resistance from wild species to cultivated species backgrounds. Kotecha and Zimmerman (1978a) developed interspecific crosses between *C. tinctorius* and *C. palaestinus* to introduce seed dormancy from palaestinus. Zimmerman and Buck (1977) identified cold tolerance segregants in interspecific derivatives between cultivated and *C. flavescens*. Backcrossing has been successfully used to transfer dominant genes

to prevent diseases like root rot brought on by Phytophthora drechsleri (Thomas and Rubis 1960; Rubis 2001) and to develop high oleic acid safflower (Knowles 1968; Hamdan et al. 2009). A study conducted by Anjani (2005) revealed that interspecific crosses with *C. oxyacantha*, *C. turkestanicus*, and *C. creticus* were found to be highly resistant to wilt disease. The backcrossing method is also employed widely in marker-assisted selection.

10.3.8 Reciprocal Recurrent Selection (RRS)

To simultaneously improve traits negatively related to seed yield, RRS is used wherein intermating of selected plants in F_2 is used as base population; phenotypically superior recombinants are selected and intercrossed that helps in breaking undesirable linkage. Intercrossed seeds are sown and superior plants are again crossed after repeated selections. The intermating of the F_2 results in the accumulation of fixable components of genetic variability, breaking unfavorable effects and linkage and resulting in the shifting of genetic correlation, thus increasing the frequency of desirable genes in the population. It is mainly used to improve traits with high heritable value. In safflower, oil content is governed primarily by additive gene action (Vijayakumar and Giriraj 1980; Rao 1983) and polygenic inheritance. Rubis and Levin (1966) improved thin hull plants' seed set and stem strengths through four cycles of recurrent selection.

10.3.9 Recurrent Introgression Population Enrichment Method (RIPE)

RIPE applies the recurrent selection principle in self-pollinated crops (Falk 2001). In safflower, a modified RIPE approach is used to generate a large number of crosses by employing the non-spiny marker linked to GMS by Anjani (unpublished data 2015). She recovered several desirable recombinants with high oil (>35%) coupled with high seed yield per plant (>60 g/plant), early duration genotypes coupled with high seed yield and oil content, and genotypes having high seed yield coupled with wilt resistance. This approach promotes the recombination between loci with the population to create high potential genotypes with favorable agronomic traits and stress tolerance.

10.3.10 Heterosis Breeding

In safflower, the studies on heterosis indicated that there is a considerable amount of heterosis for seed yield as estimated over the better parent. A high degree of heterosis for seed yield (108–182% over mid-parents) and its principal components in F_1 hybrids of safflower has been reported by several researchers (Yazdi-Samadi et al. 1975; Deokar and Patil 1978; Malleshappa et al. 1988; Pandya and Patil 1992;

Manjare and Jambhale 1995; Patil and Narkhede 1996; Anjani 1997). Heterosis for oil content (28 and 100% over mid-parent) was reported by Zemour and Adda (2021).

The discovery of dominant and recessive genetic male sterility (GMS) systems encouraged breeders to develop hybrids in safflower, which is mainly self-pollinated (Heaton and Knowles 1982; Joshi and Nerkar 1983; Ramachandram and Sujatha 1991; Singh 1996, 1997). Despite the proven potential of GMS-based hybrids, the area under safflower hybrids is negligible owing to the lukewarm response of the seed-producing agencies, mainly because of the requirement of removal of 50% male fertile plants appearing in the genetic male sterile female parent. A non-spiny marker-linked GMS system was developed by Anjani (2005), which could differentiate male sterile and fertile plants in the GMS population at the seedling stage itself.

The first CGMS system in safflower in India was developed independently from a cross between *C. oxyacantha* and cultivated species (Anjani 2005). Fourteen CGMS lines were developed by transferring the genome of cultivated species (*C. tinctorius*) into the cytoplasm of the wild species, *C. oxyacantha*. Maintainer lines were identified for each CGMS line (Anjani et al. 2012). This gives hope to improving oil content to some extent in the hybrid background by choosing appropriate parental lines. However, the challenge for improving oil content in hybrid background is the non-availability of high combining high oil parental lines. Different types of male sterility in safflower are discussed in detail in the review by Meena and Dudhe (2012).

10.3.11 Mutation Breeding

There are methods to generate genetic variation if there is no variation for a trait of interest in the existing genetic resources. Mutagenesis is one such technique that induces changes in the genomic DNA sequence, which can be done by exposing the seeds to chemical mutagens (EMS) or physical mutagen (X-rays, gamma rays, etc.). Mutagenesis is non-targeted, i.e., genes are mutated at random and are heritable. The type of mutagen and its dose will vary depending on the traits to be improved and the part to be treated. Few researchers have standardized doses and used mutation breeding to isolate desirable mutants (Mallikarjunradhaya 1978; Ramchandram and Goud 1983; Velasco and Pérez-Vich 2000; Kotcha et al. 2007; Okaz and Ahmad 2016; Rampure and Choudhary 2017; Shrivastava and Mondal 2021). TILLING (Targeting Induced Local Lesions IN Genomes) is one example of a mutagenesis technique that uses ethyl methanesulfonate (EMS) to induce short insertion/deletion (INDELS) mutations (Sikora and Chawade 2011; Kashtwari and Wani 2019). However, mutation breeding in safflower is not widely used, probably due to the availability of germplasm and cross-compatible wild relatives in *Carthmus* spp. for the potential crop improvement of safflower.

The abovementioned conventional breeding approaches can be used in conjunction with Marker-assisted selection to speed up and minimize the time it takes to introduce new crop cultivars.

10.4 Safflower Improvement in the Genomics Era

10.4.1 Safflower Biotechnology

The obstacles of conventional breeding in crop improvement can be alleviated through biotechnological approaches. The development of modern genomic resources such as genetic map using molecular markers, QTLs, association mapping, EST libraries, comparative analysis of EST data from different plant species and even from model organisms, marker-assisted selection, and genome sequencing transcriptomics and transgenics can give further comprehension in the functional annotation of unidentified genes and paves the way for the discovery of novel regulatory elements and genes involved in metabolic pathways. A detailed discussion of the use of genomic resources for the improvement of safflower till now has been discussed below.

10.4.2 Molecular Markers and Genotyping

Breeding programs must effectively use a diverse range of genetic resources in order to maximize yield and desirable genotype characteristics (Ashri et al. 1974). Safflower germplasm resources have been characterized using morphological, biochemical, and DNA markers (Zhang 2001; Bella et al. 2019; Muhammad and Ali 2020; Houmanat et al. 2021; Rahimi 2021; Zhao et al. 2021; Qin et al. 2022). Biochemical markers based on isozyme polymorphism were used to study genetic variation in safflower (Zhang 2001; Zongwen 2001). Using the cathodal peroxidase method and acid phosphatase isozyme analysis, 9 ecotypes of *C. oxyacantha* wild species and 14 safflower cultivars were identified (Bassiri 1977). Similarly, Carapetian and Estilai (1997) used 9 biochemical enzymes to examine 20 safflower genotypes for diversity studies. Zhang (2001) used isozymes to characterize 89 safflower accessions from 17 countries. Yildiz et al. (2022) examined genetic variation in 13 safflower accessions using peroxidase gene polymorphism (POGP) markers.

The use of biochemical markers for diversity studies is limited due to the limited number of enzymes and low level of polymorphism through isozymes. In the recent decade, DNA markers have been extensively used for genetic diversity studies (Küyük and Aslan 2021; Ali et al. 2020a, b; Golkar and Mokhtari 2018; Hassani et al. 2020a, b; Rahimi 2021), precisely cataloging germplasm, DNA fingerprinting (Ragab et al. 2008; Ravikumar and Priya 2005; Sehgal and Raina 2005; Yaman and Tarıkahya-Hacıoğlu 2014), phylogenetic analysis (Mahmoudi and Salari 2019; Milošević and Ignjatov 2020; Kim and Ko 2016; Nasab and Nemati 2022), linkage map development (Mirzahashemi and Mohammadi-Nejad 2015; Jegadeeswaran and Kadirvel 2021; Poodineh et al. 2021), QTL mapping (Mirzahashemi and Mohammadi-Nejad 2015; Kadirvel et al. 2020; Jegadeeswaran and Kadirvel 2021; Poodineh et al. 2021), and association mapping (Ambreen et al. 2018; Hassani et al. 2020a; Singh and Rawat 2022; Yildiz et al. 2022; Zhao et al. 2022).

Molecular markers (DNA markers) are simple to use, inexpensive, and have high reproducibility. They also have dominant/codominant characteristics. Because they are unaffected by environmental factors and reveal differences at the whole genome level, molecular markers are reliable genetic diversity indicators (Caetano-Anolles and Gresshoff 1991). Random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs), and single nucleotide polymorphisms (SNPs) are the most commonly used markers in safflower. Yazdi-Samadi and Amiri (2001) used 283 RAPDs to characterize 28 safflower genotypes, including Iranian landraces, wild, and exotic genotypes. Sehgal and Raina et al. (2005) used RAPD (36), ISSR (21), and AFLP (4) marker combinations to screen 14 Indian varieties. Thirteen ISSR markers were used to characterize 55 safflower accessions from various geographical origins (Houmanat et al. 2016). Yang et al. (2007) used ISSR makers to analyze the genetic relatedness of 48 safflower accessions collected from 32 countries, and genotypes are grouped based on geographical origin. Ash et al. (2003) studied the genetic variation of *Carthamus lanatus* samples collected from 11 South Wales, Australia, locations, using ISSR markers. *C. lanatus* was discovered to be divided into two distinct groups based on their location (northern and southern regions). Küyük and Aslan (2021) used 12 ISSR primers to examine genetic diversity, population structure, and genetic differentiation among *Carthamus* species populations.

The wild and cultivated species were separated, and *Carthamus persicus* accessions had the highest genetic diversity compared to other species. Numerous examples of ISSR markers are being used successfully to estimate the genetic diversity of safflower (Panahi and Ghorbanzadeh Neghab 2013; Yaman and Tarıkahya-Hacıoğlu 2014; Talebi and Abhari 2016; Wodajo and Mustefa 2015; Naresh and Santha Lakshmi Prasad 2012). AFLP markers were used to characterize 96 USDA collection accessions representing seven different global regions. These regions differed in all pairwise comparisons, demonstrating how AFLP markers could differentiate safflower diversity across large geographic groups (Johnson and Kisha 2007). Similarly, Zhang et al. (2006) used AFLP markers to investigate variation in 28 safflower genotypes collected in China. In another study, the genetic diversity and population structure of 531 safflower accessions from 43 countries were analyzed using 10 AFLP primer pairs, and a high level of molecular diversity was discovered among the germplasm collection. The accessions were grouped based on their similarity across regions, with accessions from the Far East and Egypt forming one group. In contrast, accessions from the Near East and Iran-Afghanistan were grouped together (Kumar et al. 2015).

Microsatellites are tandemly repeated units made up of mono-, di-, tri-, tetra-, or pentanucleotides (Powell et al. 1996; Zietkiewicz and Rafalski 1994). SSRs are mostly used for genotyping and genetic diversity studies in many crops. Kadirvel et al. (2016) used 38 SSR markers to characterize 30 Indian and 23 Mexican safflower cultivars. Structural analysis grouped the accessions from India and Mexico into two distinct groups. High levels of genetic variation were observed in the population, and significant genetic structure was supported by cultivar groups

that were highly distinct and had limited gene flow. Hassani et al. (2020a) assessed the genetic diversity and population structure of 135 globally diverse mini-core collections of safflower using 18 polymorphic SSR markers. High allelic variation (6.8 alleles/locus) and relatively high PIC (0.69) were observed.

Similarly, Usha Kiran and Mukta (2015) evaluated 148 safflower accessions using 44 SSR loci across 11 linkage groups where the average number of alleles was 3.6 per locus. Ali et al. (2020a, b) examined 131 safflower accessions collected from 28 countries with 121 SSR markers and observed a high diversity level in populations from Pakistan and Israel. Similar studies with SSRs for genetic diversity of safflower were conducted by Sehgal and Raina (2005), Mahasi et al. (2009), Lee and Sung (2014), Ambreen et al. (2015), Bahmankar et al. (2017), Talebi and Nosrati (2018), Tabassum (2018), Usha Kiran and Mukta (2015). It is a consideration that microsatellites derived from ESTs are more indicative of genetic differences than random markers due to their unique characteristic of being associated with expressed genes. It's critical to keep in mind that, even though random markers are helpful in determining divergence, the connections of markers through random drift and adaptation are two distinct processes (Holdregger et al. 2006). Through conventional EST mining, genomic library screening, or NGS technologies, a significant portion of safflower's SSR markers have been produced (Chapman et al. 2009; Mayerhofer and Archibald 2010; Hamdan et al. 2011; Yamini and Ramesh 2013). Using 24 microsatellites developed from expressed sequence tags (EST) and 2 chloroplast markers, 70 accessions from different geographical centers were analyzed by Chapman and Hvala (2010).

The target region amplification polymorphism (TRAP) is a distinct molecular marker that integrates the attributes of both EST-SSR and AFLP (Hu and Vick 2003). TRAP markers can be created to study certain genes despite producing semi-random markers at numerous loci (Miklas et al. 2006). Hassani et al. (2020a, b) employed DArT sequence technology to examine the genetic diversity and population structure of 89 safflower accessions utilizing 3431 highly polymorphic markers (1136 SilicoDArTs+2295 SNPs). Regardless of the type of molecular marker used, further characterization of safflower germplasm from different parts of the world is much needed to enhance the germplasm resources of safflower. Several safflower researchers conducted safflower diversity using molecular marker studies, which is listed in Table 10.4.

10.4.3 QTL Mapping and Marker-Assisted Selection

Molecular breeding comprises the development and application of molecular markers, development of linkage maps, and QTL mapping for the identification of markers linked to the traits to improve breeding programs' performance. The development of linkage maps enabled the identification of genomic areas encoding traits or quantitative trait loci (QTL), which have considerable effects on numerous morphological and physiological parameters of crop performance in adverse climatic conditions. When used in conjunction with marker-assisted selection, QTL mapping

Table 10.4 Genetic diversity analysis of safflower using molecular markers

S. no.	Type of markers used	No. of markers used	% of polymorphism	No. of genotypes used	Reference
1	POGP	11	79	131	Yildiz et al. (2022)
2	EST-SSR	44	24	73.68	Singh and Rawat (2022)
3	SCoT ISSR	24 10	71.32 71.7	30	Rahimi (2021)
4	DArT	19,639	89	–	Hassani et al. (2020a, b)
5	ISSR	12	90	52	Küyük and Aslan (2021)
6	ISSR	12	93.844	131	Ali et al. (2020a, b)
7	ISSR	48	82.7	22	Yang et al. (2007)
8	silicoDART	29,048		94	Ali et al. (2020a, b)
9	SRAP SSR	10 10	72.7 68	135	Hassani et al. (2020a, b)
10	iPBS retrotransposon	13	40	131	Ali et al. (2019)
11	RAPD	10	7	33.3	Giachino and Duygu (2019)
12	SSR	200	24	79.5	Beha et al. (2019)
13	RAPD	20	15	86.49	Gupta et al. (2019)
14	SCoT CDDP CAAT	10 10 10	83 83.1 87.6	48	Talebi and Nosrati (2018)
15	SRAP SCoT	12 11	55.5 36.76	100	Golkar and Mokhtari (2018)
16	SSR	32	59	105	Mokhtari and Rahimmalek (2013)
17	SRAP SCoT	20 12	30	82.7 81.75	
18	ISSR	28	118	97	Pavithra et al. (2017)
19	SSR	20	56	20	Kumari et al. (2017)
19	SSR	9	48.9	20	Bahmankar et al. (2017)
20	RAPD ISSR	19 9	20	81.08 96	Safavi and Pourdad (2017)
21	ISSR	13	55	69.64	Houmanat et al. (2016)
22	ISSR	13	69.64	55	Houmanat et al. (2016)
22	ISSR	13	56.7	25	Talebi and Abhari (2016)
23	SSR	38	53	40	Kadirvel et al. (2016)
24	SSR	44	28.4	148	Usha Kiran and Mukta (2015)
25	RAPD	35	88.7	12	Rehman et al. (2015)

(continued)

Table 10.4 (continued)

S. no.	Type of markers used	No. of markers used	% of polymorphism	No. of genotypes used	Reference
26	EcoRI/MseI AFLP	150	41.2	531	Kumar et al. (2015)
27	SSR	325	9	31.6	Ambreen et al. (2015)
28	RAPD	11	57.7	24	Neghab and Afzal (2015)
29	ISSR	50	72	102	Majidi and Zadhoush (2014)
30	EST-SSR	109	42	100	Derakhshan et al. (2014)
31	ISSR	9	39	66.7	Yaman and Tarıkahya-Hacıoğlu (2014)
32	SNP	134	70.54	134	Pearl and Burke (2014)
33	SRAP	12	76.9	62	Mokhtari and Rahimmalek (2013)
34	SSR	509	63.3	100	Lee and Sung (2014)
35	ISSR	20	57.6	18	Panahi and Ghorbanzadeh Neghab (2013)
36	AFLP RAPD	6 9	20	92.94 79.33	Panahi and Ghorbanzadeh Neghab (2013)
37	RAPD	8	32	94.6	Hacioglu et al. (2013)
38	EST-SSR	500	19	57.7	Yamini and Ramesh (2013)
39	EST-SSR	109	48	38	Barati and Arzani (2012)
40	ISSR	4	70	87.5	Wodajo (2012)
41	SRAP	12	42	75.2	Talebi and Mokhtari (2012)
42	RAPD	20	20	43	Shahbazidoorbash and Dizaj (2011)
43	ISSR	16	70	20	Golkar et al. (2011)
44	SSR	88	10	35	Hamdan et al. (2011)
45	RAPD	40	149	82.9	Sung et al. (2010)
46	EST-SSR IFLP RGC-based sunflower markers	119 48 19	22	70.6 55.5 71.4	García-Moreno et al. (2010)
47	RAPD ISSR	13 9	20	80.08 96	Safavi and Pourdad (2010)

(continued)

Table 10.4 (continued)

S. no.	Type of markers used	No. of markers used	% of polymorphism	No. of genotypes used	Reference
48	EST-SSR	24	53	76	Chapman and Hvala (2010)
50	RAPD	14	–	36	Mahasi et al. (2009)
51	EST-SSR	119	108	63	Naresh and Yamini (2009)
52	RAPD SSR ISSR	22 18 10	85	57.6 68 71.2	Sehgal and Rajpal (2009)
53	RAPD SSR AFLP	22 18 10	85	57.6 68 71.2	Sehgal and Rajpal (2009)
54	RAPD	20	92.31	29	Qingni et al. (2008)
55	RAPD	50	54	16	Amini et al. (2008)
56	RAPD	15	56.8	193	Khan et al. (2009)
57	AFLP	102	96	14	Johnson and Kisha (2007)

speeds up the breeding process when trait-based techniques are used (Collard et al. 2005). A paucity of safflower genetics and genomics knowledge hampered breeding for yield enhancement, stress tolerance, and other quality traits. The use of molecular markers in evaluating genetic diversity and phylogeny has given us a deeper understanding of the history of the *Carthamus* species (Chapman and Burke 2007; Sehgal and Rajpal 2009). Many published studies in the pasts two decades concentrated on development of molecular markers, especially SSRs (genomic and EST-SSRs) and SNPs (Chapman and Burke 2007; Hamdan et al. 2011; Usha Kiran et al. 2019), and development of linkage maps (Mayerhofer and Archibald 2010) in safflower. There is very little progress in QTL mapping and marker-assisted selection in safflower. Mayerhofer and Archibald (2010) used an intraspecific F_2 population of *Carthamus tinctorius* and an interspecific backcross population resulting from a cross of *C. tinctorius/C. oxyacantha* to develop the first large *Carthamus* species linkage map. This map included 13 linkage groups and 116 marker loci with genetic lengths ranging from 1.3 to 170 cM and included 2 to 27 loci. The yellow color of the flower was caused by a single dominant gene, *ctfcl*, which was mapped on linkage group T9. Hamdan et al. (2008) identified *Li* gene, which controls very high linoleic acid content, which was tightly connected to the male sterility gene *Ms*, both flanked by a sequence characterized amplified region (SCAR) marker. Hamdan et al. (2012) also used two F_2 mapping populations to map high oleic content loci (*ol*) and edit genes linked with the oleic acid content of safflower seed oil. In total, 15 linkage groups were included in the map. The *ol* and *Ms* genes were discovered in the same linkage group T3 at a distance of 68.3 cM.

García-Moreno et al. (2011) used RAPD, SCAR, and SSR markers to generate a linkage map incorporating *Tph2* genes for high gamma-tocopherol concentration. Ebrahimi and Majidi (2017) reported two marker loci related to oil content under drought stress and normal conditions. Hamdan et al. (2012) identified one main QTL (*Ol*3.1) on linkage group T3 that explained phenotypic variance in F_2 (99.4%) and F_3 (96.3%) populations on linkage group T2; QTLs with minimal effects (*Ol*2.1) on oleic acid concentration were also discovered. Pearl and Bowers (2014) created a safflower genetic map using 244 SNP markers clustered into 12 linkage groups. Sixty-one QTLs were discovered in the F_2 population from *Carthamus tinctorius* and the wild species *C. palaestinus* for 21 morphological and seed oil parameters (Listed in Table). Among the 61 QTLs identified in the study, 59 had low to moderate impacts, with only 2 showing significant effects, like spininess and flower color. The QTL in linkage group L explained 32.7% of the phenotypic variation in spininess, while the QTL in linkage group D explained 63.4% of the phenotypic variation in flower color.

Similarly, QTL mapping for yield-related traits in a drought-stressed F_2 population revealed four QTLs, and three groups significantly impacted drought tolerance in safflower (Mirzahashemi and Mohammadi-Nejad 2015). Karimi and Saeidi (2015) employed 71 SSR markers to map the F_2 safflower population in both saline and non-saline conditions. Under control conditions, two QTLs with a substantial impact on thousand-seed weight and biological yield (BY) were identified. With salt stress, eight QTLs with a major effect on seed yield (SY), thousand-seed weight (TSW), harvest index (HI), diameter of capitula, relative water content (RWC), membrane stability index, potassium content, and Na+/K+ ratio were observed. In linkage group 5, the QTLs linked with days to maturity, RWC, sodium and potassium content, calcium/sodium ratio, BY, capitula diameter, and H_2O_2 content overlapped. Indeed, QTLs for SY, HI, and malondialdehyde concentration were discovered in the same area in linkage group 5. QTL mapping was attempted in the F_9 population produced from Mex22-191/Goldasht under both normal and drought stress conditions for 10 agronomic traits with 69 polymorphic AFLP markers covering 556 cM of the safflower genome. Seventeen main QTLs with additive impacts and 66 epistatic QTLs with additive × additive impacts were identified. Co-localized QTLs for multiple phenotypes were found in seven major genomic locations on linkage groups (LG)-4 and LG-5 (Poodineh et al. (2021). QTLs for aphid resistance were identified in the cross CO-1 × EC-523368-2 in F_6 RIL population. A major QTL QUc-Ct3.1, located in linkage group 3, was found to be consistently linked to days to wilt after aphid infestation with 31.5% phenotypic infestation, and another minor QTL, located in linkage group, 5 was observed with 9.1% phenotypic variation (Jegadeeswaran and Kadirvel 2021). QTL mapping for different traits is listed in Table 10.5.

