Jameel M. Al-Khayri · Shri Mohan Jain
Dennis V. Johnson *Editors*

Advances in Plant Breeding Strategies: Cereals

Volume 5

Springer

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ISBN 978-3-030-23107-1 ISBN 978-3-030-23108-8 (eBook)
https://doi.org/10.1007/978-3-030-23108-8

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

Preface

Contemporary plant breeders no longer need to rely solely on traditional methodologies in their work of assuring a sustainable and elastic level of world food production. However, human population is increasing at an alarming rate in developing countries, and food availability could gradually become a serious problem. Agriculture production is severely affected because of environmental pollution, rapid industrialization, water scarcity and quality, erosion of fertile topsoil, limited remaining arable land to expand production area, lack of improvement of local plant types, erosion of genetic diversity, and dependence on only few crop species for food supply worldwide. According to FAO, 70% more food must be produced over the next four decades to feed a projected population of 9 billion people by the year 2050. Currently, only 30 plant species are used to meet 95% of the world's food requirements, which are considered as the *major crops.* The breeding programs of these crops have been very much dependent on the ready availability of genetic variation, either spontaneous or induced. Plant breeders and geneticists are under constant pressure to sustain and increase food production by using innovative breeding strategies and introducing minor crops that are well adapted to marginal lands and can provide source of nutrition through tolerance of abiotic and biotic stresses. In traditional breeding, introgression of one or a few genes into a cultivar is carried out via backcrossing over several plant life cycles.

With the development of new molecular tools, molecular marker-assisted backcrossing has facilitated rapid introgression of a transgene into a plant and reduced linkage drag. Continued development and adaptation of plant biotechnology, molecular markers, and genomics have established ingenious new tools for the creation, analysis, and manipulation of genetic variation for the development of improved cultivars. For example, molecular breeding has great potential to become standard practice in the improvement of several fruit crops. Adopting a multidisciplinary approach comprised of traditional plant breeding, mutation breeding, plant biotechnology, and molecular biology would be strategically ideal for developing new improved crop varieties. This book highlights the recent progress in the development of plant biotechnology, associated molecular tools, and their usage in plant breeding.

The basic concept of this book is to examine the best use of both innovative and traditional methods of plant breeding to develop new crop varieties suited to different environmental conditions to achieve sustainable food production and enhanced food security in a changing global climate, in addition to the development of crops for enhanced production of pharmaceuticals and innovative industrial uses. Three volumes of this book series were published in 2015, 2016, and 2018, respectively: Volume 1, *Breeding, Biotechnology and Molecular Tools*; Volume 2, *Agronomic, Abiotic and Biotic Stress Traits*; and Volume 3, *Fruits*. In 2019, the following four volumes are concurrently being published: Volume 4, *Nut and Beverage Crops*; Volume 5, *Cereals*; Volume 6, *Industrial and Food Crops*; and Volume 7, *Legumes*.

This Volume 5, subtitled *Cereals*, focuses on advances in breeding strategies using both traditional and modern approaches for the improvement of individual crops. This volume addresses important staple food crops including barley, fonio, finger millet, foxtail millet, pearl millet, proso millet, quinoa, rice, rye, tef, triticale, and spelt wheat.

Chapters are written by internationally reputable scientists and subjected to a review process to assure quality presentation and scientific accuracy. Each chapter begins with an introduction covering related backgrounds and provides in-depth discussion of the subject supported with high-quality color photos, illustrations, and relevant data. This volume contains a total of 96 figures and 50 tables to illustrate presented concepts. The chapter concludes with an overview of the current status of breeding and recommendations for future research directions. A comprehensive list of pertinent references is provided to facilitate further reading.

The book is an excellent reference source for plant breeders and geneticists engaged in breeding programs involving biotechnology and molecular tools together with traditional breeding. It is suitable for both advanced undergraduate and postgraduate students specializing in agriculture, biotechnology, and molecular breeding as well as for seed companies and policy makers.

We are greatly appreciative of all chapter authors for their contributions toward the success and quality of this book. We are proud of this diverse collaborative undertaking, especially since this volume represents the efforts of 53 scientists from 14 countries. We are also grateful to Springer for giving us an opportunity to compile this book.

Al-Hassa, Saudi Arabia — Jameel M. Al-Khayri
Helsinki, Finland — Shri Mohan Jain
Cincinnati, OH, USA — Dennis V. Johnson

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Editors and Contributors

About the Editors

Jameel M. Al-Khayri is a Professor of plant biotechnology affiliated with the Department of Agricultural Biotechnology, King Faisal University, Saudi Arabia. He received his BS in Biology in 1984 from the University of Toledo, MS in Agronomy in 1988, and PhD in Plant Science in 1991 from the University of Arkansas. He is a member of the International Society for Horticultural Science and Society for In Vitro Biology as well as the National Correspondent of the International Association of Plant Tissue Culture and Biotechnology. His graduate work resulted in the establishment of in vitro regeneration protocols for spinach and zoysia grass. For the last two decades, he dedicated his research efforts to date palm. He has authored over 60 research articles in referred international journals and 25 review chapters and edited 7 journal special issues. In addition, he edited five reference books on date palm biotechnology and utilization of genetic resources and seven volumes of the book series advances in plant breeding strategies. He has been involved in organizing international scientific conferences and contributed numerous research presentations. In addition to teaching, students advising, and research, he held administrative responsibilities as the Assistant Director of Date Palm Research Center, Head of Department of Plant Biotechnology, and Vice Dean for Development and Quality Assurance. Dr. Al-Khayri served as a Member of Majlis Ash Shura (Saudi

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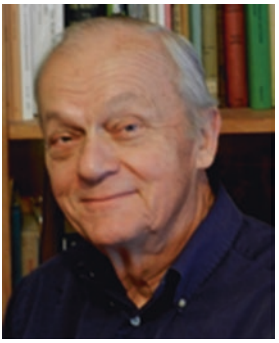
Shri Mohan Jain is a Consultant and Plant Biotechnologist, Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland; he received his MPhil in 1973 and PhD in 1978, Jawaharlal Nehru University, New Delhi, India. He was a Postdoctoral Fellow in Israel and the USA and Visiting Scientist/Professor in Japan, Malaysia, Germany, and Italy. He was a Technical Officer, Plant Breeding and Genetics, International Atomic Energy Agency (IAEA), Vienna, Austria, 1999–2005. He is a Member of the International Association of Plant Tissue Culture and Biotechnology and an Editorial Board Member of *Euphytica*, *In Vitro*, *Propagation of Ornamental Plants*, *Emirates Journal of Food and Agriculture*, and a series on *Forest Biotechnology*. His publications are more than 160 in peer-reviewed journals, book chapters, and conference proceedings, and he has edited 55 books and was invited speaker and acted as a Chairperson in several international conferences worldwide. He was awarded Nobel Peace Prize in 2005 in commemoration of the awarding to IAEA of the Nobel Peace Prize for 2005; he is also former consultant to IAEA, the European Union, the Government of Grenada, Iranian Private Company, and the Egyptian Government. Currently his research interests are somatic embryogenesis, organogenesis, haploidy, somatic cell hybridization, somaclonal variation, and mutagenesis mainly in medicinal plants, date palm, and banana genetic improvement, genetic diversity, erosion, conservation, and utilization in the context of climate change and food and nutritional security.



Dennis V. Johnson is a Consultant and former University Professor. He is a graduate of the University of California, Los Angeles, where he completed his BA (1966), MA (1970), and PhD (1972) degrees in Geography, with specialization in Agriculture and Biogeography. He has taught at several colleges and universities, including the University of Houston, and was a Visiting Professor for 2 years at the University of Ceará, Fortaleza, Brazil. Dr. Johnson also has worked extensively with international development agencies providing technical assistance to agriculture and forestry on projects and programs in Africa, Asia, Europe, and Latin America. He has published numerous articles on palm utilization and conservation and has edited or written books for FAO, IUCN, and UNEP. He has also translated into English plant science books from Portuguese and Spanish. A decade ago, Dr. Johnson began to focus his research on date palm, in particular its introduction to nontraditional areas such as Spain, North and South America, and Australia. He co-authored a book on date growing in the USA and has made presentations at five international date palm conferences and co-edited books on date palm, sago palm, and plant breeding.

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Chapter 1
Barley (*Hordeum vulgare* L.) Breeding

Essam Fathy El-Hashash and Karima Mohamed El-Absy

Abstract Barley (*Hordeum vulgare* L.) is one of the Neolithic founder crops of Old World agriculture. It is a flowering plant belonging to the family Poaceae or Gramineae (herbs) that is cultivated in temperate climates across the world at 350–4050 m above sea level, and evolved from *H. spontaneum* (K. Koch) Thell. The economically most important species of the genus is barley, *H. vulgare*. Species of barley consist of diploid (2n = 2x = 14), tetraploid (2n = 4x = 24), and hexaploid (2n = 6x = 42) cytotypes. Barley constitutes the fourth most important grain crop in the world after wheat, rice and maize. Barley grain is used as livestock feed and forage, malt beverages, human food, soil improvement and has medicinal value, but is barely considered as a highly-needed crop of the present era. Common barley hails originally from western Asia and North Africa. It is one of the earliest documented agricultural grains, dating back to the Neolithic period (8500 years ago) in the Nile Delta portion of the Fertile Crescent. Barley is a rich source of proteins, B vitamins, niacin, minerals and fiber dietary; also, it is a good source of manganese and phosphorus. Raw barley consists of carbohydrates (78%), proteins (10%), water (10%) and fat (1%). This chapter discusses the taxonomy, economic importance, origin and history, germplasm resources, traditional breeding methods and biotechnology methods, and their application for crop improvement in association with conventional breeding methods of barley.

Keywords Biotechnology methods · Hybridization · Importance · Mutation · Taxonomy · Traditional breeding

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J. M. Al-Khayri et al. (eds.), *Advances in Plant Breeding Strategies: Cereals*,
https://doi.org/10.1007/978-3-030-23108-8_1

1.1 Introduction

Cereals are annual, herbaceous plants belonging to tribe Triticeae of the grass family Poaceae (Gramineae) and grown for their edible seeds. The word *cereal* is derived from Ceres, the ancient Roman goddess of harvest. The term cereal is used to either describe the grain or the seed itself. Grain is a collective term applied to cereals. Important cereals are wheat, oats, barley, rye, maize, rice, millet and grain sorghum (Decoteau 2005). There is significant historical and archaeological evidence documenting the role of barley as a source of sustainable food in human evolution. In fact, barley was one of the most important food cereals and source of alcoholic beverages from antiquity until the beginning of the twentieth century (Newman and Newman 2008). Globally, barley grain is very important today, and it occupies fourth place in both the quantity produced and in the area of cultivation of cereal crops in the world after wheat, rice and maize. In Feb 2018/2019, the annual world harvest of barley was approximately 140,602 thousand mt from an area of about 47,009,175 ha (USDA 2019). Plant breeding has been a major scientific approach in improving crop production, with the ability to contribute to increased productivity by about 50% (Fehr 1984). Plant breeding science has remained vibrant, with continued success in the development and deployment of new cultivars on a global basis during recent decades (Gepts and Hancock 2006). To initiate a proper program of breeding, it is necessary to have a notion of the variability, nature and size, heritability degree and expected genetic advance with respect to accessible breeding materials (Chand et al. 2008). Barley is a multifaceted crop, which is tolerant to a wide range of growth conditions from dry conditions in the Middle East, to the coldness of the high Andes region. It is more productive and yields change less than most other small grain crops such as wheat. Thus, it is widely used among farmers with limited and poor resources under less favorable climate and soil conditions (Newton et al. 2011). Generally, barley can withstand unfavorable agricultural conditions such as drought, salinity, alkalinity, varied topography like plains, hills, and under irrigated and rainfed conditions. It is the farmers'favorite crop where wheat is not possible.

1.1.1 Origin, History and Distribution

Barley is a very old crop, one of the founder crops of Old World agriculture and among the first domesticated crops in the Near East (Robinson 2007). The archaeological remains of the barley grains found at different locations in the Fertile Crescent (Zohary and Hopf 1993) indicate that the crop was domesticated around 8000 BC. The crop originated in a wild state in the Middle East, and in Ethiopia. *Hordeum vulgare* ssp. *spontaneum*, wild barley, is a direct ancestor of cultivated barley and valuable genetic resource for its improvement for productivity, biotic and abiotic resistance**.** Multiple evidence has been found to indicate that most genetic

diversity in wild barley is concentrated in populations growing in the Middle East (Nevo 1992). The direct ancestors of barley and many species close to *Triticum*, *Aegilops* and *Hordeum* and other genera of the tribe Triticeae, including *Taeniatherum*, *Psathyrostachys*, *Eremopyrum*, *Elymus* and *Thinoypyrum*, are still found in the Near East. Therefore, the Near East is one of the most important genetic diversity centers for crop relatives in the world (von Bothmer 1996). Barley gained popularity in ancient times. It was the main food of the Pharaohs (ancient Egyptians) who used it to make bread and beer. Also, barley was adopted by the Greeks as a primary cereal grain because it was used as an ingredient in bread production and is a high energy food. As for Mesopotamia, it was the preferred cereal crop in many sites, because barley is more resistant to saline soils than wheat, and old irrigation practices in the Tigris and Euphrates rivers have increased soil salinity.

Chinese culture made the barley crop a symbol of male virility. European conquests introduced barley to the New World in the sixteenth and seventeenth centuries. Early farmers quickly decided that wheat was better for bread, and that barley was better for beer, because barley contains less gluten, which is why by itself it cannot be made into bread, such as wheat and rye. Similarly, it does not have the free nature of wheat and removal of the husk is difficult (Robinson 2007). The brewing of beer from barley was common among the early Egyptians and Mesopotamians, probably predating wine. The earliest known recipe of barley wine comes from Babylonia and dates back to as early as 2800 BC. It eventually became important mainly in areas, such as northern Europe, where grapes cannot be grown. Barley use patterns have not changed much, and it is now also used as food, feed and to make alcoholic beverages (Robinson 2007). Barley has a very wide geographical range, wider than almost any other grain crop. It is grown at the highest elevations in the Andes and Himalaya. And also near the deserts of Africa, the Middle East and China; and close to the Arctic Circle in the northern Asia, Europe and North America (Blattner et al. 2010). Globally, Europe is the primary continent for the cultivation of barley, followed by Asia. With respect to countries, Russia, China, Canada, USA, Spain, France, Australia, UK and India are the leading barley producers (Fig. 1.1).

1.1.2 Taxonomy

The existence of a common and well-defined crop classification system is important in crop and agricultural sciences. Plant classification precedes the simplification of plant collection, research, breeding and specialized development efforts. The presence of standardized plant names facilitates effective communication, dissemination and retrieval of scientific information. Linnaeus was the first to provide a botanical description of barley in *Species Plantarum* in 1753 (von Bothmer and Jacobsen 1985). The *Hordeum* genus belongs to the Triticeae tribe of the Poaceae family. The Triticeae tribe includes important cereal crops like wheat, rice, corn, barley, rye and triticale (Löve 1984). The principal ranks of barley classification in descending sequence are:

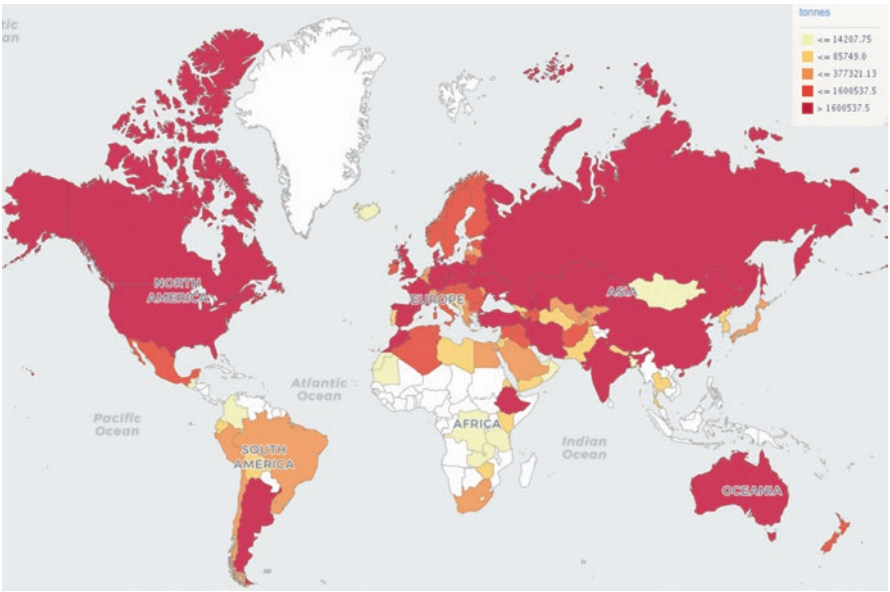

Fig. 1.1 World distribution and production map of barley: average mt, 2010–2017
Source: http://www.fao.org; accessed February 20, 2019

Domain: Eukaryota
Kingdom: Planta – Plants
Subkingdom: Tracheobionta – Vascular plants
Phylum: Spermatophyta – Seed plants
Subphylum: Magnoliophyta – Angiospermae – Flowering plants
Class: Liliopsida – Monocotyledonae
Subclass: Commelinidae
Order: Cyperales
Family: Poaceae – Grass
Genus: *Hordeum*
Species: *Hordeum vulgare*

According to von Bothmer et al. (2003), there are 32 species, for a total of 45 taxa, in the genus *Hordeum*. Blattner et al. (2010) stated that the genus *Hordeum* comprises about 33 species: 23 species in South and North America, while only 10 species occurring in other regions of the world. Jacobsen and von Bothmer (1992) documented that in *Hordeum* taxonomic studies over the years there have been many different views on the number and affinity of species in the genus. Harlan (1918) classified barley into four species, essentially on the basis of fertility of the lateral spikelets: (1) all spikelets fertile (6-row barley): *H. vulgare* L. and *H. intermedium* Keke and (2) only the central spikelets fertile (2-row barley): *H. distichon* L. and *H.*

defidens Steud. Nevski (1941) recognized 28 species of genus *Hordeum* (3 in which today are considered *H. vulgare* L.), placed into 6 sections: *Critesion*, *Stenostachys*, *Anisolepis*, *Hordeastrum*, *Bulbohordeum* and *Crithe*. Dewey (1984) and Löve (1984) divided barley into two groups; *Hordeum* s.str. consisting either of *H. vulgare* or *H. vulgare* and *H. bulbosum*, while all other species were included in *Critesion* genus. Von Bothmer and Jacobsen (1985) divided genus *Hordeum* into four sections: *Hordeum*, *Anisolepis*, *Stenostachys* and *Critesion*. Petersen and Seberg (2003) delimited four different sections from von Bothmer et al. (1995) based on phylogentic analyses of single-copy nuclear and chloroplast DNA data. Blattner (2009) further proposed a finer structure of infrageneric categories using a multitude of loci from the chloroplast and nuclear genomes. According to the analysis of Blattner (2009), the different species of *Hordeum* with their ploidy, haploid genome and distribution area are presented in Table 1.1. *Hordeum* genus differs and is unusual among the Triticeae because it contains both annual species (*H. vulgare* and *H. marinum* Huds.) and perennial species (*H. bulbosum* L.) (von Bothmer 1992).

1.1.3 Types of Barley

Barley has numerous cultivars and there are many ways to classify them. The first way to classify barley is to describe the beards (awns) covering the kernels such as long awned, short awned, (normal) hooded, elevated hooded and subjacent hooded. A second way barley can also be described by: hulled or hulless (naked), feed or malt type, height (dwarf) and seed color (colorless, white, yellow, blue). As for the third way, barley can be divided into two main types depending on the number of rows of grain observed when the heads of the stalks are viewed from the top: (1) Two-row barley, because the head of the stalk contains two rows of barley kernels (Fig. 1.2a). The central floret in this type is fertile and the two lateral florets are sterile, resulting in the presence of one seed at each node, giving the head a flat appearance (Kling 2004). When the spike is viewed from above, there appears to be two rows of kernels (Kumlehn and Stein 2014). Each stalk produces 15–30 kernels. Generally, it seems that two-row barley has the best performance of kernels with highest thousand kernel weight and starch content, while protein content is slightly lower (Bowman et al. 2011). Wild barley is two-row type. (2) Six-row barley, because the head of the stalk contains six rows of barley kernels (Fig. 1.2b). All florets are fertile. The central seeds are round and fat, but the laterals tend to be slightly asymmetric. When the spike is viewed from the top, there appears to be six rows of kernels (Kumlehn and Stein 2014). Each stalk produces 25–60 kernels. A single head of barley can produce up to 80 seeds (Kling 2004). Many sources only differentiate between two- and six-row cultivars, because the four-row barley is in reality a wide six-row barley. Most cultivated barley is of the six-row type.

Table 1.1 Taxa, ploidy, haploid genome and distribution area of the genus *Hordeum* recognized by Blattner (2009)

Taxa	Ploidy	Haploid genome	Distribution area
Subgenus *Hordeum*			
Section *Hordeum*			
H. vulgare L.			
ssp. *vulgare*	2×	H	Cultivated
ssp. *spontaneum* (C. Koch) Thell.	2×	H	SW Asia
H. bulbosum L.	2×,4×	H, HH	Mediterranean to C Asia
Section *Trichostachys* Dum.			
H. murinum L.			
ssp. *glaucum* (Steud.)Tzvel.	2×	Xu	Mediterranean to C Asia
ssp. *murinum*	4×	XuXu	NW Europe to Caucasus
ssp. *leporinum* (Link) Arc.	4×,6×	XuXu, XuXuXu	Mediterranean to C Asia
Subgenus *Hordeastrum* (Doell) Rouy			
Section Marina (Nevski) Jaaska			
H. gussoneanum Parl.	2×,4×	Xa, XaXa	Mediterranean to C Asia
H. marinum Huds.	2×	Xa	Mediterranean
Section *Stenostachys* Nevski			
Series *Sibirica* Nevski			
H. bogdanii Will.	2×	I	C Asia
H. *brevisubulatum* (Trin.) Link	2×, 4×, 6×	I, II, III	C Asia
H. roshevitzii Bowden	2×	I	C Asia
Series *Critesion* (Raf.) Blattner comb. & stat. nov.			
H. californicum Covas & Stebb.	2×	I	SW USA
H. chilense Roem. & Schult.	2×	I	Chile and W Argentina
H. comosum Presl	2×	I	S Argentina
H. cordobense von Bothmer et al.	2×	I	C Argentina
H. erectifolium von Bothmer et al.	2×	I	C Argentina
H. euclaston Steud.	2×	I	C Argentina, Uruguay
H. flexuosum Steud.	2×	I	E + C Argentina
H. intercendens Nevski	2×	I	SW USA, NW Mexico
H. muticum Presl.	2×	I	C to N Andes
H. patagonicum (Haum.) Covas	2×	I	S Argentina
H. pubiflorum Hook. f.	2×	I	S Argentina
H. pusillum Nutt.	2×	I	C+ E USA
H. stenostachys Godr.	2×	I	C Argentina
H. depressum (Scribn. & Sm.) Rydb.	4×	II	W USA

(continued)

Table 1.1 (continued)

Taxa	Ploidy	Haploid genome	Distribution area
Interserial allopolyploids of series *Critesion* (all combining genomes of an American species with most probably one derived from *H. roshevitzii*)			
H. brachyantherum Nevski	4×	II	W North America, Kamchatka,
H. fuegianum von Bothmer et al.	4×	II	S Argentina, S Chile
H. guatemalense von Bothmer et al.	4×	II	Guatemala, S Mexico
H. jubatum L.	4×	II	NE Asia, NW + W North America
H. tetraploidum Covas	4×	II	C Argentina
H. arizonicum Covas	6×	III	SW USA
H. lechleri (Steud.) Schenk	6×	III	C+ S Argentina
H. parodii Covas	6×	III	C Argentina
H. procerum Nevski	6×	III	S Argentina
Section Nodosa (Nevski) Blattner comb. & stat. nov.			
H. brachyantherum Nevski	6×	IIXa	C California
H. capense Thunb.	4×	IXa	S Africa
H. secalinum Schreb.	4×	IXa	Mediterranean, C Europe

Source: Sato et al. (2014)

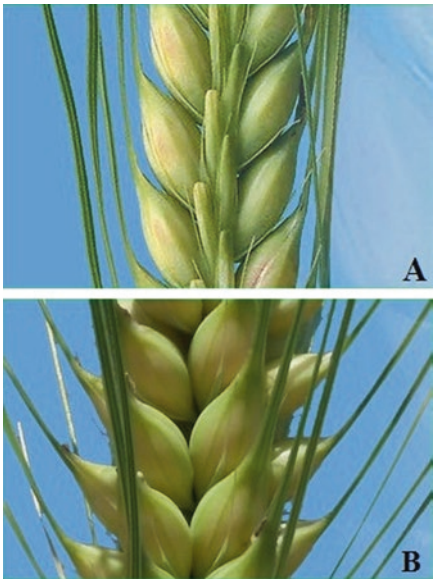

Fig. 1.2 Two-row barley (**a**) and six-row barley (**b**) Photos by E.F. El-Hashash

1.1.4 Agronomy and Trade Statistics

Barley production is important and one of the eight internationally grown cereal grains in the world (Table 1.2). The total area harvested of barley during the 2017/2018 growing season was 47.01 million ha worldwide, thereby representing the fourth most widely grown cereal crop after wheat, maize and rice and before of sorghum, oats, rye and triticale (Table 1.1) (FAOSTAT 2018). FAOSTAT (2018) records production in 243 countries worldwide. Barley production was 147.4 million mt in 2017/2018, also the estimated use of barley is 7.4 million mt as food for human use, 98.3 million mt for feed, 31.6 million mt for industrial use (mainly malt) and about 8 million mt for sowing. Because of economic, climatic and cultural influences, there are strong regional differences. Additionally, the world barley imports and exports 2017/2018 were 26.9 and 26.9 million mt, respectively (International Grains Council 2018; FAOSTAT 2018). Major production areas are in those parts of Europe, Asia, North America and Australia that share a continental climate. The top ten countries producing the most barley in 2017/2018 are summarized in Table 1.3. These countries had the largest growing area of barley, harvesting 27.48 million ha and producing 92.13 million mt in 2017, which were 58.45 and 62.51% of the total world barley production, respectively. Russia ranks first in barley production in the world, with 13.98% of the total production, followed by Australia (9.16%), Germany (7.36%) and France 7.15%). The remaining ten countries each produce between 5.62% (Ukraine) and 0.27% (Denmark) of world production. Yield levels in the Germany, France, UK and Denmark are considerably higher than the average for world production (3.14 tons/ha). Ukraine and Canada recorded a slightly higher yield and increasing rate than the world average. On the other hand, Russia, Australia, Turkey and Spain had lower than the world average. World barley production, consumption and ending stocks decreased from 149.78 million mt (2015/2016), from 150.35 million mt (2016/2017) and from 26.34 million mt (2015/2016) to 141.32, 142.67 and 17.70 million mt in 2018/2019, respectively (USDA 2018). The increase in total barley production, despite decreased harvested area, may be attributed to improved genotypes by various

Table 1.2 Area harvested, yield level and production of barley compared to other cereal crops in the world

Crop	Area harvested (million ha)	Yield level (mt/ha)	Production (million mt)
Wheat	218.54	3.53	771.72
Corn	197.19	5.75	1134.75
Rice	167.25	4.60	769.66
Barley	47.01	3.14	147.40
Sorghum	40.67	1.42	57.60
Oats	10.19	2.55	25.95
Rye	4.48	3.06	13.73
Triticale (x *Tritosecale*)	4.17	3.74	15.56

Source: FAOSTAT (2018)

Table 1.3 Barley production per continent and top 10 countries

Country	Area harvested (million ha)	Yield level (mt/ha)	Production (million mt)	Production (% of world)	Exports (million mt)
Russia	7.848	2.625	20.599	13.975	2.863
Australia	4.834	2.794	13.506	9.163	5.809
Germany	1.566	6.930	10.853	7.363	2.898
France	1.671	6.312	10.545	7.154	5.868
Ukraine	2.502	3.312	8.285	5.621	1.571
Canada	2.198	3.591	7.891	5.354	1.207
United Kingdom	1.177	6.091	7.169	4.864	1.757
Turkey	2.418	2.936	7.100	4.817	0.006
Spain	2.598	2.228	5.786	3.925	0.048
Denmark	0.665	6.000	0.399	0.271	0.694

Source: FAOSTAT (2018)

breeding methods and modern cultural practices, such as more effective weed control, balanced fertilizer application and irrigation. It is also possible that those areas that have been removed from barley production were less productive than those maintained in production (Newman and Newman 2008).

1.1.5 Economic Importance

Today barley is grown across all temperate climate regions. It is cultivated for many purposes, but the majority of barley is used for animal feed, human consumption or malting (Duke 1983). The primary use of barley is for animal feed. Barley percentage used for livestock feed in different countries ranged from >50–<90% (Zhang and Li 2009). Globally 70% of barley production is used directly or indirectly for feeding animals (Akar et al. 2004). It is a good choice as a feed ingredient as it provides energy and dietary fiber intake for domestic animals. Barley grain represents an appropriate source of starch and has a higher crude fiber and protein content than other crops such as maize (Kumlehn and Stein 2014). The second most important use of barley is for malt. Globally, 30% of the world barley production is used for malting purposes (Akar et al. 2004). Both two- and six-row cultivars are used for malting. But, two-row barleys are favored throughout most of the world (Kling 2004). Malting barley typically has a lower protein content (<11%) than feed barley (>12%) (Kumlehn and Stein 2014). Some 90% of malted barley is utilized for malting beer and the remainder for food ingredients (Akar et al. 2004). As for the third important use, barley is the principal cereal grain crop used for food consumption in several regions of the world. Regarding the healthful benefits of barley, it contains β-glucan, which has been shown to reduce cholesterol level in the liver (EFSA Journal 2011). It also has a low glycemic index and high fiber content, making it a healthy choice for diabetics (Brennan and Cleary 2005). It also stimulates fatty acid synthesis in the liver. Tocols (tocopherols, tocotrienols) are also reported to lower

the total cholesterol and the low density lipoprotein cholesterol (Wang et al. 1993). Also, the barley is used in industrial fields in making products such as paper, fiberboard, glucose and maltose syrups (Zhang and Li 2009) as well as in agriculture such as for animal bedding (Akar et al. 2004).

1.1.6 Domestication, Selection and Early Improvement

The area of domestication of barley, along with several other Old World crops, is the Near East region, beginning some 10,000 years ago (Zohary and Hopf 1993). Genus *Hordeum* is widely distributed in temperate regions of the world at diversity centers defined as locations having the largest number of wild species, such as southern South America, western North America, Central Asia and Southwest Asia (von Bothmer 1996). The economically most important species of the genus is barley, *Hordeum vulgare.* The overwhelming majority of barley cultivars are based on pure line development because it is a self-pollinating crop. Hybrid breeding is also possible and has resulted in the release of a number of hybrid cultivars using various hybridization methods (Longin et al. 2012). At this time, barley breeding represents the incorporation of conventional breeding and biotechnology methods, such as molecular marker application, transgenics, tissue culture and doubled haploid production. The integration of modern technologies with conventional breeding methods in barley breeding programs has significantly accelerated the time from first hybrid to cultivar release, while maintaining the increase of average yield and improving other traits such as biotic and abiotic stress resistances as well as quality. For the future, yield, resistance to biotic and abiotic stresses, and quality characteristics remain the important goals to be achieved to ensure the success of barley (Verstegen et al. 2014).

1.2 Cultivation and Traditional Breeding

1.2.1 Current Cultivation Practices

Planting time, seed rate and planting methods are among the key agronomic attributes which determine barley productivity. Before sowing, seeds should be pure (free of weed seed, insect pests and diseases) and should also have a good germination percentage (>85%), and particularly be free from covered smut (*Ustilago horde*), strip blotch (*Helimenthosporium graminum*) and loose smut (*U. nigra*, *U. nuda*). Seeds are usually treated with fungicide to control seed borne fungal diseases. The seed should be thoroughly washed to remove salt before sowing. For sowing in saline and alkaline areas, the seeds should be soaked in water overnight at room temperature for better and quicker germination. Normal seeding time of

barley in the Northern Hemisphere is October 15–November 15. However, the optimal periods are from the first to third weeks of November, and the third to fourth weeks of October, respectively, under irrigated and rainfed conditions. The optimal flowering date is complicated by the conflicting need to avoid frost damage around ear emergence and flowering and to complete grain filling before the high temperatures and frequent dry periods of late spring (Shackley 2000). Recent years have seen a move toward minimum cultivation techniques (min-till), which rapidly prepares a surface tilth of only a few centimeters deep and sowing the crop often in a single or reduced number of operations (Briggs 1978). Barley can be planted by broadcasting or in rows. However, it is recommended to plant in rows. The required seeding rate is 90–100 kg/ha and 80–100 kg/ha depending on number of seeds per kg and estimated establishment rate under irrigation and rainfed conditions, respectively. For saline and alkaline soils, 100–120 kg seed/ha are used to ensure desired plant population.

Barley grain yield under lower seeding rates is reduced because of higher weed infestation (Blokhin 2006). When barley is used as a cover crop for forage, its seeding rate is reduced by 1 million seeds per hectare (Lopachev et al. 2001). The highest yields of barley are generally produced from rows spaced 22–23 and 23–28 cm apart, under irrigated and rainfed conditions, respectively. In saline and alkali soils, 20 cm row spacing is recommended. Highest grain yields of barley are generally produced from rows spaced 25 cm or less. Low spike density generally occurs with rows spaced >25 cm and, although the plant will compensate with greater kernel number per spike and kernel weight, grain yield is reduced (Schillinger et al. 1999). Depending upon the initial soil moisture, seeding depth is 3–5 and 5.8 cm under irrigated and rainfed conditions, respectively. In clay soils or soils that have a tendency to form a crust, shallow planting is preferable. The method of sowing is an important aspect, particularly under rainfed conditions. Seed should be dropped with the help of a *nai* or *pora* (a wooden structure) attached to a country plough, or with the help of seed drill to ensure uniform distribution of seed at the optimum depth. In irrigated areas seed may be sown by the *kera* method (spreading seeds and turning the mould board plough), where seed is dropped by hand into the furrows. There should be adequate soil moisture for proper germination. Barley requires very little intercultural operations or weeding. In dry areas, 2–3 waterings are required after sowing (Duke 1983).

1.2.2 Current Agricultural Problems and Challenges

Agriculture faces many challenges, which have intensified in recent years due to slow increases in yields of crops, caused by biotic and abiotic stresses (Grassini et al. 2013). Climate shock, greenhouse gas emissions and increasing carbon sequestration, and consequent drought and floods, limit production and lead to higher prices worldwide (FAO 2016). Barley compared to other crops is less demanding with regard to environmental conditions. To prepare for the future and

to increase food production in the next 25 years, advanced agricultural research must now be promoted, utilizing all the available genetic resources (McCouch et al. 2013) and plant breeding techniques. Keeping this in view, efforts have been made to develop new cultivars resistant to heat and drought, and to improve soil and water management. Worldwide use of barley for feed and food is expected to remain stable for the foreseeable future.

1.2.3 Improvement Strategies

Plant breeding has several practical strategies to improve barley characteristics so that the crop becomes more desirable agronomically and economically. Barley breeders need to develop cultivars for markets which require clean bright grain with low moisture content, better disease resistance and high-quality traits (Knežević et al. 2004). Progress in improving barley depends on the availability of good candidate genes and on breeding techniques to assemble them in superior genotypes (Wiebe 1978). To produce an improved version of the barley plant by introducing and expressing one or more desirable genetic traits from a donor plant or organism to a receptor plant is the purpose of barley breeding using transgenics (Newman and Newman 2008). Researchers breeding for yield still rely heavily on traditional breeding as empirical selection and testing of elite lines and cultivars in the field. However, biotechnological strategies provide exciting opportunities to overcome many of the constraints of conventional breeding and to provide access to more diverse sources of genes.

A number of modern technical innovations have been brought to bear and influence barley breeding in recent years. The doubled haploid lines, molecular markers and genetic engineering approaches have helped accelerate the breeding process, others have made it more precise and effective (Verstegen et al. 2014), and quantitative trait locus mapping is a helpful technique for genetics and special breeding of barley to estimate the economically-important functions to specific sites of the genome (Knežević et al. 2004). Particularly important are those methods which have facilitated early generation and more targeted selection (Verstegen et al. 2014), while traditional breeding needs many years to develop a barley cultivar. Through the use of wild species in hybridization programs for barley breeding, it will be possible to produce genetic diversity among the offspring that requires selection of a combination with desirable traits and further crossing to repair the selected genotype. The success crossing of wild relatives in barley *Hordeum chilense* and durum wheat, and development of new hexaploid crop plant x *Tritordeum* Ascherson et Graebner, indicate that a wider hybridization program could be an open trend to identify new useful hybrids (Knežević et al. Knežević et al. 2004). A unique feature of barley is that the life of cultivars can easily be 10 years or more.

1.2.4 Traditional Breeding Methodologies and Limitations

Farmers, barley breeders and geneticists have developed increasingly complex breeding methods over the past 10,000 years (Patrick and Alfonso 2013). Barley breeders have used various traditional breeding methods to improve barley traits such as high yield, biotic resistance, abiotic resistance and malting quality. Most modern elite cultivars of barley were developed by traditional breeding, which is still highly effective. Traditional breeding methods to improving yield and quality traits of barley include: mass selection, pure line selection, pedigree selection, bulk selection, haploid production, doubled haploids, (anther or microspore culture, *Hordeum bulbosum*), male sterile-facilitated recurrent selection (MSFRS), diallel selective mating system (DSMS), mutation, interspecific and intergeneric crosses, backcross and single seed descent. Composite crossbreeding can be used in barley breeding. It is the result of combining a number of single crosses into one large mixture or composite as described by Harlan (1957). In barley, unlike other breeding methods, the two methods (MSFRS, DSMS) facilitate the expansion of the genetic base, break up existing linkage blocks, and provide a great amount of genetic variation (Anderson and Reinbergs 1985). The backcrossing method was used to develop strains of barley for agronomic and malt quality by Wiebe (1978) and to measure the influence of dietary fiber on the feed value of barley by Hockett (1981). Adaptation of introduced germplasm to new production areas is considered a major challenge in barley breeding. The reasons for this can range from the appearance of new diseases types to changing malt quality requirements for export markets. These production areas are often marginal for arable crops or have unique combinations of production constraints (Horsley et al. 2009). Traditional breeding products may not be able to meet the current requirements, so molecular breeding tools must be used actively in current breeding programs (Eglinton et al. 2006).

1.2.5 Role of Biotechnology

Conventional plant breeding and selection methods can be time-consuming and are often not very precise (Fig. 1.3) (Fehr 1987). Molecular mapping of the barley genome began in the 1980s (Kleinhofs et al. 1988), and since that time, biotechnology has played a vital role in the improvement of barley. This is because of its ability to overcome the shortcomings of other conventional breeding methods of barley improvement. Unlike traditional plant breeding, biotechnology techniques for genetic modifications effectively operate at organ, tissue, cell, protoplast and molecular levels. These modern techniques are considered an adjunct to traditional methods for effective and accurate plant breeding (Kang et al. 2007). Plant biotechnology includes three interacting technical components: (a) microbial bioprocessing techniques, (b) techniques for culturing somatic and reproductive cells, tissue and organs and (c) molecular and cellular techniques for the characterization and

Fig. 1.3 The difference between conventional and genomics tools used in barley breeding programs
Source: Kim et al. (2014) with modifications

modification of genomes, including techniques for the identification, recombination, cloning, transfer and expression of genetic material. Recently, barley grain has been successfully used in molecular farming as a promising bioreactor adapted to produce human therapeutic proteins or animal vaccines. In addition to development of reliable transformation technologies, it has been generating a large amount of various barley genetic resources and tools like sequence data, microarrays, genetic maps and databases (Mrízová et al. 2014). In barley, biotechnologies including molecular marker-aided technology and genetic engineering have been utilized to increase salinity tolerance (Roy et al. 2013), increase cold tolerance and germination vigor under low temperature conditions with a slight effect on plant growth (Soltész et al. 2012) and drought stress tolerance (Morran et al. 2011), high resistance to stem rust (Horvath et al. 2003), as well as increase the activity of thermostable β-glucanase (Horvath et al. 2000), thermostable β-amylase activity (Kihara et al. 2000), α-amylase activity (Tull et al. (2003), speed α-amylase and pullulanase activity (Cho et al. 1999).

