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

Smart Plant Breeding for Field Crops in Post-genomics Era

Springer

Smart Plant Breeding for Field Crops in Post-genomics Era

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

Smart Plant Breeding for Field Crops in Post-genomics Era

Springer

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

ISBN 978-981-19-8217-0 ISBN 978-981-19-8218-7 (eBook)
https://doi.org/10.1007/978-981-19-8218-7

© The Editor(s) (if applicable) and The Author(s), under exclusive license to Springer Nature Singapore Pte Ltd. 2023
This work is subject to copyright. All rights are solely and exclusively licensed by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors, and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, expressed or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

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

D. Sharma et al. (eds.), *Smart Plant Breeding for Field Crops in Post-genomics Era*,
https://doi.org/10.1007/978-981-19-8218-7_1

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

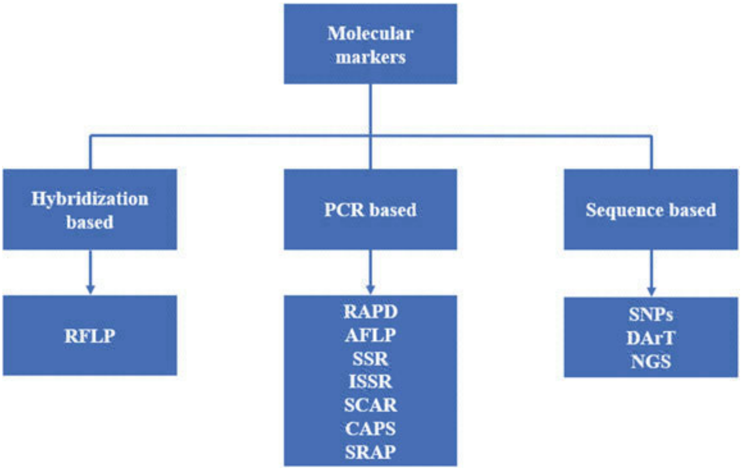

Fig. 1.1 A systematic overview of different molecular markers

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)