Marker-assisted selection in safflower was only attempted for improving oleic acid content. Liu et al. (2013) developed a multiplex test for the high oleic trait in safflower using primer pairs that created an amplicon of 315 bp from *CtFAD2-1*

Table 10.5 QTLs identified for different traits in safflower

S. no.	Trait	QTL	Population	Chromosome	LOD	R^2	Additive effect	Reference
1	Aphid resistance	QUc-Ct3.1	CO-1 × EC523368-2	3	18.3	31.5	2.5	Jegadeeswaran and Kadirvel (2021)
2	Aphid resistance	QUc-Ct5.1	CO-1 × EC523368-2	5	7	9.1	1.4	Jegadeeswaran and Kadirvel (2021)
3	Plant height	qPh6_1	Mex.22-191 × IL.111	6	2.7	17	2.3	Mirzahashemi and Mohammadi-Nejad (2015)
4	Plant height	qPh6_2		6	3.09	19	66.2	
5	Branches/plant	qBpno4_1		4	2.8	17	−185.6	
6	Branches/plant	qBpno4_2		4	2.5	16	768.66	
7	Branches/plant	qBpno6		6	2.38	15	3.66	
8	Capsules/plant	qCpno2		2	2.68	17	7.21	
9	Dry weight/plant	qDw2		2	3.46	21	34.33	
10	Dry weight/plant	qDw4		4	2.47	15	−2316.5	
11	Dry weight/plant	qDw6		6	2.72	17	53.16	
12	Seeds/plant	qSpno2		2	5.88	33	273.25	
13	Seeds/plant	qSpno3		3	2.96	18	880.94	
14	Seeds/plant	qSpno4		4	3	18	−13,917	
15	Seeds/plant	qSpno7		7	2.66	7	265.007	
16	Seeds/plant	qSpno9		9	2.91	18	−738.8	
17	Seeds/plant	qSpno18		18	2.68	17	1468.64	
18	Seed yield/plant	qSyp2		2	4.71	28	10.45	
19	Seed yield/plant	qSyp9		9	3.17	19	−21	
19	1000-seed weight	qThsw5		5	2.41	15	77.59	
20	High oleic	Ol3.1	CL-1 × CR-9	3	47.3	96.4	33.2	Hamdan et al. (2012)
21	High oleic	Ol2.1		2	3.5	27.5	0.8	
22			1		8.33	0.96	−3.73	Poodineh et al. (2021)

	No. of branches under drought	*qNB-S-LG1/LG1*		MeX22-191 × Golasht				
22	Days to heading under drought	*qDTTS-5-1*	5		3.33	10.14	1.24	
23	Grain yield under drought	*qGYS-4-1*	4		4.14	18.18	−749.19	
24	Oil yield under drought	*qOYS-4-1*	4		3.57	15.05	−208.46	
25	No. of branches/plant	*qNBS-7-1*	7		7.25	6.73	6.80	
26	No. of capitula/plant	*qNCS-4-1*	4		4.42	16.54	−1.99	
27	Days to flowering under drought	*qDTFS-2-1*	2		2.61	14.08	1.3	

intron (specific to high oleic genotypes) and 198 bp from *CtKASII* gene (positive control to check for successful PCR amplification in all the samples). To generate high oleic cultivars quickly, a low-cost, high-throughput molecular marker assay for predicting high oleic characteristics is required in safflower. Kadirvel et al. (2020) employed a collection of high oleic variants that were discovered to have the identical mutation in the fatty acid desaturase 2-1 gene *CtFAD2-1*, which was assumed to be the "*ol*" allele associated with high oleic acid content in safflower. KASP was one of the genotypic assays used. The assays were thoroughly validated in populations resulting from crossings of low and high oleic parents. The "*ol*" gene from the exotic variety Montola-2000 was inserted into the background of the popular Indian linoleic type cultivar Bhima using a marker-assisted backcrossing strategy. The MAS-generated lines demonstrated consistent expression of high oleic acid content across seasons and oil yield performance equivalent to the local check types.

10.4.4 Association Mapping in Safflower

Linkage analysis and QTL mapping, two common techniques for identifying genomic regions influencing simple/complex traits, necessitate the creation of biparental mapping populations, which is time-consuming. Furthermore, the allelic variation obtained for QTL mapping is constrained due to the use of biparental crossings, and fewer recombination events are examined, resulting in low mapping precision (Flint-Garcia et al. 2005). Alternatively, association mapping (AM) promises to be the best strategy for moving beyond the limitations of linkage mapping because it is a faster and more efficient methodology for analyzing complex features at high resolution (Abdurakhmonov and Abdukarimov 2008). Association mapping, which uses naturally occurring recombination processes to find associations between traits and genetic polymorphisms in a heterogeneous assembly of genes, enables fine-scale trait mapping. AM has evolved as an efficient strategy for detecting marker-trait relationships in many crop species. (Zhang et al. 2014; Li et al. 2011; Yang et al. 2010; Zhu et al. 2008; Blair et al. 2009). Yan and Warburton (2011) suggested that the selection of germplasm for AM is crucial and it should include a wide range of variability to capture the greatest number of historical recombination events.

Crop diversity panels derived from core germplasm collections represent maximum genetic variation available in the extant crop germplasm and have been widely used in understanding the genetic basis of agronomic traits in several crop species (Upadhyaya and Wang 2013; Zhang et al. 2014). Since such crop panels mainly consist of unrelated individuals, the possibilities of spurious marker-trait associations due to pre-existing population structure are drastically reduced, thereby enhancing the accuracy of the results. In safflower, evaluation of global germplasm collections identified significant diversity for most of the desirable traits such as oil content, fatty acid composition, and tolerance to abiotic and biotic stress (Kumar et al. 2016; Dwivedi et al. 2005). Twelve morphological descriptors along with the

geographic information were analyzed to develop a core subset of safflower germplasm from 5522 safflower accessions by Dwivedi et al. (2005).

In safflower, association mapping for eight phenotypic traits in a panel of 124 safflower accessions, including oil content, fatty acid content, plant height, number of branches, and days to flowering, was studied by Ambreen et al. (2018). A total of 96 marker-trait associations are observed through association mapping. Another association mapping study was conducted by Singh and Rawat (2022) using 89 safflower accessions to assess *Fusarium* wilt resistance. Based on 155 AFLPs and 144 SSRs, three robust marker-trait associations with phenotypic variances ranging from 4 to 6.5% were identified. It was identified that locus-128 is a promising marker-trait association for safflower fusarium wilt resistance based on its high phenotypic variance. Ali et al. (2020a, b) used silico DArT markers to assess 94 safflower accessions from 26 countries and found 3 populations from the accessions, and 2 DArT markers, DArT-45483051 and DArT-15672391, were shown to be linked to 100-seed weight. Ebrahimi and Majidi (2017) used 341 AFLP markers to perform association mapping on 100 safflower genotypes for 8 major phenotypic features.

The examination of population structure revealed three major subpopulations with considerable genetic variations. Under drought and normal conditions, the markers M51/E32-9 and M61/E2-2 were found to be consistently liked to oil content. Plant traits and genetic polymorphisms identified in a heterogeneous assembly of diverse individuals using naturally occurring recombination events aid in trait fine-mapping. The durability and utility of marker-trait relationships discovered by association mapping research must be investigated further in different environments using multi-location trials. The identified probable marker-trait connections will aid in marker-assisted breeding for crop development and the identification of candidate genes for trait variability in safflower. Zhao et al. (2021) analyzed grain yield and associated traits from eight Australian grain bank safflower accessions using genomic prediction (GP). In all traits examined, the prediction accuracy (PA) of genomic best linear unbiased prediction ranged from 0.21 to 0.86. These values were consistent with the genomic heritability (h2) estimates, which ranged from low to moderate. A low level of genome × environment interaction was observed. Based on the results, it appears that GP is feasible for safflower evaluation and can facilitate the fast introgression of desirable traits from germplasm into breeding lines.

10.4.5 Safflower Genomics

A dense genetic map aids in the accurate assembly of the entire genome of the crop. Bowers et al. (2016) sequenced 96 F6 RILs produced from a hybrid of *C. tinctorius* and *C. palaestinus* with low coverage using whole-genome shotgun sequencing. They drafted a *C. tinctorius* assembly covering 866 Mbp of the required 1.35 Gbp. A total of 57,270 scaffolds were tethered to the map, each containing 5 or more mapped SNPs. As a result, sequencing encompassing 14% of the predicted genome length was assigned to a genetic location. Safflower has the largest FAD2 gene family among any species. Cao et al. (2013) reported cloning 11 unique safflower ctFAD2

genes, each displaying divergent functionality. In recent years, advances in next-generation sequencing technologies (NGS) have reduced the price of DNA sequencing to the extent that genome-by-sequencing (GBS) has become affordable for species with large genomes and high diversity. In addition to being fast, simple, and selective, GBS has the potential to reach parts of the genome that are inaccessible to sequence capture methods. Nasab and Nemati (2022) used a GBS analysis to find closely related lineages within cultivated safflower. By phylogenetic and population genetic analyses, *C. palaestinus* was identified as the closest related and sole progenitor of *C. tinctorius*. Flow cytometry revealed that all the studied *C. oxycantha*, *C. palaestinus*, and *C. tinctorius* samples were diploid, with 2C genome sizes ranging from 4.4 to 2.7 pg. Analyses of 114 globally distributed safflower accessions yielded two to five genetic groups but no link with geographic origins. The first high-quality genome assembly (contig N-50 of 21.23 Mb) for the 12 pseudochromosomes in safflower was published by Wu and Liu (2021). In safflower, uniquely expanded gene families were found to be particularly enriched for genes that were predicted to be involved in lipid metabolism and transport as well as ABA signaling, according to comparative genomic analysis. Other research findings were tandem duplication in safflower which led to the expansion of the chalcone synthase (CHS) and fatty acid desaturase (FAD2) families.

Various methods, like transcriptomics, proteomics, metabolomics, and phenomics, are used to investigate gene functions. Compared to other oilseed crops, the quantity of studies on safflower transcriptomics appears minimal. Li et al. (2012) used deep sequencing on safflower leaves, seeds, and petals to create a de novo transcriptome. In the study, oleosin unigenes were identified, and expression studies showed differential expression in seed, leaf, and petal. Metabolic pathway analysis revealed that 23 unigenes are involved in the production of flavonoids. Lulin and Xiao (2012) assembled the safflower floral tissue transcriptome from scratch using the Illumina sequencing technology. They got 4.69 Gb of nucleotides, which included 52,119,104 sequencing reads, 195,320 contigs, and 120,778 unigenes. They annotated 70,342 unigenes using a similarity search to previously recognized proteins. Thirty-three thousand genes were assigned to 121 KEGG pathways, and 21,943 safflower unigenes were COG classified.

The transcriptome serves as a valuable platform for investigating genomics, functional genomes, and gene expression in safflower. A cDNA clone (CTOS1) encoding a novel protein from high oleic acid accessions of safflower was isolated from its genome (Mizukami and Inagaki 2000). Ren et al. (2022) studied targeted metabolomics and transcriptomics to evaluate changes in flavonoid biosynthesis in safflower flowers during color transition. The gene CtUGT9 was discovered to be strongly related to flavonoid biosynthesis, and the gene was highly expressed in the middle development of flowers. They identified 212 flavonoid metabolites. Raina et al. (2005) isolated and cloned two repetitive DNA sequences, pCtKpnI and pCtkpnI-1, from *Carthamus tinctorius*. The flavonoid biosynthesis genes in six safflower genotypes were found using gene prediction approaches, and 44 distinct isoforms were identified (Chen et al. 2018). Wei and Hou (2020) conducted transcriptome and metabolic response of two safflower genotypes (PI1560169, a

drought-tolerant, and P1401477, a drought-susceptible genotype). They identified 328 and 2260 differentially expressed genes for drought tolerance. They also identified 359 and 209 differentially expressed metabolites. Three metabolites (galactitol, neoxanthin, and arbutin) were identified to be correlated with drought tolerance. Similar transcriptome and metabolomic studies were conducted by many researchers (Lulin and Xiao 2012; Liu et al. 2015; Shinozaki and Kenmoku 2016; Ren and Wang 2020; Qiang et al. 2020; Chen et al. 2020; Hoang et al. 2021; Li and Wang 2021; Wang and Ren 2021).

10.5 Safflower Improvement in the Post-genomics Era

10.5.1 Genetic Engineering in Safflower

Extensive research on plant regeneration and transformation has resulted in the development and commercialization of transgenic plants in a variety of crop species (James 2007). Techniques for tissue culture and gene transfer in safflower and other Asteraceae plants are also established, even though they are not many advanced studies in this area. To introduce foreign genes via genetic engineering into the required crops, an effective and reproducible in vitro regeneration procedure is required (Birch 1997). The method or protocol must also be consistent and repeatable across various germplasms. Numerous reports of safflower regeneration have been published, with most regenerated plantlets derived from cotyledons and leaf tissues (Afsharshandiz et al. 2019; Gholve et al. 2015; Mendhe and Sheikh 2018; Talat and Anwar 2016). Efficient plant regeneration protocols for various safflower species, as well as spiny and non-spiny genotypes, have also been reported (Afsharshandiz et al. 2019; Dipti et al. 2015; Patial and Krishna 2016; Talat and Anwar 2016; Vijayakumar and Ponmanickam 2017), but lack of a reliable system for rooting in the safflower is a major bottleneck for the establishment of plant and absence of rosette stage in tissue culture-regenerated plants (James 2007). Details of the protocols optimized in safflower for in vitro regeneration and transformation are presented in the following sections.

10.5.2 Tissue Culture Studies

Earlier safflower tissue culture studies were mostly with young seedling tissues (Nikam and Shitole 1997; Suganya and Sujatha 1997; Zhanming and Biwen 1993). In safflower, callus initiation and plantlet regeneration from vegetative explants are also successfully achieved with different plant tissues like cotyledons, hypocotyls, leaf, roots, and embryo axis (Mandal and Gupta 2001; Mandal and Dutta Gupta 2003; Varpe and Mendhe 2021; Surbhaiyya et al. 2018; Jaychandran and Ponmanickam 2017). Callus can be initiated from the seedling explants, but the ability to regenerate plants has been limited. Sujatha and Dinesh Kumar (2007) assessed the differences in callusing ability and organogenic potential of the various

seedling explants and obtained shoots from the shoot tips and rhizogenesis from root explants, shoot, and leaf tissues. Similarly, plant regeneration that has been reported to occur in the seedling explants was reported to involve pre-existing meristematic centers like apical meristems (Nikam and Shitole 1998; Patial and Krishna 2016; Ejaz et al. 2022). The vast majority of media that encouraged shoot regeneration via organogenesis or embryogenesis comprised BA alone or in conjunction with NAA (Tejovathi and Anwar 1984; Mandal and Chatterji 1995). The shoot multiplication rates obtained from explants in most of these studies varied between 1 and 5.2 (Dhumale et al. 2016; Jaychandran and Ponmanickam 2017; Mendhe and Sheikh 2018). Callus-mediated regeneration is reported from hypocotyl sections (Surbhaiyya et al. 2018; Varpe and Mendhe 2021), young stem segments (Jaychandran and Ponmanickam 2017), young leaves (Mendhe and Sheikh 2018), and epicotyl/cotyledons (Dhumale et al. 2015; Surbhaiyya et al. 2018). However, differentiation of callus into shoots and shoot buds was reported to be either occasional or low. In most of the tissue culture studies in safflower, Murashige and Skoog (MS), the basal medium, has been found to be ideal for morphogenic response in somatic and gametic tissues (recent publications to be added (Rajendra Prasad and Khadeer 1991; Chatterjee and Singh 1993)), or B5 vitamins proved to be superior (Orlikowska et al. 1995, 1996). Multiple shoots could be proliferated when cytokinin was supplemented singly, such as BA at 0.5–2.0 mg L^{-1} (Sujatha and Dinesh Kumar 2007), 1.0–2.0 mg L^{-1} (Sri Shilpa and Dinesh Kumar 2010), 4.0 mg L^{-1} (Vijayakumar and Ponmanickam 2017), 0.2 mg L^{-1} TDZ, or 4.0 mg L^{-1} BA (Xi and Wang 2020), or in combination with an auxin 2.0 mg L^{-1} BA +0.8 mg L^{-1} NAA (Talat and Anwar 2016). Different cytokinins, viz., BAP, kinetin, 2-isopentenyl adenine, and zeatin, have been attempted singly and in combination in safflower with limited effectiveness (Radhika and Sujatha 2006; George and Rao 1982). The cytokinins, kinetin (2.0 mg L^{-1}) and BA (1.0, 2.0 or 4.0 mg L^{-1}) with NAA (1.0 mg L^{-1}) or indoleacetic acid (IAA) (0.5 mg L^{-1}), were most often used (Table 10.6). Radhika and Sujatha (2006) reported media supplemented with 2.271 mg/L TDZ +1.0 mg/L NAA combination has produced the highest response from all explants types and genotypes (American and Indian) with an increased number of shoots from explants with shoot regeneration up to 98.5%. GA_3 (1 mg L^{-1}) is sometimes added to shoot regeneration medium, although no requirement for GA3 has been demonstrated (Vijaya Kumar and Ranjitha Kumari 2008), while it has proved effective in shoot elongation (Surbhaiyya et al. 2018).

Several researchers have previously observed that direct embryogenesis in safflower fully relies on genotype, explant age, carbon, ethylene, cytokinin, and auxin supply (Mandal and Gupta 2001, 2002). Similar findings have been found for somatic embryogenesis, which is created by embryogenic cells that emerge from explant, callus, or suspension cells (Gaj 2004). Auxin concentration can influence somatic embryo development and shape (Mandal and Dutta Gupta 2003). In safflower, a high frequency of safflower somatic embryos was identified with optimal NAA, whereas IAA generated the highest number of somatic embryos per culture. On medium enriched with 2.0 mg/L BA and 0.5 mg/L NAA, safflower anthers likewise aroused morphogenic potential, and haploids were recovered with a

Table 10.6 Response of different tissues for organogenesis in safflower (*Carthamus tinctorius* L.)

Explant/s	Type of morphogenetic response	Best media combination (mg/L)	Reference
Root, hypocotyl, leaf, cotyledon	Shoot regeneration	MS + 3NAA + 5BAP	Varpe and Mendhe (2021)
Leaf	Somatic embryogenesis	MS + 2.5NAA + 1.5$AgNO_3$	Kumar and Kumari (2011)
Leaf, cotyledon	Shoot regeneration	MS + 0.5NAA + 5BAP	Dhumale et al. (2016)
Leaf	Shoot regeneration	MS + 1NAA + 5BAP	Mendhe and Sheikh (2018)
Hypocotyl, cotyledon	Shoot regeneration	MS + 5BA + 1GA	Surbhaiyya et al. (2018)
Shoot tip and node	Shoot regeneration Rooting	MS + 1.5NAA + 1.5CPPU MS + 1%sucrose +2NAA + 1.5CPPU	Jaychandran and Ponmanickam (2017)
Cotyledon	Shoot regeneration Root regeneration	MS + 3BAP MS + 2NAA	Dhumale et al. (2015)
Root, hypocotyl, cotyledon	Multiple shoot regeneration	MS + 0.2 TDZ + 0.2NAA	Shilpa et al. (2010)
Cotyledonary node, stem node	Shoot buds	MS + B5 + 19.96BA + 6.97Kn	Vijayakumar et al. (2008)
Primary seedling explants including roots	Multiple shoot regeneration	MS + TDZ (2.27–22.71) + NAA (0.53–2.69)	Radhika and Sujatha (2006)
Leaf	Multiple shoot regeneration	MS + 4.5TDZ + 5.37 NAA	Sujatha and Dinesh Kumar (2007)
Leaf, cotyledon	Somatic embryogenesis	MS + 27.5TDZ + 12.6IBA + 6.82iP	Vijayakumar et al. (2008)
Cotyledonary leaf	Adventitious shoots	MA + 2.3TDZ + 1.3IBA	Basalma et al. (2008)
Primary seedling explants, roots	Multiple shoot regeneration	MS + 5TDZ + 0.5NAA	Radhika and Sujatha (2006)
Cotyledon, stem node	Shoot buds	MS + B5 vitamin + 4.5BA + 1.5Kn	Kumar and Kumari (2011)
Cotyledon	Somatic embryos	MS + 0.5BA + 1NAA	Mandal and Dutta Gupta (2003)
Cotyledon	Adventitious shoots	MS + 2BA	

(continued)

Table 10.6 (continued)

Explant/s	Type of morphogenetic response	Best media combination (mg/L)	Reference
			Mandal and Gupta (2001)
Primary seedling explants	Direct shoot regeneration	MS + 1BA + 10CH	Nikam and Shitole (1999)
Cotyledons	Selection of calli resistant to NaCl	MS + 0.5BA + 1.5NAA	Nikam and Shitole (1997)
Primary seedling explants	Selection of calli and shoots resistant to *Fusarium oxysporum*	MS + 1BA + 1NAA for callus MS + 1BA + 0.1NAA for shoots	Suganya and Sujatha (1997)
Hypocotyl, cotyledon	Multiple shoot regeneration	MS + 0.25BA + 0.1NAA	Rani et al. (1996)
Cotyledon, hypocotyl	In vitro rooting	MS + 10 IBA (for 7 days) followed by MS + 1.5%sucrose + 1 g/L AC for 21 days	Baker and Dyer (1996)
Primary seedling explants	Adventitious shoots	MS + 5BA + 0.5NAA	Zhanming and Biwen (1993)
Cotyledons	Somatic embryos	MS + 0.5BA + 2NAA	Mandal and Chatterji (1995)
Immature embryos	Multiple shoot regeneration	MS + 0.01TDZ + 10NAA	Mandal and Chatterji (1995)
Primary seedling explants	Adventitious shoot regeneration	MS + 0.5BA + 0.1NAA	Orlikowska and Dyer (1993)
Leaf	Shoot buds	MS + 5BA + 0.25NAA	Orlikowska and Dyer (1993)
Cotyledons	In vitro rooting	MS + 2(2,4,5-D)	Tejovathi and Anwar (1993)
Anther	Multiple shoot regeneration	MS + 2BA + 0.5NAA	Rajendra Prasad and Khadeer (1991)
Leaf	Oil accumulation	MS + 1BA + 0.25NAA + 5% sucrose + 1 g/L CH + 10% coconut water	Singh and Chatterji (1991)
Hypocotyl	Multiple shoots	MS + 2BA + 0.5NAA	George and Rao (1982)
Cotyledons	Induction of capitula	MS + 0.5BA + 0.1NAA	Tejovathi and Anwar (1984)
Primary seedling		MS + 8BA + 0.5NAA + 5 adenine sulfate	

(continued)

Table 10.6 (continued)

Explant/s	Type of morphogenetic response	Best media combination (mg/L)	Reference
explants of *C. tinctorius*	Multiple shoot regeneration from leaf		Singh and Chatterji (1991)
Shoot apices of *C. oxycantha*	Multiple shoot proliferation	MS + 0.5NAA + 20GA_3 + 5 ascorbic acid	Rajendra Prasad and Khadeer (1991)

frequency of 64% (Rajendra Prasad and Khadeer 1991). The rooting of regenerated shoots from the explants and post-acclimatization and survival of the plant are the greatest challenges for safflower tissue culture. Bayer and Dyer (1996) found that a 7-day exposure to a high concentration of hormone 10 mg/L IBA, followed by a 21-day incubation in media containing 15 g/L IBA and 1 g/L activated charcoal, increased rooting frequency while decreasing shoot hyperhydricity. The root induction frequency ranged from 10 to 95%, but only shoots with less hyperhydricity and better tap roots only survived during post-acclimatization.