1.3 Germplasm Diversity and Conservation

Without germplasm, breeding is impossible to conduct, because it is the lifeblood of plant breeding. Germplasm is the genetic material that can be used to immortalize the species or the population. It has no reproductive value itself, but through plant breeding, germplasm can be improved for crop best performance (Acquaah 2007). Barley genetic resources can be divided into six main groups: (1) new cultivars actually used, (2) neglected cultivars, often elite cultivars in the past and often found in the pedigrees of new cultivars, (3) landraces, (4) wild relatives in the genus *Hordeum*, (5) genetic and cytogenetic stocks and (6) breeding lines. Conservation of biodiversity is urgent due to (1) low genetic variation in natural and cultivated populations, (2) presence of natural disasters such as wildfires and prolonged droughts and (3) human activities such as indiscriminate clearing of land, new land settlements, breeder actions in types of cultivars developed and narrow genetic base (Acquaah 2012). The collection, assessment, use, conservation and exchange of genetic resources is of great importance, particularly in view of the rapid deterioration and

utilization of available world biodiversity (Mehra and Arora 1982; Mengesha 1984). Bioversity International (BI), formerly International Board for Plant Genetic Resources (IBPGR), and germplasm banks have been active in conserving and managing plant genetic resources. BI is working with most countries around the world to promote and coordinate the establishment of genetic resource centers and, moreover, to collect, store, conserve, document and evaluate plant germplasm use (Williams 1989). This strategy provides scientists ready and quick access to germplasm when they need it. Major barley germplasm collections are presented in Appendix II.

1.3.1 Germplasm Diversity

Aleksandr Sergeevich Serebrovskii was the first to develop the concept of germplasm diversity in the 1920s, calling it *genofond*; the concept was then brought to the USA from Russia by Theodosius Dobzhansky and translated as *gene pooling* (Graham 2013). The collection of all available genes that can be transferred from parents to offspring in the population of a single species is known as the gene pool (Gp). The larger the Gp population, the greater the diversity. Gp determines which phenotypes are present in the population at any given time. The concept of Gp mainly reflects the use of crop germplasm in breeding and thus it is a broad summary of available biosystematic data (von Bothmer et al. 1992); Gp is useful to plant breeders because it guides them in selecting germplasm to use in hybridization for plant improvement. In the specialties of crop breeding and genetics, genetic resources, gene resources and germplasm are similar concepts that include cultivated and wild plants as well as any other forms of life that can be used for crop breeding purposes (Sun and Gong 2009). Barley germplasm can be divided into five groups: cultivars, landraces, breeding lines, wild *Hordeum* species and genetic stock (Sato et al. 2014). When describing the barley germplasm state, the genetic diversity can be divided into the various Gps, and within the primary Gp in wild and cultivated plants of barley. Even within cultivated barley, there should be a division into cultivars, landraces and research material (Hintum van Hintum and Menting 2003). The genus *Hordeum* has three Gps as a source of new advantageous alleles in breeding for improving many traits, including yield (Fig. 1.4).

The primary gene pool (Gp-1) of cultivated barley (*Hordeum vulgare*) consists of (a) breeding lines and released cultivars; (b) landraces, which are still available in Asia and North Africa including Ethiopia and have been used until recently in other areas (both ssp. *vulgare*) (Sato et al. 2014) and (c) the wild *H. vulgare* ssp. *spontaneum* (C. Koch) Thell. (wild progenitor of the crop). There is no biological barrier to gene transfer among the Gp-1 (von Bothmer et al. 1992). Thus, the wild *H. vulgare* ssp. *spontaneum* has been repeatedly used to improve *H. vulgare* elite cultivars (Nevo 1992). This Gp-1 includes the main germplasm in current breeding activities. Fischbeck (2003) mentioned that *H. vulgare* ssp. *spontaneum* has been used for the transfer of disease-resistance genes into barley germplasm.

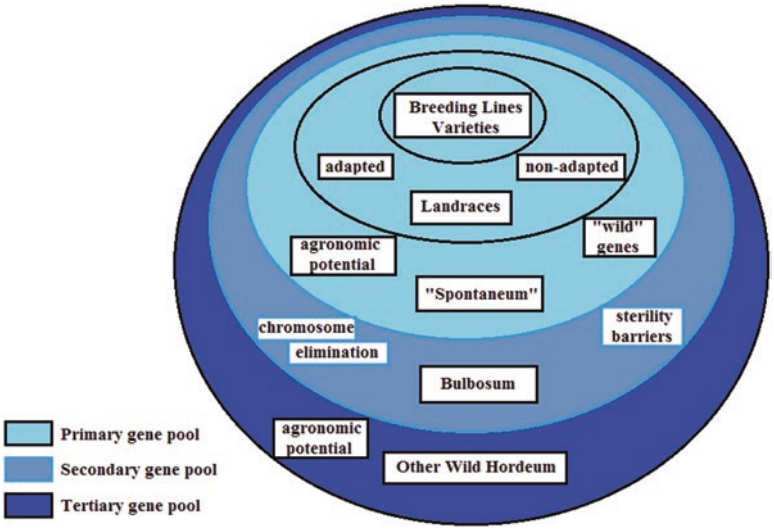

Fig. 1.4 The gene pools of barley (*Hordeum vulgare*)
Source: von Bothmer et al. (1992)

The secondary gene pool (Gp-2) is defined as containing only the single species, *Hordeum bulbosum* (bulbous barley), sharing the H genome with barley (Blattner 2009), which will cross with barley, but with some difficulty (Sato et al. 2014). Crossing barriers between *H. vulgare* and *H. bulbosum* can be overcome by modifying environmental conditions (Pickering 1984), the use of specific genotypes and biotechnology methods like embryo rescue (Kasha and Sadasiva 1971; Pickering 1983). Genes from *H. bulbosum* can be transferred to cultivated barley, thereby providing a new source for barley breeding (Pickering 2000). Also, Kasha and Kao (1970), Pickering (1984) and Chen and Hayes (1989) mentioned that it is possible to use the diploid *H. bulbosum* in production of doubled haploids by chromosome elimination. The tertiary gene pool (Gp-3) includes the other species of the genus *Hordeum* (von Bothmer et al. 1983). The species of this Gp-3 when crossed with *H. vulgare*, result in seeds that are either not viable or infertile (von Bothmer and Linde-Laursen 1989). The potential for barley improvement from this Gp-3 is therefore very limited; only through extraordinary procedures such as embryo rescue is gene transfer possible, or somatic hybridization and transformation, can be applied (Sato et al. 2014). Intergeneric hybridization between wild *H. chilense* and domesticated tetraploid durum wheat (*Triticum durum*) were used to form a new crop taxon x *Tritordeum* with new nutritional properties (Martin et al. 1999) and *H. chilense* introgressions allowed introduction of abiotic stress resistances into wheat (Forster et al. 1990).

1.3.2 Genetic Resources Conservation Approaches

In barley, plant genetic resources can be utilized to broaden the germplasm base of high-yielding improved cultivars with abiotic and biotic stress resistances (Singh 2006). There is extensive natural variation in barley, which makes it quite responsive to artificial selection (Kling 2004), but the collection and preservation of these genetic resources could make an important contribution to future barley research to improve the quality and productivity of barley. Owing to their great importance, conservation of barley genetic resources is necessary to address the global challenges that are currently facing human society, such as climate change, food scarcity, natural calamities, and the outbreak of new diseases and pests. Moreover, the number and assortment of cultivated plants and their wild species have been significantly reduced (Dzyubenko 2018). Biodiversity can be conserved away from its natural habitats and ecosystems (ex situ) like gene banks, or within them (in situ conservation).

1.3.2.1 In Situ Conservation

Wild relatives, landraces and weed barley can be maintained in their natural habitats or in locations where the plants have evolved. In situ conservation is often achieved in protected regions or habitats, and can either target the species or the ecosystem in which they occur (FAO 2010). It is an especially important way to maintain species that are difficult to conserve ex situ. Unlike the ex situ conservation methods in gene banks, where only part of the full diversity is covered, and the in situ method is able to save a greater proportion of the biological diversity (Acquaah 2012). For establishment of a global network for the in situ conservation of barley wild relative species, priority locations for genetic reserves have been identified for barley (*Hordeum* spp.) in the Americas (Chile); the highest priority locations for conserving the wild relatives of barley (*H. spontaneum* and *H. bulbosum*) are in the Near East (FAO 2010).

1.3.2.2 Ex Situ Conservation

A National Plan of Action for plant genetic resources for food and agriculture (PGRFA) conservation and management is being formulated over the next 20 years to address national development planning for the agriculture, environment and socioeconomic sectors (FAO 2018). Ex situ conservation continues to represent the most significant and widespread means of conserving PGRFA (FAO 2010). All conservation methods in which the species or cultivars are taken from their natural habitat and kept in surrounding areas created by humans are known as ex situ conservation (Acquaah 2007); for example gene banks, botanical gardens of research

institutes and experiment stations. Ex situ plant gene bank collections were created starting at the beginning of twentieth century, due to recognizing the danger due to a permanent risk of loss of the genetic variability of cultivated plants and their wild relatives in response to changing environmental conditions and cultural practices (Nagel et al. 2009). Gene banks maintained by public or private institutions act either alone or network with other institutions (FAO 2010). Barley occupies third place after wheat and rice in terms of the largest total number of ex situ accessions (Fig. 1.5). Based on figures from FAO (2010), it is estimated that about 466,531 barley accessions exist in ex situ germplasm collection, currently maintained globally, 18,469 less than were reported in 1996 FAO (1996). The number of barley accessions was adjusted to 371,000 by Hintum and Menting (2003) and to 370,796 by Global Crop Diversity Trust (2008). Accessions in a general collection include cultivars, selections, breeding lines and landraces.

The number of accessions includes all taxa of *Hordeum*: 88% of *H. vulgare* ssp. *vulgare*, 10% of *H. vulgare* ssp. *spontaneum*, 0.4% of *H. bulbosum* and 1.7% of other wild species. Of all barley accessions, Europe, North America and Middle East maintained 41, 25 and 12%, respectively. The remaining accessions are distributed among Asia, South America, Africa and Australia with 9, 7, 4 and 2%, respectively (van Hintum and Menting 2003). The genetic resources of barley i.e., landraces, breeding lines, crop wild relatives, cultivars and genetic stocks in genebank collections represent 44, 17, 15, 15 and 9%, respectively (Bockelman et al. 2010). Therefore, global barley holdings which include germplasm that has evolved in long-term interaction with local environments and farming practices, represent 59% of barley accessions, while the other three which are the result of modern plant breeding and research represent 41% (Bockelman et al. 2010). Major collections are

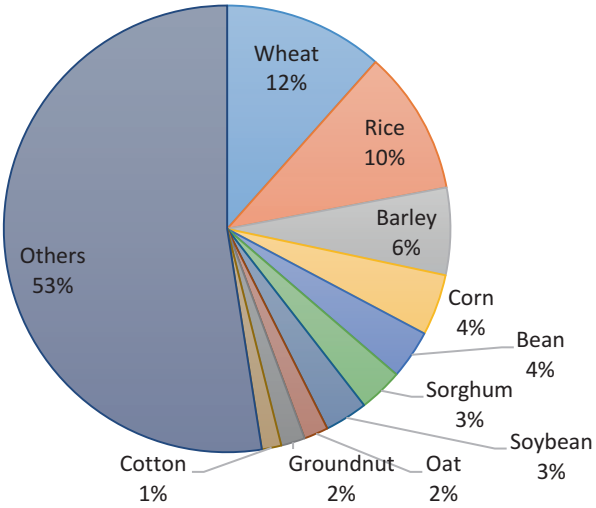

Fig. 1.5 Contribution of major crop groups conserved ex situ in the world
Source: FAO (2010)

those that contain more than 10,000 accessions of barley. The ten largest collections of barley germplasm are presented in Appendix II. Collectively, the ten collections maintain a total of about 254,386 accessions of 466,531 accessions, which represent 54.5% of the world total. The largest collection of barley germplasm is held by Plant Gene Resources of Canada (PGRC). Its holdings represent 16.4% of 254,386 and 9% of the world total. According to Hintum and Menting (2003), considerable duplications exist among the four largest global collections, i.e. PGRC, NSGC and ICARDA and CENARGEN, especially in the category of cultivars (Global Crop Diversity Trust 2008).

1.3.3 Cytogenetics

Barley is one of the most extensively studied crop plants and is used in studies dealing with cytogenetics and molecular cytogenetics (Ramage 1985). Four basic genomes, H, I, Xa and Xu have been identified in the *Hordeum* genus (Blattner 2009) (see Table 1.1). In the genus *Hordeum*, the basic chromosome number of x = 7 is represented across the 45 taxa. Multiples of this basic number are obtained also. The species *H. bulbosum*, *H. jubatum*, *H. murinum* and *H. secalinum* have been reported by different investigators as having either 7 or 14 pairs (tetraploid, 2n = 4x = 28). *Hordium nodosum* has been reported as having 7 or 21 pairs (hexaploid, 2n = 6x = 42). The economic species all have 7 pairs of chromosomes (diploid, 2n = 2x = 14) (Hayes and Immer 1942). Barley has the lowest chromosome number (2n = 14) compared with wheat (2n = 42), rice (2n = 24) and maize (2n = 20). The genome size (IC) of barley (4873–5096Mbp) is less than that of wheat (15,966 Mbp) and larger than that of maize (2292–3313 Mbp) and rice (401–466 Mbp) (Heneen 2011). The genome of barley is diploid and consists of 5.1 billion base pairs (Doležel et al. 1998) and contains over 84% of repetitive DNA (IBSC 2012). Manuel Spannagl from the Helmholtz Zentrum München, who led the gene explanation, points out that: *The barley genome contains more than 39,000 protein-coding genes, many of them present in multiple copies* (Mascher et al. 2017). During resequencing of four different barley cultivars (Bowman, Barke, Igri, Haruna Nijo), 15 million nonredundant single-nucleotide variants (SNVs) were discovered. These SNVs tend to decrease in frequency towards the peri-centromeric regions of all chromosomes (Rakshit and Ganapathy 2014).

The International Barley Genome Sequencing Consortium (IBSC) was formed to establish a practical working agenda towards physical mapping and sequencing of the genome barley, due to its importance as a primary crop and because of its model character for other Triticeae genomes including wheat, and rye comprehensive genetic and genomic resources (Stein 2014). Increasing sequence data and subsequent analyses are a valuable resource for comparative genomics and help plant breeders to develop new cultivars of improved barley.

1.4 Molecular Breeding

Molecular markers include protein markers, DNA markers (Fig. 1.6) and metabolite-based biomarkers, but the current use of molecular markers is limited to DNA markers (Singh and Singh 2015). Use of a variety of tools to manipulate the DNA of plants to improve them for specific purposes is known as molecular breeding (Acquaah 2012).

1.4.1 Molecular Marker-assisted Breeding

Plant breeders face the challenge of how to make the selection method more efficient and to accelerate the breeding progress to satisfy changing markets demands of crop cultivars (Jiang 2015). Molecular marker-assisted breeding (MAB) has enormous potential to improve conventional plant breeding and make it more efficient and accurate (Fig. 1.7). Marker-assisted breeding supplements classical breeding as a useful tool to select plants with desirable traits (Jiang 2013). It also aids in the study of genomic organization, to locate genes of interest and to facilitate the plant breeding process (Acquaah 2012). Thus it is a strategy and a powerful methodology for developing cultivars with combinations of relevant adaptive traits, including biotic and abiotic stress tolerance, along with yield and better quality in barley grain. MAB is used to describe many breeding strategies, such as marker-assisted selection (MAS), marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS) and genome wide selection (GWS) or genomic selection (GS) (Ribaut et al. 2010). With the advent of molecular marker techniques (Fig. 1.8), it became possible to carry out high-throughput genotyping to map and tag agronomically-valuable traits in barley (Verstegen et al. 2014). These markers have been used in a wide range of studies of barley cultivar diversity by Graner et al. (1991), Altinkut et al. (2003), Mylonas et al. (2014) and Ren et al. (2016). Kleinhofs et al. (1988) reported that the molecular mapping of the barley genome began in the 1980s, and since then, extensive data have been generated by many researchers that have allowed the development of detailed barley chromosome linkage maps

Fig. 1.6 Types of DNA Markers
Prepared by: E.F. El-Hashash and K.M. El-Absy

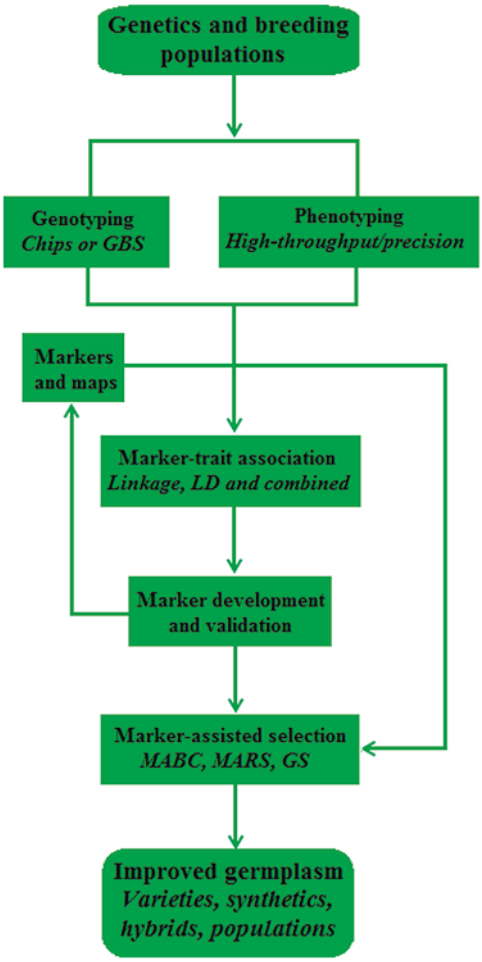

Fig. 1.7 Components of a marker-assisted breeding platform
Source: Xu et al. (2013)

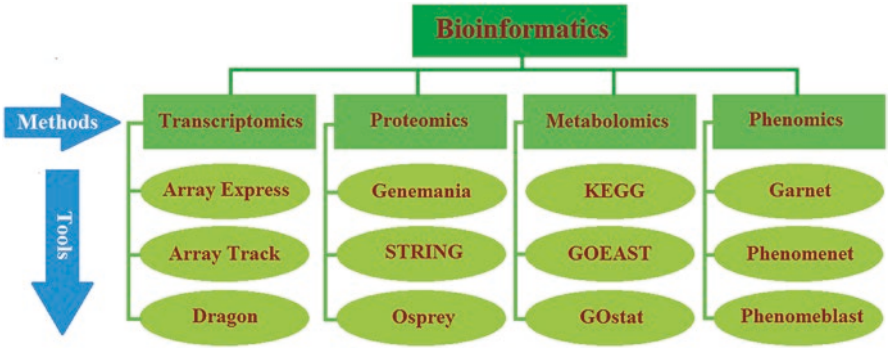

Fig. 1.8 Bioinformatics methods and tools of bioinformatics used in systems biology of plants
Prepared by: E.F. El-Hashash and K.M. El-Absy

Table 1.4 Molecular linkage map developed in barley

No.	Molecular map	Pedigree of mapping population	Markers	Agronomic trait targeted	References
1	Genetic linkage	Falcon x Azhul	SNP, DArT and SSR	Plant height, spike length and spike angle	Islamovic et al. (2013)
2	QTL map	Baudin × AC Metcalfe	SNP, SSR and DarT	Plant height	Zhou et al. (2015)
3	Genetic linkage	Huadamai 6 and Huaai 11	RAD, SNP and SSR	Plant height	Ren et al. (2016)
4	Genetic linkage	*Hordeum vulgare* x *H. vulgare* ssp. *spontaneum*	SSR and DArT	None	Hearnden et al. (2007)
5	QTL map	Mv9kr1 (wheat) × Igri (barley)	EST and PCR	Morphological traits	Ivanizs et al. (2018)

(Table 1.4). These approaches are applied very actively in current barley breeding. Approximately 1000 barley simple sequence repeats (SSRs) have been published, of which about one-half have been genetically mapped (Rao et al. 2016).

1.4.2 Functional Genomics

The term *genome* was coined by the botanist Winkler (1920). The genome is a maximum unit of a self-replicating body (Saitou 2004). Genomics is divided into two basic areas: structural genomics and functional genomics (Griffiths et al. 2005). Structural genomics focuses on sequencing of the genome; functional genomics on gene function (Acquaah 2007). In contrast genomics, functional genomics concentrates on the dynamic sides like gene transcription, translation and protein-protein interactions, compared to the static sides of the genomic information like DNA sequence or structures (Griffiths et al. 2005). The genome is basically a set of instructions for making all kinds of proteins. Because most genes are expressed as proteins, one of the common ways to understand the function of genes is by tracking protein expression by cells (called proteomics) (Acquaah 2007). The functional genomics/systems biology platform is a very complex and very strong approach to determine the function of individual genes, pathways, networks and genomes. The main hypothesis behind functional genomics is to study and evaluate the entire cell or organism as a system and to understand how various biological processes within this system occur, how to control them and how they are implemented (Mittler and Shulaev 2013). To assist functional gene analysis, insertional mutagenesis methods have been used over the last decade to create loss-of-function mutations by introducing transposable elements into a gene of interest (Zhao et al. 2006) and to use the random genomic insertion of either promoter or enhancer sequences (activation tagging) to generate dominant gain-of-function mutations (Ayliffe et al. 2007). Insertion lines are generated by creating transgenic plants carrying Ac and Ds elements, and

crossed them to induce Ds transposition (Singh et al. 2006). Ds elements were preferentially found in genic regions and exhibited a high-remobilization frequency (Singh et al. 2006). Such Ds launch pads, represented by barley lines with each harboring a one copy DS insertion at a well-defined position in the genome, were valuable for future targeted gene tagging. Similarly, dominant overexpression phenotypes (Ayliffe et al. 2007) help in the study of gene functions in the large barley genome where loss-of-function mutations often do not produce phenotypes due to gene redundancy. In order to functionally characterize candidate genes identified in functional genomic studies, a more efficient *Agrobacterium*-mediated barley genetic transformation method based on immature embryos was developed in spring barley (Hensel et al. 2008). In an effort to improve this technology, Kumlehn et al. (2006) developed a method of transformation in winter barley on the basis of infection with *Agrobacterium* of androgenic pollen cultures. During this method, homozygous doubled haploid plants can be obtained directly at high frequency by chromosome doubling. Gene family analyses in barley detect lineage-specific duplications of genes involved in nutrient transport to developing seeds and the mobilization of carbohydrates in grains. They demonstrate the importance of the barley reference sequence for breeding by inspecting the genomic partitioning of sequence variation in modern elite germplasm and highlighting areas prone to genetic erosion (Mascher et al. 2017).

1.4.3 Bioinformatics

The knowledge-based theoretical discipline that attempts to make predictions about biological function using data from DNA sequence analysis is known as *bioinformatics*. Bioinformatics is an information science application in biology through the use of supercomputers and advanced software to research and analyze accumulated databases of the genome sequencing and other similar project efforts (Acquaah 2007). It can be classified as information used in research of bioinformatics, divided into two classes: (1) Primary databases, consisting of original biological data such as raw DNA sequences and protein structure information from crystallography and (2) Secondary databases, containing the original data that has been processed to suit certain specific applications (Acquaah 2007). Recent advances in functional genomics have allowed the use of different bioinformatics methods such as transcriptomics, proteomics, metabolomics and phenomics. Figure 1.8 shows the bioinformatics methods and tools used in the systems biology of plants. Using bioinformatics, the number of miRNAs in the publicly-available miRNA database miRBase 19.0 for *Hordeum vulgare* is 69 (Saikumar and Kumar 2014). The miRNA-target pairs participate in gene expression regulation and hormone interaction in the barley embryo and provide evidence that miR393-mediated auxin response regulation affects grain development and influences gibberellic acid and abscisic acid homeostasis during germination (Bai et al. 2017). As for salinity in barley, possible contributions of *HvAKT1*, *HvAKT2*, *HvKCO1* and *HvHAK4* to regulation of K^+

relations of growing barley leaf cells were discussed by Boscari et al. (2009). Using bioinformatics, 21 *RPP13*-like genes were identified in barley. These genes all contained *CC, NB-ARC* and *LRR* domains. *MLOC_19262.1* gene is an important and promising candidate resistance gene for powdery mildew pathogenic fungus. The two *RPP13-like* genes i.e. *MLOC_57007.2* and *MLOC_5059.1*, may be involved in the regular or abiotic stress induced physiological metabolism in specific tissue or at specific developmental stages in barley; moreover, these functions may be related to specific domains. These results provide evidence of the functional diversity of plant pathogen resistance genes and would be useful in the future characterization of the *PRR 13-like* gene subfamily in barley (Cheng et al. 2018).

1.5 Genetic Engineering

Genetic engineering describes plants that are manipulated using a genetic modification technique, also called transgenics (Fig. 1.9), that introduces genes from any organism: from barley itself to other cereals or plants, bacteria and even humans (Newman and Newman 2008).

A *transgene* is a gene introduced into an organism by recombinant DNA technology; a transgenic plant is one expressing such gene(s). The transgene is integrated into appropriate plant expression vectors and then introduced into the plant cells using a transformation technique such as *Agrobacterium* coculture or particle gun acceleration (Singh and Singh 2015). Transgenic plants using *Agrobacterium* for

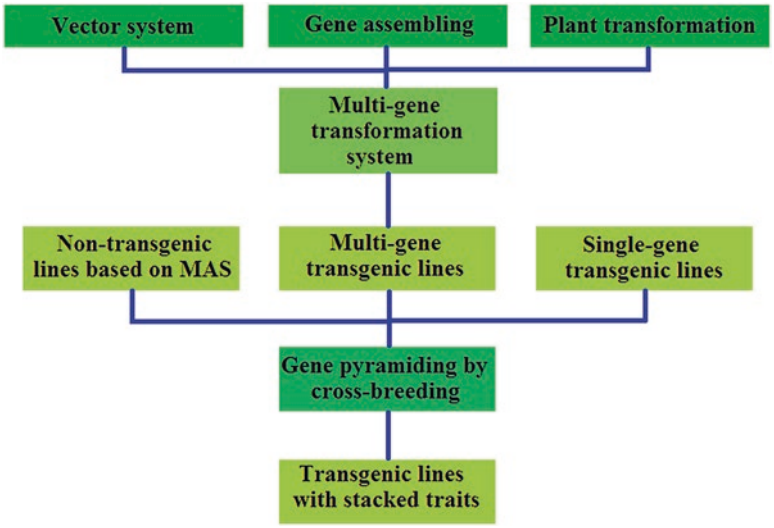

Fig. 1.9 Transgenic pyramiding methods in crop improvement
Source: Wan (2015)

plant breeding have been under development since the 1980s (Bevan et al. 1983). The Flavr Savr tomato cultivar was the first transgenic plant to be approved in 1994 for commercial cultivation (Singh and Singh 2015). The first stable barley transformation using cocultivation of immature embryos with *Agrobacterium tumefaciens* was achieved by Tingay et al. (1997). Recombinant DNA can be introduced into barley cells either by direct gene transfer, using extracted plasmid DNA, or by infection with a virus, or via the plant bacterial pathogen like *A. tumefaciens* (Kumlehn et al. 2014). Various transformation techniques such as particle bombardment, PEG-mediated, and electroporation-mediated have been used in barley (Joung et al. 2015). The expression of the *HvCBF4* gene of barley excessively in genetically modified rice has increased tolerance for low temperatures, drought and high salinity (Oh et al. 2007), while the *HVA1* gene imparted increased stress tolerance (Rohila et al. 2002). Many transgenic barleys have been produced with desired traits such as increased tolerance to low temperature, drought and high salinity; also, resistance to fungal diseases (leaf rust, stem rust) and increased grain yield (Table 1.5).

1.6 Mutation Breeding

A *mutation* is the heritable change to genetic material; individuals exhibiting modified characteristics because of heritable changes are known as *mutants* (Mba 2013). Any change of nucleotide sequences in one genome can be considered as a mutation in the broad sense (Saitou 2013). Types of mutation are shown in Fig. 1.10. The utilization of induced mutations in crop improvement is called *mutation breeding*. Breeding by plant mutation is a powerful tool for characterizing biological processes, specific gene functions and linkages between mutations and phenotypes in the mutated plant; therefore, plant mutation breeding is widely used to improve crops (Lee et al. 2015). A mutation is the ultimate source of evolutionary change; new alleles arise in all organisms, some spontaneously, others are due to exposure to radiation and chemicals in the environment (Griffiths et al. 2005). The development of semi-dwarf mutant cultivars in barley (*Hordeum vulgare*) such as Golden

Table 1.5 Transgenesis in barley for yield and certain traits

Gene	Function/stress tolerance	Reference
Rpg1	Stem rust resistance	Horvath et al. (2003)
CBF3	Cold, drought	Choi et al. (2002) and Li et al. (2011)
Rph1 – Rph20 (20 genes)	Leaf rust resistance	Golegaonkar et al. (2009)
HvCKX1	Increased grain yield	Zalewski et al. (2010)
HvGA2ox5	Frost tolerance	Soltész et al. (2013)
CYTOKININ OXIDASE/ DEHYDROGENASE	Drought tolerance	Ramireddy et al. (2018)

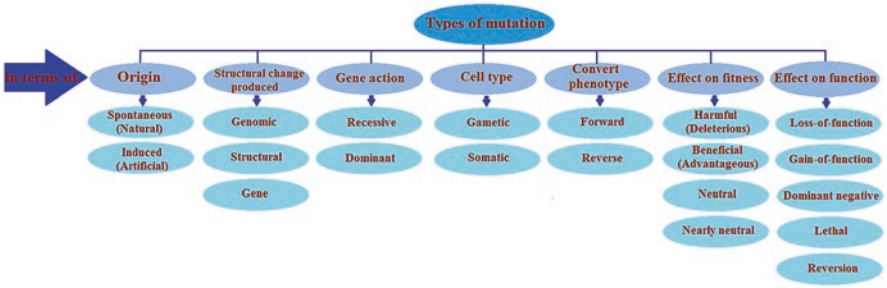

Fig. 1.10 Types of mutation
Prepared by: E.F. El-Hashash and K.M. El-Absy

Promise and Diamant in the early 1970s, are prominent examples of mutation breeding (Spencer-Lopes et al. 2018). The Cargine cultivar is the first commercial mutant produced of barley in France by irradiation of seeds with x-rays (100 Gy) (FAO/IAEA 2019). According to (FAO/IAEA 2019), 1554 mutant cultivars of cereal crops had been released to 2019. Among them, rice (821) presented the highest number with barley (312) second and wheat (260) third (FAO/IAEA 2019).

1.6.1 Conventional Mutagenesis

Mutagenesis is a valuable tool for barley breeders to directly produce cultivars or indirectly through crosses with other improved genotypes (Anderson and Reinbergs 1985) for improving the yield and quality of barley genotypes (Appendix II). Seeds are the most commonly used materials for induced mutation, thus high quality seeds must be selected. Seeds must be of the highest quality, free of disease, uniform and representative of the cultivar/line/genotype and with a high germination rate (90% or more) (Spencer-Lopes et al. 2018). Artificial mutation can be divided into two broad categories i.e. physical and chemical mutagens (Fig. 1.11). The moment that seed samples of a pure barley line has been treated with a mutagen they are designated as M0 seed and on germination, M1 plants. Selection of mutants is usually carried out in the M2 or M3 generation after mutagenic treatment of the seeds (Maluszynski et al. 1995). Some rare dominant mutants can be observed among the M1 population, but since M1 plants exhibit chimeras and physiological disorders, the selection is not usually practiced until subsequent generations (Szarejko and Forster 2007). If selection is conducted on a single plant basis in the M2 generation, the segregation ratio for a recessive mutant (in the progeny of an individual M1 plant) is seldom 3:1. Owing to the chimeric structure of M plants and the somatic effects of mutagenic treatment, there will always be a deficit of recessive mutants in M2 progeny. The homozygosity test must be performed (in the M3 or M4 generation) before the true to type homozygote mutant line is used either for direct agronomic evaluation or for a cross-breeding program (Szarejko et al. 1995). Because

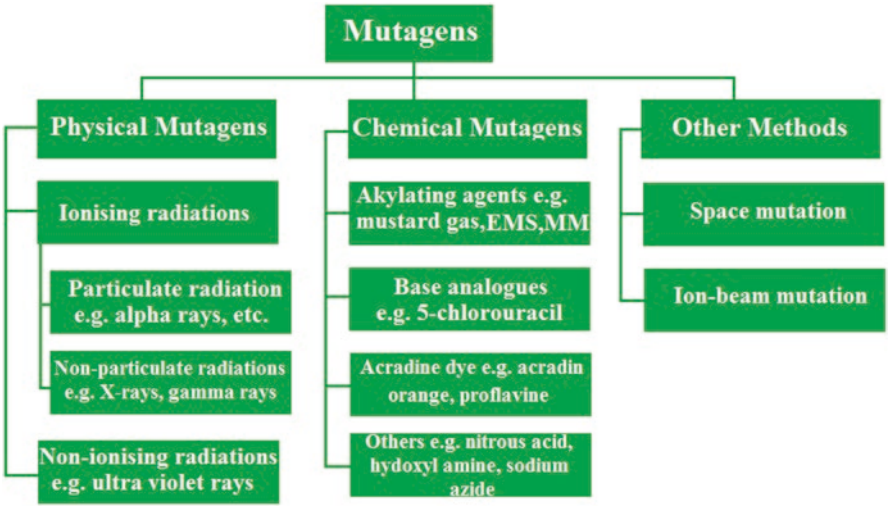

Fig. 1.11 Common mutagens used in plant mutation induction
Source: Spencer-Lopes et al. (2018) with modifications

many traits of interest to plant breeders are quantitative such as yield and quality, the process of detecting and evaluating mutations takes a long time, and can take up to four generations before introducing mutant lines into a crossing program (Szarejko and Forster 2007).

1.6.2 In Vitro Mutagenesis and Selection

Mutagenesis using in vitro plant cells and tissue cultures offers a feasible method to generate novel genetic variability (Larkin and Scowcraft 1983). The different plant material that can be irradiated/mutagenized include rooted stems, cuttings, detached leaves, dormant buds/plants, shoot apices (apical buds), axillary buds and tubers (Penna et al. 2012). It is possible to treat and examine large populations of cells before re-generating them into whole plants by using in vitro culture mutagenesis (Harten 1998). Among the various in vitro methods, the use of doubled haploids (DHs) for mutant induction and selection has been employed in many areas of barley research and breeding. The ability to repair mutations by DH is a key factor, especially since induced mutations are mostly recessive and usually cannot be detected until the M2 generation. The DH systems themselves provide an opportunity to target haploid as well as multiply haploid cells for mutation treatment and to capture the mutation in a homozygous, pure line (Szarejko and Forster 2007). Examples of DH production in M1 material of *Hordeum vulgare* plants were deployed by Umba di-Umba et al. (1991), Szarejko et al. (1991, 1995) and Vagera et al. (2004). In barley, if it is possible to carry out the mutant selection process in

vitro, the numbers of monocytes or embryos can provide a very large number of mutations generated by traditional mutations, thus increasing the likelihood of a rare mutation event. Application of the selection factor at the haploid or DH cell/embryo/callus/plant level is possible (Szarejko et al. 1995). Development of frost and disease resistance (*Fusarium* spp.) using in vitro selection has been reported in barley by Tantau et al. (2004) and Chawla and Wenzel (1987), respectively.

1.6.3 Molecular Analysis

Mutation procedures have been integrated with molecular-marker techniques thereby becoming more powerful and effective in barley breeding. To target specific nucleotide changes in any DNA fragment of interest, polymerase chain reaction (PCR) can be used, for example, genes or regulatory elements (Jost et al. 2019). Direct sequencing of PCR products can also be utilized in targeting induced local lesions in genomes (TILLING) provided that it is possible to discriminate sequencing errors from real mutations (Salvi et al. 2014). The principle for the detection of induced single nucleotide polymorphisms (SNPs) in mutagenized plants relies on the formation of hetero-duplexes at mismatch positions between pairing polymorphic DNA fragments during PCR (Jost et al. 2019). Once segregating populations are available, SNP genotyping and next-generation sequencing (NGS) platforms enable genetic and physical mapping, or even cloning of target mutant genes in single-step experiments (Salvi et al. 2014). Based on amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs) data, the effect of a mutagene leading to the development of cv. Diamant from cv. Valticky affected many genetic loci and it is therefore larger than expected (Mlčochová et al. 2004).

In barley, the chemical mutagen N-methyl-N-nitrosourea (MNU) is more effective than the physical mutagen gamma rays to induce point mutations. The average density of substitutions caused by the MNU in the selected sequences was 1 mutation per 504 kb or 2.2 mutations per 1 megabyte. The genomic barley survey with AFLP markers revealed ca. a level of AFLP polymorphism three times higher in MNU-treated than in the gamma-irradiated population. The performed bioinformatic analysis of the sequenced polymorphic AFLP products indicates that it reflects the barley genome regulation, and thus can be used for genome-wide mutation screens in this species (Kurowska et al. 2012). The use of radiation or methods of introducing larger lesions such as deletions and translocations are not appropriate for development of the TILLING population in barley due to the lack of compatibility with mutation detection by PCR (Jost et al. 2019).

1.6.4 Enhanced Traits and Improved Cultivars

The FAO/IAEA (2019) Mutant Varieties Database contains 312 barley (*Hordeum vulgare*) cultivars released worldwide. The database includes direct mutants and their hybrids. The information includes the mutant variety ID, cultivar name, country, local registration year, short description, character improvement details, usage subcategory type and mutant development type. These cultivars carry many economically-valuable traits, such as higher crop yield, malting and brewing quality, drought tolerance, salt tolerance, disease resistance, lodging resistance, cold resistance, nematode resistance, improved quality, earliness, good grain, straw stiffness, proanthocyanine-free content and genetic male sterility. Barley mutant cvs. Diamant and Golden Promise are high-yielding and short-statured and have made a large impact on the brewing industry in Europe. Golden Promise and Diamant have added billions of USD to the value of the brewing and malting industry (Ahloowalia et al. 2004). Estimates are that in Scotland itself, Golden Promise contributed USD 417 million to grain production (Ahloowalia et al. 2004). Golden Promise cv. is still common to this day as a typical cultivar resulting from transformation studies, in addition to still being grown to a limited extent in Scotland. The enormous economic contribution of the cv. Diamant, now crossed into many world barley cultivars is incalculable (Ohnoutkova 2019). Because it takes advantage of classical mutagenesis and high-throughput methods of mutation identification, TILLING has successfully been applied in barley improvement, for example, the manipulation of spike morphology, starch content and waterlogging tolerance by Gottwald et al. (2009), Sparla et al. (2014) and Mendiondo et al. (2016), respectively.

1.7 Hybridization

Hybridization is crossing or mating between two parental plants or pure lines which carry interesting traits to create new variability to initiate a breeding program. It can also create new cultivars (hybrid breeding) as has been achieved in barley and numerous crops.

1.7.1 Conventional Hybridization

Crosses may be produced in barley either in the field or the greenhouse. All the florets on a spike or panicle of each plant for use as a female parent are removed from the head before anthesis, except for about 10–15. Then the stamens of these remaining flowers are removed by small forceps before dehiscence of the anthers,

and the head is placed in a paper bag to prevent self-pollination. The crossing process is done by placing pollen grains from the male parent onto the stigma of flowers of the female parent. The paper bag is placed over the pollinated head and allowed to remain until harvest (Hayes and Immer 1942). The seeds and the progeny resulting from hybridization are known as a hybrid or first generation (F1). Segregating generations are F1 offspring and the following generations which are obtained by selfing or intermating of F1 and generations plants. Often, the term cross is used to indicate the hybridization products, i.e. the F1 and the segregating generations (F2, F3 etc.). Selection of transgressive segregants in the strain that perform better than the parents from the F2 generation and following generations are known as segregating generations and they can be handled either by the pedigree, bulk or backcross methods for new cultivar development. The constraints of this scheme are the limited number of recombinants produced in the F1, F2 and F3 generations, and the low probability of changing genotypes in other generations due to the rapid completion of homozygosity, as in the F4 generation (87.5%) (Rauf et al. 2016). Using hybridization between individuals of different species (interspecific hybridization) or genetically divergent individuals from the same species (intraspecific hybridization), a number of barley cultivars were developed in different countries (von Bothmer 1992; Pickering et al. 1995).

1.7.2 Somatic Cell Hybridization

Protoplast fusion provides a chance to circumvent barriers that prevent sexual reproduction and allows for the gene transfer of the nuclear and cytoplasmic genomes to enrich the gene pool of cultivated species. Furthermore, the fusion of protoplasts effectively generates new germplasm to breed elite traditional crosses and promotes crop improvement in current cultivars (Wang et al. 2013). Use of embryogenic cell suspension devices as a source of protoplasts for the first time in the Gramineae, led to the isolation of the protoplasts that were able to divide and produce somatic embryos and plants (Vasil and Vasil 1979). Many somatic hybrids cells and hybrid plants by protoplast fusion have been obtained between *Hordeum vulgare* and other species i.e. soybean, *Glycine max* (L.) Merr. (Kao and Michayluk 1974); carrot, *Daucus carota* L. (Dudits et al. 1976; Kisaka et al. 1997); tobacco, *Nicotiana tabacum* L. (Somers et al. 1986) and rice, *Oryza sativa* L. (Kisaka et al. 1998).