The frequency of root induction also improved by increasing sucrose concentration (9%), adding riboflavin, and incorporating 2,4,5 trichlorophenoxypropionic acid (Orlikowska and Dyer 1993; Bayer and Dyer 1996). Root induction in safflower was also tried with bacterium *Agrobacterium rhizogenes* (Baker and Dyer 1996). Root formation has been initiated when regenerated shoots were transferred to medium supplemented with auxin (IBA, NAA) alone (Radhika and Sujatha 2006; Baker and Dyer 1996), in combination with cytokinin (Orlikowska and Dyer 1993; Nikam and Shitole 1998; Dipti et al. 2015), on the shoot proliferation medium itself (Basalma et al. 2008), or with silver nitrate (Gong et al. 2005; Shah and Ali 2014). Despite different experimentations for improving the rooting efficiency, rooting problems persisted, and rhizogenesis occurred at varying frequencies depending on genotype, shoot quality, medium, and culture time. A further complication occurred during genetic transformation experiments, when regenerated shoots were exposed to bacteriostats and selective agents for identifying potential transformants.

Safflower tissue culture exhibits an intriguing feature in which capitula can be inducted in vitro on media with growth regulators (Radhika and Sujatha 2006). The type of growth regulators and the genotype strongly influence flower formation in vitro. A study by Tejovathi and Anwar (1984) found that the capitula were induced frequently on media augmented with BA + NAA and at a low frequency on media fortified with kinetin. According to Seeta and Talat (1999), an optimal concentration of BA + NAA should be present in the medium for flower production. In vitro-produced flowers were normal, with good pollen production and seed set. In vitro flowering could be used to recover interspecific hybrids and overcome asynchronous flowering problems in safflower. Hamedi and Golkar (2016) conducted an

in vitro experiment to study abiotic stresses such as salt tolerance in safflower, and callus generated from hypocotyls of different genotypes had shown varying levels of in vitro tolerance to sodium chloride. Seeta and Talat (2000) used somaclonal variation in the crop to find somaclones for several attributes such as plant height, leaf form, flower color, and oil. As genetic transformation involves several manipulations for gene introduction followed by selection for two to three subculture cycles, the efficiency of these regeneration systems for the genetic transformation of safflower needs to be established. Developing cytoplasmic genetic male sterility, a hybrid breeding system, and a beneficial outcome of ongoing efforts to use polyembryony for varietal improvement and apomixis confirmation in safflower (Mandal and Gupta 2001) can be attempted.

10.5.3 Transgenic in Safflower

Genetic engineering is commonly employed to improve crop attributes such as agronomic, quality traits, and resistance to biotic and abiotic stresses. Callus-mediated regeneration, shoot regeneration, and embryo transformation are among the transformation strategies used in safflower. Sankararao and Rohini (1999) made the first attempt to generate a broad-based genetic improvement of safflower through gene transfer using *Agrobacterium tumefaciens*. However, rooting of shoots in transgenic safflower was challenging, and so transgenic plant regeneration was poor. Ying and Dyer (1992) created the first safflower transgenic by transforming the cultivar "Centennial" with *A. tumefaciens*. Belide and Hac (2011) reported a highly efficient *Agrobacteriu*m-mediated transformation technique and improved in vitro root production by developing a grating approach. Rohini and Shankar Rao described in 2000 the development of a gene transfer system for safflower that could overcome the limitations associated with the conventional transformation approach utilizing *A. tumefaciens*. They modified the *uid*A reporter gene, directed by the CaMV 35S promoter, and the nptII gene, regulated by the nopaline synthase promoter. They demonstrated that the embryo transformation technique worked for every cultivar and genotype of safflower susceptible to *A. tumefaciens*.

Genetic transformation was attempted to incorporate resistance to biotic stresses in safflower. Matern and Kneusel (1993) and Kumar et al. (2009) attempted to develop transgenics for resistance to the fungus *Alternaria carthami* in safflower. The chitinase genes were also transferred into A1 cultivar for fungal resistance (Kumar et al. 2009). Several researchers have attempted to modify and improve the fatty acid profile of oilseed crops such as safflower (Töpfer and Martini 1995; Zhu et al. 2016; Villanueva-Mejia and Alvarez 2017; Rani and Panwar 2018). Rani and Panwar (2018) improved alpha-linoleic acid concentration in transgenic safflower by incorporating the gene that encodes the enzyme delta-15 desaturase (FAD3). Nykiforuk et al. (2011) also overexpressed 6-desaturase in high oleic and high linoleic safflower cultivars. Similarly a δ-6-desaturase gene from *Borago officinalis* was transferred into the safflower cultivar HUS-305 using the Agrobacterium-mediated gene transfer method (Devi et al. 2008). Safflower dried

Table 10.7 Genetically engineered safflower approved in different countries (Oshima et al. 2020)

OECD unique identifier	Trait	Country	Type of approval	Year
GOR-7322-6	Increased production of oleic acid	Australia	Cultivation, food, feed Processing	2018 2019
GOR73240-2	Increased production of oleic acid	Australia	Cultivation, food, feed Processing	2018 2019
IND-1000-3-4	Production of bovine prochymosin enzyme; glufosinate tolerance	Argentina	Commercial production	2017
IND10015-7	Production of bovine prochymosin enzyme; glufosinate tolerance	Argentina	Commercial production	2017
IND10003-4 × IND10015-7	Production of bovine prochymosin enzyme; glufosinate tolerance	Argentina	Commercial production	2017

petal powder (carthami flos) is used in traditional Chinese medicine to treat cardiovascular and cerebrovascular diseases (Guo et al. 2017). Later, a phytochemical study of dried safflower petal powder suggested that the disease curing property is due to bioactive metabolites hydrosafflower yellow A (HSYA) and carthamin, a quinochalcone synthase (CtCHS1). The increased expression of the genes PAL2, PAL3, CHS1, CHS4, and CHS6 using the agrobacterium-mediated pollen tube pathway technique resulted in a 20–30% rise in quinochalcone glucoside concentration, but a 48 and 63% decrease in quercetin-3-D-glucoside and quercetin in the florets, respectively.

According to Carlsson and Zhu (2014), safflower is a promising host for innovative transgenic technology for developing herbal medicines based on vegetable proteins. Markley et al. (2006) used transgenic oil body-oleosin technologies to design insulin. This method injects a transgene encoding an oleosin-insulin fusion protein into the plant. Plant-produced insulin was a cost-effective option that reduced insulin production unit costs. Safflower also contains a high concentration of pharmacological and nutritious components. Apolipoprotein Al Milano ($ApoAl_{Milano}$) serves an important therapeutic role in cardiovascular disease with high LDL cholesterol levels by boosting HDL cholesterol levels. Nykiforuk et al. created the fusion protein "apolipoprotein Al Milano ($ApoAl_{Mialno}$)" in transgenic safflower seeds (2011). During seed development, a phaseolin promoter terminator was coupled to allow for tissue- and time-specific expression. Some of the examples of genetically engineered safflower approved in various countries are presented in Table 10.7.

10.6 Conclusions

Safflower, cultivated for its highly nutritional and healthy seed oil, thrives well even with limited inputs in semi-arid regions of the world. However, safflower crop production, as is the case in any other crop species, faces numerous challenges, including biotic and abiotic stresses and adaptational challenges to changing climate. Aside from this, the spiny nature of the crop adds to the cost of cultivation, for it demands the engagement of highly skilled and costly labor. Researchers across the globe have been concerting their efforts to evolve solution(s) to safflower production problems. However, the benefit of advanced and innovative approaches in accelerated breeding and biotechnology is yet to be harnessed. Therefore, marker-assisted breeding requires high-density agronomic and phenological trait mapping which further requires the development of genomic and genetic resources. Besides this, the advantage of genome editing is yet to be realized. Thus, there is a need for globally coordinated efforts for developing metabolomic networks so that mathematical and machine learning models can be built to validate the consequence of genome editing. The need of the hour is the availability of quality reference genomes that can be used for physical mapping of traits, developing genome-editing strategies, and studying functional genomics.

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Biotechnological Approaches for Genetic Improvement of Sesame (*Sesamum indicum* L.)

11

H. H. Kumaraswamy, K. T. Ramya, Swarup Nanda Mandal, P. Ratnakumar, J. Jawahar-Lal, H. D. Pushpa, K. Ramesh, A. L. Rathnakumar, P. Duraimurugan, and Sakthivel

Abstract

Sesame (*Sesamum indicum* L.) is an important oilseed crop cultivated since the ancient past for its healthy and quality oil. However, it is only in the recent past that modern genomic tools have been developed in sesame and deployed in sesame crop improvement. Knowledge of biotechnological tools and techniques developed in sesame in the post-genomics era would help to bridge the long-stagnated yield barrier and relieve the crop from a range of biotic and abiotic stresses. In this context, an attempt has been made to collect, analyze, organize, and present information on biotechnological approaches for sesame crop improvement. Further, in the foreground of the immediate research attention required for sesame crop improvement and the background of works accomplished so far, future perspectives have been discussed. The present chapter is intended to educate stakeholders of sesame research ecosystem: researchers, academicians, scientists, policymakers, research funders, students, etc.

Keywords

Sesame · *Sesamum indicum* · Biotechnology · Molecular biology · Genomics · Genetic improvement · Crop improvement · Tools and techniques

H. H. Kumaraswamy (✉) · K. T. Ramya · P. Ratnakumar · J. Jawahar-Lal · H. D. Pushpa · K. Ramesh · A. L. Rathnakumar · P. Duraimurugan · Sakthivel
ICAR-Indian Institute of Oilseeds Research, Hyderabad, India
e-mail: hhk.swamy@icar.gov.in

S. N. Mandal
Department of Plant and Soil Science, Texas Tech University, Lubbock, TX, USA

Department of Genetics and Plant Breeding, Bidhan Chandra Krishi Viswavidyalaya, Burdwan, West Bengal, India
e-mail: swarupa.mandal@ttu.edu

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11.1 Introduction

Sesame (*Sesamum indicum* L.) is an annual herbaceous diploid plant ($2n = 2X = 26$) belonging to the family Pedaliaceae of the Tubiflorae order (Nayar and Mehra 1970). The only cultivated species among 37 species in the genus *Sesamum* has been cultivated for its unique oil, which has industrial-scale utility in culinary, pharmaceuticals, nutraceuticals, cosmetics, etc. (reviewed by Pusadkar et al. 2015). Sesame enjoys sobriquets "queen of oilseeds" and "seeds of immortality" due to the long shelf life of its seed oil, caused by resistance to oxidation and rancidity (Bedigian and Harlan 1986). The crop is grown in three regions: the Indian subcontinent, the African continent, and the Far East subcontinent (Kobayashi 1991). Globally, 70% of the sesame seeds are utilized for extracting oil and cake, whereas 30% is used for edible seeds (reviewed in Kumaraswamy et al. 2015).

The world produced 68.04 million tons of sesame seeds from 13.97 million hectares, with an average yield of 487.2 kg per hectare. The maximum production of sesame seeds was contributed by Sudan (1.53 mt), whereas Myanmar (0.74 mt) and India (0.66) occupied second and third positions, respectively. In terms of area under sesame cultivation, Sudan (5.17 Mha) occupied the first place, followed by India and Myanmar with 1.52 Mha and 1.5 Mha, respectively. While Lebanon realized the world's highest productivity of 3298.2 kg of sesame seeds per hectare, Jordan and Israel recorded the second and third highest yield levels of 2375 kg per hectare and 2041.7 kg per hectare, respectively (FAOSTAT 2020).

Even though sesame is an important oilseed crop from nutritional, industrial, and pharmaceutical viewpoints, sesame cultivation is facing numerous challenges, including biotic and abiotic stresses and stagnation of yield levels. Recent advancement in molecular biology and biotechnology is yet to be harnessed in sesame crop improvement. Development of high-density linkage maps, consensus linkage maps, marker-trait association studies, and deployment of genome editing is required to be focused as a high-priority area of research at the global level, and concerted efforts are needed worldwide to develop plant idiotypes suitable for mechanical harvesting and high-density planting; plant types with engineered quality seed oil and value addition with bioactive compounds; and sesame genotype resistance or tolerance to abiotic as well as biotic stresses. In this chapter, recent advances in sesame research, particularly from the genetic improvement point of view, are comprehensively discussed as to how they can be harnessed to enhance sesame productivity and production including genetic engineering and genome editing for securing nutritional benefits from sesame seed and seed oil.

11.2 Background

11.2.1 Sesame Origin and Evolution

The first report on the origin of sesame was made by Hilterbrandt (1932). According to him, Africa was the origin of cultivated sesame (*Sesamum indicum* L.), and the same view was concurred subsequently by Nayar and Mehra (1970), Seegler (1983), Burkill (1997), and Mehra (2000). However, a subsequent hypothesis based on archaeobotanical evidence illuminates that the Harappan civilization was the place of the first domestication of sesame and that it was subsequently spread to Egypt and Mesopotamia (Fuller 2003). In 2011, Bedigian showed that sesame originated in India and reached other parts of the world, moving along a trade route called "silk route" (Bedigian 2011, 2014). However, consensus regarding the origin and evolution of sesame species is yet to be established. Analysis of morphological, cytological, and comparative genomics may provide some convincing evidence on the origin and phylogeny of sesame (Zhang et al. 2013).

Based on the fact that India contains maximum genetic variability for cultivated species of sesame, it is believed that India is likely to be the center of origin for *Sesamum indicum* (L.), according to Bhat et al. (1999). In the present scenario, India occupies the major place in the world sesame seed export map (Ranganatha et al. 2014). The preferred seed quality parameters in the world sesame seed export market are free from pesticide residues, lack of pest infestation, high (>830 mg/100 g seed) lignan content, less than 2% free fatty acid, less than 1% oxalic acid content, the boldness of the seeds with white seed coat color, and uniform lustrousness (Ranganatha et al. 2014). The demand in the international market for sesame seeds is on an increasing trajectory. This calls for concerted efforts to improve sesame, taking advantage of recent genomics and molecular biology advancements.

Its oleaginous seed is rich in omega-6 fatty acids but lacks omega-3 fatty acids. Therefore, there is a need to undertake oil quality engineering through a genome editing approach to alter the desaturase enzyme pathways (reviewed by Pusadkar et al. 2015). Sesame seeds, as well as seed oil, contain nutrients, both mineral and vitamins: phosphorus, iron, zinc, copper, calcium, magnesium, manganese, dietary fiber, and vitamin B1, vitamin K, and vitamin E in sesame seeds (Pathak et al. 2014); and omega-3 fatty acids, sesamin, and lecithin were also found in the oil extracted from sesame seeds (Shivhare and Satsangee 2012).

11.2.2 Sesame Cytogenetics

The study of cytogenetic aspects of cultivated sesame (*Sesamum indicum* L.) is challenged by two facts: firstly, chromosomes ($n = 13$) are relatively smaller in size, varying between 1.106 μm and 3.871 μm; and secondly, they lack the morphological variations (subtelocentric, metacentric, or submetacentric (Zhang et al. 2012)). These chromosomal and morphological indistinctness further constraints investigations into structural aspects of chromosomes and evolutionary details of

sesame genome (Zhang et al. 2012; Nyonggesa et al. 2014). Based on the diploid number of chromosomes, there exists three sesame species: *S. radiatum* and *S. schinzianum* with $2n = 64$; *S. indicum* and *S. alatum*, having $2n = 26$; and *S. prostratum* and *S. angolense*, where $2n = 32$ (Nimmakayala et al. 2011; Ashi 2006). Genus *Sesamum* has two types of basic chromosome number, $X = 8$ and $X = 13$ (Ashi 2006; Zhang et al. 2013).

The genome size of cultivated sesame (*Sesamum indicum* L., $2n = 13$) was determined indirectly by deploying the flow cytometry technique and comparing it with the known genome size of other species, in addition to that of *Arabidopsis thaliana*. Based on an indirect approach, it was found to be 369 megabase pairs (Mb), whereas according to sequence data, it was observed to be 354 Mb (Yi and Kim 2011; Zhang et al. 2013).

11.2.3 Sesame Phylogenetics

Genome sequence data analysis revealed the phylogenetic position of *Sesamum indicum* (L.), where it belonged to asterids clade forming a part of core eudicotyledons that constituted the second phylogeny group of angiosperms (AGP 2, The-Angiosperm-phylogeny-group et al. 2003). Further, phylogenetic analysis using the chloroplast genomic sequence information showed that sesame (*Sesamum indicum* L.) falls under Pedaliaceae family and is a sibling genus to *Jasminum* and *Olea* (members of Oleaceae family) clade. Therefore, sesame seems to have the core lineage of the Lamiales families (Yi and Kim 2011).

11.3 Sesame Improvement in the Genomics Era

In this chapter, we tried to comprehensively summarize the recent developments in biotechnological/genomic approaches for sesame crop improvement. Omics studies and functional genomics in sesame have been reviewed explicitly by Dossa et al. (2017) and Wei et al. (2017). This chapter provides an overview of recent developments in sesame biotechnology and genomics and their potential applications in sesame crop improvement.

Research initiatives and developments in the sesame crop improvement research can be broadly viewed under three eras: (1) collection of wild and cultivars and genebank creation (Prior to 2000); (2) genetics and traditional breeding (2000–2013); and (3) genomics and omics (Since 2013, reviewed in Dossa et al. 2017). Sesame is also a very good model crop for conducting genomic research and functional genomic analyses of oilseed crops, which can be attributed to its small-sized diploid genome (Wei et al. 2015) of 354 Mb (Wei et al. 2017; Wang et al. 2014a). An overview of various resources, tools, techniques, strategies, and approaches that are deployable in sesame biotechnology are graphically illustrated in Fig. 11.1.

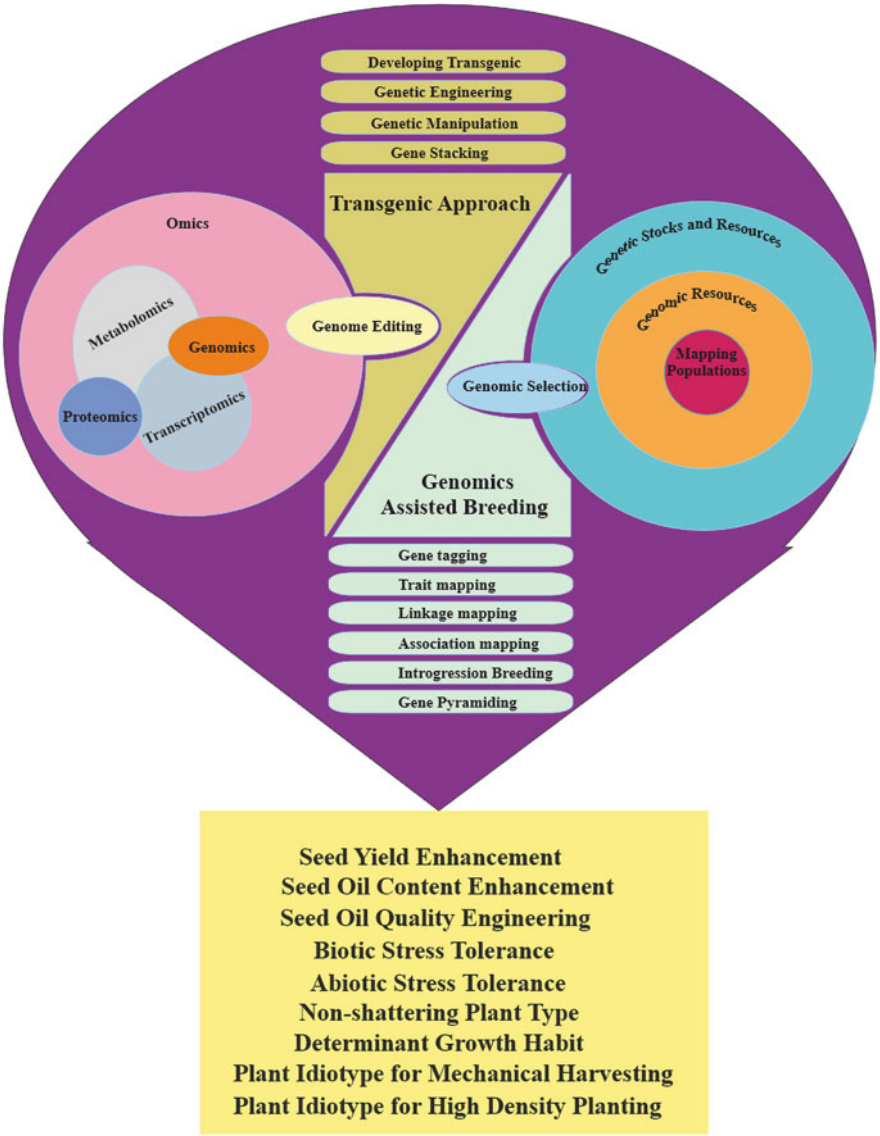

Fig. 11.1 Interrelationship among numerous genomic tools, resources, techniques, and strategies useful for sesame breeding programs

11.3.1 Sesame Genetic Resources

As stated by Murray (2017), for genetic material to be qualified as a plant genetic resource, it must be of value as a resource for present and future generations of humans. The world germplasm collection has 35,000 lines in its sesame basket, of

Fig. 11.2 Sesame crop raised under field conditions: The inserts show flowers (bottom-left) and a closeup of capsules (the right half); a closeup of a plant showing capsules is given on the right side of the figure

which 4000 are in India and China alone (Hodgkin et al. 1999). Large intra-population variations and rich phenotypic and genotypic diversity offer huge potential, and immense opportunities for genomic-assisted crop improvement in sesame are attributable to rich genotypic and phenotypic diversity with greater extent of intra-population variations (Wei et al. 2016, 2017). An aerial view of the sesame crop raised under field conditions is given in Fig. 11.2.

11.3.2 Sesame Genomic Resources

Yi and Kim (2011) sequenced the chloroplast genome of sesame for the first time. Subsequently, another chloroplast sequencing was performed from *S. indicum* cv. Yushi 11 (Zhang et al. 2013). In the same year, Wei et al. (2011) developed 86,222 unigenes, of which 46,584 showed significant similarity with protein sequences of Swiss-Prot database and NCBI nonredundant protein database. Transcriptome sequencing using the paired-end technology of Illumina led to the sequencing of 42,566 unitranscripts (Wei et al. 2011; Zhang et al. 2012). Two libraries, 57,600 BAC clones and 80,000 BIBAC clones having insert sizes of 85 kb and 120 kb, covering 13-fold and 27-fold genomes, respectively, are developed by Zhang and his team (Zhang et al. 2013). EST database of NCBI contains 45,093 sequences from *S. indicum*-expressed sequence tags. Including one full-length cDNA library of 300,000 clones, there are two seed-specific cDNA libraries of *S. indicum* (Ke et al. 2011; Suh et al. 2003). In 2009, Wei and his team constructed a first linkage map of sesame involving 284 microsatellite loci (Wei et al. 2009) which has been subsequently augmented with other 653 simple sequence repeat (SSR) markers, single nucleotides (SNPs), amplified fragment length polymorphism

(AFLP), and random selective amplification of microsatellite polymorphic loci (RSAMPL) assorted to 14 linkage groups (Zhang et al. 2013).