1.7.3 Hybrid Cultivars

The progeny of a F1 generation cross between two or more unrelated pure lines—inbreds, clones or other genetically-dissimilar populations/lines— released for commercial cultivation are designated as hybrid cultivars and carry one or more favorable traits. Hybrid cultivars of barley have the best yield, grain quality, abiotic

and biotic resistance, and important agronomic traits. For example, the hybrid barley cultivars all grow well throughout the UK, where the top five positions have been given to the Hyvido family of cultivars. Yields reached 11.93 mt/ha despite the storms and drought experienced in 2018 by farmers. The hybrid cv. Belmont yields 111%, equivalent to 2% more than the next-highest yielder, cv. Bazooka, while its specific weight and disease resistance is much improved from early six-row hybrids (https://www.syngenta.co.uk; accessed February 20, 2019). Belmont is the highest yielding hybrid barley cultivar on the AHDB (Agriculture and Horticulture Development Board, UK) Recommended List 2019/2020, combined with good grain quality and strong agronomics (https://www.ahdb.org.uk/rl; accessed February 20, 2019).

1.8 Conclusion and Prospects

1.8.1 Current Research Initiatives to Combat Global Climate Change

Climate change poses a major and increasing threat to global food security and affects every country on every habitable continent. It is very difficult to predict the precise future effects of climate change on crop yields because there are many parameters. These include: (1) physical parameters like temperature, rainfall patterns and carbon dioxide fertilization; (2) changes in agricultural environmental systems like loss of pollinators and increased occurrence of biotic stresses and (3) the adaptive responses of human systems (FAO 2016). Climate change is the essential issue of our time, and now is the decisive moment to do something about it. Tackling climate change will require an unprecedented effort from all sectors of society, but there is still time for it. As of April 2018, 175 parties had ratified the Paris Convention in 2016, 168 parties had reported their first nationally-identified contributions to the UN framework convention on Climate Change Secretariat, and ten developing countries successfully completed the submission of their first national adaptation plans to respond to climate change. Also, developed country have jointly mobilizing USD 100 billion annually by 2020 for mitigation actions. To boost motivation and accelerate actions to implement the Paris Agreement on Climate Change, the UN will host the next Climate Summit on 23 September 2019, to meet the climate challenge (http://www.un.org/en/climatechange/).

Barley has emerged as a model for achieving an excellent response to the effects of different climate change scenarios (Newton et al. 2011), because of the broad and well-formed collections of landraces, wild genotypes (*Hordeum vulgare* ssp. *spontaneum*) and other *Hordeum* species which are important sources of new alleles (Dawson et al. 2015). Therefore, barley yields suggest less variation under climate change conditions than those of wheat and most other small grains (Cossani et al. 2011). Climate change will lead to a reduction in the barley harvest in some locations

in the 2010/2029 period such as the Andean region (2.1%) (Lobell et al. 2008) and Finland (5.7%) (Peltonen-Sainio et al. 2011). Meanwhile, barley production will thrive and benefit from climate change in countries such as the UK. Projected barley production under expected land use and climate change vary from 4.6 million mt in 2030 to 9 million mt in 2050 (Yawson et al. 2017). There are already useful alleles to improve barley in landraces, wild species and in the wider *Hordeum* gene pool, thus utilization of these resources is taking place to overcome yield reductions of barley as a result of climate change. It is important that breeding to improve barley under drought and temperature conditions does not affect the quality of the barley grain crop.

1.8.2 An Overview of the Current Status

Barley grain is used both for human consumption and animal feed; it ranks fourth in world grain production after maize, wheat and rice. Barley is cultivated internationally and is more tolerant to cold, drought, saline, and alkaline soils than other important cereals. Current data on barley, which exceeded 140 million mt of world production, indicate that the production in the 2019 season decreased by 2.49%, compared to the 2018 season. According to USDA data, approximately 27 million mt of global barley production will enter international trade in 2019, a slight decrease compared 2018 season (27,455,000 mt) (USDA 2019). Globally, many research studies have been carrying out barley cultivar improvement. Future conventional and biotechnology breeding techniques should be more systematized and supplied with new technological tools to develop cultivars. Recent concerns about global warming, abnormal weather patterns, and unfavorable environments have compelled breeders to speed up the breeding process.

1.8.3 Recommendations for Future Research

A remarkable amount of genetic research has been completed on barley in terms of taxonomy, germplasm diversity, genetic resource conservation, cytology, cytogenetics, cell and tissue culture, traditional breeding methodologies, conventional and somatic cell hybridization, molecular breeding, genetic engineering and mutation breeding. The long-term ex situ conservation of barley is essential and presents a number of challenges, as its in situ conservation requires significant resources and leaves it vulnerable to natural disasters. Conventional barley breeding can profit from the use of biotechnology such as transgenics and molecular technologies for

different tasks to facilitate programs without mixing alien genes into the cultivar that is bred. It is therefore important that the entire barley research community work in concert to produce cultivars of high yield and quality as well as with resistance to biotic and abiotic stresses, so that the improvement of one trait does not adversely affect another trait, and to provide a template that can be extended to other crops.

Appendices

Appendix I: Major Institutions Engaged in Barley Research

Institution	Specialization and research activities	Contact information and website
International Barley Hub, James Hutton Institute	Barley research	Dundee, Scotland, UK, email: barleyhub@hutton.ac.uk, www.barleyhub.org
ICAR-Indian Institute of Wheat and Barley Research (IIWBR)	Increasing and stabilizing the barley production	Karnal-132001, Haryana, India, Email: director.iiwbr@icar.gov.in, http://www.iiwbr.org/
Brewing and Malting Barley Research Institute	Malting barley research	P.O. Box 39120 Lakewood PO, Saskatoon, Saskatchewan S7V 0A9, Canada, Email: gfeist@bmbri.ca., http://bmbri.ca/
Canadian Malting Barley Technical Centre	Providing technical support and market information to stakeholders of barley	1365-303 Main Street Winnipeg, Manitoba, Canada R3C 3G7, E-mail: cmbtc@cmbtc.com, http://cmbtc.com/
Institute of Barley and Malt Sciences	Promote the informational and educational activities/resources on barley and malt at North Dakota State University.	NDSU Dept. 7670, 166 Loftsgard Hall, N. Bolley Dr. Fargo, ND 58102. North Dakota State University, https://www.ag.ndsu.edu
Alberta's Field Crop Development Centre (FCDC)	The Field Crop Development Centre focuses on breeding barley for feed, malt and food	Toll-free in Alberta: 310-FARM (310-3276), Out of province 1-403-742-7901, Email: Ag Info Centre, https://www1.agric.gov.ab.ca
Global Crop Diversity Trust	International organization dedicated solely to conserving and making available barley and other crops diversity.	Platz Der Vereinten Nationen 753113 Bonn, Germany, info@croptrust.org, https://www.croptrust.org

Appendix II: Barley Genetic Resources

(A) Barley Genetic Resources Available in the Ex Situ Germplasm Collections of PGRC, NSGC and ICARDA

Species	Number of available accessions of barley		
	PGRC	NSGC	ICARDA
H. vulgare ssp. *spontaneum*	3875	1504	1696
H. vulgare ssp. *agriochriton*	–	1	9
H. vulgare ssp. *vulgare*	22,882	25,024	23,639
H. vulgare	9684	–	–
H. vulgare var. *distichon*	–	–	–
Hordeum mutant collection	–	–	–
Other species	5372	6647	6656
Total	41,813	33,176	32,000

Sources: PGRC, www.agr.gc.ca/pgrc-rpc; NSGC, www.ars-grin.gov/npgs; ICARDA, www.icarda.cgiar.org

(B) Total Barley Germplasm Accessions Available at Ten Major Centers of the World

Rank	Country, city	Name/institution	Acronym	Code	Number of accessions
1	Canada, Saskatoon	Plant Gene Resources of Canada	PGRC	CAN004	41,813
2	USA, Aberdeen	USDA National Small Grain Collection	NSGC	USA029	33,176
3	Syria, Aleppo	International Centre for Agricultural Research in Dry Areas	ICARDA	SYR002	32,000
4	Brazil, Brasilia	Centro Nacional de Pesquisa de Recursos Genéticos e Biotec.	CENARGEN	BRA003	30,000
5	Japan, Ibaraki	National Institute of Agrobiologicl Sciences	NIAS	JPN003	23,471
6	Germany, Gatersleben	Institute of Plant Genetics and Crop Plant Research	IPK	DEU146	22,106
7	China, Beijing	Institute of Crop Germplasm Resources	CAAS	CHN001	18,818
8	Republic Korea	Genetic Resources Division, National Institute of Agricultural Biotechnology, Rural Development Administration Suwon	RDAGB-GRD	KOR003	18,764

(continued)

Rank	Country, city	Name/institution	Acronym	Code	Number of accessions
9	Russia, St. Petersburg	N.I. Vavilov Research Institute of Plant Industry	VIR	RUS001	17,850
10	Ethiopia, Addis Ababa	Biodiversity Conservation and Research Institute	BCRI	ETH001	16,388
11	Others countries				212,145
Total					466,531

(C) Total Barley Germplasm Accessions Available at Ten Major World Centers

Rank	Gene bank/ Institutes	Wild %	Landraces %	Breeding materials %	Genetic stocks %	Cultivars %	Unknown status %
1	PGRC	15	35	12	15	13	10
2	NSGC	7	44	13	11	15	12
3	ICARDA	7	59	17	2	9	6
4	CENARGEN	0	0	0	0	0	100
5	NIAS	4	75	0	20	0	0
6	IPK	6	56	10	2	23	2
7	CAAS	14	82	0	0	0	4
8	RDAGB-GRD	1	24	10	0	0	66
9	VIR	1	29	10	3	54	3
10	BCRI	0	100	0	0	0	0

(D) List of Mutant Barley Varieties in World During 2000/2010 Period

No.	Variety name	Mutant type	Local registration year	Country	Character improvement details
1	Phenix	Crossing with 1 mutant variety	2000	Ukraine	Drought tolerance
2	Gama	Direct use of an induced mutant	2000	Ukraine	High yield

(continued)

No.	Variety name	Mutant type	Local registration year	Country	Character improvement details
3	Dobrynia-3	Direct use of an induced mutant	2001	Russia	Low temperature tolerance
4	Hutorok	Direct use of an induced mutant	2004	Russia	Improved adaptability
5	Pavel	Direct use of an induced mutant	2003	Russia	Stiffness
6	Stimul	Direct use of an induced mutant	2003	Russia	Early maturity
7	Cruiser	Crossing with 1 mutant variety	2001	Germany	Erected type
8	Felicitas	Crossing with 1 mutant variety	2005	Germany	Erected type
9	Landora	Crossing with 1 mutant variety	2000	Germany	Erected type
10	Penelope	Crossing with 1 mutant variety	2001	Germany	Erected type
11	Penofuli	Crossing with 1mutant variety	2001	Germany	Erected type
12	Roxana	Crossing with 1mutant variety	2000	Germany	Erected type
13	Centenario	Direct use of an induced mutant with gamma rays (300 Gy)	2006	Peru	Altered maturity and improved seed production traits
14	Centenario	Direct use of an induced mutant with gamma rays (400 Gy)	2006	Peru	Improved seed production traits
15	Sayakaze	Crossing with 1 mutant	2003	Japan	Early maturity, good quality, short culm, superior lodging resistance and resistance to barley yellow mosaic virus
16	Furat 3	Direct use of an induced mutant with gamma rays	2000	Syria	Resistance to lodging, resistance to drought and high yield

(continued)

No.	Variety name	Mutant type	Local registration year	Country	Character improvement details
17	IZ Bori	Direct use of an induced mutant	2009	Bulgaria	Tolerance to low temperatures, very good resistance to powdery mildew, as well as to brown, black and stem rust, high grain yield (15–17%), high grain protein and lysine content
18	Scope	Direct use of an induced mutant	2010	Australia	Herbicide tolerance, high yield, early maturity
19	Janus	Direct use of an induced mutant	2003	Russia	Cold tolerance
20	Taran	Direct use of an induced mutant	2003	Russia	High tolerance of cold

Source: FAO/IAEA (2019) Mutant Variety Database. http://www-naweb.iaea.org

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Chapter 2
Fonio (*Digitaria spp*.) Breeding

Suleiman Dangana Abdul and Afam I. O. Jideani

Abstract Fonio, *Digitaria exilis* (Kippist) Stapf and *Digitaria iburua* Stapf, is a hardy crop grown in West Africa. It is a staple food in some parts of the region with excellent nutritional and culinary properties. It contains high quality vitamins, minerals, fiber, and sulfur-containing methionine and cysteine. The crop originated in present-day Guinea and Mali. It is an annual plant growing to a height of 80 cm (*D. exilis*) or 150 cm (*D. iburua*). It is a free-tillering C_4 plant, with slender and glabrous culms and a life cycle of 70–150 days. Fonio is mainly cultivated on marginal soils, and soils containing aluminum. Fonio is cultivated at 400–1500 m elevation, at 25–30 °C and annual rainfall 150–3000 mm. The crop is drought tolerant, and mostly cultivated by traditional methods that make harvesting and threshing labor intensive. The crop is faced with challenges that include; poor yields (≤ 500 kg/ha), seed shattering, plant lodging, small grain size, fungal diseases and infestation by the parasitic weed *Striga rowlandi*. The cultivated species are allotetraploids ($2n = 4x = 36$). The landraces from farmers' selection show polymorphism in their morphological, reproductive, yield and molecular-marker characteristics that can be used in marker-assisted selection for crop improvement. Fonio grain is consumed in the form of porridges, fonio *jollof*, flour creams or used to prepare salads, couscous, stews, candy and alcoholic/non-alcoholic beverages. Fonio and its products are good for diabetics and celiacs. The ecological versatility of fonio has enormous potential as one of the crops that guarantee food security in the future.

Keywords Acha · Biotechnology · *Digitaria exilis* · *Digitaria iburua* · Food security · Fonio · Production · Molecular breeding

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J. M. Al-Khayri et al. (eds.), *Advances in Plant Breeding Strategies: Cereals*,
https://doi.org/10.1007/978-3-030-23108-8_2

2.1 Introduction

Fonio is a hardy and ancient crop with a long history of cultivation in West Africa for its nourishment, good taste and dietary characteristics. It is considered a major staple food in dry areas of several parts of western Africa. It has important influence on human diets due to the high methionine and cysteine content in the tiny grains. Fonio (hungry rice) is thought to be a French language corruption of the term *foño* used by Wolof-speaking people of coastal Senegal and Gambia to refer to the minor grain crop *Digitaria exilis* (Kippist) Stapf (white fonio) and *D. iburua* Stapf (black fonio) that are both cultivated in those areas. Another view is that the word *fonio* may have originated from the Mandinka people of Mali, who called it *fonyo*. Currently, the term *fonio* is used in some former French speaking colonies of West Africa, *acha* also an English corruption of the term *acca,* and *iburu* (for black fonio) are used by the Hausa-speaking people of Nigeria, *fundi* (Guinea), *findi* (Gambia) to refer to the crop. *Digitaria exilis* is known to be grown for food in the Dominican Republic (Deive 1974; Morales-Payán et al. 2002) where it is locally known as *funde*, *D. cruciata* var. *esculenta* (*raishan*) in Asia (India and Vietnam) and *D. sanguinalis* in Europe (Germany and Poland). The palatable and nutritious seeds (caryopses) are sometimes referred to as the *grain of life* due to its consumption by several millions of people in West Africa when the major crops are yet to reach maturity, and food supply is inadequate (Ibrahim 2001). In some regions of West Africa, it is more than a famine crop; it is an economic crop that serves as the staple for 3–4 million people (NRC 1996; Portères 1976; Vodouhè et al. 2003).

This chapter describes fonio crop introduction, methods of traditional cultivation and breeding, status of crop production nationally and internationally, and comparison with worldwide production of some major cereal crops, and the challenges associated with fonio production. Furthermore, the chapter includes information on the origin and early domestication of fonio, cytogenetic studies as well as different uses, morphological, genetic and nutritional variation and molecular breeding.

2.2 Botany and Distribution

Fonio belongs to the family Poaceae, subfamily Panicoideae and tribe Panicea along with other major cereal crops such as rice, wheat, maize, sorghum or guinea corn, and pearl millet. The 230–325 species of the genus *Digitaria* Haller, comprising both annuals and perennials, are widely distributed in the tropics and subtropics (Clayton and Renvoiz 1986; Henrard 1950). Fonio as a crop, comprising *D. exilis* and *D. iburua*, is cultivated in western Africa throughout the Savanna zone from the Cape Verdean archipelago in the middle of the Atlantic Ocean, and on the mainland from Senegal to the Lake Chad region, as shown in Fig. 2.1 (Ayo and Nkama 2006; Gyang and Wuyep 2005; Jideani 1990, 1999, 2012a, b; Jideani and Jideani 2011; Jideani et al. 1996; Kone 1993; Lewicki 1974; Obilana 2003; Portères 1976;

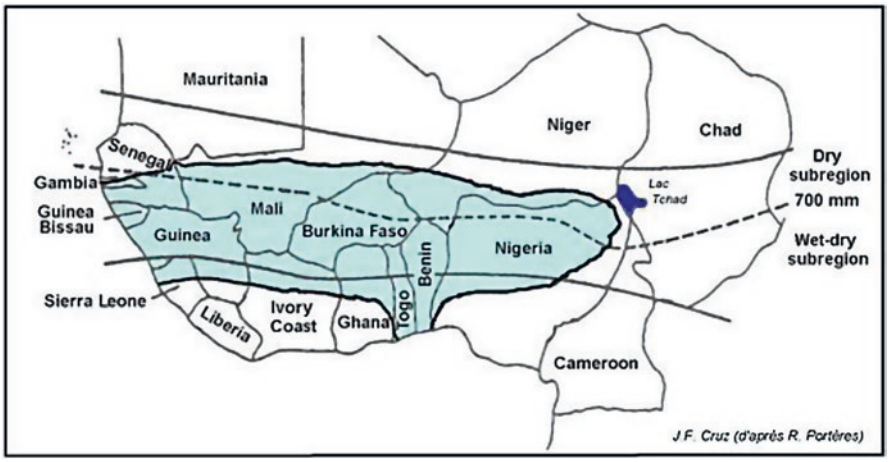

Fig. 2.1 Fonio producing area and countries of West Africa. (Source: Cruz (2001) according to Portères (1976))

Purseglove 1972). *Digitaria exilis* is cultivated throughout the region, while *D. iburua* is restricted to the Jos-Bauchi Plateau of Nigeria, the Atacora Mountains of Benin, Guinea, Cameroun, Zaire, and the northern regions of Togo and Benin (Dalziel 1937; de Wet 1989; Harris 1976; Hilu et al. 1997). These isolated focal areas of *iburu* cultivation may reflect a relic of a much wider area of production across West Africa. Some wild species of *Digitaria* were harvested in the past for food in times of famine or food scarcity and used as valuable forage (Adoukonou-Sagbadja et al. 2006; de Garine 2002; Haq and Ogbe 1995).

Digitaria exilis is a small annual herbaceous plant reaching a medium height of 30–80 cm and producing digitately-branched inflorescences with 2–5 racemes (Fig. 2.2). Fonio species are C_4 metabolizing cereals, free-tillering with erect, slender and glabrous culms and a short life cycle (Haq and Ogbe 1995). The leaves are also glabrous with a proximal sheathing base and distal strap-shaped blade. The racemes that may reach 15 cm in length, bear spikelets in pairs in the early varieties or in 3s or 4s in late varieties, on pedicels. The spikelets have a pair of flowers, one sterile and the second fertile, the latter gives rise to the fonio grain. The fertile floret bears 3 stamens with yellowish anthers, 2 lodicules and a pink or purplish stigma. The ovary is superior. The grain is tightly enclosed within 2 brown husks (lemma and palea). The lemma is almost as long as the spikelet and has 7–9 veins. The palea is slightly shorter than the lemma. *Digitaria iburua* can be distinguished from *D. exilis* by its dark-brown husks; hence it is commonly referred to as *black fonio* in contrast with *D. exilis* (*fonio* or *white fonio*). The fruit is a caryopsis, oblong to globose-ellipsoid and about 0.5–1 mm in diameter, 0.75–2 mm in length, white to pale brown or purplish color. It has long roots that draw water and nutrient from up to 3 m deep in the soil. It is therefore suited for cultivation in dry areas with poor soils. *Digitaria iburua* was first reported outside Africa in 1911 (Portères 1976)

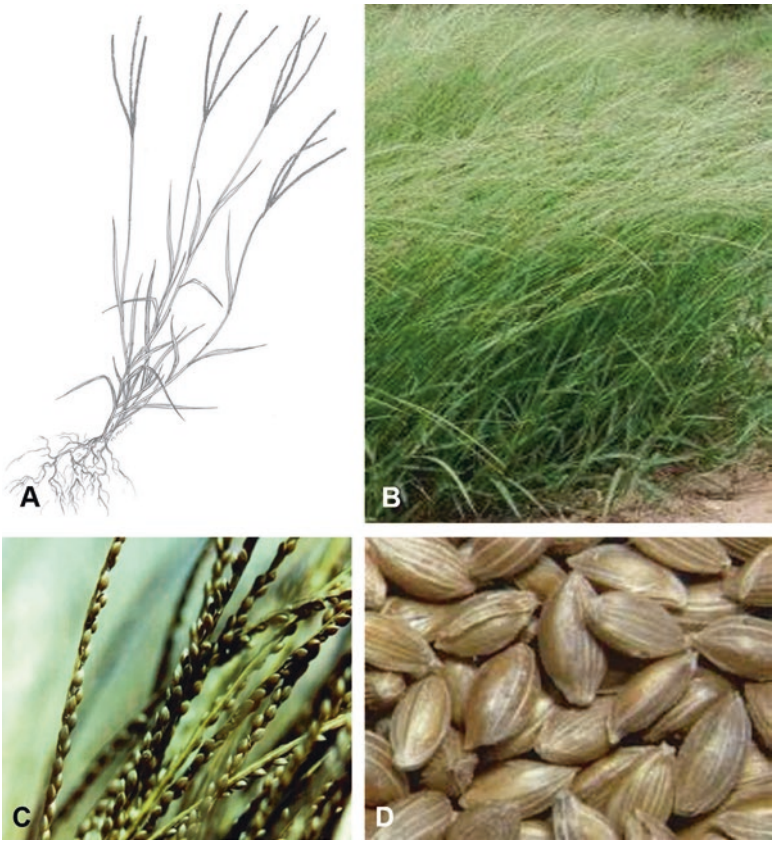

Fig. 2.2 *Digitaria exilis.* (**a**) Whole plant: Source: Cruz et al. (2016), (**b**) Crop growing in the field: Source: J.F. Cruz, (**c**) Racemes with grains, (**d**) Mature grains

when found growing in fields with pearl millet in the Zaria region of northern Nigeria. *Digitaria iburua* may grow to a height of 1.5 m, having 2–11 subdigitate racemes up to 13 cm long. The lowest raceme in *D. iburua* is somewhat distant from the others.

2.3 Origin and Domestication History

The ancestry of cultivated *Digitaria* has not been conclusively ascertained. There are several postulations by various researchers regarding the origins, evolution and domestication of fonio. On the bases of botanical affinities Stapf (1915) and Dalziel (1937) proposed *D. longiflora* (Retz.) Pers., an annual weed widely distributed in

tropical Africa, as the possible progenitor of *D. exilis*. Henrard (1950) later reported that *D. longiflora* is botanically closer to *D. fuscescens* Henr.; and went further to suggest that *D. exilis* has closer affinity with the wild *D. barbinodis* Henr., which is also commonly found in West Africa. *Digitaria barbinodis* Henr. is generally consumed as wild cereal in Nigeria and Togo (Haq and Ogbe 1995).

Regarding the origin of *D. iburua* Stapf (1915) proposed the wild *D. ternata* (A. Rich.) Stapf, while Portères (1976) considered *D. barbinodis* as its possible progenitor. Other wild species like *D. tricostulata* (Hackel) Henr. and *D. atrofusca* (Hackel) Camus are cited as being morphologically close to *D. iburua* although they are geographically remote from the areas of diversity of the crop (Haq and Ogbe 1995). Based on random amplified polymorphic DNA (RAPD) markers, Hilu et al. (1997) revealed that, in addition to morphological and other botanical characters, only *D. longiflora* and *D. ternata* displayed high genetic relatedness to *D. exilis* and *D. iburua*, respectively. Using a combination of cytological and molecular genetic data the same conclusion was arrived at by Adoukonou-Sagbadja et al. (2007a) and Adoukonou-Sagbadja (2010). Like the cultivated species, these two weedy species are cytologically tetraploids and display approximately similar genome size. The only consistent morphological distinctions observable between them and the cultivated fonio are the presence of fine pubescence on the spikelet of the wild species and heavy shattering. Arising from these it is safe to conclude that *D. exilis* and *D. iburua* have *D. longiflora* and *D. ternata* as their progenitors. It is most likely that during their domestication from these wild species traits such as spikelet hairiness and seed shattering were altered through selection.

The landraces of *Digitaria exilis* grown in Senegal come from the Fouta Djallon Highlands (Middle Guinea) and Kissi country (Forest Guinea). They rapidly spread in Senegal (natural regions of Casamance and eastern Senegal) and Gambia. Their spread across West Africa is said to follow a similar trend because of the migration of some tribes like the nomadic Fulani and Bowe Kognagui.

Information on the origin and relationships between wild and cultivated crop species is crucial in introgression of desired traits from wild relatives to cultivated genotypes. Of course, successful introgression relies on the degree of speciation and phylogenetic relationships among the gene pools concerned. It has been suggested that fonio was domesticated in the surroundings of the headwaters of the Niger River around 4500 BC (Munson 1976; Murdock 1959), in present-day Guinea and Mali. This position is further supported by the name fonio that is said to have originated from linguistic groups from the middle Niger and Senegalic areas, suggesting its domestication near the central Niger Delta (Portères 1976) in Mali. Fonio along with cereals such as pearl millet, sorghum, etc., were known to have played a pivotal role in the commencement and progress of traditional agriculture in the West African savannah (Busson et al. 1965; Haq and Ogbe 1995). Al-Omari was the first to mention, in his writing in 1342–1349, that fonio is one of the basic foods in West Africa (Lewicki 1974; Portères 1976). Also, Ibn Battuta wrote of local peoples selling the grain to travelers in Mali in 1350–1351 (Portères 1976).

2.4 Cultivation Practices

Fonio is cultivated mainly in marginal, mountainous and hilly zones and on sandy, poor and degraded soils, employing traditional and unimproved methods of cultivation. It is mostly grown in areas unsuitable for other cereals. The crop is not amenable to saline or heavy soils. Aluminum-rich soils that are toxic to other crops are known to support its production. It is cultivated mainly under rainfed condition using low input, but a few farmers grow the crop in the dry season under irrigation. Fonio can withstand drought and floods. The wide ecological adaptations and versatility of the crop confer on it a significant role in the development of agriculture and food security in traditional zones of cultivation and beyond (Adoukonou-Sagbadja et al. 2006; Eyzaguirre and Thormann 1998). Its cultivation fits perfectly into the low-input farming systems of the resource-poor African farmers.

There are different ecotypes of fonio as recognized by the length of the growing cycle and is comprised of very early (70–90 days), early (90–110 days), intermediate (110–130 days) and late (> 130 days) (Kwon-Ndung 2014). The crop is traditionally sown through broadcasting after the land has been ploughed by hand, animal traction or tractor. The broadcasted seeds, mostly landraces, are covered lightly with soil to prevent picking by birds and ants. Sowing is done usually in April or May depending on onset of the rains. In northern Nigeria black fonio is usually planted towards the end of June and harvested in November–December. The crop is a weak weed competitor; as such more attention is paid to weeding. In most cases weeds are removed manually 4–5 weeks after sowing, this is repeated after 3 weeks, and subsequently as weeds emerge up to harvest. More recently some farmers reduced weed challenges during the early stages in the development of fonio by applying nonselective post-emergence herbicides such as glyphosate at the time of land preparation before sowing. The crop grows and tillers rapidly covering the ground thus reducing competition from weeds. The few weeds that emerge subsequently, depending on the efficiency of the application and the efficacy of the herbicide, are removed manually.

Fonio is cultivated mostly by women at the subsistence level. It is grown at sea level in Gambia, Guinea-Bissau and Sierra Leone, but more broadly it is cultivated at 400–1500 m elevation. It is known to thrive at an average temperature of 20 °C at higher elevations to between 25–30 °C near sea level during the growing season. The crop flourishes in areas with average annual rainfall of 150–3000 mm, although it is mostly grown in areas with an average of 900–1000 mm. The crop's ability to resist drought lies in its fast growth and maturity with some landraces maturing within 8 weeks. This makes fonio suitable for cultivation in areas with short and unreliable rainfall. It is grown in valleys benefiting from runoff water in areas with very low rainfall. *Digitaria iburua* is a sub-alpine plant. It is often found in cultivation with white fonio and is frequently planted between rows of sorghum and pearl

millet. The crop is drought tolerant and often yields a harvest when the major cereal it accompanies fails to survive (Portères 1976; de Wet 1989).

The crop is harvested within 3–6 months depending on the genotype. Harvesting is labor-intensive, and the methods used vary with different farming communities. In some instances, mature fonio plants are uprooted at harvest. Others use traditional tools such as knives and sickles to cut straw-bearing mature panicles. Others use straw cutters for example, a special craft thimble made with a variety of calabash to protect the index fingers against laceration injuries. In recent times a mechanized thresher has been developed for fonio processing.

The grain is extracted from the digitate panicle after harvesting by beating the straw on a hard elevated surface (wood or empty drum) placed on a tarpaulin or mat or directly on a flattened rock surface to prevent contamination by sand. Others trample directly on the dry straw to extract the seeds. Because of the intense sunshine during the harvesting period, threshing is done overnight by some farmers. Some farmers harvest the grains by pulling the grains directly from the panicle in a bowl using their hands with the crop still standing. The 1000-seed weight of black fonio is about 500 g. Black fonio is difficult to husk. After threshing, grains are dried 3–4 days and stored in local granaries. According to farmers, the grains can be stored for about 5–10 years, but seed viability decreases considerably after 2 years of storage. When required for use it is mostly husked manually using a mortar and pistil. However, some fonio producing communities now have modern machines for threshing, hulling and whitening (Cruz et al. 2016) thereby reducing the drudgery and increasing productivity. In some areas the dehusked seeds are washed to give them a clean white appearance.

Generally, fonio yield compared with those of the major cereals is low; it ranges from about 200–500 kg/ha depending on the genotype, agronomic practice and the environment (Dachi and Gana 2008). Farm areas of less than 1 ha are used in its cultivation (Kwon-Ndung and Misari 1999; Kuta et al. 2003). The low yield is due to the traditional cultivation of the crop in marginal soils, with minimal input. However, recently some farmers are applying chemical fertilizers in the form of NPK or urea to their fonio crops. In an experiment using poultry manure and NPK at the rates of 5 mt/ha and 60 kg/ha, yields as high as 1.13 mt/ha and 1.03 mt/ha, respectively, were reported in Nigeria (Ndor et al. 2016).

Both fonio species have shown a relatively high level of resilience against pests and diseases. However, some pests and diseases associated with the crop include the fungi *Phyllachora sphearosperma*, *Helminthosporium* spp. and *Puccinia cahuensis*. The parasitic weed *Striga rowlandi* known to occur abundantly in West Africa affects the crop causing serious damage (Haq and Ogbe 1995; Sanou 1993). Also, significant seed losses caused by insect pests and birds are known to occur occasionally. The plants are also susceptible to smut.

2.5 Fonio Production in Comparison to Major Cereal Crops

Fonio is an important staple crop that is in high demand within the countries of production (Ayo and Nkama 2006; Jideani 1990, 2012a, b; Kone 1993; Obilana 2003) and expanding to European markets (Dury et al. 2007) due to its economic and health benefits for diabetics. This has resulted in an increased cultivation area from 285,200 in 1964 to 741,603 ha in 2016, which corresponds to an annual production of 184,645 and 645,180 mt, respectively (FAOSTAT 2018), with white fonio accounting for most of the recorded production (Ndoye and Nwasike 1993). There was a general increase in yield from 647.4 to 870.1 kg/ha during the same period (Table 2.1, Fig. 2.3).

A survey of FAO production statistics shows Guinea and Nigeria as the leading producers in 2016. There was a general trend of increase in both area under cultivation and production from 1964 to 2016 in Burkina Faso, Guinea, Niger and Nigeria. Côte d'Ivoire demonstrated significant increase in production without corresponding increase in the area under cultivation. This should be given special consideration to ascertain the cultural practices employed, agroclimatic conditions and the genotypes used. The high yield/ha of 1374.4 kg and 1073.6 kg in Côte d'Ivoire and Guinea, respectively, could be due to the attention given to the crop in national research programs in at least Guinea (Vodouhè et al. 2003). In the same year Nigeria had a low yield/ha of 434.8 kg.

On the other hand, there was a decrease in both area under production and total production in Benin, Guinea-Bissau and Mali, suggesting that fonio farmers in

Table 2.1 Fonio production, area of cultivation and yield in some West African countries

	1964			2016		
Country	Total production (mt)	Total area (ha)	Yield (kg/ha)	Total production(mt)	Total area (ha)	Yield (kg/ha)
Benin	2275[a]	8000[a]	284.4[b]	1331[a]	2193[a]	607.1[b]
Burkina Faso	11,600[a]	16,000[a]	725.0[b]	14,460[d]	18,622[d]	776.5[b]
Côte d'Ivoire	7900[a]	18,000[c]	438.9[b]	19,263[d]	14,015[d]	1374.4[b]
Gambia	6500[c]	12,500[c]	520.0[b]	–	–	–
Guinea	80,000[c]	80,000[c]	1000.0[b]	500,986[d]	466,622[d]	1073.6[b]
Guinea-Bissau	18,000[c]	24,000[c]	750.0[b]	726[d]	992[d]	731.6[b]
Mali	20,000[c]	50,000[c]	400.0[b]	16,740[a]	33,983[a]	492.6[b]
Niger	370[c]	700[c]	528.6[b]	6113[a]	10,970[a]	557.2[b]
Nigeria	38,000[c]	76,000[c]	500.0[b]	82,750[d]	190,315[d]	434.8[b]
Senegal	–	–	–	2811[d]	3891[d]	722.6[b]
Total	184,645	285,200	647.4	645,180	741,603	870.1

Note: [a]Official data; [b]Calculated data; [c]FAO estimate; [d]FAO data based on imputation methodology
Source: FAOSTAT (2018) accessed 4 February 2018

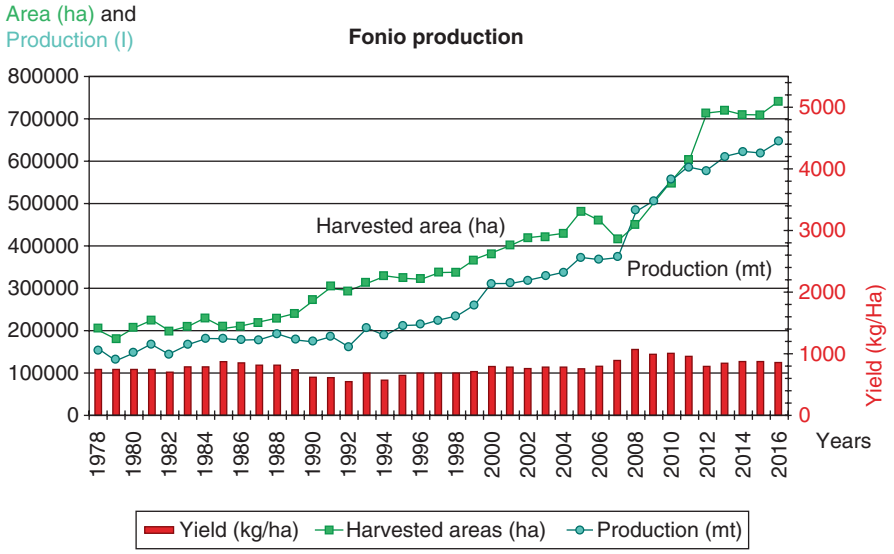

Fig. 2.3 World fonio production 1978–2016, FAOSTAT (2018)

these countries are losing interest in its production, perhaps due to the tedious production practices. In Nigeria the yield per hectare decreased marginally from 500 in 1964 to 434.8 kg/ha in 2016. This may be attributable to continued use of old traditional agronomic practices in the production of the crop by farmers, and the relegation of the crop to marginal fields by other higher-yielding crops to meet the food requirements of its rapidly expanding population. Fonio growers in Nigeria still use poor and marginal soils for its cultivation. The better yield performance of the crop in Guinea-Bissau (750 kg/ha), Guinea (1000 kg/ha) and Burkina Faso (725 kg/ha) in 1964 may be due to more suitable agroclimatic conditions, better crop husbandry practices and the use of improved genotypes. Principally, improvement in cultural practices and/or the use of better genotypes in Côte d'Ivoire and Benin was responsible for the drastic increase in yield per hectare from 284.4 and 438.9 kg/ha in 1964 to 607.1 and 1374.4 kg/ha in 2016, respectively. Yields as high as 2083 kg/ha were observed in Kolda and Kedougou provinces of Senegal using intermediate to late maturing landraces, and cultivation on gravelly soils with high fertility (Kanfany et al. 2016).

Although the area devoted to the production of fonio relative to other major cereal crops, such as maize, pearl millet and rice, is low its yield/ha compared favorably with that of millet (Table 2.2). In Senegal fonio production accounts for less than 1% of the national cereal harvest (Kanfany et al. 2016). Overall, there was an increase in fonio production from 1964–2016, while that of pearl millet was on the decrease despite the higher level of drought tolerance associated with pearl millet. This upward trend in fonio production is due to its nutritional appeal and health benefits, coupled with the premium price paid for the grains in both domestic and European markets.

Table 2.2 Comparison in total production, total area under cultivation and yield of *fonio* with other major cereal crops

	Year					
	1964			2016		
Crop	Total production (mt)	Total area (ha)	Yield (kg/ha)	Total production (mt)	Total area (ha)	Yield (kg/ha)
Fonio	184,645	285,200	647.4	645,181	741,604	870.0
Maize	215,172,627	107,925,200	1993.7	1,060,107,470	187,959,116	5640.1
Millet	27,292,542	43,354,007	629.5	28,357,451	31,705,489	894.4
Rice	262,928,956	125,056,100	2102.5	740,961,445	159,807,722	4636.6

Source: FAO (2017), data.fao.org. Accessed 4 February 2018

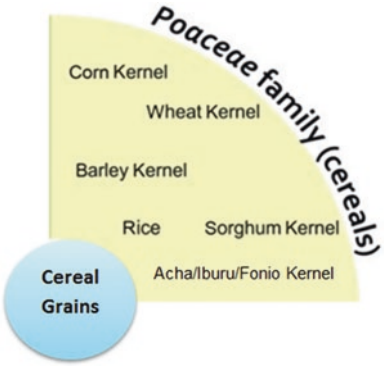

Fig. 2.4 Example of different cereal grains in the world under the family Poaceae

2.6 Nutritional Properties and Uses

Fonio, one of the different grains consumed in the world (Fig. 2.4), has excellent culinary and nutritional properties. It is known not only to contribute to the diet, wellness and economic status of peoples from the developing economies of West Africa, but also to have the potential to play an important role in food security of the peoples of such economies (Jideani 2012a, b). A return on investment of 1.81 was reported by Duniya (2014) in Nigeria, implying that for every unit of investment in fonio production, a profit of 0.81 units was achieved. The grain has excellent nutritional and culinary properties, with high quality vitamins, minerals, fiber, and sulfur-containing amino acids (methionine and cysteine). Today's major cereals (wheat, rice, maize, sorghum or guinea corn, barley and rye) are deficient in methionine and cysteine and the human body cannot produce them on its own.

Digitaria exilis and *D. iburua* grains are similar in nutritional composition, except for the morphological characteristics of the kernel (Irving and Jideani 1997). The caloric value of fonio (16.264 ± 0.245 to 16.462 ± 0.238 MJ kg^{-1}) compares well with those for most cereals (13.975 ± 1.7313 MJ kg^{-1}) (Oyenuga 1968; Temple and Bassa 1991). The grain composition showed lipid content and ash values that

Table 2.3 Proximate composition of fonio (*Digitaria exilis* and *D. iburua*) in gkg^{-1} of dry matter

Component	*Digitaria exilis*	*Digitaria iburua*
Crude fiber	19.0 ± 2.0	11.5 ± 2.0
Crude protein	106.0 ± 2.0	125.0 ± 2.0
Total lipid	22.0 ± 1.0	52.0 ± 1.0
Total ash	36.0 ± 0.3	42.0 ± 0.3
Nitrogen-free extract	817.0 ± 5.0	769.5 ± 5.0
Potassium	1.072 ± 0.06	1.097 ± 0.06
Sodium	0.0532 ± 0.003	0.0653 ± 0.003
Calcium	0.067 ± 0.002	0.087 ± 0.002
Magnesium	0.835 ± 0.05	0.875 ± 0.05
Manganese	0.027 ± 0.001	0.031 ± 0.001
Iron	0.1286 ± 0.03	0.1382 ± 0.03
Zinc	0.0417 ± 0.001	0.0422 ± 0.001
Copper	0.00153 ± 0.0001	0.00174 ± 0.0001
Lead	0.00019 ± 0.0001	0.00022 ± 0.0001

Source: Chukwu and Abdul-Kadir (2008)

are higher than those reported for most cereal grains (Chukwu and Abdul-Kadir 2008; Oyenuga 1968; Temple and Bassa 1991) (Table 2.3). Its fiber content is at similar levels with those of millet and rice but lower than the values for sorghum and maize. The fiber in fonio coupled with its low glycemic index facilitates digestion and weight loss, making it easy to be consumed by both children and adults. Fonio is richer in calcium, magnesium, iron and copper than most cereals but poorer in potassium, sodium, lead and manganese (Table 2.4). Its protein content is high compared with that of other grains (Chukwu and Abdul-Kadir 2008; Oyenuga 1968; Temple and Bassa 1991) with some high molecular weight proteins (Jideani 1993). Fonio grain has an exceptionally high methionine content relative to most grains, and the leucine, methionine and cysteine values are slightly higher than the values in the FAO reference protein (FAO 1970). Temple and Bassa (1991) reported leucine (9.8%) methionine (5.6%), and valine (5.8%) as the most abundant amino acids in the grains. Overall fonio protein contains almost twice as much methionine as egg protein. It has more of the minerals iron, calcium, magnesium, zinc and manganese serving-per-serving, than other grains. The high nitrogen-free extract content is characteristic of the non-waxy type of fonio endosperm.

Shewry (2009) observed that variation in amounts and compositions of dietary fiber and phytochemical components in cereal grains is under both genetic and environmental influence. Therefore, there is the need for extensive chemical evaluation of fonio seeds collected across their primary and secondary centers of diversity.

Miano and Augusto (2018) stated that the hydration process is a crucial step in the industrialization and direct consumption of grains and provided several beneficial effects on physicochemical and nutritive quality. Tunde-Akintunde (2010) studied hydration kinetics behavior in fonio where a downward concave shape was exhibited as opposed to the sigmoidal shape behavior.

Table 2.4 Amino acid composition of fonio (*Digitaria exilis* and *D. iburua*) in g per 16 g N

Amino acid	*Digitaria exilis*	*Digitaria iburua*	FAO (1970) reference protein
Isoleucine	1.37 ± 0.03	1.41 ± 0.03	4.20
Leucine	4.40 ± 0.02	4.43 ± 0.02	4.20
Lysine	1.90 ± 0.01	1.91 ± 0.01	4.20
Methionine	2.98 ± 0.02	3.12 ± 0.02	2.20
Threonine	1.89 ± 0.02	1.92 ± 0.02	2.80
Phenylalanine	2.37 ± 0.01	2.35 ± 0.01	2.80
Valine	2.34 ± 0.01	2.38 ± 0.01	4.20
Tyrosine	0.91 ± 0.01	0.90 ± 0.01	2.80
Tryptophan	0.95 ± 0.03	0.92 ± 0.03	1.40
Cysteine	3.10 ± 0.04	3.30 ± 0.04	2.00
Arginine	1.34 ± 0.02	1.36 ± 0.02	–
Histidine	1.33 ± 0.02	1.34 ± 0.02	–
Alanine	4.28 ± 0.01	4.24 ± 0.01	–
Serine	2.10 ± 0.03	2.22 ± 0.03	–
Proline	3.26 ± 0.02	3.23 ± 0.02	–
Glycine	1.93 ± 0.01	1.94 ± 0.01	–
Glutamic acid	6.97 ± 0.04	6.99 ± 0.04	–
Aspartic acid	3.50 ± 0.02	3.49 ± 0.02	–

Source: Chukwu and Abdul-Kadir (2008)

The uses of fonio grains have been known for centuries across West Africa and the Dominican Republic (Deive 1974, 1978). Although there is great need for improved threshing and husking methods, it is a staple food in most parts of where it is cultivated in sub-Saharan Africa. The grains should be washed thoroughly before cooking to remove sand that remains with the seed because of its small size during processing. Sand left with the seeds produces gritty foods. De-husked fonio grain may be consumed directly, cooked in porridges and flour creams, like grits and wheat cream. The processed grain can be used to prepare salads, couscous, stews and porridges or mixed with meal of other cereals, candy, alcoholic and non-alcoholic beverages or even ground into flour. The flour can be mixed with other cereal flours to make cookies, baked into bread for diabetics, those who are gluten intolerant or have celiac disease. It may also be prepared as fermented beverages. In Togo it is used for brewing beer (*tchapalo*). The brewing and malting properties of fonio have been studied by Nzelibe and Nwasike (1995). In West Africa and the Dominican Republic, it is used in traditional medicine. The grain is considered as an aphrodisiac in the Dominican Republic. Aside from everyday meals, consumption of several fonio recipes has also been associated with religious festivities in both West Africa and the Dominican Republic (Morales-Payán et al. 2002). Fonio is so closely associated with the sociocultural setup of its area of cultivation to the extent that the Dogons people of Mali believed that the universe was born from a grain of *Digitaria exilis* (Griaule and Dieterlen 1950).

Fonio is a healthy alternative to people suffering from diabetes, gluten intolerance and celiac diseases. Consuming whole grain reduces risk of cardiovascular disorders including heart disease and stroke. Fonio helps to reduce the risk of heart attacks and stroke. The high amount of iron in fonio prevents the development of anemia. It also contains folic acid and other amino acids that are beneficial for women during pregnancy (Adoukonou-Sagbadja et al. 2006; Jideani 1999; Jideani and Jideani 2011). The sulfur-containing amino acids, methionine and cystiene in fonio are important for the formation of cartilage and healthy nails and hair thereby preventing hair breakage and loss. The same amino acids aid in the detoxification process of the liver. The minerals calcium, magnesium and phosphorus in fonio are essential for the building of strong bones and teeth. The numerous health benefits of fonio are an indication of its versatility not only aimed at addressing hunger but also as a complement for standard diets.

Fonio has considerable potential in foods and beverages (Jideani 1997, 1999; Jideani and Ibrahim 2005; Jideani and Jideani 2011). It is proposed in the development of health or specialty foods like acha-bread, biscuit, cookies, sour dough, traditional drinks, breakfast cereal flour, nonfermented steamed and granulated dumpling products are gaining interest (Agu et al. 2007, 2014a, b, 2015; Alexander 1995; Ayo and Nkama 2003; Jideani et al. 2007, 2008; Nzelibe and Nwasike 1995; Nzelibe et al. 2000; Oluwajoba et al. 2013).

The use of fonio grain as animal feed is rare. However, the chaff and straw are widely used as livestock feed. Also, the straw is used to stuff mattresses or as thatch to make kitchen and barn roofs. Generally, fonio grain remains greatly appreciated by all consumers for its taste, fineness, softness, digestibility, and for its nutritional, therapeutic importance. It has potential in food product development (Agu et al. 2014a, b; Filli et al. 2014; Jideani 2011, 2012a, b; Jideani and Jideani 2013).

2.7 Current Production Problems and Challenges

Some of the major disadvantages of fonio as a crop are poor yields when compared with other cereals, shattering, lodging, small grain size, pests and diseases. Lodging arises from the fragile shoot of the plant, and makes harvest tedious and contributes significantly to yield loss. Shattering at maturity is another major drawback, although limited in the crop, it can become important if harvesting is delayed, and is responsible for up to 25% yield loss (Vodouhè et al. 2003). A survey of farmers' fonio husbandry activities in Nigeria demonstrated a lack of improved agronomic practices in its production especially in weed control, and the use of unimproved genotypes. Apart from the general poor husbandry, the husking process of the grains is very tedious and time-consuming, constituting a major bottleneck in its processing and utilization. Overall the tediousness associated with the production, postharvest techniques and food processing in comparison with other cereals represent a drawback in the cultivation of this crop.

Additionally, Sartelet et al. (1996) reported the presence of high flavonoid content in the crude fonio grains that is likely to have anti-thyroid properties.

2.8 Methods of Traditional Breeding

The prerequisite for the successful genetic improvement of any crop is the availability of genetic variation in the population. This is followed by accurate selection of the desired genotype. Variations in a plant population may arise from crossing between plants with characters of interest or mutation. Relatively high variation is associated with landraces and varieties. Wild relatives and mutant genotypes are known to have a high level of variation relative to cultivated varieties. Exotic germplasm and elite cultivars harbor low genetic variation. Usually traits earmarked for improvement may include yield, nutritional and culinary enhancement, environmental stress tolerance, improved plant structure, or pest and disease resistance.

The traditional approaches to the improvement of plants depend on an understanding of the biology of their reproduction, mode of inheritance and heritability of the traits, as well as the number of genes controlling the traits. The plant may express a vegetative form of reproduction, self-fertilization, or cross-fertilization. Some traits may be under the control of simple Mendelian genes that are qualitatively inherited, showing discrete distribution, while others may be quantitatively inherited showing continuous distribution. Quantitatively-inherited traits may have low, moderate or high heritability. Those with high heritability values are minimally influenced by the environment and vice-versa. Heritability can be partitioned into additive and non-additive components. Progress in selection is more rapid when the additive component of heritability is high.

2.8.1 Variability and Heritability

Studies have identified significantly large variability among fonio in agro-morphologic traits, such as grain weight, number of tillers produced, leaf length and width, plant height and day to heading, panicle length, etc. (Aliero and Morakinyo 2001; Sanou 1993; Sekloka et al. 2016).

There is general lack of information on heritability of both vegetative and reproductive characters of fonio except for the moderate to high (45–100%) broad-sense values reported for various characters including yield (Buba and Abdul 2016), suggesting little or no environmental influence for characters with high values.

2.8.2 Radiation Breeding

Radiation breeding is a method of mutation breeding that entails the deliberate use of radiation to induce and develop lines for crop improvement. Mutant genotypes generated through radiation have served as sources of novel genetic variability in several crops. The use of radiation to induce mutation in plants was first suggested and promoted by the Dutch botanist Hugo de Vries in the period 1901–1904, and subsequently various researchers used different types of radiation to generate new varieties in many crops including rice (Sarigabutr et al. 1989; Shen et al. 1994). Mutation breeding has been used in crops with low level genetic diversity. Radiation treatment of tissue culture has found application in the generation of mutants that can be used for crop improvement.

In fonio, gamma radiation was used by Animasaun et al. (2014) to generate variability in morphological and reproductive traits including yield in *Digitaria exilis*. The mutants created could be screened for possible utilization in the development of new varieties.

2.8.3 Cytogenetics

Members of the genus *Digitaria* express chromosomal variation, having a basic chromosome number x = 9. This characteristic is typical for most genera of the Paniceae tribe (Hunter 1934). Species in this genus are known to have very small chromosomes and polyploidy is said to have played important role in their evolution. The cultivated species (*D. exilis* and *D. iburua*) are reported to be diploid ($2n = 2x = 18$), tetraploid ($2n = 4x = 36$) or hexaploid ($2n = 6x = 54$) (Hunter 1934; Morakinyo and Awojobi 1991; Zeven and de Wet 1982). Subsequently Adoukonou-Sagbadja et al. (2007a) established tetraploidy in cultivated fonio.

The germplasm used in the studies by Adoukonou-Sagbadja et al. (2007a) was mostly sourced from French-speaking West Africa. Despite this limitation it is considered representative of the majority of fonio landrace diversity found in the region since it was collected from areas associated with extensive genetic diversity (Portères 1976). If diploid and hexaploid types of fonio really exist, their occurrence may be low in comparison to most tetraploids. Expanding the investigations to Nigerian landraces (another possible secondary center of diversity), and to other fonio producing countries like Senegal, Gambia, Côte d'Ivoire and Niger, could help to achieve a definitive conclusion on the existence of diploid or hexaploid fonio.

Adoukonou-Sagbadja et al. (2007a) used nuclear DNA and reported slight and insignificant intraspecific variations, suggesting genome size stability within the cultivated gene pool with an average 2C-values ranging from 1.848 ± 0.031 pg for *Digitaria iburua* to 1.956 ± 0.004 pg for *D. exilis*. There is similarity in the 2C DNA

contents of *D. longiflora* (1.869 ± 0.035 pg) and *D. ternata* (1.775 ± 0.070 pg) that suggests close affinity between the two wild species. This supports the close relationship of cultivated fonio species *D. exilis* and *D. iburua* with *D. longiflora* and *D. ternata*. Using 1C DNA content Marie and Brown (1993) and Bennett et al. (2000) confirmed the small genome size in some species of *Digitaria*. They reported genome size of 1.2 pg–2.3 pg in *D. setigera* Roth, *D. sanguinalis* L. and *D. ascendens* Rendle. Nevertheless, Adoukonou-Sagbadja et al. (2007a) reported larger genomes for more distant species such as *D. lecardii* and *D. ciliaris* with 2.660 ± 0.070 pg and 2.576 ± 0.030 pg per 2C nucleus, respectively.

2.9 Genetic Diversity and Conservation

The success of a breeding program, as well as conservation of fonio genetic resources, depend on sufficient knowledge of the amount, distribution and structure of genetic diversity. At present there is still much work needed in the systematic evaluation of the genetic diversity present in fonio and the classification of the landraces found in the growing areas of West Africa. Currently some efforts have gone into germplasm collection; however, the converse is the case regarding breeding programs of fonio.

2.9.1 Morphological Variations

A considerable range of variation in quantitative morphological characters exists both within and between the two fonio species (Hayward and Hacker 1980). *Digitaria iburua* appears to have taller plant height, greater stem girth, wider leaf, longer leaf, greater number of days to maturity and larger seeds (Kwon-Ndung et al. 2001). Employing morpho-botanical characters and geographic origin Portères (1976) identified a few botanical varieties (with many cultivars each) in *D. exilis*. All these have arisen from traditional selections carried out by farmers producing hundreds of fonio landraces in West Africa. The largest landraces' diversity of the crop occurs in the central swamps of River Niger in the upper Niger River basin (Murdock 1959; Portères 1959, 1976). These landraces are recognized primarily by the time period they require to mature. In addition, they are further distinguished by their production requirements, taste, color and cooking behavior. Several varieties are found in Senegal, the most common being: Yawko or Yaoko in Fulani or Momo in Mandinka, that is an extra early genotype (60–75 days), Mora (Fulani) or Dibong (Mandinka) that is early (90 days), Rane (Fulani) or Findiba (Mandinka) that is intermediate (100–120 days), and Maoko (Fulani) that is the late type (150 days). The late type, though coarser and heavier, is gradually disappearing from cultivation due to its long growth cycle. Germplasm of two landraces (Tchibam and Tripka), putatively identified as *D. iburua*, has been collected in Togo for the Institut Togolais

de Recherche Agronomique (ITRA). In Benin a survey of villages resulted in the identification of five morphotypes among 15 farmer-named landraces with four of them belonging to *D. exilis* and the remaining morphotype to *D. iburua* (Dansi et al. 2010). Similarly, Sekloka et al. (2016) detected significant variability for several characters using 22 accessions from Benin and categorized them into four morphological groups.

Several phenotypic variants of fonio that might have arisen from the selection of grain by separate growers over the years have been recognized. In Nigeria a few reports on variation (qualitative and quantitative) in vegetative and/or reproductive traits, including yield in fonio have been documented (Abdul and Zadva 1997; Aliero 2000; Aliero and Morakinyo 2001; Buba and Abdul 2016; Morakinyo and Adelekun 1997; Morakinyo and Awojobi 1991). Divergent agro-morphological types were recognized in studies of some fonio ecotypes originating from Burkina Faso and Mali (Sanou 1993).

2.9.2 Molecular Variations

Arising from the consequences of gene-environment interactions, and the intricacies of genetics control of polygenic traits, there are limitations in using only morphological attributes (especially quantitatively inherited ones) in population studies. Such limitations have resulted in an early use of biochemical markers and the application of molecular approaches to assess genetic diversity (Karp et al. 1997).

Isozymes were the first molecular markers used to determine genetic variation in organisms. These are homologous enzymes differing in their amino acid sequences but sharing the same catalytic function (Markert and Möller 1959). Isozymes that are expressed by different alleles of a gene are known as allozymes, although others can be under the control of different loci. These differences may arise either due to differences in the nucleotide sequence of the gene(s) that results in amino-acid substitutions and changes in charge of the protein or from post-translational modifications (e.g. glycozylation) which lead to changes in molecular weight. These changes are determined using electrophoresis that separates isozymes on the bases of their molecular weight and net charge. The allelic variation recognized by isozyme has found application in genetic studies, systematic and evolution studies (Crawford 1989; Gao and Hong 2000; Hamrick 1989). Isozyme markers have the advantage of usually being dominant, rarely codominant, and requiring less sophisticated equipment. However, the major constrains associated with the use of isozymes are the limited number of available enzyme systems (Avise 2004). Furthermore, it is made more cumbersome with the use of specific detection methods for each enzyme, and the fact that only genes coding for expressed proteins can be analyzed resulting in low polymorphism (Lewontin 1991). As the cheapest and quickest marker system, isozymes find ease of application in studies that identify low levels of genetic variation such as in quantifying mating systems (Adoukonou-Sagbadja 2010; IPGRI 2004; Zeidler 2000).

Modern DNA-based approaches have replaced isozymes because of their versatility. They are more informative, and they offer broader genome representation and higher prospects for selective neutrality. Other DNA marker techniques used in plant genetic studies are restriction fragment length polymorphisms (RFLP), random amplified DNA polymorphisms (RAPD), amplified fragment length polymorphisms (AFLP), inter simple sequence repeats (ISSR), simple sequence repeats or microsatellites (SSR) and single nucleotide polymorphisms (SNP). These DNA-based approaches are used exclusively or in conjunction with traditional agro-morphological markers which are often known to be subject to environmental modulation. The prospects of the application of these techniques in fonio improvement program are bright.

RAPD studies in fonio using very small number of genotypes have been carried out by Hilu et al. (1997) and Kuta et al. (2005). The genotypes employed in these studies were restricted to samples from Togo and Nigeria, therefore lacking in their coverage of the entire fonio growing zones of West Africa. This highlights the need of more comprehensive studies to consider patterns of genetic diversity in relation to the regional distribution of fonio. Opportunity for in-depth population and evolutionary genetic studies using SSRs (microsatellites) was first provided in *Digitaria exilis* by Barnaud et al. (2012) with the development of 38 nuclear microsatellites primers. These primers revealed polymorphism in the accessions of fonio studied (Barnaud et al. 2012; Nyam et al. 2017a). SSRs markers confirmed the phenotypic differences of *D exilis*, *D. iburua* and *D. barbinodis* (Fig. 2.5), and confirmed their relatedness (Nyam et al. 2017a,b). With the limited SSR primers, and slow

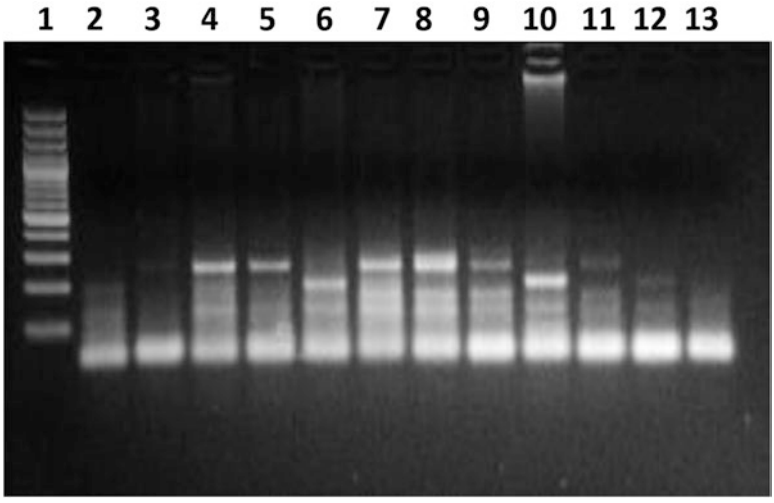

Fig. 2.5 Gel electrophoresis picture of primer De 01 on the 11 accessions. Lane 1: 100 bp marker; Lane 2: P1(*Digitaria iburua*); Lane 3 P4 (*D. barbnodis*); Lane 4 P6 (*D. exilis*); Lane 5 P15 (*D. exilis*); Lane 6: P 24 (*D. iburua*); Lane 7: P27 (*D. exilis*); Lane 8:K1 (*D. exilis*); Lane 9: K2 (*D. exilis*); Lane 10: K4 (*D. iburua*); Lane 11: T3 (*D. exilis*); Lane 12: T4 (*D. exilis*); Lane 13: negative control (using nuclease free water). (Source: Nyam et al. (2017b))

development of other more sensitive markers, amplified fragment length polymorphisms (AFLPs) have proven to be a powerful and efficient tool in population studies, molecular taxonomic classification, gene mapping and marker-assisted breeding in variable crops (Ayele et al. 1999; Carr et al. 2003; Uptmoor et al. 2003). Comparatively, AFLPs markers, overall, are more stable and reproducible vis-à-vis RAPDs markers (Rafalski and Tingey 1993) as they are based on combined use of endonuclease (to fragment the genomic DNA) and polymerase chain reaction (PCR) that allows amplification of these fragments using primers with arbitrary sequences. AFLPs markers were employed to identify genetic diversity and population differentiation among a large collection of fonio landraces (122 accessions) originally obtained from diverse producing areas of West Africa (Adoukonou-Sagbadja et al. 2007b, 2010). Koreissi-Dembélé et al. (2013) identified three genetic groups in fonio, and between-individual landrace variation was observed in one group using the AFLPs. All the genetic diversity identified in the crop was unequally distributed across West Africa. Although the genetic variability and phenotypic attributes were loosely correlated there was a clear difference not just between D. *exilis* and *D. iburua* populations, but clustering of *D. exilis* into three major groups corresponding to their geographic origin. Furthermore, *D. exilis* had a relatively moderate broader genetic background compared to the extremely narrow one in *D. iburua*. Overall sufficient phenotypic and genotypic variability were identified in fonio suggesting the availability of materials that can be used in improvement programs and germplasm conservation.

2.9.3 Germplasm Conservation

Agricultural intensification through modern practices coupled with other human activities, especially deforestation, desertification and urbanization has great pressure on biodiversity. This is more so with the replacement of the traditional crop cultivars with improved high-yielding ones. This ultimately constricts the genetic base necessary for mining novel genes for use to address emerging challenges of crop improvement. This challenge of genetic erosion is more likely to impact underutilized crops like fonio that is mainly composed of landraces with high levels of heterogeneity. Traditionally there are various methods of germplasm conservation used to address genetic erosion in crop plants. Plant germplasm can be conserved in situ in their natural habitat or ex situ through seed and field collections or in botanical gardens. In vitro germplasm collection involves allowing the collected plants to grow normally or slowly by applying low temperature or oxygen or water. Cryopreservation which entails storage of living plant tissues at ultra-low temperature (−196 °C) is also used in the conservation of plant genetic resources. More recently DNA banks are used in germplasm conservation.

Several organizations are involved in efforts to conserve fonio germplasm through national and international initiatives (Adoukonou-Sagbadja et al. 2004; Clément and Leblanc 1984; Clottey et al. 2006; Kwon-Ndung et al. 1998). A

significant number of fonio accessions across West Africa with adaptations to the diverse agricultural zones are maintained ex-situ by different National Agricultural Research Centers in West Africa and IRD (ex-ORSTOM, France). This effort needs urgent expansion to capture the available genetic diversity among cultivated landraces and wild fonio relatives using a combination of the germplasm conservation approaches adaptable to the African situation.

2.9.4 Molecular-Assisted Breeding

An appreciation and detailed information on existing landraces of fonio and farmers' preferences are a prerequisite to planning a systematic breeding program to produce elite fonio varieties that will be grown on farms. Farmers in Benin demonstrated preferences in the selection and cultivation of landraces with earliness given the highest priority, followed by culinary characteristics, ease of processing, productivity, facility for harvesting, grain size, storability and lastly drought (Dansi et al. 2010). An understanding of the reproductive system of a crop is a prerequisite for its genetic improvement in a breeding program. The reproductive system of fonio is just being unraveled. However, different hypotheses exist from claims that it is self-pollinated (Sarker et al. 1993; Watson and Dallwitz 1992) to outcrossing (Fogg 1976; Hilu et al. 1997). Using a combination of classical morphological characters along with isozyme and AFLP markers, Adoukonou-Sagbadja et al. (2010) identified pseudogamous type of apomixis as the mode of reproduction in *Digitaria exilis* and *D iburua*, with *D. exilis* demonstrating 2% outcrossing. The studies also revealed fonio crop as highly self-compatible and anallopolyploid.

Broad and in-depth knowledge of the morphology and genetic control of traits in ancestral species of crop plants are necessary prerequisites in the development of new and improved crop plants. Wild relatives of crop plants are known to hold diverse agronomic and useful traits such as resistance and tolerance to pests and diseases (Ochatt et al. 2004). Breeding of fonio varieties that combine resistance to lodging and large seed size will demand the use of genetic diversity available in both the cultivated landraces, and wild *Digitaria* species (Kuta et al. 2003).

Modern molecular techniques targeting directly DNA sequences in the plant genome have complemented classical methods in plant genetics and breeding in the improvement of crop plants. Furthermore, several population genetic studies and diversity analysis in several crop plants were carried out using these novel DNA techniques (Ayele et al. 1999; Dida et al. 2008). They were also employed in molecular taxonomy and phylogenetic investigations (Bänfer et al. 2004; Dasmahapatra et al. 2009; Milla et al. 2005), and mating-system determination (Karasawa et al. 2007; Kollmann et al. 2000; Persson Hovmalm et al. 2004) among others. Their application to the fields listed above have greatly enhanced the genetic improvement of the crops involved. DNA markers are categorized into i) non-polymerase chain

reaction (PCR) and ii) PCR-based markers. An example of the non-polymerase chain reaction marker is RFLP; while PCR- based markers include the following examples: RAPD, AFLP, SSR and SNP.

The dominant genetic marker, RAPD, has been used to elucidate genetic diversity and evolution in fonio (Hilu et al. 1997). While this marker technique provides better information on genetic diversity compared with morpho-botanical traits, its efficiency, particularly for phylogenetic analysis, is limiting (Van de Zande and Bijlsma 1995). Now most researchers prefer the use of AFLP (Vos et al. 1995) in such studies. It has been applied successfully in the study of many crops (Ayele et al. 1999; Bänfer et al. 2004; Seehalak et al. 2006). An assessment of the genetic diversity and population differentiation in the germplasm of West African fonio was carried out by Adoukonou-Sagbadja et al. (2007b) using this technique. Some of the important advantages of the AFLP technique include: wide application on all organisms, high multiplex ratio and extensive genome coverage, high reproducibility and robustness compared to other marker systems (Bänfer et al. 2004).

It is worth noting that the use of each genetic marker system has its own benefits and drawbacks. Therefore, choosing the right marker system will depend on the purpose of the studies, the stringency attached to the levels of resolution of polymorphism, the availability of technical facilities, as well as its efficiency in terms of costs and time requirements (Vendramin and Hansen 2005).

Despite the wide perspectives in utilization of fonio genetic resources, the crops are still at a primitive production level. Unlike crops of worldwide importance, little effort has been made so far to improve fonio with a few modern varieties produced in West Africa (Benin) (FiBL 2013). Improvement may start with selection among existing landraces for genotypes with lodging and shattering resistance, large seed size, resistance to pest and diseases and high nutritional attributes under low input production. The selection can be expedited using molecular markers such as RAPD, SSR and AFLP, with emphasis on SSR and AFLP due to their ability to identify heterozygotes and their codominance taking cognizance of the pseudogamous apomictic type of reproduction in fonio. This will require fixing of valuable traits through selfing.

AFLP markers are more likely to be useful in early stages in the improvement of fonio because their application do not rely on having sequence information of the crop. Their use is more likely to speed up the improvement of fonio within the shortest possible breeding cycle initially targeting increased yield, seed size, and resistance to lodging, shattering, and to pest and diseases. Subsequently, fonio's nutritional status could be enhanced by increasing the levels of existing essential amino acid, methionine and cystine and the concentration of iron and zinc. High-yielding fonio with these enhanced qualities will be strategically situated to alleviate both real and hidden hunger, by improving food security and eliminating iron and zinc deficiencies, which affects a greater percentage of world population. Fonio belongs to a genus with a wide array of species having wide geographical distribution that can be utilized as sources of vital and novel genes for introgression.

Increased productivity can be achieved by developing genotypes with a short growing cycle such that two to three harvests can be obtained in a year. This will contribute towards addressing the food needs of the rapidly expanding world population expected to move from 7.6 billion now to 9 billion by the year 2050, mostly in developing countries. The palatability and variety of ways of preparation and consumption of fonio grains, coupled with its positive nutritional qualities, especially the presence of the essential amino acids methionine and cysteine, the minerals, iron and zinc, and an absence of glutine present in major cereals makes it the grain crop of the future. Selection for high levels of bioactive components in its breeding program leading to the production of a new generation of *healthy* cereal grains should be given utmost attention.

2.10 Tissue Culture and Genetic Transformation

2.10.1 Tissue Culture Methods and Applications

Tissue culture involves the development of whole plants through culturing of seeds, organs, tissues, cells or protoplasts on nutrient media under sterile conditions. This is generally achieved by varying the ratio of the growth hormones, auxin (promotes root development) and cytokinin (promotes shoot development) plus minerals in a growth media originally formulated by Murashige and Skoog (1962).

The seeds and seedlings of intact plants may be cultured. In addition to this the embryo (immature embryo) may be cultured especially where wide crosses are involved. The genetic differences between a cultivated crop variety and its distant wild relative often results in the abortion of an embryo obtained from such crosses. Such embryos are usually rescued and allowed to develop in a culture medium. Cultures are also generated from different organs of the plant. This may require the use of the meristematic area of the shoot (shoot-tip culture) or other parts of the shoot, the root (root culture), leaf (leaf culture) or anther (anther culture). Other types of cultures include; callus, cell suspension and protoplast. Protoplast culture is created by degrading the cell wall using enzymes.

Plant tissue culture has found wide applications in various fields. Most commonly it is employed in the micropropagation of plants. Micropropagation facilitates rapid multiplication of plants without somaclonal variation, thus giving rise to true clones. Such clones are used to maintain heterozygosity. It is also used in the production of disease-free plants. Plant tissue culture is also used in the conservation and preservation of plant genetic resources, dihaploid production, protoplast fusion, genetic engineering and secondary metabolites production. The tissue culture procedure at times results in somaclonal variations that could be exploited by breeders.

Haploid plants are produced from another culture (microspore culture), ovule culture – by culturing of unfertilized ovules (macrospore culture), creation of allo-

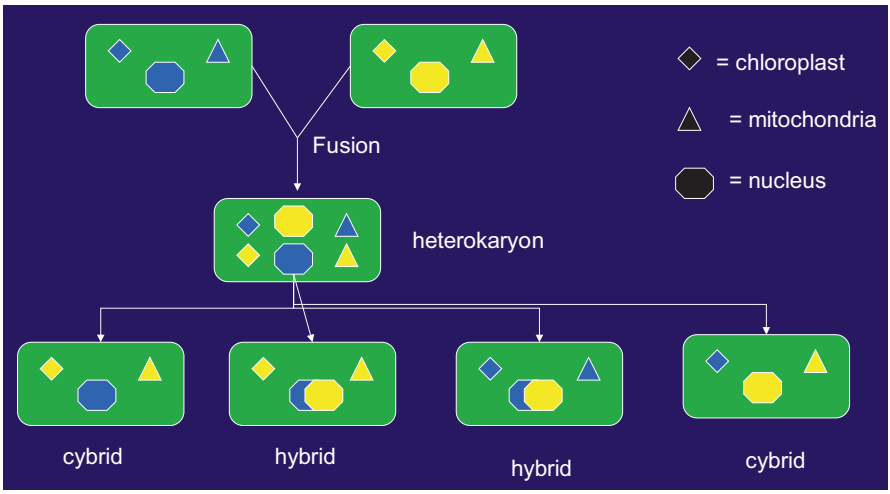

Fig. 2.6 Example of protoplast fusion showing various outcomes

polyploids arising from wide crossing between species where fertilization was achieved but embryo development failed to occur. Haploid plants are generally weak and sterile. The chromosomes of such plants are doubled spontaneously or by colchicine treatment to obtain fertile dihaploid plants that are homozygous at all gene loci. Dihaploids are used to evaluate for recessive and quantitative traits in fixed progeny for F_1, for creating permanent a F_2 family for molecular marker development, for fixing inbred lines, and theoretically for eliminating inbreeding depression. Inbreeding depression can only be selected against using dihaploids if large populations can be screened in culture for deleterious genes. This may reduce the time required for such selection.

Protoplast fusion is usually carried out between two species earmarked for crossing. The membranes of the two protoplasts are made to fuse through osmotic or electric shock or using a virus. The fusion product containing both genomes is regenerated. This procedure requires technical competence. Protoplast fusion can be used to generate allopolyploids, facilitate exchange of single or few traits between species, and transfer of mitochondria and/or chloroplast between species (Fig. 2.6).

2.10.2 Genetic Transformation

Ntui et al. (2010, 2017) developed tissue culture protocols and transformed two genotypes of *Digitaria exilis* (Agyong and Churiwe) using *Agrobacterium* (Fig. 2.7). With protocols for the transformation of the major cereal crops in place, possibilities for the transfer of genes that are responsible for the production of methionine and cysteine, as well as those associated with increased iron and zinc content, exist.

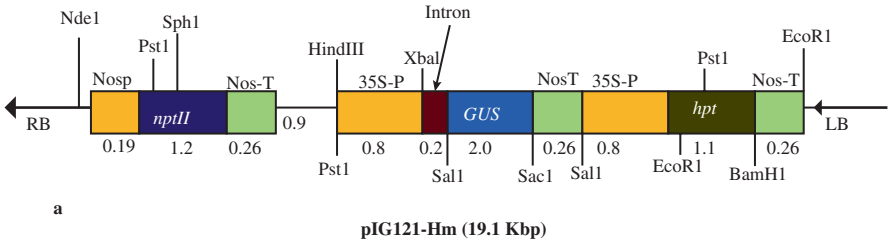

Fig. 2.7 T-DNA map of the construct used for transformation of fonio. RB: Right border; LB: Left border; nptII: neomycin phosphotransferase gene; hpt: hygromycin phosphotransferase; gus: b-glucuronidase gene

Clones carrying such genes can be used to transform the major cereal crops aimed at improving their nutritional qualities. Such transformed crops with improved nutritional value will go a long way in eliminating hidden hunger caused by deficiencies in iron and zinc that particularly affects women of reproductive age in Africa.

Considering all of the positive agricultural, nutritional and culinary properties attributed to fonio, there is the urgent need for the sequencing of its relatively small genome (2C DNA = 1.85 to 1.96 pg) with a view not only to bring about its improvement, but to the possibilities of bringing the techniques of its improvement to a par with the scientific developments, especially genomics of the major cereal crops. This will lead to mutual inter-genomic transfer of useful genes across the major and minor cereal crops. The transformation frequency of 2.1 and 2.7% (Agyong and Churiwe, respectively) that resulted in phenotypically normal transgenic plants by Ntui et al. (2017) provides useful platform for genetic improvement of fonio. Sequenced the fonio genome will open a new vista of bioinformatics that could facilitate the identification of the genes that are responsible for hardiness, versatility and excellent nutritional and culinary properties.

2.11 Conclusions and Prospects

Despite its importance in traditional agriculture, research efforts to improve fonio are still developing. There is no global research center responsible for the improvement of this crop. In consequence, the crop remains neglected, faced with diverse agronomic and technological challenges. In Sub-Saharan Africa, the production of fonio is not in serious decline compared to some cereal grains, such as sorghum. Germplasm collection, evaluation and conservation are therefore important to reduce genetic erosion and produce population sources for genetic improvement and preservation. With prevailing challenges in Sub-Saharan Africa, the preservation of fonio germplasm and seed should enjoy regional and global intervention.

The fonio landraces under cultivation are adapted to subsistence production techniques with less productivity. This subsistence approach relies on the use of low quality seeds, poor and degraded soils, poor husbandry, droughts, etc. which combined affect the productivity of the crop. Although fonio at the regional level suffers from low ranking when compared to other major cereals like pearl millet, sorghum or maize, its yield/hectare is on a par with that of pearl millet. This low level of acceptance has direct impact on its improvement through breeding. There is need for a high-performing variety which would combine good agronomic characteristics with good biochemical characteristics coupled with ease of processing. Since little information is available on the inheritance of traits of agronomic importance in fonio, genomic information arising from RAPDs, AFLP, SSRs and SNP related to major agronomic traits and QTLs can be accumulated within a short period of time for use in marker-assisted selection that can lead to the development of new varieties devoid of the production challenges associated with the crop. Quick progress is envisaged when the limitations of small floral organs, limited knowledge of its reproductive biology and organization of the genetic diversity in the crop, are addressed. Arising from all these efforts is the need to characterize and exploit fonio genetic resources for its improvement.

The high economic return to farmers associated with fonio cultivation at local and international level should provide the incentive for its improvement. An expansion of the current geographic and ecological limits of fonio cultivation using improved varieties under low agricultural input is bound to increase world food diversity as well as productivity, thus contributing to nutritional and food security of the worlds' growing population. The present race for space exploration and the planned manned voyages to other planets brought to the fore the need for further study of food supply and processing. The fact that the current space food is based on pre-processed meals, there is an urgent need for advances in grain breeding and processing (for outer space) that meet the requirements for extra-terrestrial conditions. Terrestrial conditions are subject to gravitational pull and atmospheric pressure which are lacking in outer space. The future areas of investigation will include the complex process of hydration of fonio grains using innovative technologies.