Expressed sequence tag-based simple sequence repeats (EST-SSRs), also called microsatellite markers, have been developed in sesame using transcriptome sequence information (Wei et al. 2011). Wang et al. (2012a) developed and characterized 59 polymorphic cDNA-based microsatellite markers; genic-SSR markers were developed and validated by Zhang et al. (2012) using RNA sequence information; 218 polymorphic SSR markers were developed by Wei et al. (2014) using genome-wide survey. They observed that 23,438 simple sequence repeats had at least 5 repeats, and the most common (84.24%) repeat motif had 2 nucleotides, while 3 nucleotide, 4 nucleotide, 5 nucleotide, and 6 nucleotide repeats comprised 13.53%, 1.65%, 0.3%, and 0.28% of the SSRs, respectively.

The sesame genome working group utilized these genomic resources for sequencing and assembling sesame genome (Zhang et al. 2013). Whole-genome sequence information (Wang et al. 2014b) is available in the public domain for both landrace and cultivated varieties (Wei et al. 2016). Based on what has been covered in the literature under the umbrella term "genomic resources," Kumaraswamy et al. (2022) attempted to define the term "genomic resources" as the sum total of biological samples and/or the information that provides the foundation for further study of the biological processes and genomic mechanisms of an organism aimed to be exploited for the benefit of mankind including ecological and environmental gain.

11.3.3 Development of DNA Markers and Sesame Genomic Diversity

The term "genome," initially coined by Winkler (1920), was described by Kihara (1930) as "a set of chromosomes that forms a fundamental and physiological unit which is indispensable for normal housekeeping metabolism, growth and development of the plant or organism." Subsequently, in the last decade of the twentieth century, the field of genome and genomics advanced impactfully.

The available of reports on genomic diversity studies on crop species suggests that the following criteria can be employed for genetic diversity analyses: morphological traits (Schut et al. 1997; Maric et al. 1998; Casadesus et al. 2007; Zarkti et al. 2012; Malik et al. 2014), molecular markers (Karp et al. 1996; Rao and Riley 1994; Manifesto et al. 2001; Pagnotta et al. 2005; Gogoi et al. 2018; Bhattacharjee et al. 2019; Kahsay et al. 2020), pedigree analysis (Barret et al. 1998), biochemical markers (Cox et al. 1985; Metakovsky and Branlard 1998), and

11.3.3.1 Morphological Markers

Among different markers, morphological markers are the first kind of markers available to plant breeders. They can be easily and visually characterized, for instance, pigmentation in any part of the plant, including corolla color, growth habit, seed shape, hairiness, etc. In the traditional plant breeding approach, plant breeders usually prefer to select wanted plants for advancing to subsequent generations only based on visual and/or directly measurable attributes. If any

morphological features are co-inherited with the traits of importance, then such markers are used to select the concerning traits indirectly. However, the morphological markers are not widely applicable owing to their environmental influence, low polymorphism, limited availability, pleiotropism, expressivity, etc.

The first problem in diversity studies solved by molecular marker was establishment of variability among functionally similar but structurally different proteins as allozymes synonymous with isozymes (Schlotterer 2004) which was extensively deployed by Hamrick and Godt (1990) in studying population genetics. Molecular marker property of the allozymes (or isozymes) was imparted and empowered by their ability to move differentially under gel electrophoresis according to their net charge and tertiary structure. Allozyme-based diversity enjoyed the monopoly field of molecular markers serving various purposes, including fingerprinting of plant genetic resources, assessment of genetic diversity, taxonomic and phylogenetic delineation, developmental biology and population genetics, and plant breeding (Bretting and Widrlechner 1995).

Isozymes originate due to amino acid alterations, which cause changes in net charge or the spatial structure (conformation) of the enzyme molecules and, therefore, their electrophoretic mobility. Isozyme analysis has been used for over 60 years in biology to delineate phylogenetic relationships, estimate taxonomy, and study population genetics and developmental biology (Bretting and Widrlechner 1995). Like in the case of the morphological markers, the biochemical markers are also impacted by the environmental factors and phenological (developmental) stages of the organism (Winter and Kahl 1995), apart from being not abundantly available in nature.

11.3.3.2 DNA/Molecular Markers

Plant DNA-level variations form the basis of variations in its morphological traits and can be analyzed using various types of DNA markers. Molecular markers are the DNA sequence variations that can be readily detected and whose inheritance can be monitored easily. The development and deployment of deoxyribonucleic acid (DNA) marker technology for detecting and exploiting DNA sequence diversity is one of the marvels in the advancement of molecular genetics (Semagn et al. 2006). DNA extraction can be accomplished using any parts of the plant taken from any developmental stage, and its analysis can be cheaper and non-laborious (Kumar et al. 2009). It has also been proved to be helpful in studying the genetic relationships, evolutionary trends, and fingerprinting of varieties.

The term "marker" was coined by Stansfield in 1986 (Stansfield 1986). In general, DNA markers mean any segment (locus) of genomic DNA with a defined nucleotide sequence that can be used as a reference point to specify other nearest locations (loci) on the same chromatin or the chromosome. Suppose the marker locus varies among different copies of genomes (individuals) in terms of the nucleotide sequence. In that case, the marker is said to be a polymorphic marker and is useful to distinguish the species' individuals or cultivars/genotypes. Individuals having two copies of the genome are called diploids and carry two copies of the marker locus. Two copies of the marker locus are called alleles if and only if they occur at the same

locus in the genome. Otherwise, they constitute multi-locus segments and are not useful for marker analysis. However, in terms of the nucleotide sequence, two alleles can be the same (identical) or different (non-identical). If a diploid individual carries the identical alleles of the marker, it is called homozygote for the marker locus, and the condition of the marker is called homozygosity. Otherwise, if it carries non-identical alleles of the marker locus, the individual is called heterozygote for the marker locus, and the marker locus is said to be in heterozygous condition.

Microsatellite or simple sequence repeat (SSR) markers are abundant throughout the genome. There is a possibility of high variations in their loci due to the inherent nature of their origin: replication slippage and crossing-over events. Therefore, SSR markers have been utilized for a wide spectrum of applications in plant genetic and genomic research. The most commonly applied fields of research include (1) population and evolutionary studies, (2) genome mapping, (3) genetic diversity analyses and phylogenetic relationships, (4) DNA fingerprinting and cultivar identification, and (5) gene tagging and marker-assisted selections. Various types of DNA marker-based diversity studies in different panels of sesame genotypes and the salient findings are summarized in Table 11.1.

After the entry of sesame into the omics era, various types of DNA markers, including SSRs, SNPs, and indels, have been discovered in sesame, paving new horizons for genomics-assisted sesame improvement programs (Dossa et al. 2017).

11.3.4 Genome Sequence-Driven Sesame Genomics

Genome-level variations form the basis for variability in every biological process and trait, including genetic control, biochemical processes at the cellular level, and physiological attributes at the organism level. Therefore, genome sequence information is vital to understanding and manipulating traits of agronomic and economic importance in crop species, including sesame. In addition, sesame genome sequence information is of paramount importance in understanding the genome's organization, evolution, structure, and size, which helps study comparative genomics of sesame.

After the genome sequencing (Wang et al. 2014a) was accomplished in the Chinese cultivar "Zhongi No. 13" of cultivated sesame, deep sequencing (Wang et al. 2014b) was carried out; this resulted in the dawn of sesame omics and subsequent development of a comprehensive database called SINBASE (Wang et al. 2014c). Subsequently, other cultivars and landraces were sequenced, including a cultivar "Swetha" from India (Purru et al. 2018), which led to the development of a dedicated microsatellite database "GinMicrosatDb" (Purru et al. 2018).

Table 11.1 Molecular marker-based genetic diversity studies in sesame

SN.	Marker type (number)	Genotypes (number)	Remarks	Reference
1	RAPD (24)	58 accessions from India and 22 exotic collections	Highly diverse accessions were from Rajasthan and northeastern states and low diversity among exotic lines	Bhat et al. (1999)
2	ISSR (14)	75 Korean genotypes	Narrow genetic diversity existed in Korean accessions	Kim et al. (2002)
3	RAPD (12)	38 Turkish genotypes	Analysis of molecular variance (AMOVA) revealed the presence of more variations within the region than among the region	Ercan et al. (2004)
4	SSR (50)	16 germplasm accessions	Ten of 50 SSRs showed polymorphism with PIC values: 0.34–0.8	Dixit et al. (2005)
5	AFLP (457)	32 germplasm lines	Ninety-three percent AFLPs showed polymorphism, and Jaccard's similarity coefficients ranged between 0.38 and 0.85	Laurentin and Karlovsky (2006)
6	AFLP (21)	96 accessions collected from East Asia and South Asia	Clustering of East Asian and South Asian accessions into two separate groups suggested the geographical identity	Ali et al. (2009)
7	RAPD (75)	10 lines collected from different regions of Sudan	Sixty-four of 75 primers gave reproducible amplicons of which 10 primers gave unique bands in each of the ten lines	Abdellatef et al. (2008)
8	RAPD (5) and morphological markers (20)	20 lines belonging to different agroecological zones of India genotypes obtained from Slovakia	Phenotypic markers (with a genetic similarity index of 0.88–0.99) were more discriminative than the RAPD markers (with a genetic similarity index of 078–095). Morphological grouping was similar to DNA-based grouping, based on principal coordinate analysis (PCA)	Kumar and Sharma (2009)
9	ISSR (14)	10 accessions	ISSRs discriminated the accessions with PIC values: 0.50–0.85 with an average of 0.67	Anitha et al. (2010)
10	Morphological, phenological, and reproductive traits (24); RAPD markers	27 lines (14 exotic accessions and 13 from Iran)	While morphological typing indicated the existence of a high spectrum of genetic variability in both exotic and Iranian genotypes, RAPD markers could decipher higher variability in Iranian genotypes than in exotic lines	Tabatabaei et al. (2011)
11	SSR (16)	150 sesame collected from 22 countries	One hundred twenty-one alleles were detected in the range of 2–18; average number of alleles were 7.6 per locus. The genotypes were clustered into three groups	Cho et al. (2011)
12	SSR (207)	9 genotypes	Forty-six of 150 markers were polymorphic and revealed the genetic similarity coefficient ranging from 76 to 92%. The genotypes were grouped into four clusters and one clade, revealing the presence of good variability and diverse origin of the genotypes	Rao et al. (2012)

13	SSR (156)	49 genotypes	Twenty of 156 markers showed polymorphism, with PIC values ranging from 0.49 to 0.90 with an average of 0.72	Yepuri et al. (2013)
14	SSR (14)	70 lines (43 from Korea and 27 from China)	Variability among the panel was low as there were only 2.8 alleles per marker locus; some markers were discriminative with PIC values in the range of 0.24 and 0.77 with an average of 0.51. Markers were found to be potentially useful in deciphering genetic variability in sesame	Park et al. (2014)
15	SSR (8); morphological characters (38)	60 genotypes of sesame including exotic collections, cultivars, and indigenous collections	Microsatellite marker loci showed 27 alleles with an average of 3.37 alleles per locus which ranged from 2 to 6. Based on morphological and molecular variability, dendrogram grouped the accessions into two major groups where accessions from the same geographical area were grouped into different clusters	Pandey et al. (2015)
16	SSR (10)	23 accessions	Four of the 10 marker loci had 14 alleles which were averaged to 3.5 alleles per locus. Polymorphic information continent values ranged between 0.28 and 0.78 and had an average of 0.53. Pairwise similarity coefficient values were in the range of 0.2 and 0.7, with an average of 0.45	Kiranmayi et al. (2016)
17	SSR (68); 19 morphological traits	41 lines comprising varieties and advanced breeding lines	Plant height was the most variable trait. Microsatellites revealed 29% polymorphism with an average PIC value of 0.409 and 2.8 alleles per locus, suggesting the presence of moderate diversity	Ramprasad et al. (2017)
18	SSR (50)	33 Indian genotypes	Twenty-seven of the 50 markers were polymorphic with 49 of the 78 alleles showing polymorphism with an average of 2.89 alleles per locus in the range of 1–5 alleles. Similarity coefficients were in the range of 0.931 and 0.591 with mean of 0.754	Gogoi et al. (2018)
19	SSR (32); morphological traits (12)	30 sesame genotypes	Marker-based and morphological trait-based dendrogram revealed that genotypes from same geographical area were grouped into different clusters; PIC value for the discriminative markers ranged from 0.07 to 0.87	Bhattacharjee et al. (2019)
20	ISSR (4); morphological qualitative traits (5)	10 Ethiopian genotypes	Molecular markers revealed 56.25–100% polymorphism; five sesame genotypes formed one group, while three other grouped to their own clusters	Kahsay et al. (2020)

11.4 Sesame Improvement in the Post-genomics Era

11.4.1 Sesame Genome Modification

Suppose a set of tools and techniques are used for the modification or manipulation of the genome of an organism that does not occur in nature. In that case, such a modification is called genome modification, genetic manipulation, or genetic engineering. Genetic manipulation helps mobilize gene resources across the taxonomic barriers, making it possible to create a myriad of variability by using varied combinations of genes from a wide array of biodiversity to achieve the target biological process(es) and/or product(s) to serve the humankind. Genome editing or engineering helps introduce new traits and knock out already existing undesirable ones. Advanced tools such as CRISPR/Cas9-based genome editing have allowed for achieving required genome modification and functional genomic analyses in crop plants, including sesame (You et al. 2022).

Genome editing requires prior knowledge of functional genomics of the trait to be modified. Aside from this, it involves tedious steps of vital procedural importance such as the development of gene construct having validated *cis*-regulatory elements including terminator and promoter sequences, repeatable in vitro culture and genetic transformation procedures, selection markers, and methods for hardening and acclimatization of transformed plants up to the stage of obtaining T_0 generation seeds. Stable integration of transgene is another crucial feature of successful transgenic technology, and therefore, it is needed to be confirmed through empirical molecular analyses. In addition, genomic location and genetic background influence the transgene's desired biological effect(s). Therefore. The technical advantage of genome editing approaches needs to be explored for functional analysis of gene (s) and their modification for commercial benefits.

11.4.1.1 Fundamental Prerequisites for Genome Engineering

As discussed herein before, genetic manipulation strategy involves validation and confirmation of suitable gene(s) to be modified, *cis*-regulatory or enhancer sequences including promoters and terminators to be employed, gene expression pattern and pathways involved, etc. The other key procedural requirements are strategy and protocols for transgene construct delivery for achieving stable integration into the target organism's genome, selection of transformants, and acclimatization for life cycle completion to obtain transgenic seeds. In the following subsections, we briefly discuss these requirements with special reference to sesame.

11.4.1.2 In Vitro Culturing of Sesame

Developing transgenic genotypes in sesame, as in any plant species, necessitates repeatable in vitro regeneration and transgene delivery methods. These procedural requirements are critical to the efficiency of transgene integration and realization of transgene product(s) or effect(s). Optimization of parameters, namely, nutrient media, growth condition, hormonal regime, frequency of subculturing, and plant

parts to be deployed as explants, is important for the successful in vitro culturing of sesame.

The effectiveness and the efficiency of regeneration and, therefore, that of transformation depend on the nature of the selection marker and the kind of antibiotics deployed during the selection of transformed cells against the non-transformants (Zhang et al. 2000; Kumaraswamy 2000; Penna et al. 2002). Transformed cells selectively grow on the culture media containing herbicides such as glyphosate or antibiotics such as hygromycin, phosphinothricin, and kanamycin, as transformed cells alone can neutralize the effect of these selection chemicals with the help of corresponding degrading enzymes produced by the deployed selectable marker genes "*gox*," "*hpt*," "*bar*," and "*nptII*." Thus, even in the chimeric tissue (e.g., callus), the selection agents coupled with the products of selectable marker genes integrated with the transgene in the recombinant construct assist the selective survival, growth, development, and regeneration of only transformed cells, while non-transformed cells get killed at the initial stage of selection cycle itself (Zhang et al. 2000). Besides, deployment of the selection markers helps overcoming the inherent problems associated with low efficiency of transformation (Jones 2003).

Cell, tissue, and organ culture in sesame provides a critical tool for sesame genetic improvement not only by providing means for genetic transformation and genome editing but also for embryo rescue of distant hybridization (Yang et al. 2017) and doubled haploid production through anther/ovary culture. However, highly reproducible protocols for efficient regeneration up to R_0 seed production are yet to be developed. Reported sesame tissue culture and plant regeneration works are reviewed in Miao et al. (2021). Culture-time contamination is one of the serious problems in realizing successful tissue-cultured plants. Shashidhara et al. (2011) reported that while Alternaria, Rhizopus, and Trichoderma are the major endogenous contaminants, Bacteria, Aspergillus, and Penicillium were the exogenous contaminants. Such factors must be considered while carrying out routine protocols such as disinfecting seed material and glass wares.

Different variants of protocols work for different genotypes. For instance, genotype "Darak" was used by Seo et al. (2007); Wadeyar and Lokesha (2011) used genotypes such as "DS-1," "E-8," and "W-II"; genotype "RT-54" (Kushwaha and Khan 2011) and "SVPR-1" (Raja and Jayabalan 2011) were also used in tissue culture experiments. The type and age of explants play another important role in the successful in vitro culturing of sesame in terms of developmental pathways. While culturing of de-embryonated cotyledons could give rise to multiple shoot production (Seo et al. 2007), hypocotyl leads to callus-mediated regeneration (Kushwaha and Khan 2011; Wadeyar and Lokesha 2011), and nodal explants and shoot tips resulted in shoot regeneration and flower bud formation (Raja and Jayabalan 2011).

Seo et al. (2007) reported high-efficiency sesame in vitro regeneration protocol where they used Murashige and Skoog (MS) basal medium supplemented with 5.7 μM indole-3-acetic acid (IAA) along with 22.2 μM 6-benzylaminopurin (BA) to obtain adventitious shoots. They reported that AgNO3 (29.4 μM) and abscisic acid (3.8 μM ABA) enhanced the efficiency. When cotyledon explants were cultured for 2 weeks on media containing 6–9% sucrose before exposing

them to a low sucrose concentration of 3%, an elevated frequency of adventitious shoot formation was recorded. The deployment of high sucrose concentration (6–9%) for 2-week-long pre-culturing of cotyledon explants followed by exposure to 3% sucrose resulted in further efficiency enhancement. Root induction was exhibited by 2.7 μM of α-naphthalene acetic acid (NAA). Wadeyar and Lokesha (2011) used the hypocotyl to induce callus. They sub-cultured it for 2 weeks on high sucrose (6–9%), followed by culturing it on MS media with 3% sucrose and then to MS supplemented with 20 μM silver nitrate (AgNO3), 3.5 mg/L BAP, and 2.5 mg/L NAA.

Raja and Jayabalan (2011) could get 91.8% of explants responding to shoot regeneration at an average of 25.9 shoots when shoot tips were used as explants to culture on Murashige and Skoog media carrying 0.3 mg/L NAA and 2.0 mg/L BAP. Further, they could observe rooting and in vitro flowering on MS media supplemented with 0.03 mg/L BAP and 1.5 mg/L NAA. They could successfully acclimatize plantlets under protected conditions. Kushwaha and Khan (2011) could achieve callus induction when in vitro seedling-derived hypocotyl segments of sesame cultivar RT-54 were cultured on MS basal media with a hormonal regime of 3.0 mg/L 2,4-dichloro phenoxy acetic acid. They could get shoot regeneration (85%) with 6.0 mg/L BAP and 2.0 mg/L NAA from 40-day-old callus, and shoot elongation was achieved with 6 mg/L BAP combined with 20% coconut water or a combination of 8.0 mg/L and 05. Mg/L NAA. Rooting (85–90%) was caused by 2.0 mg/L IBA, and 80–85% of seedlings survived in the natural field condition upon acclimatization.

11.4.1.3 Genetic Transformation Studies in Sesame

Globally there is limited work on sesame genetic transformation. Yadav et al. (2010) attempted to standardize agrobacterium-mediated genetic transformation protocol using a reporter β-glucuronidase (GUS) gene (*uidA*) and a selection marker gene neomycin phosphotransferase gene (*nptII*) jointly cloned but separated by an intron in a binary vector pCAMBIA2301. Cotyledons were used as explants for agroinfection with the vector, and transformants were allowed to produce green shoots on MS media carrying selection pressure of 25.0 mg/L kanamycin and 400.0 mg/L cefotaxime and supplemented with 25.0 μM BA and were further rooted with 2.0 uM IBA and 5.0 mg/L kanamycin. Transformants (T_0) were confirmed using GUS assay, Southern blotting, and polymerase chain reaction with gene-specific primers.

Jin et al. (2001) studied the effect of the SeFAD2 gene encoding a microsomal ω-6 desaturase on linoleic acid levels in sesame (*Sesamum indicum* L.) seeds and based on the phylogenetic analysis. It was found that the SeFAD2 gene might have diverged as a different member of a family. Driven by a seed-specific promoter, the SeFAD2 gene expresses 18–27 days post-bloom. They observed that levels of linoleic acid were concomitant with that of SeFAD2 transcript, changing the hitherto assumption that linoleic acid played a role in the synthesis of stored linoleic acid in sesame seed.

The seed-specific expression of the gene of stearoyl-acyl carrier protein desaturase (SACPD) was characterized by Yukawa et al. (1996) by cloning its cDNA. Interestingly, they could isolate and clone two cDNAs of the gene: CDES01 and CDES04; these differed with respect or expression pattern. While the messenger RNA of CDES01 was found at low in young levels, its products accumulated along with that of CDES04 only in developing seeds 21 days post anthesis. The existence of a distinct regulatory pattern suggests that at least two isozymes of ASCPD exist in sesame.

11.4.2 Potentials of Genome Editing in Sesame

With the help of a genome editing tool, it is possible to design tailor-made crop plants. Already witnessed soybean (Bao et al. 2020) and maize (Young et al. 2019) will create a wave of impact on crop breeding due to which it will be the most used genetic modification tool in the twenty-first century. The following four types of genetic engineering tools can be used for making an edited genome:

- Clustered regularly interspaced short palindromic repeats/CRISPR-associated protein (CRISPR/Cas, Barrangou et al. 2007; Jansen et al. 2002; Zhang et al. 2016)
- Zinc finger nucleases (ZFNs, Urnov et al. 2005; Baltes et al. 2014)
- Base editing system where nucleotide deaminase is fused with a Cas9-D10A nickase (nCas9, Chen et al. 2017; Li et al. 2017; Qin et al. 2020; Zong et al. 2017)
- Transcription activator-like effector nucleases (TALENs, Christian et al. 2010; Haun et al. 2014)

Global literature search suggested that no genome editing work has been reported in sesame. However, the first report of successful deployment of CRISPR/Cas9 tool to accomplish targeted editing of the sesame genome has most recently been made by You et al. (2022). In their investigation, they designed two single guide RNAs (sgRNAs) to target *CYP81Q1* and *CYP92B14* gene sequences for functional validation of their vital role in sesamin and sesamolin biosynthesis, respectively. Disruption of sesamin and sesamolin synthesis in transgenic tissue (hairy roots) proved the critical role of the genes in their biosynthesis. The targeted insertion-deletion (InDel) mutations were achieved to the efficiency of 93.33% and 90.63% in *CYP92B14* and *CYP81Q1*, respectively. It is imperative to note that despite mismatches, *CYP81Q1*-sg RNA did not show any off-target consequences. Their findings demonstrate that sesame functional genomics is empowered with CRISPR/Cas9 tool aided by hairy-root method of delivering sg-RNA-harboring gene construct into plant cell interior (You et al. 2022).