Appendices

Appendix I: Research Institutes and Online Resources Relevant to Fonio

Institute/Organization	Specialization and research activities	Contact and website
Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Montpellier. France. (French Agricultural Research Centre for International Development).	General All aspects of the crop	Head Office: 42, rue Scheffer, 75,116 Paris, France Tel.: +33 1 53 70 20 00 Montpellier Office: Avenue Agropolis, 34,398 Montpellier Cedex 5, France Tel.: +33 4 67 61 58 00; www.cirad.fr/en
Guinea Agronomic Research Institute (IRAG)	Agronomic, extension and marketing	Agricultural Research Institute of Guinea Bd. of Commerce, BP: 1523 Conakry Republic of Guinea Tel. (224) 30435580 e-Fax: (33) 821480314
Institut Togolais de Recherche Agronomique (ITRA)	Agronomic, extension and marketing	National Road N ° 1, Km 10, Agoè Cacavéli – BP 1163, Lomé – Togo
National Cereals Research Institute, Badeggi. Nigeria	Agronomic, extension and marketing	National Cereals Research Institute (NCRI), Badeggi, P. M. B 8, Bida, Niger State Phone No.:08069314862 www.ncribadeggi.org.ng
Institut national de recherches agricoles du Bénin (National Agricultural Research Institute, INRAB), Niaouli. Benin Republic	Agronomic, extension and marketing	01 BP 884 Cotonou, Benin http://inrab.org
Institut d'économie rurale (IER, Mali)	Agronomic, extension and marketing	MOHAMED V ST, BP 258, Bamako, Mali
Senegalese Agricultural Research Institute – ISRA	Agronomic, extension and marketing	BP 3120 Tel. +221 33,832 84 27 Fax +221 33,832 24 27 Dakar, Senegal www.isra.sn

Appendix II: Genetic Resources of Fonio

Cultivar	Important traits	Cultivation location
Afio	White seed fonio, early maturing landrace (3 months)	Northern Togo
Ayôrô (Yôrô)	White seed landrace, matured in less than 3 months	Northern Togo
Adjougouri	Produces many tillers	Northern Togo
Angim	Late maturing landrace (4 months).	Northern Togo
Ezio	White seed, easy to husk, early maturing landrace (3 months).	Southern Togo
Folom	Hairy and irritating; tiny, rounded white grains and late maturing landrace	Northern Togo
Foukmum	Produces many tillers; late maturing landrace (4 months)	Northern Togo
Gnimimbi	Produces sparkly, tiny and easy husking grains; early maturing landrace	Southern Togo
Ipowan	Reddish grain; intermediate maturing landrace (3.5 months)	Central Togo
Kopordagou	Rounded and reddish grains; late maturing landrace (4 months)	Central Togo
Yôrô	Extra-early maturing (2.5 months); harvesting, drying and conservation very difficult as it matures during the rainy season; low yield and high loss of seeds due to shattering; grains must be roasted before pounding; paste not soft and difficult to prepare	Benin
Ipoaga	Semi-late landrace (4 months); panicles with mostly 4 to 5 racemes; black grains; easy cultivation, drying and storage; drought tolerant; high yield; large and very hard chaff; husking difficult and time-consuming; paste normally soft and tasty but it quality and color are generally affected by non-removed chaff	Benin
Iporhouwan	Intermediate landrace (3.5 months); easy cultivation, drying, and storage; high yield; grains big and easy to husk; paste soft and tasty. Susceptible to drought and poor soils (reduced yield)	Benin Republic
Iporni	Late maturing (5 months); easy cultivation, drying and storage; performs well in humid zones (around shallows); low seed loss (low shattering); drought susceptible; hard bran; husking difficult and time-consuming paste normally soft and tasty but it quality and color can be affected by non-removed bran	Benin
Yawko	Extra-early growing cycle with 60–75 days	Senegal
Mora	Intermediate maturity of 90 days	Senegal
Rane	Intermediate-late maturing requiring 100–120 days	Senegal

(continued)

Cultivar	Important traits	Cultivation location
Maoko	Late maturing landrace requiring 150 days; seeds are coarse and heavier	Senegal
Jenger 1	High yield; produces many reproductive tillers; brown seeds, erect plants; leaf and glumes pubescence.	Plateau State, Nigeria
Tzaat	High yield; dark brown seeds; erect plants; leaf and glumes pubescence	Bauchi State, Nigeria
Chit Kusung	High yield; early maturing; straggling plant habit; light brown seeds; leaf and glumes glabrous	Bauchi State, Nigeria
Ibura	Very low yield; up to 8 racemes; erect plant; leaf and glumes pubescence; brown seeds	Plateau State, Nigeria
Wondat 1	Moderate yield; intermediate maturity; light brown seeds; produces many reproductive tillers; straggling plant habit; leaf and glumes glabrous	Bauchi State, Nigeria
Tswan 1	Low yield; straggling plant habit; high number of nodes; tillers profusely with few bearing inflorescence; leaf and glumes glabrous	Kaduna State, Nigeria
Tswan 2	Extra early; straggling plant habit; leaf and glumes glabrous; light brown seeds; seed shattering	Kaduna State, Nigeria
Kachang	Low yield; erect plant habit; leaf and glumes glabrous; brown seeds	Plateau State, Nigeria
Yar zheti	Late maturing; moderate yield; straggling plant habit; leaf and glumes glabrous; light brown seeds	Bauchi State, Nigeria
Lhad	Late maturing; tall and thick culm; large leaf	Bauchi State, Nigeria
Wèlè	High protein content (16.97 g/100 g DM); low carbohydrate content (70.85 g/100 g DM)	Mali
Siragué	Most popular variety; yielding 800–1000 kg/ha with 52% for harvest index	Guinea
Péazo	Low protein content (7.86 g/100 g DM); high carbohydrate content(80.03 g/100 g DM)	Mali

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Chapter 3
Finger Millet (*Eleusine coracana* L. Gartn.) Breeding

Neelofar Mirza and Soma S. Marla

Abstract Finger millet (*Eleusine coracana* (L.) Gaertn.) is a minor millet grown in the arid and semiarid tropics and subtropics of Asia and Africa. It is cultivated for food, as well as fodder and medicinal purposes. The genus *Eleusine* includes eight species of diploid and tetraploid annual and perennial herbs. The cultivated species also have several races and subraces and hence, finger millet displays great variability and diversity for most agronomically- important traits. It is a hardy crop which can withstand abiotic stress such as water scarcity and cold temperatures. Several genotypes are blast resistant and nutritionally rich especially in minerals and essential amino acids. Therefore, studying the germplasm diversity and selection of superior genotypes are prerequisites for a successful breeding program for crop improvement. The advent of tissue culture techniques, genomics and transcriptomics will further facilitate the study of genetic diversity. Increasing the finger millet production will help attain food and nutritional security especially for poor countries. Although the Green Revolution has transformed agriculture sector, the shift from traditional to modern production systems has led to the displacement of traditional landraces and genetic erosion of many crop species. It is thus vital to preserve the germplasm for the conservation of genetic diversity. The present chapter reviews the finger millet species, present diversity and recent breeding programs to improve the crop. The chapter also discusses the measures taken for genetic conservation of the species.

Keywords Blast resistant · *Eleusine* · Finger millet · Genomic resources · Improved varieties · Interspecific breeding

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J. M. Al-Khayri et al. (eds.), *Advances in Plant Breeding Strategies: Cereals*,
https://doi.org/10.1007/978-3-030-23108-8_3

3.1 Introduction

Finger millet or *Eleusine coracana* (L.) Gaertn. (Gaertner 1788; Basionym: *Cynosurus coracanus* Linnaeus) is a minor cereal millet. It is also known as bird's foot millet, coracana, African millet, ragi and kurukkan. The generic name *Eleusine* is derived from *Eleusis* an ancient city sacred to Demeter, the Greek goddess of agriculture. The term *coracana* is derived from its name *kurukkan* in the Sinhalese language of Sri Lanka. The common name finger millet is acquired from the finger-like branching of the panicle. Finger millet is an allotetraploid (2n = 4X = 36), mainly found in the arid and semiarid tropics and subtropics (Fakrudin et al. 2004). It is a robust annual grass grown as a cereal in more than 25 countries in warm temperate regions of Africa, Asia and South America (Phillips 1972). Finger millet is nutritionally comparable to rice and wheat and ranks fourth in importance among millets in the world after sorghum, pearl millet and foxtail millet (Upadhyaya et al. 2007). Around 4.5 million mt of finger millet are produced worldwide every year accounting for 12% of the global millet area (ICRISAT 2015). Africa produces 2.5 million mt and India produces 1.2 million mt annually. Finger millet accounts for about 85% all millets produced in India and is cultivated over 1.19 million ha in India according to a recent report (Sakamma et al. 2018).

Finger millet is cultivated for food, as well as fodder and medicinal uses (Oduori 2005; Phillips 1974). It is a hardy crop that can be grown in very diverse environments and can be stored for very long periods. The long duration cultivars can be grown as a hot weather crop while it can also be grown as a cold season crop using early maturing varieties (Duke 1978, 1979). Finger millet can yield as much as 5000 kg/ha of grain. It is a potentially nutritious crop for the increasing world population. Owing to its purported medicinal properties, finger millet has long been used as a folk remedy in Africa and India for several diseases such as liver disease, measles, pleurisy, pneumonia and small pox (Bachar et al. 2013). Various landraces possess genes for robust growth, early vigor, large panicle size, high finger number and branching and high-density grain as well. It is also reported that finger millet is tolerant to alkali, salt, slope, drought, laterite, disease, fungi, insects, mildew, viruses and pH variation (5.0–8.2) (Duke 1978). Some of the landraces are water-efficient types with high carbon dioxide fixation and low leaf area that could perform well in semiarid climates. Others have genes for tolerance to heat, cold, drought, blast resistance and some also for lodging as well as an ability to mobilize phosphorus and utilize nitrogen efficiently (NRC 1996).

Several finger millet cultivars or genotypes have high dietary fiber content, more than 10% protein and a good source of essential amino acids including tryptophan, cysteine and methionine (Iyengar et al. 1945, 1946; Jideani 2012; Mirza et al. 2014b). The micronutrient density of finger millet is also higher than the staple crops like rice or wheat (Rao and Deosthale 1983). It is rich in minerals such as Mn, Cu, Mg, Se, Mo and P and is particularly high in Ca content (Barbeau and Hilu 1993; Jideani 2012; Mirza et al. 2014a; NRC 1996; Vadivoo et al. 1998). The grains are also rich in vitamin B complex such as thiamine, riboflavin, folic acid and niacin (Gull et al. 2015; Saleh et al. 2013). Due to its reasonably high grain calcium content

and nutritionally good quality, finger millet can be used for formulating diets for pregnant and lactating women as well as for growing children and can be utilized in preventing or lowering the risk of osteoporosis (Antony and Chandra 1998; Poutanen 2012; Verma and Patel 2013). Millet is also recognized for its health benefits such as hypoglycemic, hypocholesterolemic, anti-ulcerative, nephroprotective and anti-cataractogenic characteristics (Chethan and Malleshi 2007; Shobana et al. 2010). Consumption of a finger millet-based diet has been reported to significantly lower plasma glucose levels in hyperglycemic adults suffering from non-insulin dependent diabetes mellitus (NIDDM) (Kumari and Sumathi 2002; Muthamilarasan et al. 2016) attributed to its higher fiber content. Condensed tannins extracted from finger millet have been shown to exert significantly higher antioxidant and anti-diabetic activities than other food ingredients (Kunyanga et al. 2011). Finger millet is regarded as the *wonder grain* due to its excellent nutritional properties and termed a *famine crop* owing to its long storability, ensuring food and nutritional security (Fakrudin et al. 2000; Gupta et al. 2017; Mgonja et al. 2007; Takan et al. 2004). In 2015, ICRISAT added finger millet as its sixth mandate crop.

To design a successful breeding program, it is essential to understand the biology of the plant and identify the existing variability and diversity in the genus. This chapter gives an up-to-date review of the information and resources generated for finger millet including the plant biology, species characterization, wild relatives, and achievements in breeding and transgenic developments. The chapter also details the agricultural limitations related to the crop and recommendations for future research.

3.2 Taxonomy

Finger millet was firstly documented by Linnaeus (von Linnaeus 1759) in *Systema Naturae, Editio Decima II* where he identified it as *Cynosurus coracan* hence, the basionym *Cynosurus coracanus* L. The genus *Eleusine* was later described in detail by Gaertner (1788) in *De Fructibus et Seminibus Plantarum* and hence the appellation, *Eleusine coracana* (L.) Gaertn.

Kingdom:	Plantae
Subkingdom:	Tracheobionta
Superdivision:	Spermatophyta
Division:	Magnoliophyta
Class:	Liliopsida
Subclass:	Commelinidae
Order:	Cyperales
Family:	Poaceae
Subfamily:	Chloridoideae
Genus:	*Eleusine* Gaertner
Species:	*Eleusine coracana* (L.) Gaertn.

The genus *Eleusine* includes nine annual and perennial species as recognized by Phillips (1972), with eight African species and one New World species (*E. tristachya* Lam.) native to Argentina and Uruguay (Lovisolo and Galati 2007). The range of the genus has been extended by widespread introduction of the crop (*E. coracana*) throughout the tropics, and the common weed often associated with cultivation, *E. indica* (L.) Gaertn. East Africa is considered the center of diversity of the genus and eight species (*E. africana, E. coracana, E. kigeziensis, E. indica, E. floccifolia, E. intermedia, E. multiflora and E. jaegeri*) (Table 3.1) occur in this region (Mehra 1963; Phillips 1972). The species of *Eleusine* Gaertn. are distributed in the tropical and subtropical areas (Fig. 3.1) of India, Myanmar, Sri Lanka, Nepal, China and Japan in Asia; while in Africa, it is grown in Uganda, Kenya, Tanzania, Ethiopia, Eritrea, Rwanda, Zaire and Somalia (Upadhyaya et al. 2010). It is an annual allotetraploid (2n = 4X = 36, AABB) that includes two distinct subspecies: *E. coracana* ssp. *coracana* (L.) Gaertn. and *E. coracana* ssp. *africana* (Kennedy-O'Byrne) Hilu & de Wet (Hilu 1994; Hilu and de Wet 1976). *Coracana* is the cultivated ssp. while *africana* is the wild ssp. Wild finger millet is native to Africa and is believed to have migrated from there to Asia and the Americas. The cultivated ssp. *coracana* was domesticated from wild populations of *E. coracana* ssp. *africana* as suggested by morphological and cytogenetic evidence, and through molecular studies (Chennaveeraiah and Hiremath 1974; Hilu and de Wet 1976; Hilu and Johnson 1992). Both cultivated and wild species are important from the point of germplasm collection, conservation and utilization, since they collectively form the primary gene pool. Dida et al. (2008) recently demonstrated the gene flow between ssp. *africana* and ssp. *coracana* through genotypic analysis of microsatellites. However, hybridization between the wild and cultivated populations has given rise to many morphological intermediates that are completely fertile. These hybrids are aggressive colonizers and are grouped under the race spontanea (De Wet et al. 1984; Kennedy-O'Byrne 1957; Mehra 1962; Phillips 1995). The diploid wild species *E. indica* (L.) Gaertn., *E. floccifolia* Spreng. and *E. tristachya* (Lam.) Lam., supposedly form the secondary gene pool while the tertiary gene pool includes *E. intermedia* (Chiov.) S.M. Phillips, *E. jaegeri* Pilg., *E. kigeziensis* S.M. Phillips and *E. multiflora* A. Rich. (Guarino 2012) (Fig. 3.2).

3.2.1 Eleusine coracana *ssp*. africana

The *africana* ssp. is found along the highlands of East Africa and the grasslands of Southern Africa. It has also been reported from India and the UK. *Africana* was first recognized as a distinct species by Kennedy-O'Byrne 1957). Afterwards, considering the morphological similarities, it was designated a subspecies of *E. indica* (Lye 1999; Phillips 1972, 1974). *Eleusine africana* is comparatively robust as compared to *E. indica*, with longer leaves, thicker and longer spikes, larger bracts etc. but has very similar morphology. However, attempts of hybridization between the tetraploid *E. africana* (2n = 4x = 36, AABB) and the diploid *E. indica* (2n = 2x = 18, AA)

Table 3.1 Other *Eleusine* spp. of possible genetic value

Species	Notes	References
Eleusine floccifolia	Perennial diploid (2n = 2x = 18); distinguishing white hairs on leaf margins; mid to high elevations; avoided by livestock	Phillips (1972, 1974, 1995) and Sisay and Baars (2002)
Eleusine intermedia	Perennial diploid (2n = 2x = 18); stout rhizome; softer leaves with sparse, soft straight hairs on the smooth margins (pilose); laxly arranged spikelets; florets with 3-nerved lemmas with distinct 1-nerved keel or ridge	Grassland Index (2009) and Phillips (1972)
Eleusine jaegeri	Perennial diploid (2n = 2x = 20); most robust; common in grasslands of east African mountainous regions; invasive culms branch abundantly form thick and coarse tussocks or tuft; whitish overlapping leaf-sheaths; tough pale green glabrous leaves with rough margins; unpalatable to livestock; used for basket making	Grassland Index (2009) and Phillips (1972, 1974, 1995)
Eleusine kigeziensis	Perennial tetraploid (2n = 2x = 38); occurrence-east African uplands; short slender ascending rhizome; long open inflorescence; fairly soft leaves sometimes pilose lacking hair; lemma with a central and two lateral inconspicuous nerves; distinguishing lemmas with a central 3-nerved keel	Phillips (1972, 1974, 1995)
Eleusine multiflora	Annual diploid (2n = 2x = 16); distinct three or more short oblong-ovate spikes generally at a distance of 1 cm or more from each other alternating at the top	Hilu and Johnson (1997), Neves (2011) and Werth et al. (1994)
Eleusine tristachya	Annual diploid (2n = 2x = 18); only species native to South America; important forage grass; digitate inflorescence tightly clustered at the top of the axis; short and oblong spikes with spikelets neatly arranged perpendicular to the spike axis	Clayton et al. (2006-onwards), Ellis et al. (2004), Hansen (1980), Hilu (1980, 2003), Sanz Elorza et al. (2001), Lovisolo and Galati (2007), Phillips (1972, 1974, 1995) and USDA NRCS (2009)
Eleusine indica	Annual diploid (2n = 2x = 18) known as goosegrass; major weed worldwide; small plant with narrow rachis and thin stems; 3-nerved lemmas with a 3-nerved keel; short shattering spikelets; small seeds enclosed in relatively short glumes and lemma and thin racemes	Phillips (1972) and Neves (2011)

resulted in sterile plants (Channaveeraiah and Hiremath 1974; Hiremath and Salimath 1992). Whereas, it was observed to hybridize ssp. *coracana* in nature and hence classified as a subspecies of *E. coracana*.

Later, studies of chromosome numbers and genome size clearly distinguished *Eleusine africana* from *E. indica*. It is an annual plant that grows in dense tufts. They have geniculately ascending culms and branching at the lower nodes. The inflorescence branch is long and flowering culms are up to 135 cm tall. The inflores-

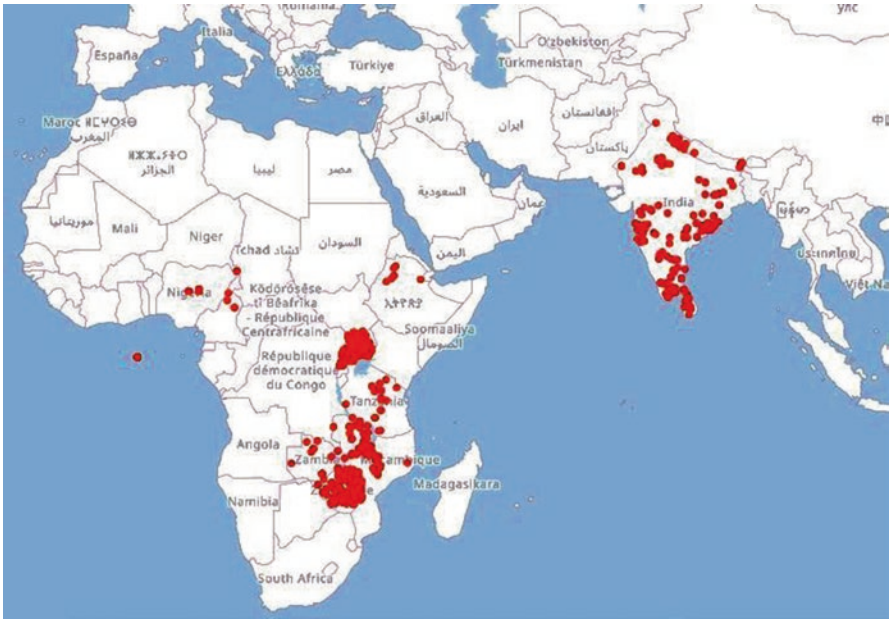

Fig. 3.1 Distribution of *Eleusine* accessions map generated in GENESYS, a global portal to information about Plant Genetic Resources for Food and Agriculture (PGRFA)
Source: https://www.genesys-pgr.org

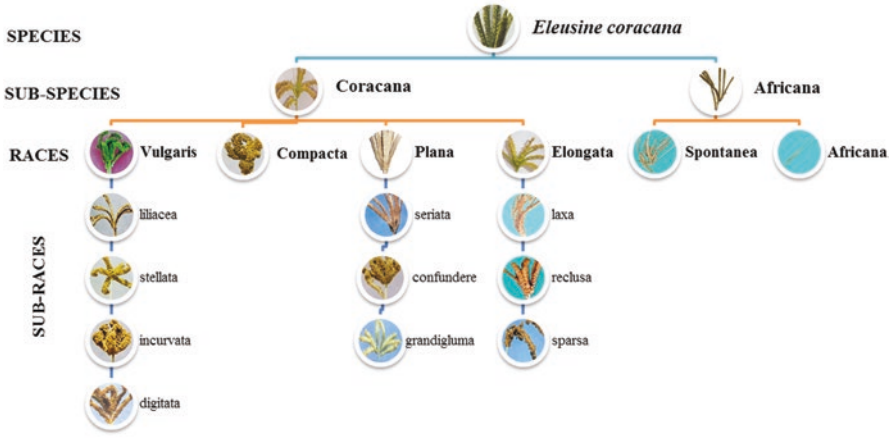

Fig. 3.2 Races and subraces of the species *Eleusine coracana*

cence or spike is long and open. The spikelets are arranged in two rows on one side of the rachis. Glumes are shorter than spikelet, lanceolate-oblong, rarely more than 5 mm and narrow-winged along the keel (Bharathi 2011). The ligule and grain characteristics can differentiate *E. africana* from *E. indica*. In *E. africana*, the ligule has

a clear ciliate fringe while in *E. indica*, the ligule is a truncate, scarcely ciliolate membrane. The *E. africana* grain has very fine granular surface with barely visible ridges whereas in *E. indica,* the grain has well-marked, oblique ridges (Hilu et al. 1979; Phillips 1972). *Eleusine coracana* ssp. *africana* has two races, Africana and Spontanea (Fig. 3.2).

Race Africana is found in Burundi, Uganda, Malawi, Tanzania and Ghana. Plants may be erect, prostrate or decumbent and can reach a height up to 165 cm. Race Africana has the characteristic long open inflorescence. The panicle has 4–14 fingers and a length up to 14 cm. The grains are brown to dark brown in color.

Race Spontanea is found in Kenya, India and also in the UK. The plants are erect and both green and pigmented. The maximum height recorded for Spontanea is 120 cm. The fingers are generally 6–9 cm and incurved. The inflorescence generally consists of 6–8 fingers however, up to 10 fingers have also been reported. The grains can be light to dark brown.

3.2.2 Eleusine coracana *ssp*. coracana

The *coracana* ssp. is an annual tetraploid (2n = 4x = 36, AABB). It includes all the four cultivated races (elongata, plana, compacta, vulgaris) and subraces (Fig. 3.2) that show considerable morphological variation of the inflorescence and more frequently referred simply as *E. coracana* (De Wet et al. 1984; Dida and Devos 2006; Hilu and de Wet 1976; Upadhyaya et al. 2007). The morphological characteristics of the each of the subraces are discussed below (Bharathi 2011).

Plants are annual, erect or geniculately ascending culms and can reach a height of 165 cm high (Fig. 3.3). Culms are commonly branched from the upper nodes to produce secondary inflorescences. Inflorescences are difitate or subdigitate, often with one or more racemes some distance below the main cluster of 4–19 branches.

Fig. 3.3 *Eleusine coracana* ssp. *coracana*

Inflorescence branches are slender to robust sometimes with secondary branches. Each spikelet comprises of 6–9 overlapping flowers which are 6–10 mm long and mostly arranged in two rows along one side of the rachis. It is unique in its grain characteristic as it bears a utricle instead of a true caryopsis like other cereals (McDonough et al. 1986a). The globose grains (after removal of the pericarp) may be blackish, brown, reddish or even whitish while the grains of other *Eleusine* species are typically blackish (Phillips 1972, 1974, 1995). The grains are exposed between the florets in the nonshattering spikelets, when ripe whereas in other *Eleusine* species (including ssp. *africana*), spikelets dis- articulate (between the florets) at maturity and grains remain enclosed (Chen and Phillips 2006; Chennaveeraiah and Hiremath 1973; Phillips 1972, 1995).

3.2.3 Eleusine coracana *ssp.* coracana*, Races and Subraces*

Race *Elongata* Of the four races, Elongata is morphologically the most distinct (Rao et al. 1993). It is grown in India, Nepal, Zimbabwe, Nigeria, Uganda and South Africa. Plants can grow to be erect or decumbent. The inflorescence is digitate. Spikelets are long, slender, spreading and become curved during maturity (Fig. 3.2). The grains are of different colors, from light brown to reddish and dark brown. It comprises 3 subraces, laxa, reclusa and sparsa. In laxa*,* both erect and decumbent plants are found. The spikelets are arranged in narrow rows on the inflorescence branches, like the wild race Africana*.* It has 5–11 open and long fingers (up to 19 cm) that can be top curved or incurved. Reclusa has comparatively short fingers (12 cm) and are open or top curved. The panicle branches are 5–11 with fingers as long as 12 cm. Sparsa inflorescences are generally pendulous (drooping). It has long fingers up to 14.8 cm and the branches are quite numerous (7–13).

Race *Plana* This race is found in India, Zimbabwe, Kenya, Nigeria, Uganda and Ethiopia. Plants are either erect or decumbent. Plants are green and pigmented. Large spikelets (6–17 cm) arranged in two or more rows along the rachis giving the inflorescence branch a flat ribbon-like appearance is characteristic to this race. Plana also comprises 3 subraces: seriata, confundere and grandigluma (Fig. 3.2). Spikes are mostly top curved in seriata with 6–15 fingers, however, short open are also reported. The grains are light brown, reddish brown to dark brown. In confundere, the spikes are top curved and generally has 6–7 fingers. But, number of panicle branches as high as 23 have been reported from Uganda. The fingers surround the rachis at maturity, giving a compact look to the panicle. The grains are mostly reddish brown. The grandigluma subrace, the spikes are again top-curved with 5–10 fingers, generally is characterized by long, pointed glumes and very long fingers up to 17 cm. The grains are light brown to reddish brown.

Race *Vulgaris* is mostly cultivated in Asia. Plants are both green and pigmented. They show both erect and decumbent growth habit. They can have 5–14 spikelets

(generally 6–8) and the tips are generally in-curved (Fig. 3.2) giving the inflorescence a semi-compact appearance at maturity (Guarino 2012). The fingers are generally 7–10 cm long. It has four subraces. The fingers are in-curved in both stellata and incurvata subraces, more in subrace *incurvata* giving it a fist like appearance. The grains of both subraces are mostly reddish brown or ragi brown. The fingers are reflexed or short open in subrace liliacea while top-curved in subrace digitata. The grains are ragi brown, reddish brown to dark brown, mostly dark brown in subrace digitata.

Race *Compacta* It is mostly found in India, Kenya and Uganda. The members of this race are generally referred to as *cockscomb* finger millet in both Africa and Asia. The erect or decumbent plants are both green and pigmented. The inflorescence axis is divided at the base ascending and the fingers (4–11) are in-curved at the top to give large fist-like appearance (Fig. 3.2). The grains can be light brown, reddish or dark brown in color.

3.3 Breeding Biology

Eleusine coracana is a self-pollinated member of family Poaceae and subfamily Chloridoideae. Cross-fertilization by wind or insects is reported to contribute less than 1% (Seetharam 1998). Since then, several groups have described the genus and floral biology in finger millet (Ayyangar 1932; Ayyangar and Wariar 1934; Chavan and Shendge 1957; Chavan et al. 1955; Coleman 1920; Dodake and Dhonukshe 1998; Gupta et al. 2011; Phillips 1972; Rachie and Peters 1977). Different parts and stages of developing inflorescence have been recently documented in Mirza et al. (2014a). Four principal growth stages (S1, S2, S3, S4) were identified for the developing spike based on a decimal code developed by Zadoks et al. (1974), as shown in Fig. 3.4.

The stage when about one fourth of the inflorescence has emerged is designated as S1 and called the *booting* stage. The inflorescence is light green in color, the spikelets in the fingers are compactly arranged and florets are not identifiable at this stage. The second stage is anthesis stage. The stage when anthesis is halfway is designated as S2 stage. The inflorescence appears yellowish due to the emergence of anthers. The florets became clear. The S3 stage is the grain-filling stage. It is the late milk stage, once increase in solids in liquid endosperm is notable, when the caryopsis is crushed between fingers. The inflorescence and the developing grains are green in color. The developing grains became swollen but remain covered with lemma and palea. In the S4 stage, 50% of spikelets have ripened and the caryopsis has hardened enough so that it is difficult to divide by thumb-nail. The inflorescence is dried, its color changes to yellow-brown and the grains are clearly visible between the gaped florets (Fig. 3.5g).

The Finger millet inflorescence or spike consists of a whorl with 2–11 (average 5–7) digitate, slightly curved or straight spikes or fingers, with an odd one a little

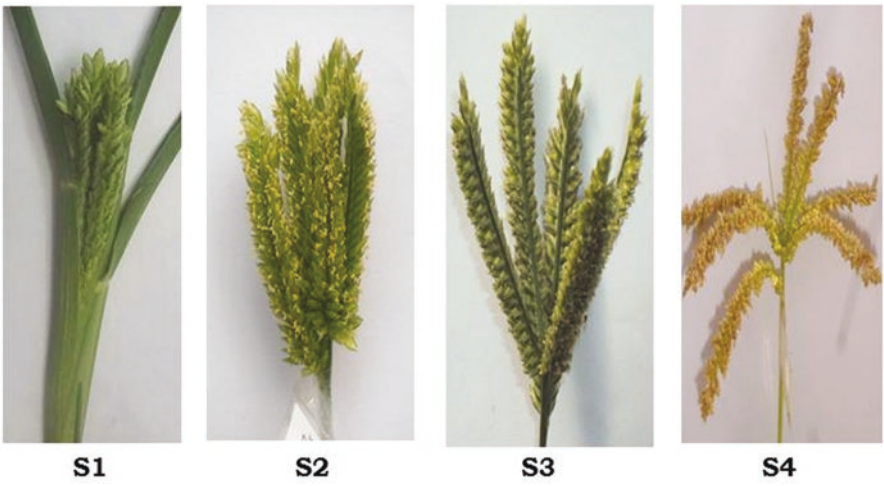

Fig. 3.4 Principal growth stages of the developing finger millet spike. **S1** Booting stage, **S2** Flowering or anthesis stage, **S3** Grain-filling, **S4** Ripening stage
Source: Mirza et al. (2014a)

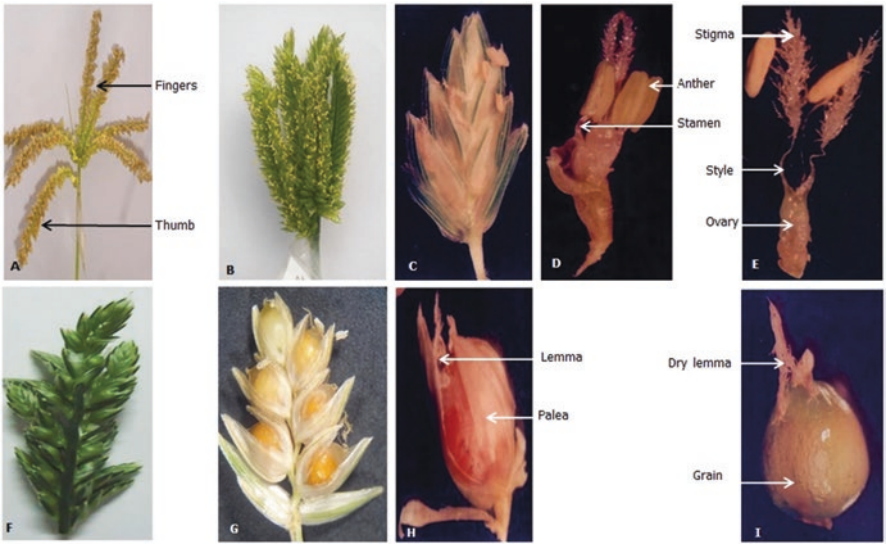

Fig. 3.5 Different parts of inflorescence in *Eleusine coracana* (L.) Gaertn. (**a**) Complete spike or inflorescence, (**b**) Inflorescence showing flowers on the edge of fingers, (**c**) Floret (spikelet), (**d**) Developing embryo in S1 stage, (**e**) Developing embryo in S2 stage, (**f**) Finger with developing grains in S3 stage, (**g**) Single spikelet with maturing grains in S4 stage, (**h**) Grain with dried lemma and palea still attached, (**i**) Developed grain with remnants of stigma and stamen attached at the top
Source: Mirza et al. (2014a)

lower known as a *thumb*, giving the inflorescence a bird-foot appearance (Fig. 3.5a). The complete emergence of the inflorescence may take up to 10 days. Each spikelet consists of 3–13 florets. Flowering takes place simultaneously in all fingers and begins from the top (Fig. 3.5b,c). Usually, flowering is complete by third day; however, it varies from place to place depending on temperature and humidity. The terminal floret is mostly sterile. Florets are hermaphroditic having boat-shaped lemma, a smaller palea with two lodicules (Fig. 3.5g, 6h). Before anthesis florets are compact, the androecium and gynoecium are very small, closely arranged and pale in color (Fig. 3.5d).

The androecium consist of 2–3 stamens with long filaments and short oblong anthers to form the ovary with 2 styles and a plumose stigma forms the gynoecium. After anthesis, the anthers and feathery stigma are visible at the tip of the florets (Fig. 3.5c); anthers, filaments, stigma and style increase in size, anthers appear yellowish due to pollen grains and the ovary is swollen (Fig. 3.5e). Pollen viability is very short in finger millet, only 10–15 min and the stigma is receptive for up to 5 h (Dodake and Dhonukshe 1998). Opening of the florets and grain filling starts from bottom to top within the spikelets (Fig. 3.5f). The nonshattering spikelets bear plump grains, usually enclosed in a thin brown pericarp that is exposed between the lemma and palea (Fig. 3.5g, 6h). Variation in head shapes and grain shapes helps to distinguish closely related species (De Wet et al. 1984).

3.4 Domestication, Selection and Early Improvements

Finger millet originated in the highlands of Uganda and Ethiopia and domestication began there around 5000 years ago, as evident from the archaeological records of early African agriculture (Hilu and de Wet 1976; FAO 1995; NRC 1996). Finger millet arrived in India probably more than 3000 years ago; India has been debated as its origin for a long time due to the presence of several cultivars in different regions. However, Fuller (2002, 2006) did an exhaustive review of the work on the origin of *Eleusine* and confirmed its African origin. Fuller reported that most of the claims of Indian origin of finger millet are widely based on misidentified material of other species. *Eleusine indica* is evidently the maternal diploid genome donor (AA genome) of both *E. coracana* subspecies resolved through various cytological, isozymes, RAPD, chloroplast DNA and genomic in situ hybridization (GISH) studies (Bisht and Mukai 2001a; Chennaveeraiah and Hiremath 1974; Hilu 1988, 1995; Hilu and de Wet 1976; Werth et al. 1994;). Using GISH, Bisht and Mukai (2001b) also suggested *E. floccifolia* as the B genome donor. However, Neves et al. (2005) rejected the assertion based on their nuclear internal transcribed spacers (ITS) and plasmid trnT-trnF sequence analysis. The sequence divergence and population structure analyses of 14 wild *E. coracana* ssp. *africana* lines and 79 cultivated finger millet accessions (*E. coracana* ssp. *coracana*) from African and Asian countries using SSR markers by Dida et al. (2008) supported the African origin of finger millet. They suggested that finger millet was first domesticated in the African

highlands, then moved to the southern lowlands, and finally was brought to India. The gene flow between the African wild and cultivated subpopulations indicate the natural hybridization among these sympatric subspecies. The B genome donor most likely has become extinct (Liu et al. 2014).

3.5 Germplasm Biodiversity and Conservation

3.5.1 Cytogenetic Analysis

The haploid chromosome number in the annual species of finger millet was reported first by Krishanswami and Ayyangar (1935) to be 9 in *Eleusine indica*, 18 in *E. coracana*, 18 in *E. brevifolia* Wall. and 17 in *E. aegyptica* (L.) Desf. Bisht and Mukai (2000) counted the diploid chromosome number in annuals as 36 in *E. coracana*, 18 in *E. indica* and *E. tristachya*, and 16 in *E. multiflora* while the perennials, *E. floccifolia* has 18, *E. intermedia* 18, and *E. jaegeri* 20 chromosomes. The DNA content in the leaves and roots was measured through laser flow (Mysore and Baird 1997). The 2C DNA content of *E. indica*, *E. tristachya*, *E. jaegeri*, *E. multiflora* and *E. floccifolia* ranged from 1.51–2.65 pg while that of the polyploid species *E. coracana* ssp. *coracana*, *E. coracana* ssp. *africana* ranged from 3.34–3.87 pg. In a series of fluorescent in situ hybridization (FISH) studies based on the hybridization of a 5sRNA probe of the chromosomes, Bishit and Mukai (2000, 2001a; b) reported that there were 3 diploid species, *E. indica, E. intermedia* and *E. floccifolia* and 2 tetraploids, *E. africana* and *E. coracana*. They also suggested that *E. multiflora* is a distinct species, the 2 tetraploids are related and that the 2 diploid species *E. indica* and *E. floccifolia* are probably the donors to the tetraploid species. This hypothesis was later refuted when the ITS (internal transcribed spacer) based phylogenetic analysis of species sequence data showed that *E. coracana* and *E. floccifolia* are not related (Neves et al. 2005). *Eleusine indica* was concluded as the progenitor of *E. coracana* ssp. *coracana* and *E. coracana* ssp. *africana* on the basis of the restriction analysis of chloroplast DNA (Hilu 1988). The RFLP data of Hilu and Johnson (1992) appeared to corroborate that *E. indica* is one of the parent species of finger millet. The RFLP and ISSR analysis by Salimath et al. (1995) and ITS based-phylogenetic analysis further helped in differentiating the species.

3.5.2 Germplasm and Genetic Diversity

For success in any breeding program and crop improvement effort, it is crucial to understand the amount and distribution of variability present in a gene pool. As finger millet is cultivated under diverse climatic conditions in Asia and Africa, understanding the genetic diversity is vital to identifying genotypes resilient to climate change (Mercer and Perales 2010). Genotypes tolerant to various biotic and

abiotic stresses have more allelic variation compared to susceptible types and thus are very useful for breeding programs. The DNA-based markers such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), inter-simple sequence repeats (ISSR) and simple sequence repeats (SSR) have frequently been used for the analysis of genetic diversity in finger millet (Babu et al. 2014a; Bezaweletaw et al. 2011; Das and Misra 2010; Fakrudin et al. 2004; Gupta et al. 2010; Parani et al. 2001; Patil and Kale 2013). It is reported that large variation exists for agronomically- and nutritionally-important traits.

Barbeau and Hilu (1993) observed wide variations in the protein (7.5–11.7%), calcium (376–515 mg/100 g) and iron (3.7–6.8 mg/100 g) content in 2 wild and 8 domesticated cultivars. The wild cultivars were significantly higher in protein, calcium and iron than many domesticated accessions. The wild species were also found to be higher in the essential amino acids including lysine. Das et al. (2007) used RAPD markers to distinguish lines from Orissa from those originating from the southern states of India. Bezaweletaw (2007) analyzed over 64 landraces from Ethiopia and Eritrea and found great variation in several characters such as plant type, seed color, seed shape and persistence of pericarp. Plants with decumbent and prostrate types were found in Ethiopia, while erect types were found in the Eritrea collection. Srinivasachary et al. (2007) aligned the finger millet probes (332 loci detected by 266 probes or primer pairs) on a rice linkage map and compared 9 linkage areas and found duplication, deletion and sequence translocation. Dida et al. (2008) indicated that wild and cultivated accessions differed by a range of domestication-related characters, such as tiller number, plant height, peduncle length, seed color and grain yield. They also identified that the Asian and African subpopulations significantly differed in plant architecture and yield and the Indian alleles most likely contributed to the varietal enhancement. The genetic diversity and population structure were assessed in a number of Indian and non-Indian genotypes and collected from various geographical regions using 25 RAPD and 72 genomic SSR markers (Ramakrishnan et al. 2016a, b). Molecular variance and population structure in 42 genotypes of finger millet collected from different geographical regions of southern India were analyzed using 10 RAPD, 9 ISSR and 36 SSR markers (Rajendran et al. 2016).

Gimode et al. (2016) identified 10,327 SSRs and 23,285 nonhomologous SNPs from 2 cultivated finger millet genotypes KNE755 and KNE796. A number of markers of each type were analyzed across a diverse set of wild and cultivated finger millet germplasm for polymorphism. Polymorphism was shown by 95% (76 out of 80) of the SNP markers across 30 wild accessions while only 27.5% (22 out of 80) were polymorphic across the 59 cultivated genotypes revealing low variability within the cultivated finger millet. Most wild accessions were new collections that were expected to be cross- compatible with cultivated species, but the SNP markers clearly discriminated the wild species from cultivated ones and enabled correct classification of unknown genotypes. The analysis showed higher levels of homeologous SNPs which might suggest independent segregation of the AA and BB subgenomes. The recently released WGS will further help in SNP based diversity analysis in finger millet accessions.

3.5.3 Genetic Resource Conservation

For crop genetic improvement and subsequent utilization, conservation of the germplasm, evaluation and characterization of the existing diversity is vital. *Ex* situ conservation prevents the loss of genetic diversity and resources crop breeding programs. For ex situ conservation, seeds are preserved in national crop diversity collections, international genebanks such as those of the Consultative Group for International Agricultural Research (CGIAR), the Millennium Seed Bank, Royal Botanic Gardens Kew and the Svalbard Global Seed Vault (SGSV). SGSV holds 22,000 accessions of millets. Gene banks altogether hold more than 29,000 finger millet germplasms.

The International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) Genebank is one of the largest international genebanks that serves as a world repository for the six mandate crops i.e. sorghum, pearl millet, chickpea, pigeonpea, groundnut, finger millet and five small millets with over 126,830 germplasm accessions collected from 144 countries. In addition, the ICRISAT Genebank has also deposited over 111,000 accessions at SGSV, Norway. ICRISAT currently has a collection of 7519 germplasms (http://genebank.icrisat.org/IND/Passport?Crop=Finger+millet) from several Asian and African countries. Most of the collection constitutes traditional cultivars and landraces (7121). The collection also includes 143 advanced or improved varieties 205 wild varieties and 50 accessions of breeding/research material. The Indian germplasm collection in general has early-maturing varieties combining high grain yield, quality and stover yield whereas African germplasm are said to possess higher level of resistance to blast (Babu et al. 2013a).