With the successful demonstration of CRISPR/Cas9-mediated genome editing (You et al. 2022) and the availability of tissue culture (Kushwaha and Khan 2011; Raja and Jayabalan 2011; Wadeyar and Lokesha 2011) and non-tissue culture (Ellison et al. 2020; Maher et al. 2020) modes of DNA delivery in sesame, concerted

and coordinated research efforts are required to be directed towards genome editing of sesame for functional genomics, metabolic engineering, and sesame crop improvement. Further, the possibility needs to be explored in sesame for developing sesame genotype resistance or tolerance to biotic and or abiotic stresses as well as meeting other breeding objectives.

11.5 Biotic Stress Tolerance in Sesame

11.5.1 Biotic Stress

11.5.1.1 Insect Pests

Several pests and diseases in sesame have emerged as serious problems. Antigastra, caused by *Antigastra catalaunalis* Dup. (Pyralidae), is one of the devastating pest problems in sesame cultivation. Leaf webber-infested sesame plant is depicted in Fig. 11.3. Mukherji (1947) reported that the food preference of the larvae depends on the total soluble salts of cell sap contents. Therefore, he created a synthetic hybrid of *S. orientale* and *S. prostratum* and demonstrated their resistance to the larva.

11.5.1.2 Diseases

A fungal species *Macrophomina phaseolina* causes charcoal rot in sesame. Yang et al. (2017) developed inter-specific crosses between wild sesame *S. indicatum*, a cultivar Zhongzhi 14, and an autotetraploid of Zhongzhi 14 (Yang et al. 2017). They confirmed the hybrid nature of the progenies using cytological and molecular marker

Fig. 11.3 Leaf webber-infested sesame under field condition

techniques. The degree of the disease resistance was assessed using the artificial inoculation method. The inter-specific hybrid of the cross: *S. indicatum* X Zhongzhi 14 exhibited the maximum degree of charcoal rot resistance (measured by infection lesion length of 6.65 cm) compared to those of other combinations of the crosses. However, it was of intermediate degree compared to that of *S. indicatum* (4.80 cm), diploid Zhongzhi 14 (14.30 cm), and autotetraploid Zhongzhi 14 (11.46 cm). Phyllody, Macrophomina, and Fusarium wilt are the other serious diseases in sesame, and concerted efforts are required to develop resistant or tolerant sources of sesame genotypes. In addition, pre- and post-emergence herbicides are required to be developed to reduce the cost of sesame cultivation.

11.5.2 Abiotic Stress Tolerance in Sesame

Drought tolerance, waterlogging tolerance, salt tolerance, and heavy metal tolerance studies in sesame are limited. Waterlogging is among the most significant factors constraining sesame production (Van Rheenen 1973; Khidir 1997; Osman 1985; Islam et al. 2016; Li et al. 2017). Changing climate poses the risk of heavy and continuous rainfall, resulting in waterlogging-induced damage to sensitive crop plants, particularly sesame. The loss of sesame seed yields due to waterlogging ranges from 30 to 100% worldwide (Wang et al. 2012b, 2016; Li et al. 2017) and 15 to 80% in India (Athul 2016; Sangeeta et al. 2019; Sreepriya and Girija 2020), depending on the duration of waterlogging, the growth stage of the crop, and type of soil (Sarkar et al. 2016). In the last 2 years, excess rainfall caused 75% of crop loss in Gujarat and the Saurashtra region in India (Faldu 2019; Sanghavi and Lashmi-Patel 2021).

Sesame is a crop of choice for small and marginal farmers who cultivate it on soil with poor and marginal fertility (Kumaraswamy et al. 2015). On soil with poor soil aeration, waterlogging due to excess rainfall further negatively impacts plant growth (Boru et al. 2001) due to oxygen deficiency (Kozolwski 1984). Sesame is more sensitive to waterlogging at the seedling establishment stage (Sarkar et al. 2016). Since waterlogging is a complex mechanism, a holistic and comprehensive understanding of the underlying mechanism is a prerequisite for initiating sesame breeding programs for waterlogging tolerance in sesame.

11.6 Applications of Genomics and Post-genomic Approaches in Sesame

11.6.1 Seed and Seed Oil Quality Engineering in Sesame

Unfortunately, in nature, nutrient factors mostly go hand in hand with antinutritional factors in the seeds of crop species, including sesame, which requires biotechnological intervention to separate them. Aside from this, desirable nutritional traits are needed to be included as value addition to enhance the nutritional gain of sesame

oils. This necessitates the modification of nutritional aspects of seeds and seed oils in sesame. For instance, genome editing tools CRISPR/Cas9 offer technological empowerment for seed and seed oil quality engineering in sesame. For example, modification of oil biosynthetic pathway to achieve enhanced levels of unsaturated fatty acids and reduced levels of saturated fatty acids is vital for securing nutrition through engineered sesame seed and seed oil.

There are different ways of modifying fatty acid quality: physico-chemical methods, including partial fractionation and hydrogenation of oils (Thimm et al. 2004). However, these methods are costlier and result in unwanted components in the final products. Therefore, genetic modification of sesame for nutritionally enhanced oil quality is a viable option, not only from the nutritional security point of view but also for the economic profitability of the sesame farmers, for it may help them fetch premium market prices. Efforts are being made to alter bioactive compounds, including antioxidants, namely, sesamolin and sesamin, in sesame by employing conventional breeding (reviewed in Kumaraswamy et al. 2015) as well as genome editing (You et al. 2022). While the breeding approaches are limited to naturally available variability within the sesame species, genetic engineering helps appropriation of gene wealth from other taxonomic units, and genome editing offers the creation of targeted and desirable variabilities that are naturally not present in sesame.

The current global trends suggest that the increasing demand for vegetable oils with nutritional value addition will be on an accelerated trajectory. This warrants that concerted global research efforts must be directed towards functional genomics focused on investigating the individual role of gene sets and metabolic engineering, particularly for oil quality and value addition, using advanced genome editing tools and accelerated breeding approaches.

The biotechnological method of quality oil engineering provides efficiency and ecological and economic advantages against physico-chemical methods (Hosur et al. 2020). For specific modification of fatty acid composition, genetic modification strategies need to be so oriented that unintended or adverse effect(s) and off-targets remain unaltered. However, unforeseen favorable effect(s) rather contribute(s) to extra value addition. In sesame seed oil, for instance, elevated tocopherol and lignan levels may cause favorable effects of enhanced oil quality, ultimately resulting in better keeping quality of the oil.

Using molecular marker-assisted back-cross breeding approach, nutritionally vital traits, including high antioxidant quality, must be transferred from wild relatives to popular cultivars. Sesame seed oil comprising 45–50% of the total mass of the seed contains numerous bioactive compounds that add health and nutritional values to the product. A detailed investigation into the metabolic network leading to the biosynthesis of different kinds of bioactive compounds needs to be undertaken before venturing into metabolic engineering for oil quality (Pathak et al. 2014; Kumaraswamy et al. 2015).

Sesame seed is naturally endowed with health-benefiting compounds with wide spectrum of applications (Pathak et al. 2014), including health foods (Cheng et al. 2006). In addition, oil extracted from sesame seeds also contains various beneficial

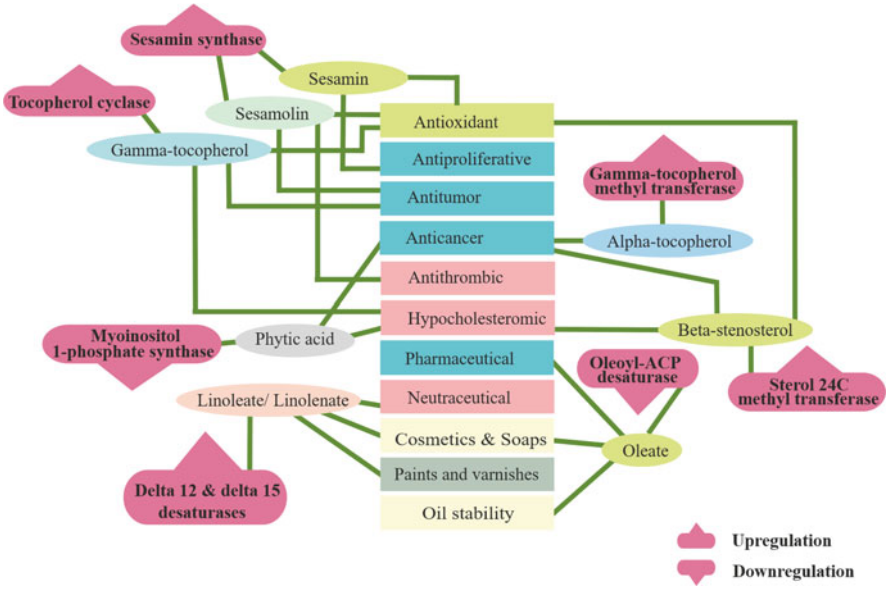

Fig. 11.4 Strategies for oil quality engineering in sesame. Industrial applications (represented by rectangular shapes) of bioactive compounds (represented by oval shapes) are given in the central column, and respective enzymes to be upregulated or downregulated to produce corresponding compounds are represented by upward and downward callout shapes, respectively

compounds such as sesamin, sesamolin, gamma-tocopherol, alpha-tocopherol, oleate, linoleate (linolenate), beta-sinosterol, and phytic acid. Through upregulation or downregulation of rate-limiting enzymes taking part in the biochemical pathways leading to production of respective enzymes, it is possible to enhance required compound and diminish undesired components in engineered sesame oil (Pathak et al. 2014; Kumaraswamy et al. 2015), and overview of the strategy is illustrated in Fig. 11.4.

11.6.2 Utilization of Sesame Oilcake/Meal

The by-product obtained after oil extraction from oleaginous material is called oil cake/meal. It is economically important as it is rich in minerals, protein, and other nutrients (Table 11.2). Sesame cake is rich in dietary fiber, essential amino acids, antioxidants, and health enhancers such as glucosides of triglucosides of sesaminol and sesamolinol (Sarkis et al. 2014; Shu et al. 2019).

Valorizing sesame cake is a viable option to utilize lipids and proteins from the sesame seed. By this method, what is otherwise waste can be efficiently as well as effectively used in the food chain (Nunes et al. 2018; Hosur et al. 2020; Melo et al. 2021).

Table 11.2 Nutrient components of sesame seed (reviewed by Pathak et al. 2014)

Constituent	Composition %
Moisture	6–7
Proteins	20–28
Oil	48–55
Sugars	14–16
Fiber content	6–8
Minerals	5–7

11.7 Conclusions

Even though sesame is an important oilseed crop from nutritional, industrial, and pharmaceutical viewpoints, the benefit of advancement in molecular biology and biotechnology is yet to be harnessed in sesame crop improvement. Development of high-density linkage map, consensus linkage maps, marker-trait association studies, and deployment of genome editing is required to be focused as high-priority area of research at the global level, and concerted efforts are needed worldwide to develop plant idiotypes suitable for mechanical harvesting, high-density planting, plant types with engineered quality seeds oil, value addition with bioactive compounds, and sesame genotype resistance or tolerance to abiotic as well as biotic stresses.

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Sugar Signaling and Their Interplay in Mitigating Abiotic Stresses in Plant: A Molecular Perspective

12

Vishal Varshney, Jawahar Singh, and Prafull Salvi

Abstract

Recently, carbohydrates and/or sugars have emerged as crucial components for improving plant tolerance to abiotic stress. Abiotic stressors such as drought, salinity, severe temperature, and so on can create an accumulation of soluble sugars as well as sugar alcohols or polyols. In particular, sugars function as storage compounds, energy reservoirs, structural components, and plant signaling molecules. In addition to their accumulation, sugar transport via transporters performs important functions in overall plant growth and development at different levels. Several studies have shown their important role in plant adaptation to various abiotic conditions. We tried to include and emphasize the significance of sugar(s) signaling and their various roles in plant abiotic stress tolerance. This chapter also examines some of the key regulatory aspects of sugar metabolic pathways and the challenges and impediments to enhancing abiotic stress tolerance by manipulating sugar metabolism. Several biotechnological research in the post-genomics age can assist in developing climate-resilient crop plants under various abiotic stressors. Such techniques for agricultural enhancement, sustainable agriculture, and producing stress-tolerant crops were considered. In a

V. Varshney
Govt. Shaheed Gend Singh College, Charama, Chhattisgarh, India

J. Singh
Laboratorio De Genómica Funcional De Leguminosas, Facultad De Estudios Superiores, Iztacala, Universidad Nacional Autónoma De México, Tlalnepantla, Estado De Mexico, Mexico

P. Salvi (✉)
Agriculture Biotechnology Department, National Agri-Food Biotechnology Institute, Mohali, Punjab, India
e-mail: prafull.salvi@nabi.res.in; https://loop.frontiersin.org/people/268859/overview; https://publons.com/researcher/AAP-1007-2020/

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demanding context, we also highlight potential scientific challenges and future research directions in the involvement of plant sugar biology in enhancing abiotic stress.

Keywords

Sugars · Abiotic stress · Sugar transporters · Signaling · Genetic engineering · Crop improvement

12.1 Introduction

Owing to the sedentary nature of plants, their exposure to environmental constraints is inevitable. These stressful environmental conditions, which include drought or low water accessibility; extreme temperature (heat or cold); inadequate light; soil pH, structure, or texture; and the availability of ions in the soil, are commonly called abiotic factors (Rosa et al. 2009a, b; Lunn et al. 2014; Salvi et al. 2022). These factors are expected to reduce global food yields by more than half and harm more than 80% of the world's land surface (Cramer et al. 2011). Majorly the mechanism of any abiotic stress in plants involves three basic stages: sensing, signaling, and response (Gangola and Ramadoss 2018). When any of the abiotic variables are experienced by plants, their first response is to sense the change or adverse condition through numerous physical and biochemical processes. After sensing, with the aid of secondary messengers like calcium, reactive oxygen species (ROS), ADP, etc. trigger and amplify the plant cell's signaling cascade, which activates the resistance or responsive machinery and leads to the third phase, i.e., response. The third phase encompasses the alterations in the physiological activities of plant cells. Persisting unfavorable or extreme conditions result in sets of changes like reduction in photosynthesis ability, inhibition of water transport, deficiency symptoms, overaccumulation of ions, ROS outburst, etc. that collectively affect the plant growth and development (Van den Ende and El-Esawe 2014). As a result, abiotic stress is one of the most severe threats to agricultural crop productivity, and it must be addressed on a priority basis to feed the world's rising population (Bevan et al. 2017).

New strategies for designing varieties or cultivars with desirable traits which can endure and tolerate maximum production potential have become important. Even though most abiotic stressors are complicated and multigenic regulated, significant progress in breeding resistant crops has been accomplished. However, climate change-related issues have forced the use of new technologies to understand better stress perception, signal transduction, and plant stress tolerance systems (Zhang et al. 2018c; Vats et al. 2022). Carbohydrates and/or sugars have emerged as promising components for enhancing or boosting plant tolerance to abiotic stress in recent years (Sami et al. 2016; Kaur et al. 2021; Salvi et al. 2022). Carbohydrates are the fundamental cellular elements, characterized by the basic chemical formula [Cx (H_2O)y], and contain carbon hydrates (Hernandez-Marin and Martínez 2012).

Sugars are polyhydroxy aldehydes or ketones that have been classified mainly by molecular size, individual monomer properties, degree of polymerization (DP), and type of linkages. Based on the characteristics above, sugars are divided into four groups: monosaccharides (DP 1), disaccharides (DP 2), oligosaccharides (DP 3–9), and polysaccharides (DP >10) (Cummings and Stephen 2007). Sugars have a role in various metabolic, structural, and physiological aspects of a plant's growth and development. They function as storage compounds as reserve energy, energy reserves to sink organs, and as a precursor for various metabolic activities (Gangola and Ramadoss 2018). They also function as osmoprotectants and a regulatory molecular switch for regulating many genes involved in the abiotic stress tolerance mechanism (Rosa et al. 2009b). So, they have been highly investigated for their crucial function in abiotic stress resistance and/or tolerance in the recent decade. Carbohydrate partitioning is the sugar absorption, transport, and distribution process from the source (leaves) to sink or storage organs that requires energy (Slama et al. 2015; Kaur et al. 2021). Plants may also govern glucose partitioning via several transporters, which coordinate signals in different stress responses, including biotic and abiotic stress (Diehn et al. 2019). Sucrose transporters (SUT), monosaccharide transporters (MST), and sugars will be exported transporter (SWEET) are examples of these (Chen et al. 2010; Salvi et al. 2022). At multiple levels, sucrose transporters are closely controlled, allowing plants to adjust to environmental stimuli such as light regime, temperature, pathogen attack, etc. These findings highlight the need to combine abiotic stress and sugar signaling into a functional paradigm and develop techniques to improve abiotic stress tolerance using biotechnological technologies (Saddhe et al. 2021).

This chapter highlights the importance of sugar(s) signaling and their diverse role as well as sugar partitioning via sugar transporters during plant abiotic stress tolerance. This chapter also discusses some important regulatory facets of sugar metabolic pathways and the challenges and obstacles in engineering the metabolic sugar process for improving abiotic stress tolerance. Several biotechnological studies can aid in developing climate-resilient crop plants under different abiotic stresses in the post-genomics era. We discussed such approaches for crop improvement, sustainable agriculture, and developing stress-tolerant crops. We also discuss possible scientific problems and future research paths in plant sugar transporter biology in a stressful environment.

12.2 Sugar and Its Associated Components in Plant: An Overview

Plants use light energy to fix water and carbon dioxide in their chloroplasts via photosynthesis, and sugars are formed. The plant produces various sugars that can be used for structural and non-structural purposes. Like cellulose and hemicelluloses, long-chain molecules are made up of structural carbohydrates that contribute to plant structure and biomass (Hartmann and Trumbore 2016). On the contrary, monosaccharides (trioses, tetroses, pentoses, and hexoses), disaccharides (sucrose,

trehalose, and maltose), oligosaccharides (stachyose, raffinose), and polysaccharides (raffinose, stachyose) are non-structural or soluble sugars that regulate a variety of functions like energy reserve, precursors for many metabolic compounds, a signaling molecule, as well as an osmoprotectants (Salmon et al. 2020). Sucrose is the most important storage and transport molecule in most plants due to its non-reducing and little chemical activity. It consists of one glucose and fructose molecule that are connected by (1–2) glycosidic bond (Chibbar et al. 2016). Sucrose can be transported in either a symplastic or apoplastic manner to sink tissues and phloem cells. It can be maintained in the vacuole by tonoplast transporters or metabolized into glucose and fructose by invertase (Rosa et al. 2009b). Sucrose, along with proline and glycine-betaine, is the most prevalent osmolyte among monocot halophytes (Slama et al. 2015). In contrast, many soluble sugars like glucose, fructose, maltose, sucrose, and galactinol and sugar alcohols like mannitol, ononitol, pinitol, etc. are all prevalent osmolytes in dicot halophytes (Slama et al. 2015; Salvi et al. 2018). Next to sucrose, raffinose family oligosaccharides (RFOs) are the most prevalent soluble sugars that are found to be derivatives of galactosyl sucrose, and mainly include raffinose, stachyose, and verbascose (Martínez-Villaluenga et al. 2008; Salvi et al. 2016, 2020, 2021a). RFOs are essential photosynthetic transporter among the family members of Verbenaceae, Cucurbitaceae, Scrophulariaceae, Lamiaceae, and Oleaceae (Gangola and Ramadoss 2018).

Several abiotic stresses like drought, salinity, extreme temperature, low availability of nutrition, etc. can cause the accumulation of several soluble sugars like glucose, sucrose, trehalose, and sugar alcohols or polyols sorbitol and mannitol (Gangola and Ramadoss 2018). Sorbitol and/or mannitol are the major suitable solutes and antioxidants that protect *Apium graveolens* (celery) and many species of woody Rosaceae from different abiotic stresses. Glucose is a versatile signaling molecule and a metabolite that is involved in the control of various processes (Kiba et al. 2019). Hexokinase (HKX) detects glucose levels through a glucose HXK sensor, modulates cellular functions, and phosphorylates hexose carbohydrates for metabolic activity. The target of rapamycin (TOR) kinase signaling cascade controls the metabolism of stress-responsive carbohydrates such as glucose, sucrose, and starch. Also, it contains effector genes implicated in abiotic stress responses (Ahmad et al. 2020). Through HXK activity, glucose is converted to glucose 6-phosphate (G6P), which is then used to synthesize polyols such as mannitol, sorbitol, and inositol.

Similarly, sucrose is the most abundant sugar transportable between source and sink in plants, impacting physiological and cellular signaling pathways (Sakr et al. 2018). Several abiotic stimuli activate sucrose catabolic enzymes such as invertase and sucrose synthase (SUS), which generate sugars like fructose and glucose. Likewise, trehalose is an important disaccharide formed by two glucose molecules connected with the α-1-1 alpha bond and helps in maintaining the membrane lipids by acting as an osmolyte (Saddhe et al. 2021). Additionally, trehalose has been shown to preserve protein structure and scavenge ROS (Zulfiqar et al. 2019). Trehalose-6-phosphate (T6P) is an intermediate metabolite that plays a role in photosynthesis, sugar metabolism, and environmental response. G6P and T6P can

inhibit snRK1 activity. T6P levels in cells are precisely proportional to sucrose concentrations, suggesting that T6P can act as an endogenous stimulus and control sucrose levels via a negative feedback regulation (Sakr et al. 2018). In the vacuole, fructosyltransferase (Fts) synthesizes fructans, which interact directly with the lipid group of the membrane to maintain lipid phase transitions and fluidity, contributing to cold and drought tolerance (Ahmad et al. 2020). Sugar and its associated components have a prominent and promising role in acquiring abiotic stress tolerance and can be used for further study (Fig. 12.1).