A core collection of 622 accessions was developed in 2004 from the entire global collection, 5940 accessions at that time, based on origin and data related to 14 quantitative traits (Upadhyaya et al. 2006). Later, the data of this core collection were evaluated for 20 morphological descriptors at 5 agro-ecologically diverse locations in India during the 2008 rainy season (Upadhyaya et al. 2010). The hierarchical clustering of data based on phenotypic distances resulted in 40 clusters and about 10% or a minimum of 1 accession was selected from each cluster to form a mini-core. The mini-core collection is comprised of 80 accessions. Two more gene banks in India at the National Bureau of Plant Genetic Resources (NBPGR), New Delhi and the National Active Germplasm Collection Site (NAGS) located at All India Coordinated Small Millets Improvement Project (AICSMIP), Bangalore have collections of 10,507 and 7070 accessions, respectively. Other institutes involved in finger millet research such as the Kenya Agricultural Research Institute (KARI) (2875), Institute of Biodiversity Conservation (IBC), Ethiopia (2156), Serere Agricultural and Animal Production Research Institute (SAARI), Uganda and the USDA Agricultural Research Service (USDA-ARS), United States (1452) also have reasonable collections of germplasm. The ARS-USDA in Griffin, Georgia, maintains 766 finger millet accessions from 11 countries (Ethiopia, India, Kenya, Nepal, Pakistan, South Africa, Tanzania, Uganda, Zaire, Zambia, Zimbabwe), of which 17

are wild relatives (*Eleusine floccifolia*, *E. indica*, *E. jaegeri*, *E. multiflora*, *E. tristachya*).

Genetic erosion of millets occurs mostly due to their underutilization, poor yield, and a policy shift that has focused on staple crops such as rice, corn and wheat or cultivation of only a small number of improved millet cultivars. Genetic erosion however, can also occur at the level of germplasm collections and genebanks due to inadequate management and regeneration failures. In situ conservation involves the rescue and maintenance of species in their natural environments, thus ensuring continued evolution in the field, including the natural exchange of genes with each other and their cultivated relatives. The Global Crop Diversity Trust, along with the Millennium Seed Bank, are working towards in situ conservation of 29 crops including finger millet, under the ten year project named Adapting Agriculture to Climate Change: Collecting, Protecting and Preparing Crop Wild Relatives (CWR). It is supported by the Norwegian government. Numerous partners all over the world (Fig. 3.6) are implementing the project, which supports CWR prioritization, collecting, conservation and pre-breeding.

Fig. 3.6 Crop Wild Relatives (CWRs) Project Map depicting the partner institutes involved in collection and conservation of finger millet CWRs across the globe **1.** Instituto de Investigaciones Agropecuarias (INIA), **2.** Embrapa Genetic Resources & Biotechnology **3**. Museu Nacional de Historia Natural e da Ciéncia, **4**. International Center for Agricultural Research in Dry Areas (ICARDA), **5**. Plant Genetic Resources Research Institute, **6**. National Centre for Genetic Resources and Biotechnology (NACGRAB), **7**. Plant Genetic Resources Centre (PGRC), **8**. Kenya Agricultural and Livestock Research Organisation (KALRO), **9.** Ethiopian Biodiversity Institute (EBI), **10**. Agricultural Plant Genetic Resources Conservation and Research Centre (APGRC), **11**. Plant Genetic Resources Institute (PGRI) and **12.** National Agriculture Genetic Resources Center Source: www.cwrdiversity.org

3.6 Cultivation Practices

Finger millet cultivation practices in different regions depend upon the climate, variety, soil type and water availability. It may be grown as a hot weather crop, from May to September, using long-duration cultivars and as a cold season crop, from November and December, using the early-maturing cultivars. Finger millet is cultivated under both irrigated and rainfed conditions. It requires only moderate rainfall and under a rainfed system, it is cultivated either as a direct sown crop or is transplanted. Under irrigated conditions, it is cultivated by direct sowing, transplanting or using a system of crop intensification (SCI) methods. The frequency of irrigation varies with the season. Finger millet is monocropped under irrigation or transplantation and intercropped under rainfed conditions. In India, the common crops grown in association with finger millet are lablab bean (*Lablab purpureus* (L.) Sweet), pigeon pea (*Cajanus cajan* (L.) Millsp.), cowpea (*Vigna sinensis* (L.) Walp.), and niger (*Guizotia abysinnica* (L.f.) Cass.). Finger millet is grown as the subsidiary crop with groundnut (*Arachis hypogaea* L.). In Nepal, it is grown as a relay crop, intercrop or mixed crop along with maize, wheat and barley under rainfed conditions (MoFSC 2002; Sharma 2001).

3.6.1 Crop Intensification (SCI)System

On the basis of practices and experiences with the system of rice intensification (SRI) (SRI International Network and Resources Center, Cornell University; http://sri.ciifad.cornell.edu), a system of crop intensification (SCI) has been developed in recent years in several Asian and African countries. SRI is based on a set of principles and practices for increasing the productivity of irrigated rice by changing the management of plants, soil, water and nutrients. SRI is not a fixed set of technical stipulations, but a system that mainly focuses on altering certain conventional agronomic practices with regard to four main components: soil fertility management, planting method, water (irrigation) management and weed control. However, the fundamental principles remain more or less the same. These four components are:

1. Development of healthy young plantlets for transplantation, avoiding any shock or trauma to the plant and taking care to conserve and nurture their potential for root system growth,
2. Significant reduction in crop density; keeping wider spacing between individual plants, giving them more room to grow both horizontally and vertically,
3. Avoiding synthetic fertilizers, pesticides and herbicides and enriching the soil with organic matter by using compost or farmyard manure,
4. Maintenance of proper soil aeration and water to avoid hypoxic conditions or flooding and to support better growth of plant roots and of beneficial soil microorganisms.

SCI management has now been widely applied to finger millet and is known as System of Ragi Intensification (SRI) or System of Finger Millet intensification (SFMI). SCI methods have demonstrated numerous benefits in finger millet cultivation and production. SRI practices lead to higher head recovery and increased grain and straw yield. It reduces the crop duration by about 10 days and reduces the number of chaffy grains. Under SRI practices, only 1.25 kg/ha was utilized as compared to 3–5 kg/ha employed in conventional methods. It also improved cold tolerance in plants.

India In India, conventional cropping by broadcasting finger millet seed on a tilled field, yields 1.25–2 mt/ha and up to 3.75 mt/ha with good irrigation and fertilizers. However, with SRI-like practices developed by the farmers at Haveri, Karnataka, known as the *guli vidhana* (pit system) methodology, yields increased to 3.75–6.25 mt/ha. Farmers transplant 12-day-old seedlings in shallow furrows at wide spacing. Between 15–45 days after transplanting, a light board is pulled over the plants in the field in different directions. The bending causes moderate stress leading to growth of new roots and tillers from the crown meristematic tissue. It also loosens the soil, increasing aeration and cuts the roots of young weeds. A similar practices called the System of Finger Millet Intensification (SFMI) developed by farmers with the help of the NGO PRADHAN (2012) in the eastern state of Jharkhand, yields increased in their rainfed crop from 1 mt/ha to ≥3 mt/ha. The People's Science Institute (PSI) introduced these practices in the Himalayan state of Uttarakhand leading to a 60% increase in grain yield. SCI practices increased the yield from an average of 1.5 to 2.4 mt/ha.

Africa Farmers started practicing these methods in Tigray, Ethiopia in 2003, call it *planting with space* (Araya et al. 2013). The yield in finger millet increased to 3.5–7.8 mt/ha from 1.4 mt/ha in fields established by broadcasting and 2.8 mt/ha with liberal use of compost. Farmers are also implementing these methods in other crops.

3.6.2 SRI/SCI Constraints

SRI is labor intensive and requires certain skill levels. Planting and weeding are initially the most labor intensive parts of SRI. Hence, farmers are hesitant to adopt it due to higher cost and risk, especially for large-scale production. It is difficult to monitor water levels and avoid flooding in the rainy season due to unpredictable weather. However, the labor and cost is reduced over time. While SCI initially increased cost of production by about 25%, the higher yields reduce their costs of production by 60%. Proper training and knowledge of the benefits and availability of low-cost tools will encourage the farmers to adopt these practices.

3.7 Current Agricultural Challenges

Finger millet production is affected by both biotic and abiotic stresses. Also, various fungal and bacterial diseases and pests cause considerable damage to the crop.

3.7.1 Biotic Constraints

Insect pests and diseases are the greatest challenge to agriculture and food security. There are at least 120 insect pest species recorded on finger millet in Asia and Africa. Table 3.2 lists the major biotic constraints affecting finger millet production worldwide.

3.7.2 Abiotic Constraints

In semiarid and arid regions, low and erratic rainfall and periodic drought are major abiotic stresses affecting crop productivity. Drought intensity and frequency has increased in the recent past accompanied by serious reductions in rainfed agricultural outputs. Global warming and changing climate will only exacerbate the conditions especially in these regions by reducing the grassland productivity by 49–90% by 2020 (UN 2011). A 10% decline in the level of rainfall in Ethiopia resulted in an average drop of 4.2% in cereal yields (Webb and Braun 1994). A study on finger millet landraces in which a drought treatment was imposed 4 weeks after sowing resulted in 100% yield loss and over 30% loss in biomass (Maqsood and Ali 2007). As much as half of the world's irrigated lands and more than 20% of its cultivated area are affected by salinity (Rhoades and Loveday 1990). The osmotic potential of soil solution decreases at high salt concentrations creating water stress in plants and finally causing ion toxicity. Finger millet is generally grown in marginal areas with low soil fertility and nutrient stresses, especially in Sri Lanka and African countries. In future, the N and P demands may also affect the production of finger millet in low-input agricultural systems of Asia and Africa since the crop is largely grown by resource-poor farmers who cannot afford to buy expensive fertilizers (Thilakarathna and Raizada 2015). In addition to these abiotic constraints, harvesting and postharvest handling is still a labor intensive practice in several finger millet growing countries.

Table 3.2 Major finger millet pests and diseases

Pest/Pathogen	Symptoms	References
Pink stem borer (*Sesamia inferns*)	Larvae eat central leaves causing "pin holes", bore into stems and shoots causing empty panicles (white ears)	Baladhiya et al (2018), Sasma (2018), www.agritech.tnau.ac.in, www.aicrpsm.res.in, http://cropgenebank.sgrp.cgiar.org
White borer (*Saluria inficita*)	Larvae bore into the stem at the base close to the soil level and cause "dead heart"	www.agritech.tnau.ac.in, www.aicrpsm.res.in
Ear head caterpillar (*Euproctis subnotata*)	Adults infest the inflorescence at the milky stage turning spikes chaffy and covered with silky webs	Kalaisekar et al. (2017), www.agritech.tnau.ac.in
Brown aphid (*Hysteroneura setariae*)	Aphid feeding causes yellowing of leaves, severe infestation covers whole plant and causes stunted growth	Kalaisekar et al. (2017), www.agritech.tnau.ac.in, www.aicrpsm.res.in, http://cropgenebank.sgrp.cgiar.org
Root aphid (*Tetraneura nigriabdominalis*)	Aphids feed externally on the sap, plants turn pale, stunted, wilted and finally dry up	Gadiyappanavar and ChannaBasavanna (1973), www.agritech.tnau.ac.in, www.aicrpsm.res.in, www.plantwise.org
White grub (*Phyllophaga* sp.)	Feeds externally on the roots, inflorescence and leaves, causing wilting and finally death in seedlings and young plants; reduced vigor and loss in grain yield in large plants	www.plantwise.org, www.aicrpsm.res.in, http://cropgenebank.sgrp.cgiar.org
Root grub (*Holotrichia consanguinea*)		
Defoliators	Defoliating insect pests damage plants by eating away the leaves. They feed on the green matter leaving only the veins. A substantial loss of photosynthetic tissue leads to critical damage to the plant growth, increased susceptibility to attack by other insects and pathogens and even plant death	Kalaisekar et al. (2017), www.aicrpsm.res.in, www.agritech.tnau.ac.in
Cut worm (*Spodoptera exigua* Hübner) Armyworms (*Spodoptera* sp.)		
Black hairy caterpillar (*Estigmene lactinea*)		
Leaf folder (*Cnaphalocrocis medinalis*)	Larvae fold a leaf blade together with its silk strands, feeds inside creating longitudinal white patches and transparent streaks	Murthy et al. (2015), Kalaisekar et al. (2017), www.plantwise.org, www.aicrpsm.res.in, www.cabi.org
Grasshopper (*Chrotogonus trachypterus*)	Nymphs and adults feed by marginal notching of the leaves. In case of severe infestation, they defoliate entire leaves and graze over the entire field	www.agritech.tnau.ac.in
Leaf hoppers/Ragi Jassid (*Cicadulina* sp.)	Suck the plant sap causing withering and drying	Kalaisekar et al. (2017), www.aicrpsm.res.in, www.agritech.tnau.ac.in

(continued)

Table 3.2 (continued)

Pest/Pathogen	Symptoms	References
Blast *(Magnaporthe oryza)*	Causes blast in young leaves; also affects neck, spike; small brown circular to elongated spots on leaves elongate to spindle-shaped areas, coalesce and cause drying of foliage; conidia produced at the center give spots a smoky appearance; neck region turns black and shrinks obstructing grain formation, partially or completely reducing spikelet length, grain number, grain weight; infected seeds show reduced germination	Rath and Mishra (1975), Ekwamu (1991), Nagaraja et al. (2007), Singh and Kumar (2010), Kumar and Kumar (2011), Babu et al. (2013b), Klaubauf et al. (2014) and Gashaw et al. 2014
Blight (*Helminthosporium nodulosum*)	Causes seed blight; can infect all plant parts, throughout the life cycle; several minute, oval, light-brown lesions on the young leaves coalesce to form large dark brown patches killing the seedlings prematurely; in mature plants, it causes linear oblong and dark brown spots on the leaves, prominent brown to dark brown discoloration in neck region leading to heavy chaffiness, breakage of head; secondary infection can occur through air borne conidia	Kalaisekar et al. (2017), www.aicrpsm.res.in, www.agritech.tnau.ac.in
Sclerotium rolfsii Sacc.	Causes foot rot or wilt on the basal region of stem, causing large dark brown lesions and become soft; small, spherical, dark-colored sclerotia appear on the surface of the lesions; hindered transport of water and nutrients ultimately causes plant death. The plants become stunted	Nagaraja et al. (2007), www.aicrpsm.res.in
Pseudomonas syringae pv. *syringae*	Causes bacterial brown spot; small, water-soaked lemon yellow spots that enlarge, coalesce, turn brown and become necrotic; often light-cream or silver exudates are produced on the lesions under moist conditions	Nagaraja et al. (2007), www.aicrpsm.res.in

(continued)

Table 3.2 (continued)

Pest/Pathogen	Symptoms	References
Xanthomonas sp.	Causes leaf streak disease; narrow, water-soaked, transparent lesions on leaves that turn red, opaque and coalesce to form long irregular streaks and blotches extending across the leaf blade, characterized by dead tissue bordered by narrow, dark margins; light-yellow exudate dries to form thin white or cream scales; infected plant debris also transmits the bacteria	Nagaraja et al. (2007), http://cropgenebank.sgrp.cgiar.org, www.aicrpsm.res.in

3.8 Traditional Breeding Methodologies and Limitations

Plant breeding is cultivar development, crop improvement and seed improvement of various agriculturally- and horticulturally-important crops, conventionally by selective mating or hybridization. Early finger millet breeding was largely confined to India, particularly in the southern states of Tamil Nadu, Karnataka and Andhra Pradesh. Later, it spread to other Indian states such as Maharashtra, Gujarat, Orissa, Bihar and Uttarakhand. The East African countries involved in finger millet breeding include Uganda, Zaire, Malawi and Zimbabwe. Finger millet breeding is also reported from Sri Lanka, Malaysia and the Philippines. In the European colonial period, indigenous crops were largely ignored. Yield levels were very low due to lack of inputs, poor soil fertility, rainfed farming, low-yielding cultivars and lack of improved agronomic practices. Initial breeding efforts in finger millet were limited due to its self-pollinating nature. Development of emasculation and pollination techniques created the opportunity to improve the crop and create new hybrids. Later, various breeding approaches such as pure-line selection, recombination breeding and mutation breeding were extensively used for the genetic improvement of finger millet. The breeding strategies for selection and genetic improvement have greatly improved since the availability of genomic data and genome editing tools.

3.8.1 Pure-Line Selection

The earliest reports of finger millet improvement are from India, where crop improvement was initiated by Leslie C. Coleman, the second director of agriculture of Mysore State in Karnataka. He initiated the work on pure-line selections from indigenous cultivars such as Hullubele, Gidda and others in 1913 at the Zonal Agricultural Research Station, V.C. Farm, Mandya and Hebbal farms, Bangalore. He contributed the first finger millet cv. H-22 in 1918 and his concerted efforts resulted in the release of several other cvs. such as K-1, R0870, ES-11, ES-13 CO-1

and H-1. For pure-line selection, single superior plants are selected from a population and the superior progeny selected on the basis of characteristics such as superior spikes, high yield, earliness, and pest and disease resistance. Any progeny superior to an existing cultivar is then released as a new pure-line cultivar. The line can be tested in multiple locations and released. Pure-line selection resulted in the development and release of several other cultivars of finger millet in India such as CO-2, CO-3, CO-7, CO-8, PLR-1, K-22, ES-11, RO-786, AKP6, VZM2 and Aruna. Three improved pure lines D-11 (early), D-31 (mid-late) and A-16 (late) were released at Hathkamba, Konkan in 1921.

In Africa, selection and preservation of superior heads from indigenous cultivars formed the basis of seed for the subsequent season and a major step toward crop improvement (Jameson 1970; Khizzah 1985). Research work on finger millet improvement began with the initiation of a millet breeding program at Serere Research Station, Uganda, and to a limited extent in Tanzania, Malawi and Zambia (Peters et al. 1967–1971). Several high-yielding cultivars were identified including Phagalala and Fumbat (Nyasaland-AQ 1951) from Malawi; Vuri, Lango and Omidu from Zaire (INEAC 1956, 1958); Kiyaka and the Kisozi from Rwanda, Burundi; Kiega and 1-M strains of Mphunsi and 0–9 and B.K. strains of Agahagarika (Bruyere 1958) from Zaire and Gulu E and Serere-1 from Uganda (Peters et al. 1970). Later, Engeny, P283, WC65 and P224 (PESE1) cvs. were introduced (Zake and Khizzah 1986). In Ethiopia, KNE 409, KNE 1098, Acc 100057 and KNE 479 (Mulatu et al. 1985) and in Kenya, Gulu E, P224, KA2 and KATFM1 were identified as high-yielding cultivars (KARI 1990).

Although the early efforts largely focused on the development of pure breeding strains and cultivars, Ayyangar (1932) proposed the development and improvement of composite cultivars. He proposed that this would help in developing cultivars with hybrid vigor and a much greater range of adaptation to erratic weather due to the natural crosses among the different genotypes. This paved the way for hybridization techniques for finger millet improvement.

3.8.2 Hybridization Breeding

Finger millet genetic improvement got a boost after the establishment of hybridization techniques and several new cultivars were released. The aim of hybridization is to combine desirable genes found in two or more different plants or cultivars and to produce pure-breeding progeny superior to the parents.

3.8.2.1 Hybridization Techniques

Contact Method Successful hybridization in finger millet was achieved through the contact method (Ayyangar 1934) in the 1950s. Ayyangar suggested the exploitation of dominant characters in the male parent. This method involves

removal of all except a single ear or finger in the desired plants and the panicles of the selected parent plants are tied together before flowering, to enhance the chances of natural cross-pollination. The two panicles are enclosed in a bag to prevent unwanted pollen. This results in low frequency of true hybrids, which can be identified with the help of dominant characteristics of the parents as a marker in the F1 generation such as pigmentation in the nodes of male parent (Gupta 2006). The results obtained with contact method were generally inconsistent.

Hot Water Treatment Emasculation is essential for successful hybridization in self-pollinated plants. Manual removal of the immature anthers using forceps or needles is very difficult in finger millet due to the small size of the florets. As an alternative, Rao and Rao (1962) suggested the hot water treatment for emasculation where the florets which are likely to flower in the next few (2–3) days are immersed in hot water. They emasculated the ears by immersion in water at 47 °C for 10 min, or at 48 °C for 7 min. This treatment did not damage other floral parts. Furthermore, Raj et al. (1964) found a hot treatment at 52 °C for 5 min quite successful. However, the procedure requires standardization depending on temperature and climatic conditions. These techniques have limitations in small millets probably as the delicate pistils are largely protected by glumes (Riley et al. 1989). Also, a combination of hot water and contact pollination was used which proved effective in obtaining reasonable quantities of crossed seeds.

Cold Water and Plastic Bag Method Later, a technique was developed at the University of Agriculture Sciences, Mandya, Bangalore, under the All India Coordinated Small Millets Improvement Project, to use cold temperature and humidity to induce flower opening. The fingers are sprayed with cold water and covered tightly with a polythene bag. The high humidity created in the plastic bag prevents anther dehiscence and anthers emerge without shedding the pollen (House 1985). The glumes slowly open and the premature anthers are exposed and thereafter removed carefully without injuring the stigma. The emasculated fingers are again sprinkled with cold water to prevent drying of the pistil.

3.8.2.2 Hybrid Cultivars

Using hybridization, four high-yielding cultivars were developed in India: Poorna (Co-1 x Aruna), Udaya (K-1 x Aruna), Annapurna (K-1 x Aruna) and Cauvery (Hulluble x H22). These cultivars showed up to 50% increase in yield potential and met to the needs of different finger millet growing seasons for a long time. Two more cultivars were developed through crossing, namely Shakti (Ro 013 x H22) and 5–6 (Co-1 x H22).

The development of high-yielding, white-grained finger millet also started in India at Coimbatore, Tamil Nadu (Wariar and Divakaran 1956). The first improved cv. E.C. 4310 was created by a cross between E.C. 1540 (low-yielding, white-grained strain, high vitamin-13 content) with male parent E.C. 985 (high-yielding,

brown-grained). A coordinated finger millet improvement program was initiated in India in 1963 to evaluate, screen and catalogue the 947 stocks of world collections.

The contributions of Indian breeder C.H. Lakshmanaiah to finger millet crop improvement are unparalleled. At the VC Farm, Mandya, Karnataka in 1964 he created new recombinant cultivars by crossing Indian cultivars with African ecotypes. A few African donor parents such as IE-927, IE-929, IE-980, IR-810 and IE-902 were identified by screening the available world collection of germplasm over 8 years. He crossed these lines with the local cvs. such as Hallubele, K1, Annapurna, Purna, Cauvery, Shakti, Co-1 and Hamsa. The hybridization resulted in 16 Indo-African cultivars with substantially more yield potential and these were designated as "Indaf" cultivars. These can be grown under both irrigated and rainfed conditions (Bhat et al. 2018; Lakshmanaiah 1967; Madhusudan et al. 2015; Zake and Khizzah 1986).

3.8.2.3 Male Sterility Breeding

Male sterility can effectively be achieved through male gametocides, chemical hybridizing agents such as maleic hydrazide, gibberellic acid (GA), ethyl methane sulfonate (EMS), ethrel, and physical agents such as fast neutrons. Hot water treatment of inflorescences at 52 °C for 5 min was found effective in inducing male sterility in finger millet (Raj et al. 1964). Male-sterile mutants in cv. Gulu E were created using fast neutrons at the Serere region under the East African Agriculture and Forestry Research Organization, Uganda (Mukuru et al. 1974).

A GMS line, INFM 95001, was developed for the male sterility locus ms1 using EMS (1.5% aqueous solution, for 6 h at 25 °C) jointly by ICRISAT and the Department of Agronomy, University of Nebraska, USA (Gupta et al. 1997). INFM 95001 is a white finger millet and is a medium maturity (94 days) cultivar. It was recommended in composite breeding and heterosis studies. A male-sterile plant is easily distinguished from a male-fertile plant at anthesis. As compared to using chemical hybridizing agents and cytoplasmic male sterility, nuclear-encoded recessive male steriles (ms) offer major advantages for hybrid breeding.

3.8.3 Mutation Breeding

Mutation breeding has been around since the 1930s. It is a powerful means of creating useful genetic variability. Mutation breeding simply accelerates the process of mutation in plant genetic material which otherwise is underway in nature. Mutation breeding is based on selfing of mutants instead of crossing as in conventional breeding, until the induced character has a stable expression in the subsequent mutant generations. It is cost effective, quick, robust, transferrable and ubiquitously applicable. There are more than 3200 mutant cultivars of more than 210 plant species from over 70 countries, including 2 cultivars of finger millet (FMM165, FMM175)

from Zambia, registered in the FAO/IAEA Mutant Varieties Database (https://mvd.iaea.org/) and released for commercial use. The most common method of mutation breeding is to treat seeds with physical, chemical or a combination of both mutagens and selecting from the subsequent population the desirable mutants which are superior to their parents.

Mutation breeding in finger millet started in southern India when Krishnaswami and Ayyangar (1941) irradiated seeds with X-rays. Cultivar Hagari-1, released in 1941, was the first successful mutant cultivar of finger millet developed using X-ray irradiation. Later three more cvs. CO-3 (1942, dwarf), Dibyasinha (1976, early maturing) and K-6 (1982, dwarf and early maturing) were released for commercial use. Goud et al. (1969, 1971) studied the effects of gamma irradiation and EMS on cvs. Purna and Hamsa and several viable mutations of potential breeding value were produced in the cv. Purna. Sinha and Sahoo (1971) developed three early-maturing mutant lines by treated seeds of finger millet cv. AKP-7 with chemical mutagens ethyl methane sulfonate (EMS) and nitroso guanidine (NG). Among these, the early maturing (90 days) cv. Dibyasinha was released for commercial use. Another promising mutant M21 of cv. HES 927 was created using gamma irradiation of the seeds (Shivashankar et al. 1973). This mutant was high yielding and blast resistant. Nayar et al. (1974, 1979) treated different cultivars of finger millet with gamma-rays, fast neutrons and EMS. Mutants (fast neutrons) for ear shape and type namely, semi-compact, open and lax were isolated for cv. Hamsa which otherwise has very compact and fist-like ears, thus harbors many insect pest and diseases. Tikka (1985) isolated a number of dwarf and early-maturing mutants of six cultivars of finger millet (PR 202, Co10, Indaf-9, HR 24, IE 744, GN_1) by treating the seeds with physical (gamma-rays) and chemical mutagens such as EMS, methyl methane sulfonate (MMS) and diethyl sulfate (DES). High-yielding mutants of white finger millet cv. Co 9 were obtained with low doses of EMS and NG (Devkota 1987), short-duration mutants were obtained for cv. Sarada and bold grain mutants for MS2698 with NG (Raveendran et al. 1982). Devkota (1987) also suggested that there was scope for improvement through a second cycle of mutagenic treatment. Several dwarfs, early types and high tillering type mutants were developed from PR 202, HR-911, Indaf-8 and TNAU-294 cvs. Using gamma-rays (Gowda and Seetharam 1983).

Sreekantaradhya (1979) estimated the LD_{50} dose of gamma-rays and EMS in finger millet. Mahishi and Seetharam (1983) concluded that lower doses of gamma-rays and chemical mutagens EMS, MMS, nitroso methyl urea and 1-methyl-3-nitro-l-nitrosoguanidine are more effective. Gamma-rays, X-rays, EMS and DES have been widely used since for developing mutant lines. Several studies support these findings and for further calibrating the dosages for different mutagens. More recently, Eswari et al. (2014) identified desirable mutants for plant height, tillers/plant, productive tillers, finger number, finger length and 1000-grain weight for cv. TRY 1, and suggested a dosage of 0.15% of EMS to be ideal. Mutant cvs. FMM165 and FMM175, currently registered in the FAO/IAEA Mutant Variety Database (https://mvd.iaea.org), were developed using X-ray irradiation at the Zambia

Agriculture Research Institute (ZARI). They were isolated for increased finger length and number of fingers.

In many instances the alternate alleles e.g. resistant and susceptible gene vary by only a few bases (Bryan et al. 2000). Gene editing technology (GenEd) can be used to mutate the susceptible alleles to resistance directly rather than by a series of crosses and backcrosses (Georges and Ray 2017). Mutants can also be developed in polyploid plants using the CRISPR/CAS9 GenEd where mutants are often difficult to isolate, particularly in recent polyploids where all homeologues of a gene may be expressed (Georges and Ray 2017).

3.8.4 Improved Cultivars

Several coordinated projects have been undertaken recently by major international and national research institutions in India, African countries, Nepal and Sri Lanka by ICAR, KALRO, NaSARRI, EIAR, ICRISAT and ICARDA, to develop new improved cultivars of finger millet. Currently 143 advanced/improved cultivars are registered at ICRISAT.

3.8.4.1 India

Between 1986 and 1999 in India, the main focus was on developing cultivars with high grain and straw yield, as well as drought and disease resistance. Several hybrids as well as pure-line high-yielding cultivars (1500–5000 kg/ha) such as MR-2; MR-6; Indaf-15; VL124; HR911 (UAS 1 x IE 927); L-5 (Malawi x Indaf 9); Gautami (PR 1158–9) (PR 202 x U22) and Gujarat nagli 2 (NS 109) (Pureline selection) were released from different research centers. Blast-resistant cvs. GPU 28, Indaf 5 (Indaf 9 x IE 1012) and KM 65 and two drought tolerant cvs. RAU 8 (BR 407 x Ranchi Local) GN 3 (KM 13 x GN 2) were released. Two cultivars, a pureline Suraj (VR 520) and a hybrid Saptagiri (or PR 2614) (MR 1 x Kalyani), were developed in Andhra Pradesh with both blast and drought tolerance. Another cultivar, PR 230 (or Maruthi) with both blast and blight resistance was developed through pure-line selection at ANGRAU, Paleru, A.P. A salinity tolerant cultivar, TRY 1, was developed at TNAU, Coimbatore.

In the period 2000–2018, with the establishment of the AICRP on small millets, emphasis was on developing hybrid cultivars involving productive lines with elite backgrounds. Both early-maturing and long-duration cultivars with high yield potential and suitable for irrigated or rainfed conditions were released. Most of the cultivars were resistant to blast (neck and finger) disease. Numerous blast resistant cultivars of *GPU* and *KMR* series i.e. GPU-26, GPU-28, GPU-45, GPU-48, GPU-66, KMR-204, KMR-301 and KMR-340, with average yields of 2000–4000 kg/ha were released by AICRP (1986–2018) (Appendix II). A semi-dwarf, non-lodging cv. GPU-67 was also released which is suitable for cultivation in all finger millet

growing regions. Breeders also focused on tolerance to brown spot disease, stem borers and aphids. A somaclonal cv. Dapoli-2 (SCN-6) was developed through tissue culture at Dr. BSKKV, Dapoli, Maharashtra and released in 2017. The parent cv. Dapoli-1 (1985) was mid-late (125–135 days), non-lodging and responsive to nitrogenous fertilizers and with reddish brown grain color. The somaclone Dapoli-2 is a high-yielding cultivar rich in iron and calcium, moderately resistant to blast and tolerant to aphids and tobacco cutworm (*Spodopter littura*).

3.8.4.2 Africa

In Africa, a systematic regional finger millet program was initiated in 1985 by SADCC/ICRISAT (Southern African Development Coordination Conference) and ICRISAT (Gupta et al. 1986a, b). During 1985–1986, the program evaluated a total of 394 accessions collected from Zimbabwe (374), Zambia (14), Malawi (4) and Tanzania (2), to be used in crossing for hybrid development. From this program a new non-lodging cv. Steadfast was created by crossing the local lodging susceptible cv. M144 with Line 197. The local finger millet cultivars gave an average yield of 1600 kg/ha. With the establishment of regional cereal programs and concerted efforts of different centers, several high yielding improved finger millet cultivars such as Engeny, Gulu E, Serere 1, Pese 1, Seremi 1, Seremi 2, Seremi 3, SX 8, SEC 915 and SEC 934 were released over the years (Alimu 1985). These cultivars are also blast resistance, have good grain quality and early maturing (mostly within 90 days).

In 1993 two lines, KNE#1098 and KNE# 409, were identified as better yielding out of the 57 lines introduced by the East African Regional Sorghum and Millets Network (EARSAM), now renamed the East and Central African Sorghum and Millets Network (ECARSAM), across the intermediate to high elevation areas in Ethiopia. These lines were later released for commercial production in 1998 and named Tadesse and Padet, respectively. Due to severe drought in 2002, almost all crops in the dry districts of Ethiopia failed except finger millet cv. Tadesse (KNE# 1098). Another line, KNE# 411 was identified and released with the name Boneya by the Bako Agricultural Research Center in the western Ethiopian region in 2002. In Zimbabwe, a new high-yielding cv., SDEV 87001, was developed (Gupta and Mushoga 1994), which yields up to 3500 kg/ha.

The Kenya Agricultural Research Institute (KARI) developed new superior finger millet cvs. U-15, Gulu and Okahale-1 in 2011 that guaranteed higher yields. The cultivars are also tolerance to drought, striga weed and blast disease. Two more cultivars, a red-seeded Katumani and a brown-seeded P224, have been released recently. Katumani is a dwarf cultivar and is drought tolerant. It matures in 3 months and gives an yield of 700–1000 kg/ha. Cultivar P224 is tall, however, tolerant to lodging and blast. P224 matures in 3–4 months and yields 1000–1500 kg/ha of grain. With the help of ICRISAT and the Harnessing Opportunity Productivity Enhancement (Hope) Foundation, these new cultivars are being introduced in different provinces all over Africa.

3.8.4.3 Finger Millet Improvement in Other Countries

Nepal In Nepal, five varieties have been released by the Hill Crops Research Programme (HCRP), Kabre; Climate Change, Agriculture and Food Security (CCAFS) and CGIAR Center, for cultivation by local farmers (Table 3.3). Three of these cultivars originated in India. All the cultivars are non-lodging, drought tolerant and resistant to *Cercospora* leaf spot and blast diseases, except cv. Dalle-1.

Ukraine A somaclonal cv. Yaroslav-8 is listed in *Register of Plant Varieties Suitable for Dissemination in Ukraine* (Radchuk et al. 2012). It was obtained from the genetically-stable somaclonal variant line SE7 created by Bayer et al. (2007). The cultivar has several agronomically-important features, such as the most reduced plant height, high grain yield and green biomass, rapid germination at low temperatures and reduction in duration.

3.8.5 Interspecific Hybridization

Wild relatives of cultivated crops serve as a gene pool that could potentially be used to improve them. But to date, only cultivated species of *Eleusine coracana* have been used; there are almost no reports of using their wild relatives for crop

Table 3.3 Improved varieties released in Nepal

Variety	Pedigree	Institute where developed	Year of release	Av. yield (kg/ha)	Special features
Dalle-1 (IE-980)	Unknown	Origin-India; maintained at- HCRP, Kabre	1980	3300	Drought tolerant; non-lodging; susceptible to blast and *Cercospora*
Okhle-1 (NE1304-43)	Selected from local cultivar of Okhaldhunga	HCRP, Kabre	1980	3300	Drought tolerant; non-lodging; resistant to finger and neck blast, *Cercospora* leaf spot
Kabre Kodo-1 (NF-6401-26)	Selected from local cultivar of Surkhet	HCRP, Kabre	1990	1800–4800	Drought tolerant; non-lodging; resistant to finger and neck blast, *Cercospora* leaf spot
Kabre Kodo-2 (GE-5176)	Unknown	SAARC-RVT, India	2015	2530	Drought tolerant; non-lodging; resistant to finger and neck blast, *Cercospora* leaf spot
Sailung Kodo-1 (GE-5016)	Unknown	SAARC- RVT, India	2015	2490	Non lodging; moderately resistant to finger and neck blast, *Cercospora* leaf spot

Source: Joshi et al. (2017)

improvement. This may be due to availability of vast genotype variability in cultivated species, being robust and having higher grain quality then the wild types. Recently, Akech et al. (2016) identified blast-resistant lines from interspecific crosses between the wild (*E. kigeziensis* and *E. africana*) and cultivated types. From the F4 generation of crosses *E. kigeziensis* x Pese 1, *E. africana* x Seremi 1 and *E. africana* x Seremi 3; two lines resistant to all three forms of blast diseases were identified. They found 13 lines resistant to neck and head blast and moderately resistant to leaf blast. They also identified six lines were resistant to neck and head blast but susceptible to leaf blast. In view of the continual climate change and the need for germplasm suited to extreme environmental conditions, the necessity to conserve finger millet wild relatives for future breeding research cannot be ignored.

3.9 Role of Biotechnology

Conventional plant breeding is tedious, time consuming and affected by environment conditions that cannot be controlled. Nonetheless, biotechnological or molecular techniques such as genetic engineering and genome editing provide powerful tools for genetic manipulation of crops and speed up the process of crop improvement. In order to utilize these techniques, the inception of efficient in vitro regeneration systems for the transformation and regeneration of cereals is a vital prerequisite (Shrawat and Lörz 2006; Yemets et al. 2013). The earliest attempts at callus initiation and regenerating finger millet through tissue culture was made by Rangan (1976) and Mohanty et al. (1985) using leaf-base segments. Eapen and George (1990) utilized different explants such as shoot tips, leaf sheath fragments and undeveloped inflorescences for somatic embryogenesis. Since then, different plant tissues have been used as explants such as leaf sheaths, root mesocotyls, embryogenic seed, and mature and immature embryos (Ceasar et al. 2018). Shoot apexes have proved to be an efficient explant owing to its easy availability, accessibility, ease in handling and rapid regeneration of multiple shoots (Arockiasamy and Ignacimuthu 2007; Ceasar and Ignacimuthu 2008; Dey et al. 2012). Recently, Ngetich et al. (2018) reported an efficient protocol for somatic embryogenesis and plant regeneration in six African finger millet cultivars using shoot tips from 3-day-old in vitro grown plants and achieved a 97% survival rate.

Research on finger millet transformation was initiated by Gupta et al. (2001) using the biolistic method to compare the efficiency of five gene promoters on the expression of the β-glucuronidase (GUS) reporter gene. Latha et al. (2005) utilized the biolistics method to develop transgenic plants with resistance to leaf blast disease, which was further optimized by Jagga-Chugh et al. (2012). Several reports followed on attempting and optimizing protocols for *Agrobacterium tumefaciens*-mediated genetic transformation of finger millet. Ceasar and Ignacimuthu (2011) and Sharma et al. (2011) optimized *Agrobacterium*-mediated transformation using shoot apex and embryogenic seed. The desired gene is cloned in a binary vector and introduced into *Agrobacterium*. This recombinant *Agrobacterium* is co-cultivated

with the explants. The bacteria infect the plant cells and the gene of interest along with a suitable selectable marker is integrated into the plant genome. A mature plant regenerated from these transformed cells will contain the cloned gene in every cell and will pass the cloned gene to its progeny.

Direct plant organogenesis is also an effective method to produce multiple shoots with reduced somaclonal variations. It minimizes the culture duration and mutations by omitting callus formation and reducing sub-culturing cycles (Satish et al. 2015). Further optimization of the transformation technique using direct-plant regeneration was reported by Satish et al. (2017) (Fig. 3.7).