12.3 Sugar Signaling in Plant's Metabolism

During abiotic stress tolerance, sugars serve as signaling molecules in plants and act as storage compounds, energy reservoirs, and structural molecules (Li and Sheen 2016). Sugar signaling also involves the same three basic phases of signaling mechanism sensing, signal transduction, and target gene(s) expression modulation. In plant cells, sugars are detected primarily by hexokinase (HKX)-dependent or HKX-independent mechanisms. HKX-dependent mechanisms can sense sugars with phosphorylation, whereas HKX-independent pathways can sense sugars without phosphorylation (Van den Ende and El-Esawe 2014). HKX is a multigenic family found in almost all plant species, including *Arabidopsis thaliana* (6), *Zea mays* (9), *Solanum tuberosum* (2), *Nicotiana tabacum* (9), *Oryza sativa* (10), *Vitis vinifera* (5), etc. (Paulina Aguilera-Alvarado and Sanchez-Nieto 2017; Gangola and Ramadoss 2018). Based on their subcellular location, HXKs are divided into four groups: type A HXKs (having one 30-amino-acid (aa)-long hydrophobic sequence with an N-terminal chloroplast signal), type B HXKs (having one 24-aa-long hydrophobic helix that attaches to the mitochondria), type C HXKs (lack signal peptide and membrane attachment), and type D HKX (mitochondrial HKX, but possess different peptide sequences from type B HKXs) (Paulina Aguilera-Alvarado and Sanchez-Nieto 2017). Among all four classes of HKXs, type B HXKs are the most investigated ones, commonly with nuclear-directing signals, and are critical for sugar signaling under normal and stressful environmental circumstances in plants. When glucose levels are high, the nuclear-localized HXK in collaboration with the 26S proteasome forms a glucose-signal complex that inhibits photosynthesis. However, low glucose level disrupts the HXK-mediated signal from abiotic stress. But the HXK's intracellular sugar sensing location is still being investigated or unexplored; new findings will shed more light on the mechanism underlying (Valluru et al. 2016).

A sucrose-specific signaling route has been established to influence photosynthesis and the formation of fructan sugar and anthocyanin pigment. The balance between sucrose synthesis and degradation, which is controlled by circadian clocks and hormones in plants, determines sucrose buildup. Sucrose signaling has also been linked to additional signaling pathways activated by phytohormones like ABA and light that have been linked to calcium signaling in plants. Although no sucrose sensor has yet been found in plants, sucrose signaling is believed to be transduced to

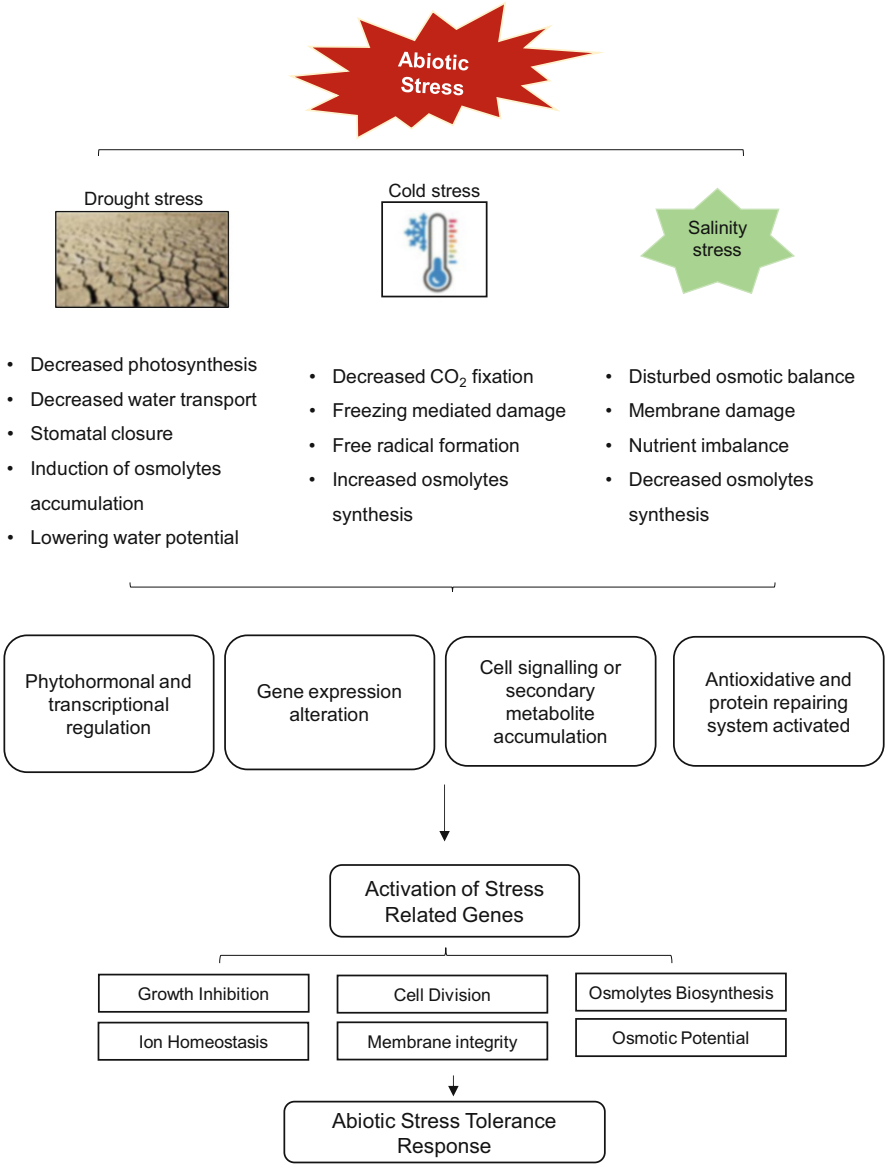

Fig. 12.1 A schematic representation of cellular responses and physiological functions of sugars and their associated processes in acquiring abiotic stress tolerance in plant

T6P signaling that controls anthocyanin production via MYB75, a transcription factor implicated in anthocyanin biosynthesis regulation (Van den Ende and El-Esawe 2014). Interestingly, HXK activity is maintained by glucose generated via invertase-catalyzed processes in the mitochondrion and cytoplasm, which

supports the homeostasis of ROS (Valluru and Van den Ende 2011). SnRK1 is also a key regulator of carbon metabolism, serving as a backup supply of carbon, energy, and metabolites under abiotic stress tolerance (Emanuelle et al. 2016). SnRK1 has been demonstrated to be influenced by sugars or their derivatives, particularly glucose, G6P, and T6P. SnRK1-binding proteins have been demonstrated to regulate SnRK1 function in plant cells in a glucose-dependent manner, whereas G6P and T6P regulate SnRK1 activity via modifying SnRK1 confirmation via an unidentified intermediate molecule. Long-distance signaling in plants might be enabled via sugars and hormones (Salvi et al. 2021b). Hexokinase (HXK) and SnRK1 both interact with plant hormones, help protect plants from abiotic stressors, and are two major components of the sugar signaling cascade (Ljung et al. 2015). Transcription factor-like ABI4 and ANAC060 are two critical components of the sugar-ABA relationship. ABI4 binds to the promoters of sugar-responsive genes to control their expression. The sugar-ABA signaling route also uses ABI4 to induce the production of ANAC060, whose nuclear localization inhibits the sugar-ABA signaling pathway (Ljung et al. 2015). Auxin synthesis and signaling in plants depend on sucrose and glucose, respectively. Sucrose also links the sucrose-GA signaling cascade to brassinosteroids (BRs) and stabilizes the DELLA protein, a negative regulator of GA signaling important for plant development and stress response. In addition, starch metabolism is associated with amylase-mediated BR signaling, which functions as a maltose sensor in plant cells (Ljung et al. 2015; Gangola and Ramadoss 2018).

12.4 Molecular Roles of Sugars in Stress Tolerance

Sugars are chemically active macromolecules that play a key role in plants' physical and chemical processes, such as respiration as respiratory agents, seed germination as energy reserve, photosynthesis as assimilatory compounds, and blooming and senescence as transporting molecules. Consequently, due to their multipurpose roles, any alteration in the sugar content in plants may help provide tolerance to several abiotic stress responses or adaption. Previous studies have also identified the sugars as playing various roles in abiotic stress, helpful in scavenging reactive oxygen species and as osmoprotectants.

12.4.1 Sugars as Scavenging Reactive Oxygen Species (ROS)

Hydrogen peroxide (H_2O_2), hydroxyl radicle (OH^-), superoxide ion radical (O_2), and singlet oxygen (O_2) are the ROS examples in the living world. They are formed as a by-product of aerobic respiration, and their accumulation is in equilibrium with the plant cell's antioxidant system under normal or stress conditions (Kwak et al. 2006). Abiotic stress, on the other hand, causes an increase in the generation of ROS in the cell, disrupting cellular redox equilibrium and leading to the breakdown of essential macromolecules such as proteins or peptides, lipids, or nucleic acid, and it

represents the oxidative stress inside the plant cell (Torres et al. 2006). On the other hand, plant sugars have recently emerged as a novel class of antioxidant compounds. Monosaccharides are rarely found to act as direct antioxidants; instead, they are more likely to influence a plant cell's antioxidative properties indirectly, either through polymerization or acting as second messengers, which increases the production or activity of various antioxidants. Sugars like sucrose, lactose, maltose, and trehalose are common disaccharides with a strong in vitro free-radical quenching effect (Bolouri-Moghaddam et al. 2010). Fructans were shown to have a higher capacity to scavenge ROS than the disaccharides studied (Peshev et al. 2013). However, while disaccharides such as sucrose appear to have the moderate antioxidant capacity, their small size and portability can play a major role in ROS control.

In addition, fructans are associated with increased accumulation of ascorbic acid salt and glutathione, suggesting that they are associated with the cytoplasmic antioxidant network (Bolouri-Moghaddam et al. 2010; Negi et al. 2017; Saxena et al. 2020). During abiotic stress, the vacuolar vesicle-derived extracellular pathway (TVE) can be used to transfer fructans from the vacuole to apoplasts, directly capturing hydroxyl radicals (Van Den Ende and Peshev 2013). In a nutshell, sucrose combines with hydroxyl radicals to generate sucrosyl radicals, which can go through four processes. Sucrosyl radicals can be converted into monosaccharide radicals and nonradicals with and without keto groups in two reactions; however, in the third reaction, sucrosyl radicals can be oxidized, giving hydrate products. Sucrosyl radicals may rejoin in the fourth step to generate distinct oligosaccharides with a greater degree of polymerization. The same principles may apply to other sugars found in plants, although no experimental evidence supports this (Gangola and Ramadoss 2018).

12.4.2 Sugars as Osmoprotectants

Major abiotic stresses like drought and salinity cause dehydration and osmotic stress to plant cells, which can cause hydrophilic connections to be disrupted, biomolecule structural breakdown (especially protein denaturation), organelle collapse, and cell membrane instability (Ozturk et al. 2021). Salt stress causes particular ions like Na^+ and Cl^- to become poisonous, reducing the intake of important minerals, including nitrogen, phosphorus, calcium, and potassium. The Na^+/K^+ ratio in the plant cell is also disrupted by Na^+ toxicity, which is critical for regular cellular processes (Singh et al. 2015). Sugars like sucrose, RFOs, fructans, etc. are the osmoprotectants found in plants (Slama et al. 2015; Salvi et al. 2016). Sugar hydroxyl groups may substitute water molecules in plant cells to sustain hydrophilic contacts, which is critical for maintaining membrane integrity and structure and the native structure of macromolecules (Pukacka et al. 2009). The buildup of osmoprotective carbohydrates is thought to aid in ion partitioning and homeostasis in the plant cell, hence assisting in maintaining correct cell functioning and improving abiotic stress tolerance. Trehalose is the most promising osmotic protective sugar in terms of required concentration (Nahar et al. 2015) and can be replaced with sucrose and

other sugars in plants. Sugar also helps plants develop drought-tolerant structures such as seeds and pollen. As mentioned earlier, the first way sugar provides drought tolerance is by replenishing water. "Vitrification" or glass formation in plant cells is another mechanism of desiccation tolerance. Cell solutions behave like solid plastic or highly viscous solutions. Vitrified cell solutions ensure cell stability by preventing diffusion (Angelovici et al. 2010). RFOs, coupled with LEA proteins and small HSPs, create a glassy cytosol that inhibits monosaccharide production, resulting in lower respiration and inhibition of the Maillard process (Pukacka et al. 2009; Salvi et al. 2016).

12.5 Regulation of Diverse Sugar Transporters Under Abiotic Stress

Sugar transporters play critical roles in plant growth and development at the cellular, tissue, and organ levels. Several studies have shown that they play an important role in plant adaptation to a variety of abiotic conditions (Chen et al. 2015; Saddhe et al. 2021; Salvi et al. 2022). As a result, learning their structure and function contributes to a better understanding of sugar transporters and their underlying mechanisms for developing stress-tolerant plants. The role of different sugar transporters in providing or enhancing the different abiotic stress tolerance has been summarized in Table 12.1.

12.5.1 SWEET Transporters

In plants, SWEET transporters belonging to the sugar efflux or bidirectional transporter family are known to play essential functions in pollen and seed development and nectar production (Chen et al. 2010). Significant progress has been made in understanding their distribution, phylogenetic relationships with other transporters, and structural and functional variations in several groups of plants, from algae to angiosperms, which were higher over the past decade (Doidy et al. 2012, 2019). An optical glucose sensing approach was used to identify this new family of sugar transporters in *Caenorhabditis elegans*, *Homo sapiens*, *Arabidopsis thaliana*, and *Oryza sativa* (Chen et al. 2010). Based on the number of existing MtN3 domains, all SWEET proteins should be classified into two large groups: one with two salivary MtN3 domains and the other with one salivary MtN3 domain.

The participation of the SWEET plant family in the control of sugar transport, abiotic stress tolerance, overall plant growth, seed and fruit development, and nectar secretion has achieved remarkable progress over the past decade (Jeena et al. 2019). Abiotic stressors disrupt metabolic and photosynthetic activities, disrupting sugar homeostasis. In a typical situation, plants maintain tight control over photosynthesis, sugar production, and the distribution of these substances to sink organs (Chen et al. 2012). AtSWEET15 is localized in the plasma membrane of *Arabidopsis thaliana*,

Table 12.1 Functional role of sugar transporters imparting abiotic stresses in plants

Plant species	Sugar transporter	Enhanced tolerance to	References
Arabidopsis thaliana	*AtSWEET16 and AtSWEET17*	Cold stress	Klemens et al. (2014)
	AtSWEET11 and *AtSWEET12*	Cold stress	Le Hir et al. (2015)
	AtSUC4	Salt stress	Gong et al. (2013)
	AtSUC1	Drought stress	Durand et al. (2016)
	AtSUC2 and *AtSUC4*	Salt, osmotic, and low temperature	Gong et al. (2015)
Oryza sativa	*OsGMST1*	Salt stress	Cao et al. (2011)
	OsMST6	Drought and salt stress	Monfared et al. (2020)
	AtSWEET4	Cold stress tolerance	Liu et al. (2016)
Brassica oleracea	*BoSWEET11b*, *11c*, *12b*, *16a*, and *17*	Cold stress	Zhang et al. (2019)
Glycine max	*GmSWEET6* and *GmSWEET15*	Drought stress	Du et al. (2020)
	GmSUC2	Drought stress	Du et al. (2020)
Solanum tuberosum	*StSWEET10b*	Drought stress	Aliche et al. (2020)
	StSUT2	Drought stress	Aliche et al. (2020)
Saccharum spontaneum, *S. robustum*, *S. officinarum*	*SaSUT1-6*	Drought stress	Zhang et al. (2016)
Vitis vinifera	*VvSUC11*, *VvSUC12*, and *VvSUC27*	Cold and osmotic stress	Cai et al. (2021)
	VvSUC27	Salt, oxidative, and drought stress	Cai et al. (2017)
Gossypium hirsutum	*GhSWEET20* and *GhSWEET51*	Heat, drought, cold, and salt stress	Li et al. (2018)
Populus	*PtaSUT4*	Drought stress	Frost et al. (2012)
Medicago truncatula	*MtSWEET1a*, *2b*, *3c*, *9b*, *13*, *15c*, and *16*	Cold, drought, and salt stress	Hu et al. (2019)
Camellia sinensis	*CsSWEET16*	Cold stress	Wang et al. (2018)
Musa acuminata	*MaSWEET4b*, *14c*, *4c*, *and 14d*	Cold, drought, and salt stress	Miao et al. (2017)
Dianthus spiculifolius	*DsSWEET12*	Osmotic and oxidative stress	Zhou et al. (2018a)
	DsSWEET17	Salt, osmotic, and oxidative stress	Zhou et al. (2018b)

whose transcriptional levels are significantly higher during drought, meaning that it plays a role in the release of sucrose apoplasts (Hennion et al. 2019).

AtSWEET15 is activated during leaf aging and osmotic stressors such as salt, cold, and drought via abscisic acid-dependent pathways (Julius et al. 2017). Plants that overexpress *AtSWEET15* have faster leaf aging and are more susceptible to high salt stress, while *AtSWEET15* variants are less susceptible to salt stress (Chen et al. 2015). Under cold, low-nitrogen conditions, studies demonstrated that *AtSWEET16* and *17* largely regulate glucose or fructose levels in *Arabidopsis* leaf and root stem cells (Klemens et al. 2014). The single and double mutants of *Arabidopsissweet11 sweet12* were more cold-tolerant than the wild type (Le Hir et al. 2015).

Wild-type Arabidopsis plants showed dramatically altered electrical conductivity compared to gene knockdown and *AtSWEET4*-overexpressing lines. In addition, increased hexose sugars (glucose and fructose) have been shown to protect plants from cold stress (Salvi et al. 2022). Salt stress has been reported to alter the expression of sucrose synthase (*SUSY1*) and several sugar transporters such as TMT and SWEET (Sellami et al. 2019). Hu et al. (2019) found that the *M. truncatula* genome contains 25 SWEET genes, and half showed a significant increase in transcripts during cold, salt, and drought stress. Drought, salt, and cold treatment dramatically changes the transcriptional levels of seven *MtSWEET* genes (Hu et al. 2019). Thirty *SWEET* genes have been found in *Brassica oleracea*, and their expression patterns suggest that five *BoSWEET* members are downregulated in response to cold stress (Zhang et al. 2019).

Gossypium hirsutum genome contains 55 *SWEET* genes, and transcript profiling reveals six *GhSWEET* genes with significant upregulation in heat, drought, cold, and saltwater conditions (Li et al. 2018). Transcript analysis revealed that *GmSWEET6* and *GmSWEET15* are highly upregulated under drought stress among 52 *SWEET* members of soybean (Patil et al. 2015; Du et al. 2020). Tomatoes (*Solanum lycopersicum*) overexpressing *MdSWEET17* of apples (*Malus domestica*) showed increased fructose accumulation and drought tolerance (Lu et al. 2019). The *SWEET* gene family as a whole plays a variety of roles in stress responses and other physiological processes as well.

12.5.2 Sucrose Transporters (SUT)

The sucrose transporter is a member of one of the most important facilitator superfamilies, the glycoside pentose hexuronide (GPH) cationic symporter family (Reuscher et al. 2014). Members of the GPH family have a 12-transmembrane helix, having cytoplasmic facing N- and C-terminus. Plant growth, biomass degradation, pollen germination, fruit size control, and ethylene biosynthesis are all regulated by SUT. Nine sucrose transporter genes (SUT or SUC) have been found in *Arabidopsis*, but only five SUT members are in the rice genome (Kühn and Grof 2010). The sucrose transporter is involved in phloem loading in source tissue, sucrose absorption in sink cells, and migration of stored vacuoles (Slewinski et al. 2010). Several studies have also been conducted to functionally evaluate sucrose transporters for

their use as candidate genes for abiotic stress tolerance (Julius et al. 2017). Low sucrose levels and salinity, osmolality, cold stress, and other abiotic stressors all cause alternation in the expression of *AtSUC9* (Jia et al. 2015). In addition, the *Atsuc9* mutant showed low levels of endogenous ABA under stress and suppressed ABA-inducible gene expression. Under salt stress, the *Atsc4* mutant had higher levels of glucose, fructose, and sucrose in the shoots than in the roots, leading to an imbalance in sugar distribution (Gong et al. 2013). Salinity, osmotic stress, low temperature, and extrinsic abscisic acid promote *AtSUC2* and *AtSUC4* (Gong et al. 2015).

Rice *OsSUT2* is upregulated in photosynthetic tissues under drought and salt stress, improving sucrose distribution in plants (Zhang et al. 2016). In response to drought, CBL-interacting protein kinases (CIPKs) phosphorylate the sucrose transporter MdSUT2.2 in Ser381 and Ser254 to improve salt tolerance (Chincinska et al. 2008). Overexpression of SUC27 in tobacco reduced abiotic stress by increasing the activity of reactive oxygen species and abscisic acid-related genes. Under water stress, SUT1 and SUT2 were downregulated with *S. robustum*, while SUT4 and SUT5 were upregulated with the leaf tissue of three *Saccharum* species. Drought stress has a significant impact on carbon uptake, partitioning, and tuber output in *Solanum tuberosum*. Under drought stress, the expression of key genes such as the sucrose transporter (*StSUT2*) was shown to be upregulated (Aliche et al. 2020).

12.5.3 Monosaccharide Sugar Transporter (MST)

MST is a member of the major facilitator superfamily and is involved in carbohydrate flux. These transporters contain 12 transmembrane domains. In *Arabidopsis*, the MST-like gene family comprises 53 genes divided into 7 subfamilies (Büttner 2010). MST regulates various physiological activities, including the distribution of sugars at the intracellular level, and is expressed in response to stress (Kong et al. 2019).

12.5.4 Sugar Transporter Protein (STP)

The STP of plants is a well-studied MST group. It is a sugar/H^+ symporter in plants because it is a multipass transmembrane transporter (with 12 TM helices) (Büttner 2010). During phloem unloading, they are engaged in the absorption of hydrolyzed sucrose in the apoplast area. STP's regulation functions under abiotic stress are well documented in the literature (Kong et al. 2019). The involvement of rice *STP* genes in floral development and abiotic and biotic challenges was revealed by expression analysis. *OsSTP1*, *OsSTP3*, *OsSTP14*, and *OsSTP28* were upregulated in response to submergence, whereas *OsSTP8*, *OsSTP11*, *OsSTP20*, and *OsSTP21* were increased in response to high temperatures. Any extremes in temperature on either side, like heat or cold and submergence stress, demonstrated upregulation of *OsSTP14* (Kong et al. 2019). In a gene expression investigation, one study

discovered that *OsSTP2*, *OsSTP3*, *OsSTP4*, *OsSTP11*, *OsSTP19*, *OsSTP25*, and *OsSTP28* were upregulated in several abiotic responses like drought, salinity, and osmotic stress. *OsSTP10*, *OsSTP1*, and *OsSTP14* were solely upregulated in response to osmotic stress (Deng et al. 2019). These investigations showed that STP has a variety of functions in drought and osmotic stress and also impacts overall plant growth and development.

12.5.5 Polyol Transporters

Polyols (also known as sugar alcohols) are sugar derivatives that can be classified as cyclic (myo-inositol, pinitol, and ononitol) or acyclic (inositol, myo-inositol, mannitol, and sorbitol) (Saxena et al. 2013; Bhattacharya and Kundu 2020). They provide a variety of physiological tasks, including carbon transfer between source and sink organs, osmoprotectant, and antioxidant defense against biotic and abiotic stressors (Noiraud et al. 2001; Bhattacharya and Kundu 2020). Polyols are thought to have osmoprotective properties by generating a hydration sphere around macromolecules, avoiding metabolic deactivation at low osmotic potential (Williamson et al. 2002; Schneider 2015). Under abiotic stress, the polyol transporters (PLT and INT) have distinct expression patterns.

In rice, *OsPLT4* expression was shown to be greater in salt and drought stress than osmotic stress, whereas *PLT13* expression was found to be higher in salt and osmotic stress than drought stress. In the case of *OsPLT4* and *14*, a similar differential expression was found. *OsPLT14* was considerably upregulated during salt stress compared to osmotic and drought stress, but *OsPLT3* was found upregulated under all salt, drought, and osmotic stresses (Deng et al. 2019). Similarly, under salt and osmotic stress, *OsPLT13* was much more upregulated than under drought stress. Under salt stress, *OsPLT14* upregulation was greater than under osmotic and dry stress. Under the three abiotic stressors, *OsPLT3* was considerably upregulated. One study examined transcriptome data from two drought-tolerant *Eruca vesicaria* subs. sativa lines and found ERD6-like 12 transcripts were considerably upregulated when PEG treatment was applied (Hu et al. 2019). Although there are 19 ERD6-like members in *Arabidopsis*, only a handful have been functionally described. The varied functions of ERD6-like members in plant growth development under stress situations would be intriguing to investigate. Three TST genes are encoded by the *A. thaliana* genome and are found on the tonoplast membrane (Schulz et al. 2011).

In cold, drought, and salt stress, AtTMT1 and AtTMT2 were shown to be significantly upregulated. The research investigated the *Beta vulgaris TST2.1* member, which is found in the vacuolar membrane and controls sucrose transport in taproot tissues via proton gradient energy (Klemens et al. 2014). Proteomics technique has been used to quantify abiotic stress-induced alterations in low abundant vacuolar transporters such as tonoplast monosaccharide transporter 2 (TMT2) and found that salt stress increased TMT2 abundance (Julius et al. 2017). Furthermore, TST2 transcript abundance was found to be highly sensitive to diverse abiotic

stressors (salt, drought, and cold) (Hu et al. 2019). TST is a proton/sugar antiporter protein found in the vacuole that primarily transports glucose, fructose, and sucrose.

Furthermore, it is involved in fruit storage, organ growth, and sugar buildup in vacuoles. TST also plays an important function in maintaining cellular osmotic adjustment during abiotic stress by collecting excess carbohydrates in the vacuole. Few plant TST members have been functionally described under abiotic stress tolerance, yet additional research is needed to understand their functional diversity. In *Arabidopsis*, the plastid sugar transporter (pSuT) is involved in the export of glucose and sucrose (Klemens et al. 2014; Salvi et al. 2022). Chloroplast function, plant growth, and stress tolerance are all dependent on pSuT expression. This shows that, in addition to vacuolar sugar transfer, plastid sugar transport may play a role in stress tolerance development. Because there are so few studies on plastid glucose transporters, greater research on their physiological and functional insights under varied stress circumstances is essential.

12.6 Biotechnological Approaches for Developing Climate-Resilient Crop Plants in the Post-genomics Era

World agriculture faces issues as the human population grows, as well as the decrease in the agricultural land owing to industrialization, urbanization, climate change, and desertification. So far the breeding of agricultural crop plants has been beneficial in feeding an ever-increasing population; yet, 44 million metric tons of food would be required each year to feed the 9 billion people expected by 2050 (Godfray et al. 2010; Kaur et al. 2021). These yield differences are even more difficult to reconcile when it comes to the expected effects of global warming. As discussed here, sugar has an important and potential role in acquiring tolerance/resistance to different abiotic stresses. Sugar buildup in plants has long been thought to respond to abiotic stressors. It has also been well documented that to enhance stress response, abiotic stressors affect gene expression and the distribution of sugars (Gangola and Ramadoss 2018; Salvi et al. 2022).

Initially, traditional breeding methods were used to develop resistant cultivars by utilizing the genetic heterogeneity of crops at distinct gene pools. As a result, only a few abiotic stress-tolerant breeding lines in various crop species have been developed or created, most of which have failed to perform well in field testing (Manna et al. 2021). It makes traditional breeding procedures for developing stress-resistant cultivars of various agriculturally important crops more challenging (Saddhe et al. 2021). One approach was to use wild ancestors as the donor for resistance gene/s for agricultural crop manipulation to boost abiotic stress resistance. However, transferring tolerant genes for any specific abiotic resistance from wild varieties to domesticated crops is time-consuming and labor-intensive (Gangola and Ramadoss 2018; Manna et al. 2021).

Furthermore, reproductive barriers prevent beneficial genes from being passed down from wild relatives. As a result, genetic engineering has emerged as a viable option, and it is now being applied to increase abiotic stress tolerance worldwide.

Recent research addressing these sugar genes' molecular and functional control for building climate change resistance agricultural plants in various abiotic conditions are discussed in the coming sections.

12.6.1 Salt Stress

Plant physiology is altered by salt stress, which reduces cell division, photosynthesis, and nitrogen uptake, eventually affecting the plant's overall development (Salvi et al. 2016; Kaur et al. 2021). Salinity affects 850 million hectares of land worldwide. Furthermore, salinity issues are growing at a 10% yearly rate worldwide, mostly in Asia (Ashraf and Foolad 2007). Moreover, modern agriculture and ineffective agronomic practices have resulted in increasing soil salinity of agricultural land. In most situations, saline soil has excessive Na^+ and Cl^- ions, which reduces water potential ion imbalance and overall plant development. Plant sugars operate as osmolytes, mitigating the negative effects of salt stress. Increases in glucose, fructose, and sucrose concentrations caused by salinity are critical for osmoprotectant, carbon storage, and ROS scavenging (Rosa et al. 2009a, b). Rice transgenics that express the trehalose gene are more resistant to several abiotic stresses, including salt, cold, and drought stress (Ashraf and Foolad 2007). Rice plants with the chimeric gene Ubi1:TPSP accumulated more trehalose, improving their resilience to salt and cold stresses (Jang et al. 2003). Mainly, trehalose-producing transgenic plants, on the other hand, exhibited pleiotropic effects that influenced other plant development pathways (Ashraf and Foolad 2007). In tobacco and wheat plants, the *mt1D* gene was shown to enhance salt stress resistance and mannitol accumulation (Abebe et al. 2003).

12.6.2 Drought Stress

Drought resistance breeding is undoubtedly the most challenging and time-consuming endeavor scientists face when striving to improve the genetic potential of various crop species. Drought accounts for more than 40% of crop failures, accounting for 89% of crop failures (Iordachescu and Imai 2008). Glucose improves plant resilience to drought and heat by promoting stomal closure (Osakabe et al. 2014). Furthermore, multiple investigations have discovered RFO buildup in seed desiccation events such as raffinose, verbascose, and stachyose (Bolouri-Moghaddam et al. 2010). Additionally, sugar accumulation under drought stress inhibits cell membrane oxidation (Arabzadeh 2012). Sugars also help to maintain leaf turbidity, membrane water levels, and osmotic potential (Sawhney and Singh 2002). Rice has bi-functional genes for trehalose biosynthesis that express TPP and TPS enzymes and help in the accumulation of more trehalose, which in turn is reported to increase drought, cold, and salinity tolerance in many plants (Jang et al. 2003).

12.6.3 Cold Stress

Another important ecological variable limiting plant distribution and its associated yield is temperature. Low temperatures impact the rates of reactions involved in biochemical processes differentially, resulting in metabolic pathway imbalances between partial processes. Furthermore, plants' cold tolerance has been demonstrated to be influenced by changes in soluble sugar levels. Many soluble sugars, including sucrose, glucose, RFOs, etc., are known to give cold tolerance in plants (Jia et al. 2017). Soluble sugars also aid in acclimatization under cooling stress by interacting with lipid bilayers and aiding in their stability (Garg et al. 2002). For example, trehalose is generally present in very low concentrations, but it rises rapidly when subjected to cold stress (Fernandez et al. 2010). Moreover, sugars also influence the functions of housekeeping genes that are important throughout plant development. Advanced technologies might be employed to do more study on the role of specific or combination sugar in the cold response. These findings might help researchers better understand how sugar response pathways function during the cold stress response.

12.6.4 Heat Stress

Photosynthesis is the physiological function that suffers more when crop plants are subjected to heat stress, inhibiting overall plant development. The allocation of photoassimilates is also disrupted as a result of reduced photosynthesis. Indeed, when subjected to heat stress, the soluble glucose contents in the source leaves of many plants often decrease (Zhou et al. 2017). Sucrose transport and loading into the phloem were equally repressed in both maize and tomato plants under heat stress, suggesting that SWEETs and SUTs restrict phloem sucrose transport (Frey et al. 2015). However, in heat-stressed lemon and cucumber, glucose or fructose levels decreased, while sucrose levels increased, most likely due to increased sucrose biosynthesis (Aung et al. 2001). Heat shock proteins (HSPs) play a crucial role in how plants respond to heat stress. As per studies, sugars have a vital role in the modulation of HSP proteins, and these HSPs, in turn, regulate sugar metabolism. Heat-resistant tomato cultivars, for example, have higher invertase activity and sugar in tomato fruit (Li et al. 2012). Similarly, overexpression of the *SlCIF1* gene coding for a small HSP protein resulted in a 1000-fold increase in SlHSP17.7 expression.

Furthermore, the silencing of SlCIF1 in tomatoes resulted in a drop in fructose and sucrose levels and the downregulation of numerous genes related to sugar metabolism (Zhang et al. 2018a). As a result, the plant's response to heat stress is defined as a decrease in carbohydrate absorption followed by a drop in sugar levels in the leaves, resulting in altered sugar transporter performance (Julius et al. 2017). Heat stress regulates sugar transporters differently at different stages of development. As the temperature increased, the expression of the sucrose transporter 4 gene (OsSUT4) in embryo germination and pollen development increased. However, under prolonged heat treatment, the OsSUT4 transcript was downregulated in

leaves, stems, and ears (Chung et al. 2014). They also discovered that the assimilate distribution between leaves and panicles was changed and that juvenile panicles were more susceptible to heat stress than fully matured panicles. Plasmodesmata deformation may cause delayed sucrose transport in plants under heat stress (Zhang et al. 2018b). Sugars, such as sucrose, play essential roles in thermo-tolerance control by modifying heat shock protein induction via the TOR-E2F signaling module, where E2F regulates the transcription of several HSP genes by regulating their promoters (Sharma et al. 2019, 2021).

12.7 Limitations and Challenges

Most of the studies and research were carried out on model plants like Arabidopsis and tobacco, which have demonstrated substantial resistance to various abiotic stressors. On the other hand, these model plants cannot anticipate the agriculturally significant crop plants. Although rice and wheat have been employed in different studies, they were all done under strictly controlled conditions. Most of the experiments were done when the plants were in the early stages of germination or vegetative growth. So, to better understand the significance of specific sugars and their associated gene or the signaling in crop plant abiotic stress tolerance, the practical strategy is to apply and reproduce the results directly to a crop of interest to access the gene's true potential in the desired and natural environment (Salvi et al. 2018, 2022; Manna et al. 2021).

Furthermore, multilocation studies with the target crops are required to comprehend the activity and expression profile under natural conditions. Despite substantial efforts to produce abiotic resistant cultivars of varied agricultural plants using traditional plant breeding procedures, little progress toward the stated goal of creating viable variants has been made. It was believed that with the advent of molecular genetics and gene modification techniques, grown varieties resistant to diverse abiotic stresses and reasonably high throughput might be created, but the results are expected. Abiotic resistance features are likely to be complicated and controlled by several genes, with various biological, molecular, and physiological processes involved in abiotic resistance mechanisms.

Several studies have shed light on the significance of sugar signaling and its involvement in plant metabolism during the last few decades. The molecular basis of sugar transport, on the other hand, remains largely unknown. Despite research indicating that overexpression or downregulation of sugar transporters improves responses to a variety of abiotic stresses, efficient transformation of transporters depends on an understanding of their specific role and a virtual network with the linked biological mechanism (Salvi et al. 2016; Kaur et al. 2021). Sugar transporter modulation for increased abiotic stress responses is difficult because sugar transporters' biological importance has been extended beyond just transporting sugar from source to sink. Some sugar transporters discovered so far also transport other substrates such as AtSWEET13 and AtSWEET14 that aid in transmitting

gibberellin along with sugarMtN3/SWEET type (Kanno et al. 2016; Julius et al. 2017).

Transporter proteins play an important role in regulating many physiological processes by transporting various sugars and other metabolites. As a result, altered expression of the genes involved can adversely affect related cellular functions and developmental factors (Chen et al. 2015). Sugar signaling comprises a sophisticated network of phytohormone signaling, several transcription factors, and secondary messengers; therefore, altering sugar transporter genes may appear to have pleiotropic effects. Similarly, excessive sugar levels inside the leaves as a function of sugar exporter inhibition or downregulation could have detrimental implications on plant growth and mechanisms like photosynthesis. Reduced photosynthesis may eventually have a detrimental effect on the plant yield and also the associated environmental factors. Transforming C3 to C4 plants increases photosynthesis and output possibilities in field crops such as rice by improving CO_2 fixation efficiency (Zhu et al. 2010; Baker et al. 2016). However, such a transformation would need a better knowledge of sugar transport.

12.8 Conclusions and Future Outlook

Sugars play diverse roles in plant development and mitigating unfavorable conditions. Due to their coordinated participation in stress resistance as osmoprotectants/antioxidants, role in several signaling pathways, and noteworthy relationship with photosynthesis or source-sink association, they are considered a potential target for balancing plant resilience to abiotic stresses. Sugars' protective effect against abiotic stress has been studied to generate crop varieties with enhanced abiotic stress tolerance by altering their biosynthesis route (Kaur et al. 2021; Salvi et al. 2022). The challenge of discovering vital molecules or the genes involved, directly or indirectly, in abiotic stress tolerance has been improved by recent developments in molecular biology, particularly utilizing next-generation sequencing. However, there are few examples of generating a stable crop variety against some abiotic stress. As a result, agricultural and plant scientists must convert existing whole-genome data and omics approaches like transcriptomics, proteomics, and metabolomic data into abiotic stress-tolerant crop cultivars.

Environmental extremes caused by climate change have a recurring stress effect on plants, which has become a critical worry for maintaining high yield and plant production. Abiotic stress-tolerant cultivars have improved defense and yield due to both traditional and biotechnology techniques. Plants will need to adjust sugar transport and metabolism to counteract the detrimental effects of abiotic stressors and possess a defense arsenal. Under stress, research on the kinetics of starch to sucrose conversion has revealed multiple roles of sugars, including osmoprotectants; movement in various tissue, including sources and sink organs; and resources for long-term consideration (Kaur et al. 2021; Salvi et al. 2022). It's also critical to understand how plants perceive and modify their cellular environment in response to specific stress such as drought, heat, or salt and how it can be interconnected when

the plant senses more than one stress at a time. Stress-induced starch-sugar transformation, translocation, and relocation are also of interest, both topographically and transiently (Manna et al. 2021). Diverse pathways in these processes, both hereditarily and metabolically, might be ideal candidates for stress resistance development in agricultural plants. In any case, increased sugar accumulation might have various unintended consequences for plant development; stress-specific and tissue-specific acceptance should be addressed. In a nutshell, sugars and sugar transporters may play an important role in fine-tuning abiotic stress tolerance and agricultural productivity.

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Epigenetics for Crop Improvement: Challenges and Opportunities with Emphasis on Wheat

13

Gautam Saripalli, Vijay Gahlaut, Tinku Gautam, and Hemant Sharma

Abstract

Rice, wheat and maize are the three major cereal crops that are imperative to food security and nutrition. Out of the three cereals, wheat has the most complex and largest genome (~16 GB) and is a staple food for most people worldwide. Therefore, continuous efforts are being made to improve the production of important cereals, including wheat. Breeding these cereals for major biotic and abiotic stresses and nutritional quality has been an important area of research. Further, with the advent of next-generation sequencing technology, a tremendous wealth of genomic resources is now available, paving the way for modern genomic approaches for crop improvement. Recently, epigenetics is also becoming popular as an important area of research, and some efforts have been made in this direction to understand what part of the cereals' genome is actually regulated through epigenetic factors, which mainly include DNA methylation, histone modifications, and noncoding RNAs (including microRNAs or miRNAs and long noncoding RNAs or lncRNAs). The available literature, to some extent,

G. Saripalli (✉)
Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD, USA
e-mail: gautams@umd.edu

V. Gahlaut
Biotechnology Division, CSIR-Institute of Himalayan and Bioresource Technology, Palampur, Himachal Pradesh, India

Department of Biotechnology, University Center for Research and Development, Chandigarh University, Gharuan, Mohali, Punjab, India

T. Gautam · H. Sharma
Department of Genetics and Plant Breeding, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India

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suggests that epigenetics is a highly complex mechanism. Therefore, more efforts are certainly needed in this direction so that it may prove helpful in the breeding of cereals for resistance against important biotic and abiotic stresses. Some attempts have also been made to identify important epialleles in rice; however, they have not been used in breeding for the development of stress-tolerant varieties using epigenetic markers. The present chapter provides an overview of the research conducted worldwide to understand the epigenetic component involved during different environmental stresses in important cereals, with special emphasis on wheat. Further, it also highlights different challenges and future strategies that may help in development of cereal genotypes that are resistant to different environmental stresses.

Keywords

Epigenetics · Wheat · DNA methylation · EWAS · Epialleles

13.1 Introduction

Epigenetics is emerging as an important area of research during the current scenario of changing climatic conditions which is adversely affecting crop yield. Continuous efforts are being undertaken to understand the importance of epigenetic regulation in plants (Pikaard and Mittelsten Scheid 2014). Epigenetic changes are mainly the heritable changes that arise independently of DNA sequence variations, and these epigenetic changes are often associated with changes in gene expression (Kakoulidou et al. 2021). The role of epigenetics during biotic and abiotic stresses is being explored in agronomically important crops like rice, maize, wheat, etc. with a view to using it for crop improvement (Kakoulidou et al. 2021). During the last decade, several studies have been conducted on these crops to understand the molecular mechanism of stress resistance in more detail. It has now become evident, to some extent, the role of epigenetic components in addition to the genetic components in controlling the genes that are involved in biotic and/or abiotic stress responses (Guarino et al. 2022). This has certainly opened more avenues to geneticists working in the area of genetic improvement of crop plants. However, compared to the knowledge on the genetic mechanism, the knowledge of the epigenetic mechanism is still very limited, especially in crops with a complex polyploid genome like wheat.

Epigenetics mainly involves three components: DNA methylations, histone modifications, and sRNAs. The techniques that are used to explore these components differ from each other and have been applied to crops to some extent. Similarly, there are even variants for each of these techniques. For instance, the study of DNA methylation initially started with methylation-sensitive amplified length polymorphism (MSAP; Ruiz-García et al. 2010), which is a relatively low-resolution technique; later, advanced techniques like bisulfite sequencing (BS-seq) and reduced representation bisulfite sequencing (RRBS) were developed and are being widely used in recent days. In fact, the DNA methylation changes in plants are dependent on

the cytosine, which actually gets methylated, and based on the context, CG, CHH, and CHG methylation patterns are observed. The later techniques (BS-seq and RRBS) can identify context-specific methylation patterns. Similarly, for histone modifications and sRNA analysis, techniques like chromatin immunoprecipitation (ChIP)-sequencing and sRNA sequencing are used, respectively. These techniques are also helpful in understanding gene-specific epigenetic changes. For instance, methylation-specific PCR (MS-PCR) can be used to study gene-specific DNA methylation changes, whereas ChIP-qPCR is used to study gene-specific histone modifications.

The present chapter mainly deals with the challenges and opportunities involved in using epigenetic techniques to explore the role of the abovementioned epigenetic components in different biotic and abiotic stresses in cereal with special emphasis on wheat. Wheat, being a hexaploid crop, has a complex huge genome of about 16 GB (IWGSC 2018), which is a great challenge. Therefore, compared to other crops, there are very limited studies in wheat, where epigenetics has been explored. However, there are still studies available in wheat where the epigenetic components have been explored. Some of the important examples have been explained in different sections involving abiotic and biotic stresses. Further, some information on the use of epialleles in crop improvement has also been highlighted. We believe that this chapter will be a useful resource for the researchers and students working in the area of crop epigenetics.

13.2 Epigenetics for Abiotic Stress

13.2.1 Drought Stress

Drought stress significantly impacts wheat yield, and frequent drought incidents have been expected to increase yield loss by at least 12% by the end of the twentieth century (Helman and Bonfil 2022). Therefore, there arises a need to implement breeding strategies that may aid in developing wheat cultivars with increased drought tolerance. Molecular breeding using conventional molecular marker approaches has already been employed for drought stress improvement (Gautam et al. 2021; Rai et al. 2018); however, the use of epigenetic markers is still limited due to the limited information on epigenetic mechanisms of drought tolerance in wheat. Some studies have already been conducted in this direction, and efforts are being made to understand the epigenetic components of drought tolerance. For example, tissue and genotype-specific cytosine DNA methylation changes have been reported under drought stress in seedlings (Duan et al. 2020) as well in roots and leaves (Kaur et al. 2018) in two different wheat cultivars differing in drought tolerance, thereby providing some evidence of epigenetic response under drought stress in wheat. Some evidence indicating the role of histone acetyltransferases during drought stress is also available using genome-wide analysis of histone acetyltransferases and deacetylases (Li et al. 2021). In this study, six important genes encoding for HATs/HDACs were identified through comparative expression proofing using three wheat cultivars; these HATs/HDACs could be further explored

for their use in wheat improvement for drought tolerance. Histone variants (*TaH2A.7* and *TaH2B.7D*) involved in drought tolerance have also been characterized in wheat, either using overexpression in Arabidopsis (*TaH2A.7*; Xu et al. 2016) or knockdown through virus-induced gene silencing (*TaH2B.7D*; Wang et al. 2019).

13.2.2 Epigenetics for Heat Stress

Compared to other abiotic stresses like drought and salinity, the efforts for the development of heat-resilient crops are relatively recent, and therefore, major efforts are already underway to understand the genetic and epigenetic mechanisms of heat stress tolerance in crops like wheat, rice, and maize. Several QTLs and candidate genes have already been identified for heat stress tolerance in wheat. These can be potential resources for developing improved wheat cultivars for heat tolerance (Singh et al. 2021; Kumar et al. 2021). However, to date, epigenetic markers have not been explored; therefore, efforts need to be made in this direction. Relative to other crops like cotton (He et al. 2022; Harkess 2018), tomato (Singh et al. 2021; Pu et al. 2020), rice (Li et al. 2022; Zheng et al. 2017), and maize (Guo et al. 2021; Qian et al. 2019), the information in wheat is very limited which may be attributed to large and complex genome size as mentioned earlier. However, due to the advent of NGS technologies, efforts can certainly be made in this direction. Some information on the epigenetic regulation involving sRNAs is available in wheat during heat stress; however, till date, no efforts have been made to understand the epigenetic control due to DNA methylation and histone modifications. In one of the studies, one of the epigenetic components, involving sRNAs, was explored during heat stress in wheat. Some ncRNAs like tRNAs, rRNAs, and snoRNAs were identified, which showed upregulation due to heat stress in wheat seedlings (Wang et al. 2016). In another study, targets for miRNAs (miRNA156, miR166, and miR393) were identified, encoding for superoxide dismutase, F-box proteins, and leucine zipper-like proteins. These proteins were found to be involved in important stress responses like ubiquitination and antioxidant activities (Ravichandran et al. 2019). Another study identified a gene encoding histone acetyltransferase (TaHAG1) in wheat under heat stress. In this study, the transcripts of three TaHAG1 homeologs were induced quickly under heat stress and then gradually increased in similar pattern with the time of stress prolonging, indicating its role in heat stress. Such genes involved in heat stress may be further validated and then used for developing epigenetic markers for heat stress tolerance. Thus, there seems to be tremendous scope to explore epigenetic control of heat stress in wheat. Some information on the epigenetic control of heat stress is available in crop plants like rice, maize, and wheat.