Recently, a regeneration system via direct organogenesis was reported using in vitro-derived shoot apical meristems (Mukami et al. 2018). The highest shoot induction was observed in MS medium supplemented with 1.75 mg/l benzylaminopurine (BAP). Yemets et al. (2013) reported an advanced and rapid method of plant regeneration through callus cultures of *Eleusine coracana* seeds. White nodulated calli were formed on medium with N6 macro-salts, MS micro-salts 2, 4-D (2 mg L^{-1}), kinetin (0.4 mg L^{-1}), NAA (2 mg L^{-1}) and certain additives (Fig. 3.8). They found NAA beneficial in view of callus regeneration capacity,

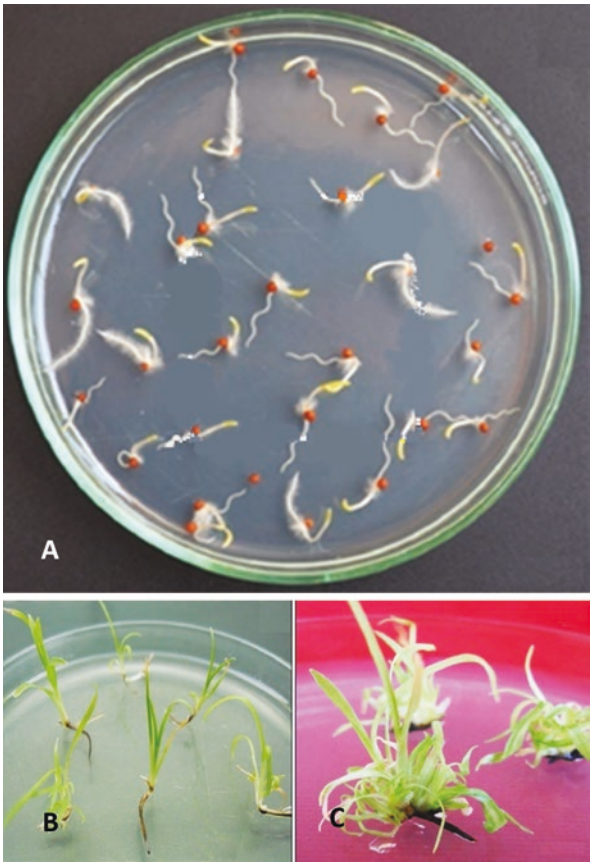

Fig. 3.7 Typical in vitro shoot apical meristems (SAM) explant preparation and direct shoot regeneration from a finger millet cv. CO (Ra). (**a**) 14 seeds were germinated on MS germination medium, (**b**) Germinated seed derived SAMs produced shoots, (**c**) multiple shoots were induced within 12 days of incubation in light within 4 days Source: Satish et al. (2017)

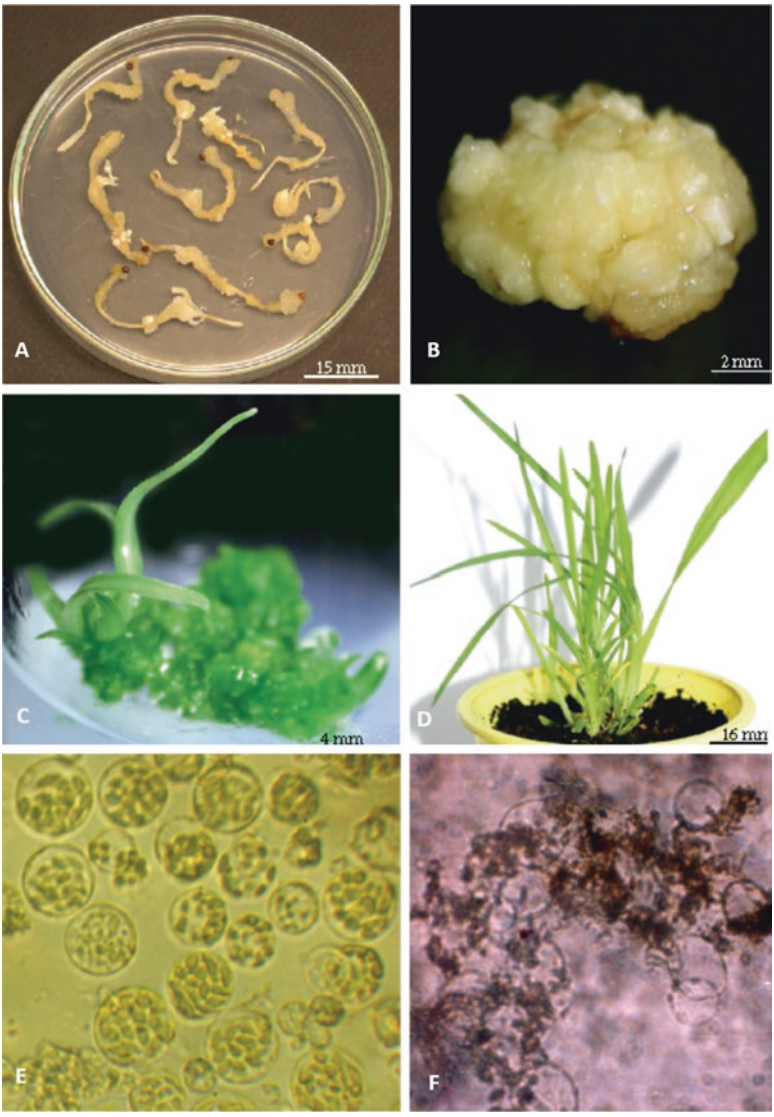

Fig. 3.8 (**a**) Callus induction from mesocotyl of *Eleusine coracana* from seed callus culture, (**b**) Morphology of compact, well-structured callus, (**c**) Plant regeneration of *E. coracana* from seed callus culture, (**d**) Rooted *E. coracana* plants in vitro, (**e**) *E. coracana* protoplasts isolated from seedlings, (**f**) Microcolonies formation in liquid KM8p medium
Source: Yemets et al. (2013)

although it is not commonly used in cereal callus induction. Yemets et al. (2013) also reported an efficient procedure for finger millet protoplast culture and induction of micronuclei with subsequent microprotoplast isolation of finger millet. Seedling coleoptiles were better sources of tissue for protoplasts isolation compared to callus cultures. An osmotic medium of mannitol and $CaCl_2$ (pH 5.5) and enzyme

solution of Cellulase (Onozuka R-10) and Pectolyase resulted in the best protoplast isolation. Microcolonies were visible 20–25 days after incubation on KM8p medium supplemented with amino acids, glutamine and proline.

The microprotoplast-mediated chromosome transfer (MMCT) technique is an asymmetric somatic hybridization method used for transferring alien chromosomes and genes (Ramulu et al. 1999). Polygenic traits or traits with unknown biochemical or molecular mechanisms (e.g. resistance to certain diseases or stresses and other economically important traits) are still recalcitrant to transfer using genetic engineering. This technique can be used for improvement of monocot plants by introducing agronomically-important genes, especially those that are recalcitrant to transfer i.e. polygenic traits or pathogenicity related traits, resulting in the production of chromosome addition lines (Saito and Nakano 2002; Yemets and Blume 2008). Yemets et al. (2013) established an effective method for induction (by antimitotic agents) and isolation of microprotoplast from finger millet somatic cells. The antimitotic drugs isopropyl N-(3-chlorophenyl) carbamate (CIPC) and cytochalasin B were used for microfilaments disruption and micronuclei induction in cells of finger millet seedlings.

3.10 Molecular Breeding

Crop improvement through conventional breeding is slow, especially for traits controlled by quantitative gene action like drought tolerance. Hence, the use of modern crop improvement tools such as genomics to transfer information about genes from model species to the species of interest, and genetic mapping in order to identify genes controlling traits of interest, can provide a more timely and robust response to crop production threats (Sorrells et al. 2003). It also provides added opportunities to develop crop cultivars with multiple stress tolerance.

Allele identification for agro-morphological traits and stress resistance is a major concern for improving productivity of finger millet. DNA-based microsatellite markers have been used to identify the agronomically-important traits such as days to flowering, plant height, peduncle length, ear exsertion, ear length, finger number, grain yield, disease resistance, drought resistance and nutritional quality. As finger millet is a hardy crop, it exhibits great morphological variation and is resistant to storage pests, it can prove important to identify QTLs related to climate resilience, storage-related and other important traits. Association-based QTL mapping gives a high resolution and several other advantages over the linkage mapping (Mott et al. 2000). Anchored, genomic and genic simple sequence repeat (SSR) markers have been used to map important QTLs e.g. 9 QTLs associated with Ca content were identified using 23 anchored SSR markers in 113 genotypes of finger millet (Kumar et al. 2015), 2 QTLs (OM5, FM8) for tryptophan content and 1 QTL (FMO2EST1) for protein content linked to opaque2 modifiers (*Opm*) gene in 190 genotypes using 120 genomic SSR markers (Babu et al. 2014b). Several agro-morphological trait-related QTLs have also been identified such as basal tiller number, days to 50%

flowering, flag leaf blade width, plant height, productive tillers, seed yield, leaf and neck blast resistance and number of tillers (Babu et al. 2014a, c; Ramakrishnan et al. 2016c). Ramakrishnan et al. (2017) identified 4 QTLs associated with root dry weight, shoot dry weight, and root length under P deficient and P sufficient conditions providing information to breed low P-tolerant genotypes in finger millet (Fig. 3.9). Recently, Babu et al. (2018) analyzed 66 accessions of the mini-core collection using 46 genomic and 58 genic SSRs markers. Significant associations were found for 20 agro-morphological traits (days to flowering, plant height, peduncle length, ear exsertion, ear length, length of longest finger, finger number, grain yield, finger blast). Two SSR markers designed from the blast resistance *Pi21* gene sequence of rice (FMBLEST35, FMBLEST36) were also found to be associated with blast disease resistance in finger millet.

3.11 Genomic Resources and Whole Genome Sequencing

The genomic resources available for *Eleusine coracana* are inadequate when compared with the major cereals crops. Less than 2000 expressed sequence tags (ESTs) are available, which is significantly lower than that of maize, rice and barley. A limited number of protein and nucleotide sequences are available (Table 3.4). Only 3 protein structures and 135 PopSets (sequence sets from phylogenetic and population studies) can be found. No complete gene, Unigene sequence or SNPs have yet been reported. A limited number of transcriptome studies have been carried out mainly for grain Ca content and a few stress conditions; however, most of these studies lack validation of sequence information and characterization of key genes. The recently released whole genome sequences (WGS) of finger millet will serve as a major basis to build these resources.

Recently, the whole genome sequence of about 1.2 Gb of two finger millet genotypes ML-365 (Hittalmani et al. 2017) and PR-202 (Hatakeyama et al. 2018) have been reported. Genotype ML-365, from the University of Agricultural Sciences, Bengaluru is a blast resistant and drought tolerant genotype with good cooking quality. Several drought responsive genes (2866), disease-resistance genes (R-genes) (1766) and genes related to calcium transport and accumulation (330) were identified. Genotype PR-202 (IC: 479099, NBPGR India accession number) was obtained from the University of Agricultural Sciences, Gandhi Krishi Vignan Kendra (GKVK), Bangalore and is resilience to drought and high-temperature stresses. The gene EcNAC1, the *Eleusine coracana* gene related to the drought response was also analyzed. The WGS availability can effectively be used for several strategies such as SNP identification, allele mining, identification and functional characterization of candidate genes related to agronomically-important traits and marker-assisted breeding programs.

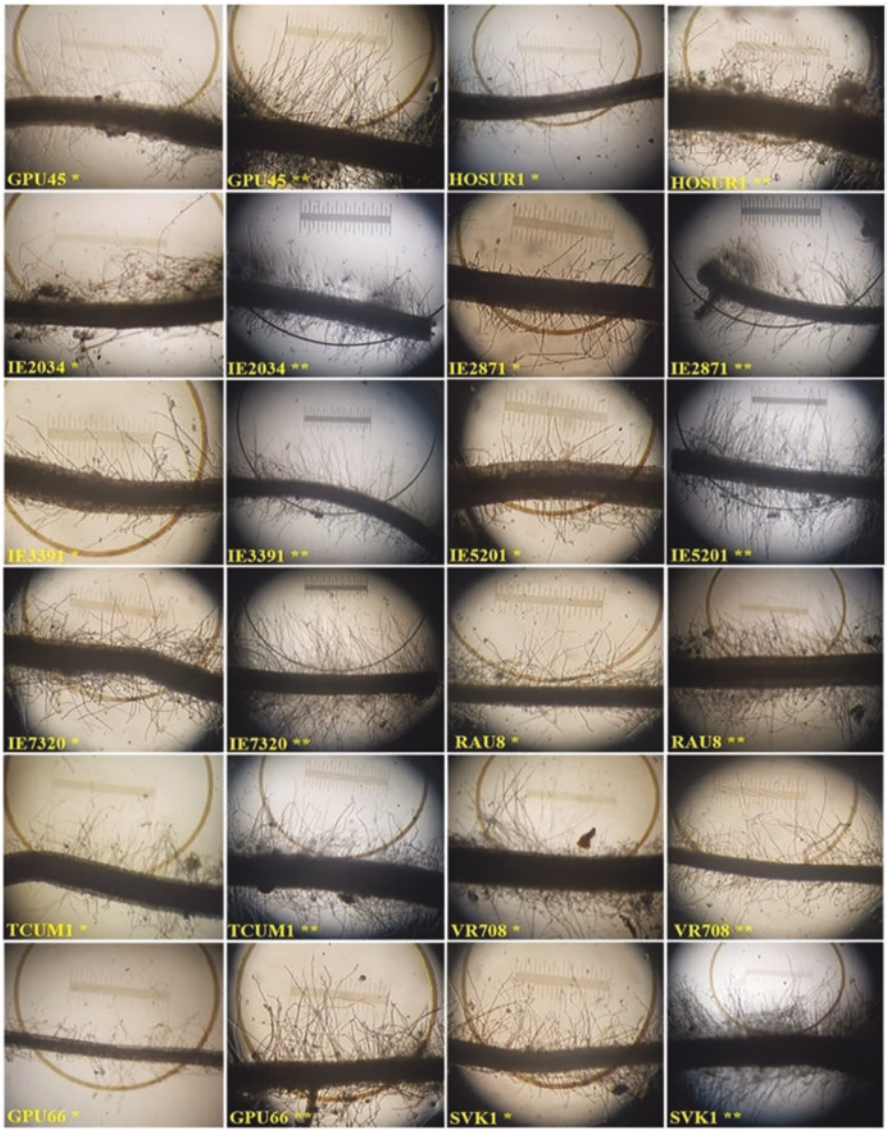

Fig. 3.9 Low phosphorous responding finger millet genotypes show low number of root hairs under P*suf* (∗ = P sufficient) and high number of root hairs P*def* (∗∗ = P deficient) conditions, respectively
Source: Ramakrishnan et al. (2017)

Table 3.4 Details on genomic and proteomic resources available for *Eleusine coracana*

Sequences/Resource	*Eleusine coracana* (No. of entries)
Expressed sequence tags (EST)	1934
Nucleotide	1111
Protein	559
PopSet	135
Protein structure	03
Identical protein groups	364
GEO datasets	06
BioProject	27
BioSample	243
Sequence Read Archive (SRA)	249
Probes	265
BioChem BioAssay	18

Source: NCBI (www.ncbi.nlm.nih.gov). Accessed September 2018

3.12 Genetic Improvement of Finger Millet Traits

3.12.1 Genetic Improvement for Herbicides Resistance

Bayer et al. (2014) developed *Eleusine coracana* lines resistant to dinitroaniline herbicides such as trifluralin, using mutant α-tubulin gene (*TUAm1*) isolated from dinitroaniline-resistant biotype of goosegrass *E. indica* (Blume et al. 2003; Yemets and Blume 2007). The mutant *E. indica* α-tubulin 1 (result of a single unique point mutation in both the alleles) confers an intermediate to high level of tolerance to a number of antimicrotubule herbicides, for example, dinitroanilines and phosphoroamidates. These herbicides have high affinity to plant tubulins. The team developed biobalistic (using tungsten particles with 0.7 μm diameter) and *Agrobacterium*-mediated systems for effective transformation of embryogenic calli of cvs. Tropikanka and Yaroslav 8. They demonstrated that the herbicide trifluralin resistance gene itself could be used as a selective marker gene in the selection of transgenic lines.

3.12.2 Genetic Improvement for Blast Resistance

The first successful transgenic finger millet plants were reported by Latha et al. (2005). They developed blast-resistant finger millet plants against the fungal pathogen *Pyricularia grisea* using a chemically synthesized gene based on prawn antimicrobial peptide (*PIN* gene). The shoot-tip derived embryogenic callus was transformed by the particle-inflow gun-mediated method. The stable integration of

multiple copies exhibited a high level resistance to leaf blast fungus. In a more recent report, leaf blast disease resistance transgenic finger millet plants have been developed by introducing the rice chitinase (*chi11*) gene (Ignacimuthu and Ceasar 2012). The rice chitinase gene has been frequently used for the production of fungal-resistant transgenic plants and not just in rice but in other crops as well such as peanut, mustard, cucumber, strawberry and American ginseng. The gene was introduced through *Agrobacterium*-mediated transformation using a plasmid pHyg-Chi.11 under the control of maize ubiquitin promoter. In both reports, the first progeny of transgenic lines produced resistant and susceptible plants in the ratio 3:1 confirming the normal Mendelian pattern of transgene segregation in both cases. No reports are available for neck and finger blast resistant or other disease-resistant transgenic finger millet. Screening of potential pathogenicity related genes and gene pyramiding will help in developing transgenic plants for a wider spectrum of diseases.

3.12.3 Genetic Improvement for Abiotic Stress Tolerance

Due to erratic weather and increasing climatic stress globally, it is important to develop increased stress tolerance in crops by overexpressing the gene of interest. Although finger millet is a hardy crop, it can be susceptible to drought, salinity and associated-oxidative stress especially at seed germination and early stages of seedling development. Jayasudha et al. (2014) developed salinity-tolerant finger millet GPU28 by expressing a double gene construct of *PgNHX1* (from *Pennisetum glaucum*) and *AVP1* (from *Arabidopsis thaliana*). At high salt concentrations, Na^+ causes ion toxicity (Tester and Davenport 2003) hence sequestration of excess cytosolic Na^+ into vacuoles is important to maintain ion homeostasis. The uptake of Na^+ ions is mediated by vacuolar Na^+/H^+ antiporter (NHX1) driven by the electrochemical gradient of protons generated by different vacuolar transporters such as H^+-PPase (AVP1) (Yamaguchi and Blumwald 2005). Several studies in different plant species indicate the importance of vacuolar antiporters, NHX1 and AVP1 in plant salt tolerance. The finger millet co-expressing PgNHX1 and AVP1 exhibited higher level of salinity tolerance (300 mM) compared to the wild type plants.

Osmotic adjustment and efficient scavenging of free radical generated during various abiotic stresses are important components of stress tolerance mechanisms in plants. Mannitol is an osmolyte known to scavenge hydroxyl free radicals and thereby minimize stress damage in several species. Hema et al. (2014) developed transgenic finger millet plants expressing the mannitol biosynthetic pathway gene from bacteria, mannitol-1-phosphate dehydrogenase (mtlD). The transgenic plants showed better growth, osmotic adjustment and chlorophyll retention under drought and salinity stress compared to wild types. The transgenic plants in both cases were developed using *Agrobacterium*-mediated transformation.

3.13 Conclusions and Prospects

Because of the increasingly dwindling agricultural land due to burgeoning population and industrialization, the world is expected to face a severe food demand by the end of 2050 (Gupta et al. 2017). Hence, there is an urgent need to increase the production of cereals. Finger millet should be given high priority in research and breeding programs as it can help meet all the challenging scenarios of malnutrition, water scarcity, extreme climatic conditions due to global warming and increasing disease susceptibility due to erratic weather. This C4 plant outperforms the C3 plants in harsh conditions and hence is an ideal crop for climate-resilient agriculture. It is a low-input crop and is often grown in infertile soils. This demands the need for identification of genotypes which have high fertilizer use efficiency, particularly N and P. There are only a few early-maturing cultivars which can mature in 90–95 days. Germplasm screening has provided some early flowering (50–52 days) cultivars and there is need to utilize these resources to develop early-maturing, photoperiod-insensitive cultivars suitable for different cropping systems. Breeding of dwarf varieties to avoid lodging and increase grain yield is also important. Finger millet is rich in micronutrients, especially calcium, iron and zinc, protein (especially the white grained varieties) and a good source of essential amino acids and antioxidants. The most cost-effective approach of mitigating *hidden hunger* is to introduce varieties with high Ca, Fe, Zn and protein content. The stress (drought, salinity, lodging) tolerant lines identified from multi-locational screening should be used to introgress the traits through breeding programs. Also, most of the cultivars of Africa origin are resistant to blast and storage pests. These cultivars are being used to transfer resistance to the susceptible cultivars through breeding. There is need of extensive and systematic work to identify the genes of interest and understanding the underlying genetic control and molecular physiological mechanisms of resistance and mineral accumulation. The availability of new genomic resources (WGS) of finger millet and knowledge of important trait governing genes can be combined with recent genome editing technology to develop nutritionally rich, climate resilient cultivars not only in finger millet but also in other important cereal crops.

Replacement of local cultivars by modern cultivars, a policy shift towards rice and wheat and other causes such as environmental degradation, urbanization, deforestation and bush fires have led to genetic erosion of indigenous species. Conventionally, efforts have been concentrated on conserving seeds in crop genebanks. However, it has become clear that the best strategy now is to combine ex situ approaches with the in situ conservation in their native agro-ecosystems and conservation of crop wild relatives. On-farm conservation ensures climate adaptability and evolution and is crucial for food and nutritional security. People world over are recognizing the health benefits of these once neglected coarse grains and hence are willing to pay high prices. Development of local niche markets for the indigenous landraces and their products can aid in their availability and popularity and can also ensure a premium price to conservationist farmers.

Appendices

Appendix I: Research Institutes Relevant to Finger Millet

Institute	Specialization and research activities	Website
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)	Research Program Dryland Cereals, Genebank repository	https://www.icrisat.org
All India Coordinated Research Project (AICRP), Indian Council of Agriculture Research (ICAR), India	Research program on Small Millets, Genebank repository	http://www.aicrpsm.res.in
National Bureau of Plant Genetic Resource (NBPGR), India	Research program on crop plants including small millets, Genebank repository	www.nbpgr.ernet.in
National Semi-Arid Resources Research Institute (NaSARRI), Uganda	Research in crops production for semiarid production systems in the areas of seed research and production management	www.nasarri.go.ug
Kenya Agricultural & Livestock Research Organization (KALRO), Kenya	Generating and promoting crops knowledge, information and technologies suitable for the region	www.kalro.org
Ethiopian Institute of Agricultural Research (EIAR), Addis Ababa, Ethiopia	Research to generate, develop and adapt agricultural technologies suited to Ethiopian Agricultural Research System	www.eiar.gov.et
Consultative Group for International Agricultural Research (CGIAR)	Research program on dryland cereals; crop improvement, crop management, postharvest technologies and market access to dryland cereal crops	http://gldc.cgiar.org/finger-millet/
Eastern and Central African Regional Sorghum and Millet Research Network (ECARSAM)	Enhance productivity, value adding and competitiveness of the ECA Subregional agricultural production system through increased production and productivity of sorghum and millet in quality and quantity	https://uia.org/s/or/en/
United States Agency for International Development (USAID)	Accelerated Value Chain Development (AVCD) Program -Drought Tolerant Crops (DTC) Value Chain	https://www.usaid.gov
International Livestock Research Institute (ILRI)	Bioversity social seeds – promoting open source seed systems for beans, forage legumes, millet and sorghum for climate change adaptation in Kenya, Tanzania and Uganda	https://www.ilri.org; http://data.ilri.org/portal/

Appendix II: Finger Millet Genetic Resources

Cultivar	Important traits	Cultivation location
GPU 26	Early, blast tolerant, suitable for late sowings and summer	India
GPU 45	Early, blast resistant	India
Chilika (OEB 10)	Moderately resistant to blast, resistant to stem borer	India
VL 315	Moderately resistant to finger and neck blast	India
GPU 48	Early, high yield, blast resistant	India
PRM-1	Resistant to blast	India
Bharathi (VR 762)	Moderately resistant to blast	India
GPU-67	Nonlodging (semi dwarf)	India
Srichaitanya (VR-847)	Moderately resistant to blast	India
KMR-301	High grain and straw yield, tolerant to blast	India
KOPN-235	Resistant to blast	India
OEB-526	Moderately resistant to leaf, neck and finger blast	India
OEB-523	Moderately resistant to blast; nonlodging; nonshattering	India
KMR-204	Early duration variety	India
VR936	Responsive to nitrogenous fertilizers	India
PPR2700 (Vakula)	Resistant to leaf blast and tolerant to drought	India
Indira Ragi-1	Non-shattering, nonlodging, responsive to fertilizers	India
VL-352	Moderately resistant to blast	India
Chattisgarh Ragi-2	Blast resistance; suitable for rainfed conditions	India
VL-376	Responsive to fertilizer; moderately resistant to blast	India
GNN-6	Moderately resistant to leaf and finger blast	India
GN-5	White grained; moderately resistant to leaf and finger blast	India
VL Mandua-348	Suitable for organic cultivation; resistant to neck and finger blast; tolerant to lodging	India
KMR-340	White grained; resistant to blast and blight diseases, tolerant to stem borer and aphids	India
Dapoli-2 (SCN-6)	High yielding, rich in iron and calcium; moderately resistant to blast; tolerant to aphids and *Spodopter littura*	India
CO-15	Highly responsive to nitrogenous fertilizer; non lodging; resistant to leaf, neck and finger blast	India
GNN-7	High mineral content (Ca, P, Mg)	India
VL-379	Resistant to neck and finger blast; moderately resistant to banded sheath blight; responsive to fertilizers	India
Chattisgarh Ragi-2 (BR-36)	Moderately resistant to neck and finger blast; tolerant to stem borer and other major pests	India
DHFM-78-3	Resistant to finger and neck blast; suitable for contingency planting	India
Engeny	High yielding	Africa
U15	High yielding; drought tolerant; blast resistant; *Striga* weed tolerant	Africa
P224	High yielding; blast resistant; tolerant to lodging	Africa

(continued)

Cultivar	Important traits	Cultivation location
Katumani	High yielding; dwarf variety; drought tolerant	Africa
Gulu E	High yielding	Africa
Okahale-1	High yielding; drought tolerant; blast resistant; *Striga* weed tolerant	Africa
Seremi 1	High yielding	Africa
Seremi 2	High yielding	Africa
Seremi 3	High yielding	Africa
Pese 1	High yielding	Africa
SX 8	High yielding	Africa
KNE 648	High yielding	Africa
SEC 915	High yielding	Africa
SEC 934	High yielding	Africa
IE 4115	High yielding	Africa
Tadesse	High yielding, drought resistant	Africa
Padet	High yielding	Africa
SDEV 87001	High yielding	Africa
Dalle-1 (IE-980)	Drought tolerant, nonlodging, susceptible to blast and *Cercospora* in rainy regions like Kaski	Nepal
Okhle-1 (NE1304-43)	Drought tolerant, nonlodging, field resistant to finger blast, neck blast and *Cercospora* leaf spot	Nepal
Kabre Kodo-1 (NF-6401-26)	Drought tolerant, nonlodging, field resistant to finger blast, neck blast and *Cercospora* leaf spot, tolerant to heavy rainfall	Nepal
Kabre Kodo-2 (GE-5176)	Drought tolerant, nonlodging, field resistant to finger blast, neck blast and *Cercospora* leaf spot	Nepal
Sailung Kodo-1 (GE-5016)	Nonlodging, moderately resistant to finger blast, neck blast and *Cercospora* leaf spot	Nepal
Yaroslav-8 (SE7)	Early; short height; high yielding; tolerant to low temperature	Ukraine

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Chapter 4
Foxtail Millet (*Setaria italica* L.): Potential of Smaller Millet for Future Breeding

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Abstract Millets are considered nutri-cereals which play a crucial part in overcoming malnutrition and have a significant role in improving the status of human health. Foxtail millet (*Setaria italica* L.), also known as Italian millet or German millet, belongs to Poaceae family and is cultivated globally, including in India. It is also a staple food and feed in several regions of Asia and Africa. There is a great genetic diversity with a large number of germplasm collections maintained in countries like China, Japan, the USA and India. The crop is nutritionally superior as the grains contain high amounts of proteins, essential amino acids, minerals and vitamins and micronutrients like iron and zinc. Thus, foxtail millet can be useful for biofortification programs aimed at combating malnutrition. Foxtail millet is a relatively drought-tolerant crop and hence genomic interventions can be in place for genetic engineering for abiotic stress tolerance. Recent advancements in a draft genome sequence of this millet has spawned great enthusiasm in unraveling genetic and genomic intricacies, genome-wide molecular marker development, genomics-assisted breeding, identification and validation of stress-associated gene families. There have been great research efforts in the creation and facilitation of genomics databases. In this chapter, we present an overview of the importance, genetic diversity, potential and genomics interventions for foxtail millet improvement.

Keywords Abiotic stress · Biotechnology · Diversity · Genetic improvement · Genomics · Molecular markers · Nutritional importance

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J. M. Al-Khayri et al. (eds.), *Advances in Plant Breeding Strategies: Cereals*,
https://doi.org/10.1007/978-3-030-23108-8_4

4.1 Introduction

Foxtail millet (*Setaria italica* L.) is a minor cereal crop belonging to family Poaceae, subfamily Panicoideae. It is widely cultivated in Eurasia, the Americas, Africa and Australia. With a relatively small genome (470 Mbp, 2n = 18), the crop is rapidly becoming a model organism for the grasses (Lata et al. 2012). Foxtail millet is a rich source of nutrients, minerals, fiber, protein and phyto constituents. Antinutritional factors such as tannin and phytic acid are reduced by using suitable processing methods (Sharma and Niranjan 2018). Small stature, short life cycle, small diploid genome size, low-glycemic index, antioxidant properties and drought-tolerant features make foxtail millet a model grass (Brutnell et al. 2010).

Foxtail millet is the ancestor of green foxtail with a small genome size (470 Mb) and is now being considered a model species for functional genomics research (Diao et al. 2014; Li and Brutnell 2011). Molecular markers like single nucleotide polymorphisms (SNP) and simple sequence repeats (SSR) have been used to elucidate the population genetics of different foxtail millet germplasm (Jia et al. 2013; Wang et al. 2012). A foxtail millet haplotype map has been made available based on 0.85 million SNPs identified from sequences of 916 cultivars from around the world, and 512 quantitative trait loci (QTL) (Jia et al. 2013). It is important to rapidly identify each locus or the major locus of QTLs for efficient crop breeding by marker-assisted selection (MAS) (Wang et al. 2017a, b).

The advances of genetic engineering technology can be harnessed for the improvement of traits such as nutritional quality and resistance to abiotic stresses (i.e. drought, salinity). The biolistic method was mostly employed for millet transformation. Since the first report of *Agrobacterium*-mediated transformation in foxtail millet (Liu et al. 2005), progress has been rather slow as compared to the great success achieved in other cereals like rice (Bajaj and Mohanty 2005; Ignacimuthu et al. 2000; Shrawat and Lörz 2006) and sorghum and barley (O'Kennedy et al. 2006; Shrawat and Lörz 2006). Researchers have been trying to extend the *Agrobacterium*-mediated transformation to foxtail millet and, transgenic millets expressing agronomically-important foreign genes will soon be developed. This will greatly help to improve millet improvement for resistance to biotic and abiotic stresses.

The foremost objectives of foxtail millet breeding are directed to breed cultivars for higher nutritional quality, yield, disease resistance and drought tolerance. In this regard, efficient use of the hybridization methods, marker-assisted breeding, in vitro and genetic engineering technologies can have relevance. Furthermore, genomics-based methods of efficient transcriptional and post-transcriptional regulation of traits of interest could augment research efforts aimed at the development of high-yielding cultivars. In this chapter, we present an overview of the crop genetic diversity, nutritional importance, conventional breeding and biotechnological approaches for foxtail millet improvement.

4.1.1 Taxonomy

Foxtail millet (*Setaria italica* L.) is an important food and fodder grain crop in arid and semiarid regions of Asia and Africa. The genus *Setaria* belongs to the tribe Paniceae, subfamily Panicoideae and family Poaceae, the grass family. There are about 125 *Setaria* species widely distributed in warm and temperate parts of the world. The genus contains crop, wild and weed species with different breeding systems, life cycles and ploidy levels (Benabdelmouna et al. 2001).

Cultivated foxtail millet was initially identified as *Panicum italicum* by Linnaeus (1753); however the variants were synonymized into *Setaria italica* (foxtail millet) (Beauvois 1812), along with the weedy *P. viride* L. (green foxtail). Prasada Rao et al. (1987) compared foxtail millet accessions based on their morphology and identified three races: (1) moharia from Europe, Southeast Asia, Afghanistan and Pakistan; (2) maxima, common in eastern China, Georgia (Eurasia), Japan, Korea, Nepal and Northern India and (3) indica, found in the remaining parts of the India and Sri Lanka. Subsequently Li et al. (1995) proposed a new classification system with the recognition of four races, i.e., maxima, moharia, indica and nana, of foxtail millet. The classification of foxtail millet (*Setaria italica*) is as follows:

Kingdom: Plantae
Group: Monocots
Order: Poales
Family: Poaceae
Subfamily: Panicoideae
Genus: *Setaria*
Species: *italica.*
Botanical name: *Setaria italica* L.

4.1.2 Foxtail Millet Gene Pool

The foxtail millet gene pool shows that the genus *Setaria* is organized into three gene pools: the primary gene pool has species which are diploid with 2n = 2x = 18 composed of *S. italica* and its putative wild ancestor *S. viridis* (L.) P. Beauv. (Harlan and de Wet 1971); the secondary gene pool consists of *S. adhaerans* (Forssk.) Chiov. with 2n = 2x = 18 having two allotetraploids, S. *verticillata* (L.) P. Beauv. and *S. faberii* Herrm. (2n = 4x = 36) (Benabdelmouna et al. 2001; Li et al. 1942) and the tertiary gene pool of *S. glauca* (L.) P. Beauv. (or *S. pumila* (Poir.) Roem. & Schult., 4x to 8x) and several wild species (Zangre et al. 1992).

4.1.3 Origin, Evolution and Distribution of Setaria italica

Setaria italica is among the oldest crops whose farming possibly began about 5900 BC in the Gansu Province of Northwestern China (Barton et al. 2009). Geographically, the region ranging from Afghanistan to India is believed to be the crop's origin for domestication. Afterwards, the crop spread is thought to have been both eastward and westward (Sakamoto 1987). According to Vavilov (1926), the principal center of diversity for foxtail millet is East Asia, including China and Japan. Several hypotheses concerning the origin and domestication of foxtail millet have been proposed (De Wet et al. 1979; Kawase and Sakamoto 1987; Vavilov 1926) but the multiple domestication hypotheses (De Wet et al. 1979; Li et al. 1995) are widely accepted based on three centers of origin: China, Europe and Afghanistan-Lebanon (Li et al. 1995).

4.1.4 Morphology of Foxtail Millet

Foxtail millet is an annual grass with slim, vertical, leafy stems which can reach a height of 120–200 cm. The inflorescence is a spike with short side branches bearing spikelets and bristles. Each spikelet consists of a pair of glumes that embrace two minute flowers; the lower one sterile and the upper one is bisexual, with three stamens and a long oval smooth ovary with two long styles, which terminate in a brush like stigma (Hector 1936). One to three bristles develop at the base of each spikelet (Vinall 1924). Anthesis in foxtail millet generally takes place near midnight and in the morning but varies significantly with environment (Malm and Rachie 1971). The seed head is a dense, hairy panicle, 5–30 cm long. The seeds are small, about 2 mm in diameter. Seed color varies greatly among cultivars and ranges from pale yellow, through to orange, red, brown and black.

4.1.5 Nutritional Importance

Foxtail millet has many nutritional and health benefits. Akin to buckwheat and quinoa, foxtail millet is non-glutinous, and it is soothing and easy to digest. The nutrient composition of foxtail millet is presented in Table 4.1. The grains are least allergenic compared to other available grains (Prashant et al. 2005; Xue et al. 2008). Millet bran is extensively used as animal feed in China (En et al. 2008). Similar to other cereals, foxtail millet is deficient in lysine, with amino acid scores similar to corn. It is relatively high in leucine and methionine. The starch in some foxtail millet cultivars contain 100% amylopectin, and the starches contained in foxtail, proso and barnyard millets are more digestible than maize starch, because they release sugars slowly and thus have a low glycemic index.

Table 4.1 Nutrient composition of foxtail millet (100 g^{-1}).Values are at 12% moisture level

Cereal	Energy(kcal)	Carbohydrate(g)	Protein(g)	Fat (g)	Crude fiber (g)	Iron(mg)	GI
Foxtail millet	331	60.9	12.3	4.3	8.0	2.8	52.0
Little millet	341	67.0	7.7	4.7	7.6	9.3	52.1
Finger millet	328	72.0	7.3	1.3	3.6	3.9	60.9
Rice	362	76.0	7.9	2.7	1.0	1.8	76.0

Source: Hulse et al. (1980)

4.2 Genetic Diversity and Conservation

4.2.1 Genetic Diversity

Analysis of genetic diversity is critical to breeding and crop improvement programs. A survey of *Setaria italica* has revealed rich germplasm with phenotypic variation related to grain size, anther color, panicle density, bristles length, panicle attitude and tiller number (Fig. 4.1). About 1535 *S. italic* collections from 26 countries were analyzed at the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) for their variation in plant height, flowering time, inflorescence architecture and seed morphology. The outcrossing rate was found to vary considerable (0.3–4%) implying that there is sufficient opportunity for gene flow among *Setaria* species, particularly from crop to weedy species (Wang et al. 2010). Various studies have analyzed the genetic diversity in foxtail millet, including pedigree, morphological, biochemical analyses (Li and Wu 1996; Li et al. 1995; Murugan and Nirmalakumari 2006; Nirmalakumari and Vetriventhan 2010), isoenzymes and seed protein analyses (Jusuf and Pernes 1985) and molecular marker data (Fukunaga et al. 2002; Jia et al. 2009; Schontz and Rether 1999; Van et al. 2008).

4.2.2 Genetic Resources Conservation

Foxtail millet is a rich source of nutrients, essential amino acids, non-starchy polysaccharides, proteins and therefore, is considered as one of the nutria-cereals (ICAR 2006). Farmers mostly prefer and value the small millets because of their drought tolerance, nutritional content and health benefits, along with their adaptability to grow under low input and water conditions (Goron and Raizada 2015). Foxtail millet ranks second in total world millet production; 6 million mt of grain throughout areas in Southern Europe and Asia (Li and Wu 1996; Yang et al. 2012). The Chinese Academy of Agriculture Science (CAAS) has the largest collection of foxtail millet germplasm. Accessions collected before 2000 were landraces and improved cultivars which were then included in the National Crop Genebank of China and most of

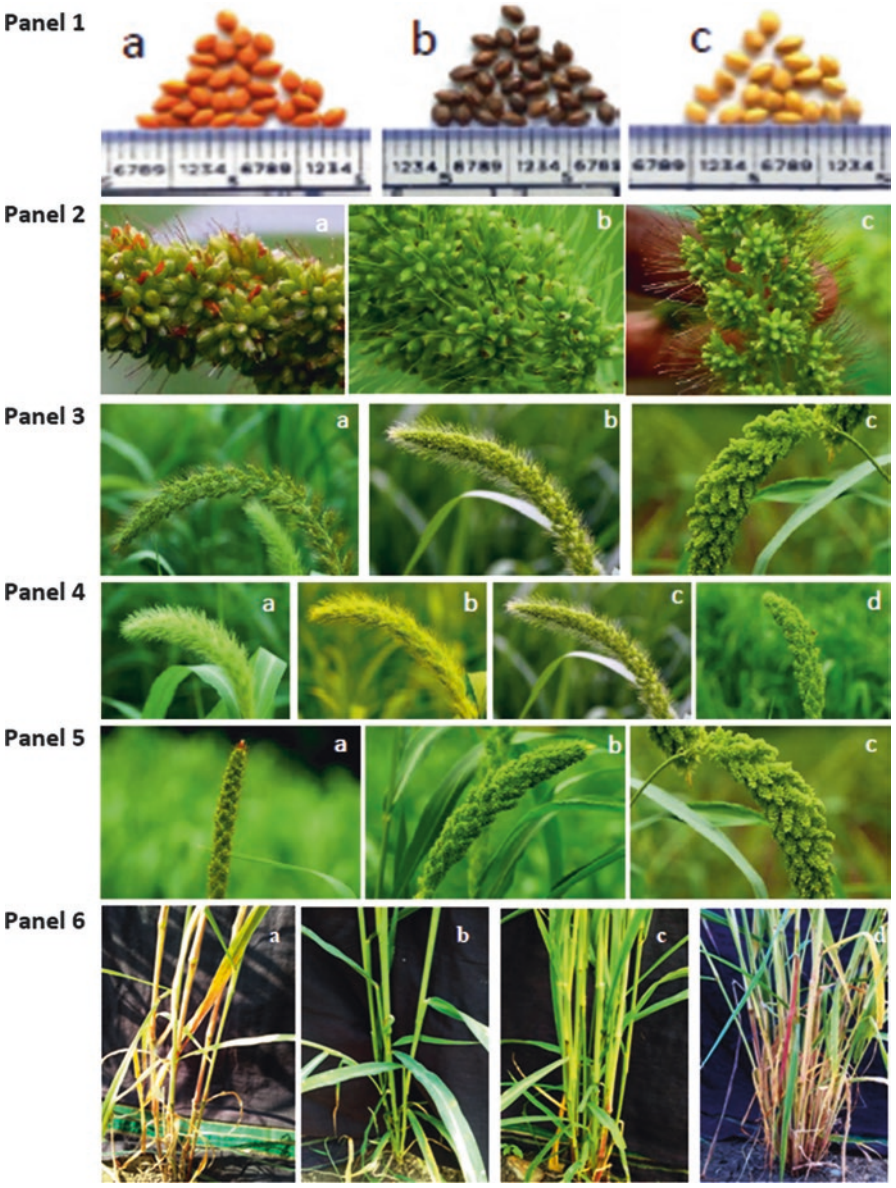

Fig. 4.1 Genetic diversity of various morphological attributes of foxtail millet. **Panel 1** Grain variation in color, size, shape and different foxtail millet accessions. (a) Ise 160 (red, narrow, ovate) (b) Ise 31 (black, medium ovate) (c) IC 97189 (yellow round). **Panel 2** Variation in anther color (a) Orange, Prasad (b) black/brown, IC 120139 (c) white, IC28439. **Panel 3** Panicle density/ compactness of different foxtail millet accessions (a) IC 41883 (max) (b) IC 97087 (medium) (c) IC 120239 (dense). **Panel 4** Bristle variation (a) IC 120139 (very long) (b) 97109 (medium long) (c) IC 97187 (medium) (d) IC 120408 (very short). **Panel 5** Panicle attitude of different foxtail millet accessions (a) IC 120159 (erect) (b) IC120408 (semi-erect) (c) IC 120239 (moderately drooping). **Panel 6** Variation in tiller numbers different foxtail millet accessions (a) Control Prasad (5–7/plant) (b) IC 120148 (low tillers, 2–5/plant) (c) IC 120159 (medium tiller, 5–14/plant) (d) IC 41898 (long tiller, 12–16/plant)
Source: Sunil (2015)

the germplasm was found to be drought tolerant (Li et al. 1991). Currently, the Chinese National Genebank (CNGB) maintains an abundance of 26,670 germplasm accessions (Wang et al. 2012). ICRISAT has germplasm collected from 26 countries and the USA (Plant Genetic Resources Conservation Unit, PGRCU) and a gene bank in Japan (National Institute of Agrobiological Sciences, NIAS) has diverse germplasm collections (Upadhyaya et al. 2008, 2011). The available germplasm collections are listed in Table 4.2.