13.3 Epigenetics for Biotic Stress

Epigenetic mechanisms play an important role in controlling the expression of genes that provide resistance to crop plants against different biotic stresses involving fungal, bacterial, and viral pathogens. The role of epigenetics in biotic stress resistance is critically reviewed, and efforts are being made to understand the in-depth mechanisms. However, based on the studies available, it can be speculated to some extent that compared to genetic components, only a small portion of the genome is actually regulated by epigenetic components, which has been observed in the case of leaf rust resistance in wheat (Singh et al. 2022; Saripalli et al. 2020a, b; Jain et al. 2021; Sharma et al. 2018). The general mechanism of biotic stress resistance involves either basal defense or host-specific defense response. Basal defense involves the activation of pathogen-activated molecular patterns (PAMPs) due to the attack by different pathogens like nematodes or fungus.

In contrast, the host-specific response involves the activation of canonical and non-canonical R genes or QTLs (Zheng et al. 2021). The molecular mechanism of the biotic stress response is widely known in cereal crops like wheat; however, the information on the epigenetic mechanism is very limited, which may be attributed to different reasons: (1) sophisticated techniques involved in exploring epigenetic mechanisms; (2) complexity of epigenetic mechanism (for instance, DNA methylation is known to exhibit both repressions and activation of gene expression which depends on the location of the cytosine methylation in the genome); and (3) methylation changes, which are observed in different contexts in plants, i.e., CG, CHH, and CHG, and the consequences of the different contexts also differ for changes in gene expression due to DNA methylation. These aspects are elaborated below in some more detail for nematode, bacterial, and fungal resistance in crop plants like wheat.

13.3.1 Epigenetics for Nematode Resistance

Cereal cyst nematodes are the most important class of nematodes, drastically impacting the yield of cereal crops like wheat and barley. In fact, these nematodes alone are known to cause a yield reduction of up to 10% globally in important crops (Whitehead 1998). Efforts are being made to understand the molecular mechanism of nematode resistance which may help in improving the crop cultivars like wheat against nematode diseases. Several differentially expressed genes (DEGs; using RNA-seq) have been identified in wheat that are actually involved during wheat-nematode interaction. Most studies involving transcriptome analysis have been carried out using *Heterodera avenae*, which seems to be the most important nematode infecting wheat genotypes. In one of the study involving wheat-*Heterodera avenae* interaction, 93 differentially expressed genes (with many involved in biotic stress response) were identified in wheat, whereas 867 DEGs were identified in nematode with several putative effector genes (Chen et al. 2017). Similarly, there are more studies involving the same nematode (Qiao et al. 2019; Kumar et al. 2014) where DEGs have been identified, which helped in speculating a pathway operating during wheat-nematode interactions. A single study identifies some important

marker-trait associations and candidate genes in a wheat association panel screened against a root lesion nematode *Pratylenchus thornei* (Kumar et al. 2021).

The above studies provide some knowledge about the molecular mechanism of nematode resistance in wheat; we believe that at least some of the important genes identified using RNA-seq analysis may be regulated through epigenetic components like DNA methylation, histone modifications, or sRNAs. Information on epigenetic changes due to nematode infection in plants is already available in different crops like tomatoes (Leonetti and Molinari 2020) and soybean (Rambani et al. 2015) and also in cereal crops like rice (Atighi et al. 2020, 2021). In rice, the role of global DNA methylation was revealed during the infection of the rice plant with a nematode (*Meloidogyne graminicola*) associated molecular pattern. In this study, the evidence for the causal impact of hypomethylation on immunity was revealed by a significantly reduced plant susceptibility upon treatment with DNA methylation inhibitor 5-azacytidine. Similarly, the role of another epigenetic component, i.e., histone modifications, was also investigated in rice plants infected with the same nematode as above. Here, three histone marks, i.e., H3K9ac, H3K9me2, and H3K27me3, were studied explicitly for their effect on gene expression, and differential binding of two of the three histone marks (H3K9ac or H3K9me2) showed expected changes in gene expression as revealed through RNA-seq analysis. Some of these genes were also involved in defense response (Athigi et al. 2021).

The above information provides a base to plan experiments in wheat to understand the role of epigenetic modifications during wheat-nematode interactions, either at individual- or genome-wide levels. For instance, some of the important defense response genes identified through RNA-seq analysis could be subjected to MS-PCR (methylation-specific polymerase chain reaction) or ChIP-qPCR (chromatin immunoprecipitation quantitative PCR) to examine the role of epigenetics for changes in expression of these genes. Similarly, genome-wide studies could also be planned, like in the case of rice (see above).

13.3.2 Epigenetics for Fungal Resistance

The most serious fungal diseases affecting wheat yield worldwide include rust (stem rust, leaf rust, and strip rust), fusarium head blight, Septoria leaf blotch, and powdery mildew. While FHB alone is ranked as the second most challenging disease in the United States, Canada, Brazil, Paraguay, Uruguay, and Argentina are next to the tan spot; both FHB and LR were ranked the topmost diseases in China and across the globe (Savary et al. 2019). Therefore, major efforts have been undertaken to understand the molecular mechanism of FHB and LR diseases. Some recent reports which decipher the molecular mechanism of FHB resistance are highlighted as follows: (1) in a recent review, an attempt was made to link different multi-omics with different resistance mechanisms, and the pathways or genes involved in providing resistance against FHB were emphasized (Wu et al. 2022). (2) The role of alien introgression in FHB resistance was also reported recently. In this study, the chromosome 7EL of alien wheat species Thinopyrum elongatum was sequenced. This chromosome 7EL carries FHB resistance which was also introgressed in wheat

cv. Chinese spring (CS) and the differentially expressed genes were studied between CS-7EL and CS through transcriptome analysis (Konkin et al. 2022). (3) The role of multiple phytohormone pathways was identified using a combination of transcriptome and hormone profiling in a resistant wheat variety Sumai3 and three Canadian wheat cultivars (Wang et al. 2018).

For LR, to date, ~80 Lr genes have been identified (McIntosh 2017, 2020), out of which 6 have been cloned (Lin et al. 2022; Moore et al. 2015; Huang et al. 2003; Feuillet et al. 2003; Cloutier et al. 2007; Krattinger et al. 2009). Similarly, several studies have also been conducted during the last 5 years where important resistant genes have been identified, and pathway during wheat-leaf rust interaction has also been speculated. For instance, a transcriptome analysis was conducted in a pair of NILs differing for *Lr28* gene, and a pathway operating during wheat-Lr was speculated. Even a putative candidate gene encoding Lr28 was also precited based on the DEGs identified during this study (Sharma et al. 2018). Similarly, transcriptome analysis was also conducted in wheat varieties for the adult plant-resistant (APR) *Lr48* gene (Jain et al. 2021). Continuous efforts are also being made to map important genes providing resistance against leaf rust disease, and in the past 5 years, some important novel Lr genes/QTLs (*Lr65*, Zhang et al. 2021; $LrTs_{276\text{-}2}$, Dinkar et al. 2020; *LrLC10*(*Lr13*), Qui et al. 2020) have been mapped.

The knowledge on the role of epigenetics in regulating the gene expression during biotic stress in crop plants is very limited, especially in the case of FHB, where only a solitary study is available where the regulation of gene expression during FHB infection in durum wheat was shown to be influenced by genome-wide DNA methylation (Kumar et al. 2020). However, there are at least seven studies in wheat where the epigenetic components for leaf rust resistance have been examined, and epigenetics was partly shown to control gene expression during wheat-Lr interactions. In the past decade, detailed studies have been carried in a pair of NILs differing for *Lr28* gene in wheat, and studies on miRNAs, DNA methylation, and ChIP-qPCR and genome-wide ChIP-Seq have been conducted, and a number of miRNAs, differentially methylated regions, and histone methylation marks were identified which partly controlled the expression of genes involved during *Lr28*-mediated leaf rust resistance and/or susceptible reactions. Similarly, genome-wide DNA methylation was also conducted in wheat genotypes differing for *APR-Lr48* gene and important genes. Therefore, this information could be used for developing epigenetics markers for leaf rust resistance in wheat. Further, the above studies also provide a framework for understanding the epigenetic mechanism of other important *Lr* genes that have been identified.

13.4 Future Opportunities in Epigenetics

13.4.1 Epialleles

Primarily the variations in DNA sequences regulate the phenotypic variations in species. But other factors like changes in DNA methylation may affect the gene expression and thus regulate the phenotypic trait variations (Becker and Weigel

2012). The methylation changes can be inherited to the next generations, and these stably inherited epigenetic variants are known as "epialleles." The number of epialleles was reported in different plant species that regulate various phenotypic traits, i.e., flower morphology, flowering time, fruit ripening, plant architecture, root length, biomass, sex determination, vitamin E accumulation, etc. (For details See Table 13.1). The clark kent (*clk*) epiallele, a classic example of an epiallele, was discovered in the Arabidopsis. Similarly, other epimutants including *SUPERMAN* (*SUP*), *Phosphoribosyl Anthranilate Isomerise* (*PAI2*), *AGAMOUS* (*AG*), *Flowering Wageningen* (*FWA*), *BAL1*, *BONSAI* (*BNS*), and *Folate transporter 1* (*FOLT1*) associated with different traits like flowering traits, plant height, starch accumulation, etc. have also been reported (Table 13.1). Besides model plants, epimutants and epialleles are also characterized in different crop plants. For instance, in *Zea mays*, four independent epialleles, i.e., *red1* (*r1*), *booster 1* (*b1*), *purple plant* (*pl1*), and *pericarp color* (*p1*), are found to be associated with pigmentation (Brink 1956; Patterson et al. 1993; Hollick et al. 1995; Cocciolone et al. 2001); and one epiallele "low phytic acid 1 (*lpa1*)" is characterized for high inorganic phosphate in maize seeds (Pilu et al. 2009). In rice, two epialleles, *Dwarf1* (*D1*) and *Fertilization-Independent Endosperm 1* (*FIE1*), were found to be associated with the dwarf phenotype (Miura et al. 2009; Zhang et al. 2012). Two epialleles *Squamosa promoter binding protein-like* (*SPL14*) and *Epigenetic short panicle* (*ESP*) resulted in short panicle (Miura et al. 2010; Luan et al. 2019). Epiallele of *RAV6* [*Related to Abscisic acid insensitive 3 (ABI3)/Viviparous 1 (VP1) 6*] gene alters the lamina inclination and grain size (Zhang et al. 2015), and epigenetic mutation in *Adenylate Kinase 1* (*AK1*) reduced the photosynthetic capacity in rice (Wei et al. 2017). Two important epigenetic mutations are also reported in the case of tomatoes; one is *colorless non-ripening* (*CNR*) associated with fruit ripening (Manning et al. 2006), and the other is *Vitamin E 3* (*VTE3*) associated with vitamin E content (Quadrana et al. 2014). Epigenetic mutation in *Pollen S-determinant gene* (*SP11*) caused self-incompatibility in *Brassica rapa* (Shiba et al. 2006) and in *WASP/N-WASP-interacting protein 1* (*WIP 1*) produced female flower in *Cucumis melo* (Martin et al. 2009). Epiallele involving transposable elements also affects the trait. In the case of oil palm, hypomethylation in Karma retro TE caused abnormal DEF gene splicing and produced parthenocarpic fruit and lower yield (Ong-Abdullah et al. 2015). These epigenetic variations reported in different plants and other gene pools may provide new opportunities for crop improvement programs.

13.4.2 Epigenome Wide Association study (EWAS)

EWAS is an emerging approach to understanding phenotypic variation. EWAS is similar to a genome-wide association study (GWAS), but here instead of genetic variations, epigenetic variations are associated with phenotypic traits (Fig. 13.1). Epigenetic marks can be transferred across the generations through mitosis or meiosis. EWAS studies are available in the case of humans, particularly for diseases like Parkinson's disease (Chuang et al. 2017), type 2 diabetes (Cardona et al. 2019),

Table 13.1 Details of some important epialleles were reported in various plants

Species and gene/locus	Phenotypic traits	References
Arabidopsis thaliana		
SUPERMAN (SUP)	Stamen and carpel number	Bowman et al. (1992)
Phosphoribosyl Anthranilate Isomerise (PAI2)	Gene expression affected; without any specific phenotype change	Li et al. (1995)
AGAMOUS (AG)	Flower structure	Jacobsen et al. (2000)
Flowering Wageningen (FWA)	Flowering	Soppe et al. (2000)
BAL1	Dwarfing and disease resistance	Stokes et al. (2002)
BONSAI (BNS)	Stunted growth	Saze and Kakutani (2007)
Folate transporter 1 (FOLT1)	Fertility	Durand et al. (2012)
Qua-Quine Starch (QQS)	Higher starch accumulation	Silveira et al. (2013)
Pheophytin Pheophorbide Hydrolase (PPH)	Leaf senescence and climate adaptation	He et al. (2018)
Histidinol-phosphate aminotransferase 1 (HISN6B)	Hybrid incompatibility	Blevins et al. (2017)
Brassica rapa		
Pollen S-determinant gene (SP11)	Self-incompatibility	Shiba et al. (2006)
Cucumis melo		
WASP/N-WASP-interacting protein 1 (WIP1)	Sex determination	Martin et al. (2009)
Elaeis guineensis		
DEFICIENS (DEF1)	Mantled fruit	Ong-Abdullah et al. (2015)
Linaria vulgaris		
Linaria cycliodea (Lcyc)	Floral symmetry; dorsiventral flower axis	Cubas et al. (1999)
Oryza sativa		
Drawf1 (D1)	Dwarf	Miura et al. (2009)
Squamosa Promoter binding protein-Like (SPL14)	Panicle branching and grain yield	Miura et al. (2010)
Fertilization-Independent Endosperm 1 (FIE1)	Dwarf	Zhang et al. (2012)
RAV6 [Related to Abscisic Acid Insensitive 3 (ABI3)/Viviparous1 (VP1) 6]	Lamina inclination and grain size	Zhang et al. (2015)
Adenylate Kinase 1 (AK1)	Defects in photosynthetic capacity	Wei et al. (2017)

(continued)

Table 13.1 (continued)

Species and gene/locus	Phenotypic traits	References
Epigenetic Short Panicle (ESP)	Short panicle	Luan et al. (2019)
Solanum lycopersicum		
Colorless non-ripening (CNR)	Fruit ripening	Manning et al. (2006)
Vitamin E (VTE3)	Tocopherol accumulation in fruit	Quadrana et al. (2014)
Zea mays		
red1 (r1)	Reduced pigmentation	Brink (1956)
Booster 1 (b1)	Reduced pigmentation	Patterson et al. (1993)
Purple plant 1 (pl1)	Reduced pigmentation	Hollick et al. (1995)
Pericarp color 1(p1)	Reduced pigmentation	Cocciolone et al. (2001)
Low phytic acid1 (lpa1)	High inorganic phosphate in seeds	Pilu et al. (2009)

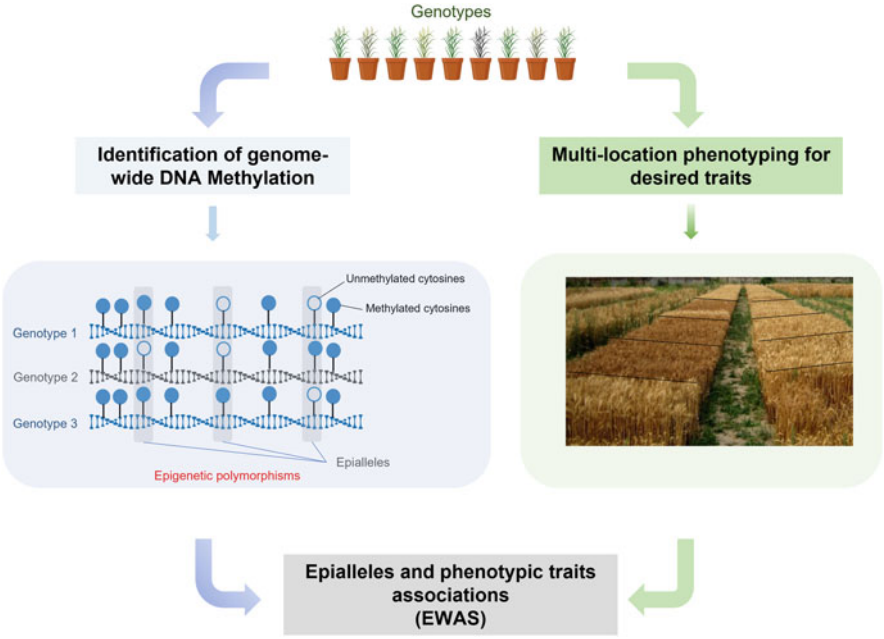

Fig. 13.1 Epigenome-wide association study

coronary artery disease (Xia et al. 2021), and Alzheimer's disease (Smith et al. 2021); EWAS Atlas has also been established to compile the developed information (Li et al. 2019). In the case of plants, limited studies are available on the topic. EWAS study in oil palm identified epigenetic modification associated with a mantled abnormality (Ong-Abdullah et al. 2015). Using somatic clones (diverse for mantled abnormality and oil yield), a locus MANTLED was identified where hypomethylation in LINE retrotransposon leads to alternate splicing and premature termination. In *Quercus lobata*, EWAS established the correlation between DNA methylation pattern and climate gradient (Gugger et al. 2016). A pipeline to study epidiversity and EWAS in plants has also been developed (Can et al. 2021).

The plant has huge potential for epimutations, and some of the well-characterized epigenetic variations are also summarized in an earlier section, providing a vast opportunity for exploring the epigenetic marks in natural populations and studying their role in trait variations. The characterized epigenetic variation can be utilized in epibreeding programs for crop improvement.

13.5 Challenges in Epigenetics Research

Research in epigenetics is rapidly evolving, and new advances in this area are constantly reported. However, the research in this area has some limitations due to which the knowledge generated is still not translated into the development of new crop varieties. Therefore, efforts are still needed, which may help to translate the knowledge into useful products like climate-smart crops, which can tolerate the stiff challenges due to several biotic and abiotic stresses. The possible applications of epigenetics in climate-smart crop breeding were recently discussed in a review (Varotto et al. 2020). Compared to cereal crops, epigenetics mechanisms are widely known in model crops like Arabidopsis and tomato (Varotto et al. 2020). Therefore, attempts need to be made to apply the knowledge to cereal crops like rice, wheat, and maize, which are major food crops for people worldwide. Several genes have been identified in the model crops whose regulation is controlled through epigenetic components. Some information on the role of epigenetics in cereal crops like wheat and barley is also known, which has been discussed in later sections.

Epigenetic regulation of genes is highly complex than expected as reported in several studies in wheat involving abiotic stress tolerance like drought (Duan et al. 2020) and heat and biotic stress resistance like leaf rust (Singh et al. 2022; Jain et al. 2021; Saripalli et al. 2020a, b; Sharma et al. 2018) and fusarium head blight (Kumar et al. 2020). Some of the complexities associated are as follows: (1) *both, suppression and activation of gene expression due to epigenetic modifications*: It is generally believed that epigenetic modifications suppress gene expression; however, this actually depends on the location of epigenetic modifications in the genome. For instance, the cytosine methylation in the promoter regions generally suppresses gene expression, but the same in the genic region (exons) sometimes even activates gene expression (Wang et al. 2016; Sun et al. 2014; Liang et al. 2014). This is attributed to its role in alternative splicing rather than controlling gene expression (Shayevitch

et al. 2018; Maunakea et al. 2013). (2) *Methylation in different cytosine contexts*: Unlike animals, where CG methylation mainly plays a role in gene expression, in plants, there are three different methylation contexts, i.e., CG, CHG, and CHH play an important role. In fact, CHH is known to be more important than CG and CHG in plants (Gallego-Bartolomé 2020; Bartels et al. 2018). (3) *Sophisticated techniques to estimate DNA methylation*: Whole-genome bisulfite sequencing (WGBS) is the most popular technique for the identification of cytosine DNA methylation changes; however, it suffers from some limitations that need to be taken care off while analyzing the data generated using WGBS. Ideally, bisulfite treatment is expected to deaminate cytosine to uracil (Fig. 13.1a) and leave 5-methylcytosine unchanged. However, the conversion of cytosine to uracil often fails or is inappropriate due to which the false positive differentially methylated regions may result. Therefore, the most reliable data analyses from bisulfite-treated DNA account for both types of conversion error: failed conversion and inappropriate conversion (Genereux et al. 2008). Several different software are available to analyze the WGBS data; therefore, proper care should be taken while selecting the appropriate software for WGBS analysis. (4) *Huge genome size*: Large genome size (especially in the case of wheat) and a huge fraction of repetitive elements also makes the study of epigenetic components a difficult task which is also discussed in a recent review (Varotto et al. 2020). (5) *Non-dependence of epigenetic phenotypes on DNA sequence*: It is well-known that epigenetic-dependent phenotypes are not strictly dependent on DNA sequence. This makes studying their transgenerational behavior challenging due to its dependence on the plant propagation methods (sexual versus clonal). Histone PTMs (post-translational modifications) are particularly useful for clonally propagated crops, such as potatoes, due to the potential erosion during meiosis. Identifying the heritable alleles is also often challenging; natural heritable epialleles are a useful source of variation. However, they might not be created as fast as necessary to meet the demand for breeding programs. The use of epigenome editing may be a promising in such a scenario (Gallusci et al. 2017; Springer and Schmitz 2017).

13.6 Conclusions

Epigenetics is an important area of research that is now gaining importance in recent days due to the evidence that explains its role in biotic and abiotic stress tolerance in crop plants. In crops like wheat, the information is still limited relative to other crops like maize, tomato, rice, etc. However, in the past decade, several reviews have been available that elaborate on the role of epigenetic control during biotic and abiotic stress tolerance in cereal crops like wheat, barley, and other crop plants. Therefore, we believe that there is a lot of scopes to explore this area in crops with highly complex genomes like wheat, and the developments in the NGS technologies will certainly help to explore it. At least some evidence are available in wheat where the role of epigenetic components like DNA methylation, chromatin modifications, and sRNA has been shown to control gene expression. These include biotic stress-related

traits like fusarium head blight and leaf rust resistance and important abiotic stresses like heat and drought. Further, the concept of epialleles is also gaining importance, and some evidence of the identification of epialleles are available in crops like Arabidopsis and rice (Table 13.1). Overall, we believe this chapter will be a useful resource for researchers working in the area of epigenetics research. Future efforts in the area of epigenetics will certainly help us in the development of epigenetic markers that will help in the development of wheat cultivars for improved stress tolerance.

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