4.2.3 Crop Growth Under Stress

Drought affects all stages of plant growth, especially in the initial stages; impaired germination and poor stand establishment are the common effects (Harris et al. 2002; Kaya et al. 2006). Using a high throughput screening method based on rate of seedling survival under multiple drought stress treatments, drought tolerant foxtail millet genotypes were selected from 17,313 accessions (Li 1997). More than 200 lines were classified as having the highest drought tolerance, including the reference cv. Yugu 1. Li (1997) also categorized the foxtail millet accessions into five grades of drought tolerance using seedling survival following repeated drought stress treatment. In another study, Karyudi and Fletcher (2002) measured the osmoregulative capacity of 11 accessions of *Setaria italic* and found that four accessions (108042, 108463, 108541, 108564) demonstrated high drought tolerance capacity. The plant's ability for osmoregulation was associated with osmotic potential as it showed a decrease with decline in leaf water potential. Seedling stage screening based on relative water content and germination rates was developed for drought tolerance using polyethylene glycol (PEG-6000) and mannitol as the selection agents (Zhang et al. 2005; Zhu et al. 2008). Subsequently PEG-6000 has been used for germination screening for drought tolerance in foxtail millet (Zhu et al. 2008).

Among the physiological processes that affect plant growth, the most sensitive to water deficit are cell division, cell enlargement and differentiation. Decreased turgor limits cell growth under stress conditions (Ashraf 1994) whereas disrupted water flow from xylem inhibits cell elongation (Nonami 1998; Taiz and Zeiger 2006). A cumulative negative effect of drought stress on growth-related traits such as plant height, leaf area, number of leaves per plant, cob length, and fresh and dry shoot weight has been shown in maize (Kamara et al. 2003). In foxtail millet, reduced shoot length, photosynthetic pigments and increased root length and compatible solute accumulation are reported as drought-adaptive mechanisms (Paul and Panneerselvam 2013).

In the case of drought, severity, duration and timing of stress assume importance, and most significantly stress at a particular stage of development can have detrimental effects on overall growth and yield (Plaut 2003). Prevailing drought can hamper flower production and grain filling which is the result of reduced assimilate partitioning and related sucrose and starch synthesis enzyme activities. Drought at pre-anthesis can decrease the time to anthesis, whereas during post-anthesis it can

Table 4.2 Significant germplasm collection of the foxtail millet

Institution	Country	Number of accessions	Reference
Chinese National Genebank (CNGB)	China	26,670	Wang et al. (2012)
National Bureau of Plant Genetic Resources (NBPGR)	India	4330	Dwivedi et al. (2012)
ORSTOM-MONTP	France	3500	Dwivedi et al. (2012)
All India Coordinated Minor Millet Project (AICMMP)	India	2512	Dwivedi et al. (2012)
International Crops Research Institute for the Semi-Arid Tropics(ICRISAT)	India	1535	http://exploreit.icrisat.org/profile/Smallmillets/187
National Institute of Agrobiological Sciences (NIAS)	Japan	1299	http://www.gene.affrc.go.jp/index_en.php
North Central Regional Plant Introduction Station, USDA-ARS	USA	1000	Dwivedi et al. (2012)
Biologie Végétale Appliquée, Institut Louis Pasteur (IUT)	France	850	Dwivedi et al. (2012)
Kenya Agricultural Research Institute (KARI)	Kenya	772	Dwivedi et al. (2012)
USDA Agricultural Research Service (USDA-ARS)	USA	762	Dwivedi et al. (2012)
Estación de Iguala, Instituto Nacional de Investigaciones Agrícolas (INIA)	Mexico	350	Dwivedi et al. (2012)

Source: Modified after Goron and Raizada (2015)

shorten grain-filling in triticale (Estrada-Campuzano et al. 2008). Under water stress, it is shown that decreased grain growth rate in wheat resulted from reduced activity of sucrose synthase, while cessation of growth resulted from inactivation of adenosine diphosphate-glucose-pyrophosphorylase (Ahmadi and Baker 2001).

Drought at flowering stage commonly results in sterility, while stress at post-anthesis stage can be detrimental to grain yield (Samarah 2005). One of the major factors responsible for this is the reduction in assimilate flux to the developing ear (Yadav et al. 2004). Lower grain set, kernel growth rate and decreased rate of endosperm cell division are associated with high abscisic acid in wheat and maize (Morgan 1990; Ober et al. 1991). It has also been shown that declining yield under water stress is due to perturbed leaf gas exchange properties that limit the size of source and sink, impairs phloem loading, nutrient uptake and dry matter partitioning (Farooq et al. 2008). In foxtail millet, grain filling and enhanced grain yield could be maintained under water stress by the lessening of leaf senescence and antioxidative defense (Dai et al. 2012). Among different millets, *Panicum miliaceumm* L. *P. sumatrense* Roth ex Roem. & Schult., *Setaria glauca*, *S. italica*, greater drought tolerance to water deficit was shown at vegetative and reproductive stages in foxtail millet compared to the other three millets (Matsuura et al. 2012).

4.2.4 Breeding Objectives and Limitations

The primary objectives of foxtail millet breeding include breeding cultivars for high nutritional quality followed by yield, disease resistance and stress tolerance. Complete utilization of germplasm often depends on two factors: (1) evaluation and characterization and (2) identification of useful gene sources. This has received considerable attention during the last 25 years, and the majority of the accessions have been screened for agronomic, physiological, pathological and even important grain quality parameters. The breeding value of many germplasm lines has been assessed through repeated field trials and a good database has been made available for most accessions, and germplasm catalogs have been published (Seetharam et al. 2006). The efficiency of utilization of germplasm can be further improved by forming core subsets and making them available to breeders. Despite such advancements, however, a major constraint is the negligence of foxtail millet area under cultivation. The realizable genetic potential is much lower as compared to other dry land crops. As a result, these crops are lagging behind and getting more and more marginalized year after year. This trend needs to be reversed and breeders should intensify efforts to improve productivity and resilience to adverse climates (Seetharam 2006). There is an immediate need to increase small millets cultivation which is economically viable to harness the yield advantages. The blast fungus (*Pyricularia grisea*) a serious threat that hampers the foxtail millet yield, is a serious problem. The blast pathogen is variable and is highly specialized in its host range (Sharma et al. 2014). The proper utilization and deployment of desirable germplasm in a given breeding area can become beneficial in improving grain yield, grain quality and composition,

drought tolerance, pest and disease résistance and, as well, meeting the emerging needs.

4.3 Conventional Breeding Methods

Plant breeding ensures the selection of better phenotypic traits among variants, in terms of high heterotic value of hybrids, nutritional quality, tolerance to environmental stresses and breeding for resistance against biotic and abiotic stresses. The various breeding methods which play a significant role in improving all the desired traits include mass selection, pedigree selection, mutational breeding and hybridization. The mass selection method is extensively employed for the multiplication of cultivars bred by pedigree or through pure-line selection and also for the purification of primitive cultivars (landraces). However, mass selection has provided very little improvement in the performance of finger millet cultivars such as Gidda ragi of Karnataka, and Murky and Nangkatna of Sikkim (Patil 2017).

Hybridization-based pedigree selection became popular in the 1970s and it remains the main breeding method in many Chinese institutions. During the 1970s, hybridization-based pedigree selection was the most popular and is still more popular today in most of Chinese institutions. Mutation breeding, both radiation and chemical-induced mutations, has been employed for the development of new cultivars or hybrids in several crop plants (Suprasanna et al. 2015). In the 1980s, significant improvement in yield was achieved with the development of new cvs. Yugu 1 and Zhaogu 1. Since 1950, 870 cultivars have been released in China and among these some prominent cultivars are mentioned in Table 4.3. Lately, the prime objective of foxtail millet breeding has shifted from high yield to high quality traits (Pan et al. 2012; Sang 2011; Wang 2008). A few cultivars such as Jingu 21 and Jingumi have been developed with a view of high yield, along with high nutritional-quality aspects (Diao 2011). Changgu 1 and Changnong 35 cvs. were developed through crossbreeding of Jingu 21 with high quality cvs. Qitouhuang and Ninghuang 1, respectively (Wang et al. 2008).Foxtail millet research began with the development of male sterile lines through hybridization of *Setaria verticillata* with foxtail millet with a view to enhance the heterosis (Zhu and Wu 1991), but neglected for hybrid seed production because of some unknown factors. Through the mutation breeding approach, a partial CMS line (3–5% seed setting rate by self-fertilization) named Suanxi 28 was developed and hybrid seeds were produced using hybridization of partial CMS with the restorer line (male fertile) (Tsui et al. 1979). Subsequently, herbicide-resistant cultivars were used as the restorer line for the development of herbicide-resistant foxtail millet cultivars (Wang et al. 1996). In 2014, a new prospective cultivar of *S. italica*, Ruberit, was bred in the Czech Republic suitable for biomass, human consumption and livestock nutrition (grain and forage) (Hermuth et al. 2016).

Table 4.3 Some prominent foxtail millet cultivars released in China during 1955–2012

Cultivar	Cross	Year	Adaptation areas
Xinnong 724	Mihuanggu reselection	1955	Henan, Hebei, Shandong
Moligu	Jiansuijinmiaohuang reselection	1958	Hebei, Beijing, Shanxi
Hualian 1	Huaidehualiangu reselection	1959	Jilin
Angu 18	Daqingmiao reselection	1965	Heilongjiang
Lugu 2	60 days Huancang reselection	1971	Shandong
Jingu 1	Baimujizui reselection	1973	Shanxi
Zhaogu 1	Shuangguayin reselection	1977	Inner Mongolia, Liaoning
Baisha 971	Baishagu reselection	1978	Jilin
Jigu 6	Japan 60 days × Xinnong 724	1982	Hebei, Henan, Shandong
Yugu 1	Japan 60 days × Tulong	1983	Henan, Hebei, Shandong
Longgu 25	Harbin 5 × Longgu 23	1986	Heilongjiang
Yugu 2	An 30 × Xiaoliugen	1989	Henan, Hebei, Shandong
Jingu 21	Jinfen 52 mutation	1991	Shanxi, Shaanxi, Inner Mongolia
Lugu 10	Yugu 1 × 5019–5	1995	Shandong, Hebei
Jigu 14	Lusuigu mutation	1996	Hebei, Henan, Shandong
Gufeng 2	95,307 × 8337	2002	Hebei, Henan, Shandong
Jigu 19	Ai88 × Qingfenggu	2004	Hebei, Henan, Shandong
Yugu 18	Yugu 1 × Bao 282	2012	Henan, Hebei, Shandong, Xinjiang, Beijing

Source: Modification from Cheng and Dong (2010)

4.4 Biotechnological Tools

4.4.1 Molecular Markers

A molecular marker is defined as an allele or a chromosomal landmark used to trace a specific DNA region, or it can also elaborate the specific portion of a known DNA segment (King and Stansfield 1997). Molecular markers are mostly employed in the field of biotechnology and molecular genetics for varied applications such as the assessment of genetic diversity, linkage map construction, phylogenetic analysis, marker-assisted selection and molecular-assisted backcrossing and in population genetics (Kesawat and Das 2009). DNA markers have immense potential for crop breeding and improvement (Roychowdhury et al. 2013). Molecular markers such as expressed sequence tag-simple sequence repeats (EST-SSR), intron length polymorphic markers (ILP), simple sequence repeats (SSR) and microRNA-based molecular markers developed using the genome of foxtail millet show cross-genera transferability >85% among the different millets (Kumari et al. 2013; Muthamilarasan et al. 2014c; Pandey et al. 2013; Yadav et al. 2014). All the physically mapped 327 eSSR markers were placed are publicly available in the NCBI Probe Database.

The first genetic map from an intervarietal cross was developed in foxtail millet using the molecular marker RFLP (Devos et al. 1998). Ajithkumar and Paneerselvam (2013) evaluated 25 random amplified polymorphic DNA (RAPD) markers and 10 ISSR markers for genetic diversity among the 5 landraces of *Setaria italica* collected from various places in the Erode District of Tamil Nadu, India. RAPD markers produced 27.5% polymorphic bands and ISSR markers produced 35% polymorphic bands between the landraces of foxtail millet. Gupta et al. (2011) exploited the EST sequences of dehydration and salinity stressed suppression subtractive hybridization and developed 98 potential intron length polymorphism (ILP) markers. Muthamilarasan et al. (2015b) reported that b225 and p61 SSR markers showed strong association with grain shape and inflorescence compactness and could be used as candidate gene markers for the traits like grain quality and yield. Dipti (2017) used SRAP markers to analyze two contrasting foxtail millet accessions under dehydration stress (Fig. 4.2).

Das et al. (2017) reported that there are many stress resistance genes which are tightly linked to molecular markers such as SSRs, STS and SNPs. QTLs associated with salt tolerant rice varieties have been mapped using microsatellite markers (Singh et al. 2007). *OsRAN1* (Ran gene) is essential for the development of cold tolerant rice varieties (Xu and Cai 2014). Marker-assisted selection is mostly useful for the rice cultivars improvement with cold tolerance (Shinada et al. 2014). Similarly in hexaploid and durum wheat the genetics of stem resistance was

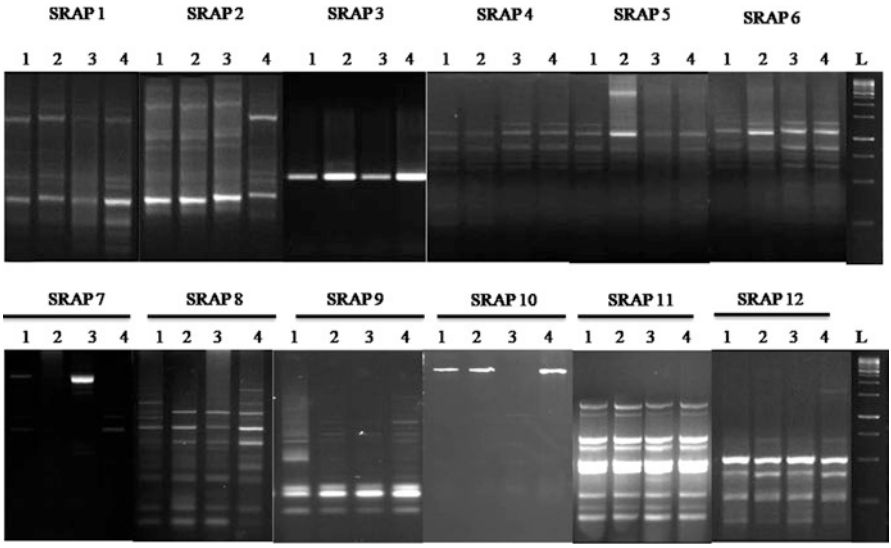

Fig. 4.2 Sequence related amplification polymorphism (SRAP) marker profiling in two contrasting foxtail millet accessions, IC-97019, IC-97189 under dehydration stress
L- Ladder, Lane 1- IC97109 control; Lane 2- IC 97109 stress; Lane 3- IC 97189 control; Lane 4- IC 97189 stress; Primers – SRAP 1 – SRAP 12
Source: Dipti (2017)

extensively studied mostly using the SSRs (*Xgwm247, Xgwm340, Xgwm547, Xbarc77, Xgwm181, Xgwm114*) and incorporated in the wheat breeding program for the wheat improvement (Clarke et al. 2002; Cook et al. 2004; Houshmand et al. 2007). The advent of high-throughput sequencing technology has generated abundant information on DNA sequences for genomes. A foxtail millet genome sequence has been independently reported by the Beijing Genomics Institute and the Joint Genomes Institute by Bennetzen et al. (2012) and Zhang et al. (2012). The open web resources in foxtail millet have been developed by NIPGR (Table 4.4).

In *Setaria,* researchers have developed a good resource population of mapping populations, fosmid libraries and mutagenized populations. Wang et al. (1998) first reported marker development in foxtail millet. QTL mapping populations have been generated in *Setaria italica* (Devos et al. 1998; Wang et al. 1998) and the first SSR-linkage map of foxtail millet was reported by Jia et al. (2009). Gupta et al. (2011) reported 98 potential intron length polymorphic ILP markers exploiting the EST sequences of dehydration- and salinity-stressed suppression subtractive hybridization (SSH) libraries. A total of 447 EST-derived SSR (eSSR) markers were successfully designed, of which 327 were mapped physically onto 9 chromosomes (Kumari et al. 2013). Gupta et al. (2011) developed (ILP) markers in foxtail millet using sequence information of the model plant rice and used as functional markers. Repeat junction markers (RJMs) are unique and cover both transposable elements (TEs)

Table 4.4 Open web resources in foxtail millet developed by NIPGR, New Delhi

Web resource	Description	URL	Reference
Foxtail Millet Marker Database (FmMDb)	Complete online large-scale marker database for retrieval, visualization and management. It provides complete marker resource (SSRs, eSSRs and ILPs) to the foxtail millet scientists	http://www.nipgr.res.in/foxtail.html	Suresh et al. (2013)
Foxtail Millet Transcription Factor Database (FmTFDb)	A database with 2297 FmTFs through query interfaces, analysis tools, BLAST search, annotation and tools for identification of enriched Gene Ontology terms and visualization of physical maps	http://59.163.192.91/FmTFDb	Bonthala et al. (2014)
Foxtail Millet miRNA Database FmMiRNADb)	This database provides complete resource of 355 foxtail milletmiRNAs. It also contains marker related information of these 123 miRNA-basedfor genotyping and molecular breeding of millets	http://59.163.192.91/FmMiRNADb	Khan et al. (2014)
Foxtail Millet Transposable Elements-based Marker Database (FmTEMDb)	FmTEMDb has thedata of ~30,000 foxtail millet transposable elements and 6 types of markers for large-scaleg enotyping use in millets, cereals and bioenergy grasses	http://59.163.192.83/ltrdb/index.html	Yadav et al. (2014)

and genic regions and, therefore, are mostly employed in functional genomic studies and used as informative markers to assess genetic diversity in plant breeding programs (Yadav et al. 2015b).

Foxtail millet is an important crop in arid and semiarid regions and is considered a model grass system for research on abiotic response and, therefore, deciphering the phenotypic and agriculturally-important attributes can provide valuable information for studies on plant functional genomics (Lata and Prasad 2012). The use of integrated omics technology with functional genomics is essential to elucidate the genomics of stress tolerance in foxtail millet, as it is mandatory for the development of the climate change resilient crops to meet ever-increasing demands of food and feed (Muthamilarasan and Prasad 2015). Yadav et al. (2014) reported microRNA (miRNA) as a functional marker that can be used in some crop plants. The pre-miRNA sequences of foxtail millet and other crops such as maize, sorghum, wheat and rice were retrieved and aligned for identifying the conserved regions. These researchers used 176 markers, among which 66 markers showed a high level of cross-genera transferability potential with an average of ~67% in millets. Currently, this miRNA marker is considered to be a novel candidate and may be useful in the molecular breeding approaches for the improvement of the foxtail millet and other non-millet species.

4.4.2 Differential Gene Expression in Foxtail Millet in Response to Abiotic and Biotic Stresses

Advances and technological developments in genomics, bioinformatics and *functional genomics* made it possible to address the complexity of stress response on a global level through genome wide *expression profiling* (Reymond and Farmer 1998; Richmond and Somerville 2000). These technologies have enabled high-throughput gene expression analysis to characterize and define the functional roles of all the genes, essential, important and ancillary, to the stress response of tolerant genotypes. Generation of in-depth understanding of the metabolic and genetic pathways will be useful in fine-tuning the stress responses towards improving tolerance. In recent years, there is a great interest in the use of genomic tools to identify and isolate genes involved in the tolerance of crop plants to various abiotic stress factors. The first step to understand and evaluate such genetically complex responses is to sequence randomly selected cDNA clones or expressed sequence tags (ESTs) from the plants exposed to the environmental stress (Zhang et al. 2001). Various molecular techniques have been used for studying differential gene expression in many plant species which includes representational difference analysis (RDA), suppression subtractive hybridization (SSH), differential display, differential hybridization, subtractive library construction, serial analysis of gene expression (SAGE), cDNA-RAPD, cDNA-AFLP and cDNA microarrays (Diatchenko et al. 1996; Lisitsyn et al. 1993; Velculescu et al. 1995) and SRAP (Li and Quiros 2001).

The salt-tolerant foxtail millet cv. Prasad showed high expression of the *PHGPX* gene suggesting its crucial role in stress-induced defensive reactions (Sreenivasulu et al. 2004). Differential expression profiles of salt stress-associated genes like glutamine synthetase (GS) and pyrroline-5-carboxylate (P5C) reductase were observed under salinity stress in salt sensitive (Lepakshi) and salt tolerant (Prasad) cvs. of foxtail millet (Veeranagamallaiah et al. 2007). Zhang et al. (2007) reported the construction of subtracted cDNA libraries from foxtail millet seedlings under dehydration stress and the expression profile analysis showed upregulation of ESTs by dehydration stress (Zhang et al. 2007). Later, Puranik et al. (2011) constructed two suppression subtractive hybridization cDNA libraries (forward and reverse) in foxtail millet under salinity tolerance and observed differential expression of some unknown genes. Using cDNA-AFLP technique, Jayaraman et al. (2008) compared gene expression profiles of a salt-tolerant and a salt-sensitive cultivar of foxtail millet (*Setaria italica*) and identified early responsive differentially-expressed transcripts that accumulated upon salt stress. Lata et al. (2011) reported analysis of early- and late-induced differentially expressed transcripts in foxtail millet cv. Prasad after dehydration stress. Through extensive transcript profiling using qRT-PCR on two *S. italica* cultivars, significant upregulation of SiDREB2 and SiNAC2 genes was revealed in tolerant cultivar cv. Prasad compared to susceptible cv. Lepakshi, suggesting that these are the putative genes playing important role in stress-responsive mechanism.

Zhang et al. (2007) found the expression of *SiOPR1*(12-oxophytodienoic acid reductase1) specifically in foxtail millet roots and reported that this was not influenced by ABA, NaCl and MeJA treatments throughout the plant, suggesting its significant role in drought stress tolerance. *SiMYB* and *SiC2H2* have been shown as the candidate genes in response to abiotic stresses and hormone treatments. *SiMYB124*, *SiMYB126* and *SiMYB150* are the putative transcription factors which showed significant upregulation during drought stress and can be used as novel candidates for crop improvement programs (Muthamilarasan et al. 2014a). Lata et al. (2014) analyzed expression profiling of *AP2/ERF* genes against drought, salt and phytohormones and characterized a differentially expressed EST encoding a putative *DREB*2 gene, *SiDREB*2 (Lata et al. 2011). Mishra et al. (2013) found that genes *SiWD40*, *SiWD40-028*, *SiWD40-037*, *SiWD40-063, SiWD40-106, SiWD40-156* and *SiWD40-203* showed upregulation in foxtail millet and found responsive to drought, salinity and cold stresses. Dipti (2017) analyzed expression profiling of drought responsive genes *DREB1, DREB2, aquaporins* and *C2H2* zinc finger proteins and found differential expression among the contrasting cultivars (Fig. 4.3).

It is also interesting that several genes responsive to water belonging to 1517 foxtail millet-specific gene families have been identified through genome annotation data of *Setaria italica* cvs. Zhang gu and A2 (Zhang et al. 2012). The study also indicated various unknown genes in response to abiotic stress in foxtail millet. Zhang et al. (2012) identified 586 genes that were predicted to have roles in stress responses. Qi et al. (2013) analyzed the whole transcriptome of foxtail millet by

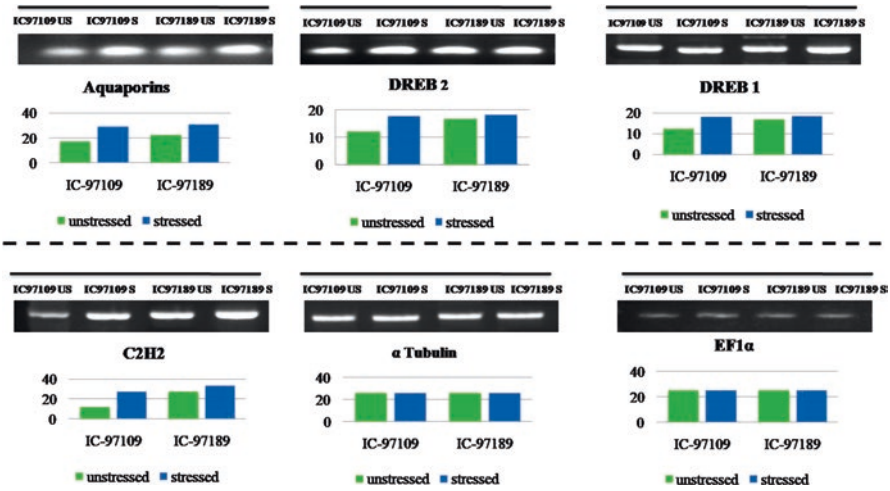

Fig. 4.3 Semi-quantitative RT-PCR of drought related candidate genes. Foxtail millet accessions, IC-97109, IC-97189 in response to water stress, differential expression of *Aquaporin*, *DREB2*, *DREB1* and *C2H2*; α tubulin and EF1α used as internal control; US-unstressed; S-stressed
Source: Dipti (2017)

using the next-generation deep sequencing technology and identified a total of 2824 genes with drought-responsive expression patterns.

Differentially expressed signaling pathway genes in foxtail millet were studied and significantly upregulated genes were identified in cv. Yugu-1 in response to rust in foxtail millet (Li et al. 2015). Differential expression of five miRNA-target genes was validated under dehydration stress suggesting the role of miRNA in dehydration stress-associated post-transcriptional regulation and their targets (Yadav et al. 2014). Similarly, transcriptomic analysis of various TFs like MYB genes (Muthamilarasan et al. 2014a); SiNAC genes (Puranik et al. 2013); C_2H_2 type of zinc finger (Muthamilarasan et al. 2014b) and ADP-ribosylation factors (ARFs) have also been performed in foxtail millet(Muthamilarasan et al. 2016).

4.4.3 Genomics Interventions

Genome-assisted breeding (GAB) and genetic engineering approaches have emerged as powerful tools for precise selection for the trait(s) of interest. Different genomic approaches such as structural genomics, comparative genomics and functional genomics, including transcriptomics and metabolomics, have been reported in the past decade (Wang et al. 2017a, b).These advances are beginning to realize their potential to increase the efficiency of crop improvement programs. The U.S. Joint Genome Institute (JGI) Department of Energy has conducted complete sequencing of the foxtail millet (*Setaria italica*) genome using a highly commercial

inbred line Yugu1 in April, 2009. The accessions of foxtail millet showed partial male cytoplasmic infertility (Wang et al. 1998) which was used to generated an interspecies genetic map. The Clemson University Genomics Institute constructed 113 bacterial artificial chromosomes library from inbred line Yugu1 and the sequences helped to assemble and orient scaffolds of the shotgun sequence. The different tissues were used to assemble the several thousands of ESTs which will help to annotate the genomic sequence and for construction of microarray chips (Doust et al. 2009).

With the two genome sequences published in 2012,the functional genomics research including microRNAs (Yi et al. 2013), transcriptional regulatory factors and metabolic pathways has generated interest (Bennetzen et al. 2012; Zhang et al. 2012). The functional genomic databases (SIFGD) of foxtail millet with URL http://structuralbiology.cau.edu.cn/SIFGD/ are currently used for bioinformatics studies such as analyzing gene function and regulatory modules. The Gbrowse (genomic software) is used for the integration of foxtail millet genomic sequences, protein sequences, miRNA-seq and RNA-seq data, transcript sequences, expressed sequence tags (EST), from the public databases including phytazome, Beijing Genomics Institute and NCBI. Gbrowse has some gene functional analysis tools such as for the identification of gene family and motif analysis (You et al. 2015).

Chai et al. (2018) provided evolutionary insight and functional analysis of HD-Zip genes involved in environmental stress responses in foxtail millet. The morphological features of the foxtail millet were modified during environmental stress due to this HD-Zip transcription factors. For the identification of HD-Zip genes in a protein databases Chai et al. (2018)further used Hidden Markov Model of Homeobox-associated leucine zipper (PF02183) coupled with BLASTP program. The proteins were then examined by Pfam and SMART for HD and Zip domain presence (Finn et al. 2014; Letunic et al. 2012). The physical parameters such as molecular weight of proteins (kDa), length of proteins and isoelectric point of the predicted HD-Zip protein were analyzed using online ExPASy programs (http://web.expasy.org/protparam/). Gene Structure Display Server (GSDS; http://gsds.cbi.pku.edu.cn) used to determine the intron-exon organization via alignment of the CDS sequences with their corresponding genomic sequences and MEME program (http://meme-suite.org/tools/meme) used to perform the structure of motif (Bailey et al. 2009). The Phyre2 server (Protein Homology/AnalogY Recognition Engine; http://www.sbg.bio.ic.ac.uk/phyre2) was used for the identification of HD-Zip proteins three dimensional structure (Kelley et al. 2015). The orthologous genes were identified using OrthoMCL (http://orthomcl.org/orthomcl/) (Li et al. 2003) and relationship among the genes was plotted using Circos (http://circos.ca/) (Krzywinski et al. 2009). Hetero-trimeric G-protein subunits and their interacting partners have been implicated in stress signal transduction (Urano et al. 2013).

Chakraborty et al. (2015) suggested that G-protein signaling component plays an important role in abiotic stress tolerance in foxtail millet. It has been found that the overexpression of the *SiLEA14* homologue of late embryogenesis abundant (*LEA*) protein confers resistance to salt/drought tolerance (Wang et al. 2014). Similarly, Li et al. (2014) cloned an abscisic acid (ABA)-responsive DREB-binding protein gene

Table 4.5 Summary of the outcomes of foxtail millet genome sequencing efforts

Information	Beijing Genome Initiative, China (Zhang et al. 2012)	United States Department of Energy Joint Genome Institute (Bennetzen et al. Bennetzen et al. 2012)
Accessions sequenced	Foxtail millet cvs. Zhang gu and A10	Foxtail millet cvs. Yugu1 and green foxtail A10
Platforms used	Illumina second-generation sequencer	ABI3730xl capillary sequencer; 454 FLX; Illumina Genome Analyzer II platform
Sequence generate	40 Gb	4 Gb forYugu1 and 3.5 Gb for A10
Genome coverage	86% (81% excluding gaps)	80%
Genome size	423 Mb	400 Mb
Total number of genes	38,801 (82% expressed)	24,000–29,000 expressed genes

Source: Muthamilarasan et al. (2017)

from foxtail millet to mediate response to drought and high salinity stress. Recently, *DCL*, *AGO* and *RDR* (Yadav et al. 2015a), WRKY (Muthamilarasan et al. 2015a) and ADP ribosylation factors (Muthamilarasan et al. 2016) involved in stress-responsive molecular machinery. The genome sequencing outcome is presented in Table 4.5.

Millets hold great promise for nutritional food security, climate change and burgeoning populations to feed worldwide (Saxena et al. 2018). They have inbuilt tolerance to biotic and abiotic and possess health benefits. High throughput genomic approaches such as genotyping-by-sequencing (GBS) and genome-wide association mapping studies (GWAS) can be useful for the discovery of the putative and novel genes/alleles or QTLs for stress and nutritional quality traits (Muthamilarasan et al. 2016; Varshney et al. 2014).

4.4.4 In Vitro Culture of Foxtail Millet

Plant biotechnology has emerged as an exciting new area of plant sciences as it can create exceptional opportunities for plant improvement. An important aspect of all plant biotechnology tools is the culture of either the plant cells, tissue or organs in an artificial medium. Plant propagation through tissue culture provides a platform to understand various factors which are responsible for growth, differentiation and morphogenesis. Plant tissue culture improves plant breeding techniques by providing methods capable of achieving objectives not possible by other means.

In millets, indirect regeneration is routinely used to establish plants in vitro whereas direct regeneration is rare. Indirect regeneration involves both organogenesis from callus culture or induction of somatic embryogenesis. Somatic embryogenesis plays an important role in clonal propagation. Integrated with conventional breeding programs and molecular and cell biological techniques, somatic embryogenesis provides a valuable tool to enhance the pace of genetic improvement of commercial crop species (Stasolla and Yeung 2003). There are reports of somatic embryogenesis in finger millet (Mekbib et al. 1997; Patil et al. 2009; Sivadas et al. 1990), kodo millet (Arockiasamy et al. 2001) and foxtail millet (Xu et al. 1984). Iriawati et al. (2017) successfully reported in vitro regeneration of foxtail millet using MS (Murashige and Skoog 1962)basal medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D), kinetin, 6-benzylaminopurine (BAP) and 1.5 mg/L NiSO4; results suggested that MS medium containing 0.5 mg/L kinetin, 2 mg/L BAP, and 0.1 mg/L 2,4-Dwas favorable for shoot induction (Fig. 4.4). Callus was formed in MS medium (Fig. 4.4) containing 0.5 mg/L 2,4-D, 1 mg/L BAP and 1 mg/L kinetin and regenerated shoots spontaneously developed roots (Fig. 4.4) when transferred into MS basal media without growth regulator.

Most in vitro culture studies use MS medium. However, N_6 medium was also used for regeneration in millets (Chu et al. 1975) and hence, this medium became popular in millets since increased frequency of embryogenic callus was obtained even during long-term culture (Lambe et al. 1999). In the case of foxtail millet, only two reports are available on genetic transformation using the

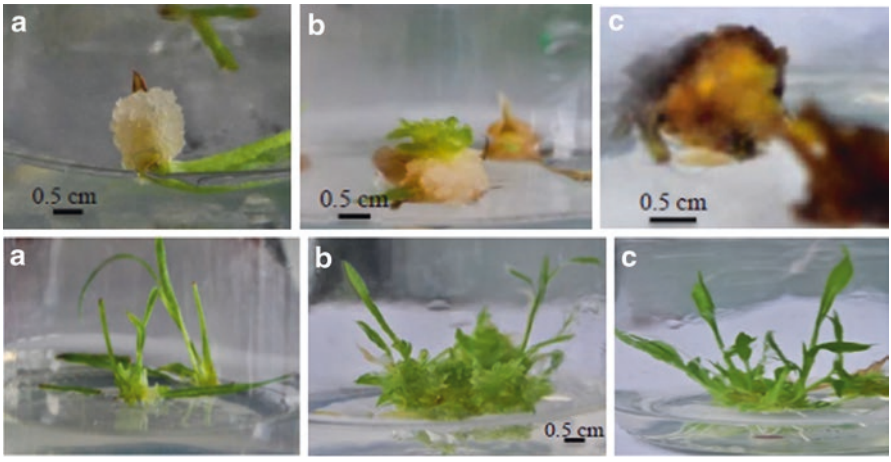

Fig. 4.4 Foxtail millet tissue culture system. ***Top row:*** (**a**) Compact calli, (**b**) Indirect shoot organogenesis developed on greenish area of callus, (**c**) Browning calli. ***Bottom row:*** (**a**) Shoot induction, (**b**) Shoot elongation**, c** Root formation on MS medium without plant growth regulators
Source: Iriawati et al. (2017)

Agrobacterium-mediated method. In a first report, Liu et al. (2005) successfully presented *Agrobacterium*-mediated transformation and obtained 6.6% transformation frequency. Fang et al. (2007) utilized the same protocol with pollen specific gene *Si401* for the transformation of foxtail millet under the control of pollen specific promoter (*Zm13*) and cloned into plasmid pBIZm13Si401 containing gene *nptII* and kanamycin antibiotic for the transformants selection and found that expression of pollen specific gene *Si401* in foxtail millet showed multiple abnormalities which could be useful for the development of important sterile cereal crops. However key parameters like explant, in vitro culture system and growth regulators affect the frequency of foxtail millet regeneration (Liu et al. 2005; Qin et al. 2008; Wang et al. 2011; Xu et al. 1984).

4.4.5 Post Genomics Applications

The whole transcriptome analysis of foxtail millet (*Setaria italica*) using deep sequencing led to the identification of 2824 drought responsive genes. Among them, 48.23% were found upregulated and 51.77% were of down regulated (Qi et al. 2013). Upregulated proteins mostly involved late embryogenesis abundant (*LEA*), dehydrins, heat shock proteins (HSPs), phosphatase 2C and aquaporins suggesting their probable role in drought tolerance. Differential gene expression profiling and genome-wide screening of different transcription factors (TFs) such as C_2H_2 zinc finger, AP2/ERFs, NAC and MYB (Lata et al. 2014; Muthamilarasan et al. 2014a, b; Puranik et al. 2013) and genes conferring to stress response such as AGO, DCL, RDR polymerases. WD40 and ALDH (Mishra et al. 2013; Yadav et al. 2015a; Zhu et al. 2014) have also been investigated in foxtail millet for the analysis of gene families implicated in stress tolerance and for the identification of the putative/candidate genes and gene regulatory networks for abiotic stress responses.

Foxtail millet is a rich source of genes/QTLs for cereal improvement, as a number of genomic resources have been developed which include intron length polymorphisms (Muthamilarasan et al. 2014c), miRNA-based markers (Yadav et al. 2014), foxtail millet transposable elements-based marker database (FmTEMDb) (Yadav et al. 2015a) and foxtail millet miRNA database (FmMiRNADb) (Khan et al. 2014). Lata et al. in 2011 developed allele-specific markers from SNP in *SiDREB2* gene for the allele mining and MAB for drought tolerance in foxtail millet. C_4 photosynthesis is found more effective than C_3 in terms of precise carbon fixation mostly in the arid region (Sage and Monsoon 1999; Xu et al. 2011). In the C_4 pathway CAβ catalyzes first reaction by hydrating atmospheric CO_2 to bicarbonate and was highly expressed in the cytosol of mesophyll cells. The CAβ genes of foxtail millet, *Ft_CA1* (*Millet_GLEAN_10030850*) expressed in mesophyll

cells having FPKM value 22,970 was found important for the C_4 photosynthesis pathway in foxtail millet.

Nutrient deficiency is a major threat that mostly depends on a cereal-based diet. After cereals, millets are considered as the primary source of energy in drought and semiarid regions. Millets are a rich source of proteins, minerals, amino acids and vitamins. Foxtail millet also has good amounts of iron and zinc. Biofortification of millets can become an economically important approach to overcome micronutrient malnutrition. But the major constraint in biofortification of millets is the presence of the antinutritional factors like tannins, polyphenols and phytic acid. In this regard, genomics tools like RNA interference and gene targeting/genome editing tools such as (ZFNs), (TALENs) and (CRISPR) will have to be applied to reduce the antinutrients to enhance the bioavailability of micro- and macronutrients (Vinoth and Ravindhran 2017).

4.5 Conclusions and Prospects

Foxtail millet and other millets have several desirable characters including hardiness, shorter growing cycle, tolerance to drought and other stresses, and are known to release less greenhouse gases. All these traits make them favorable candidates in view of the imminent threat of climate change. Millets, often considered orphan crops, might be extensively cultivated in future due to better environmental adaptation, especially an increase in global temperature. Millets thus provide an alternative to meeting the climate change and considered as climate-smart crops, as their adaptation to challenging environment is better than the currently grown major crops of the world. Millets are a rich source of micronutrients and could be incorporated in the hybrid breeding program for the development of biofortified cultivars to contribute to food security and combat malnutrition. Genomics advances such as RNA interference and genome editing tools have a great role to play in millets for the reduction in the antinutrients while enhancing the bioavailability of micro- and macronutrients. Established protocols of in vitro and genetic transformation can be useful in applying the abovementioned gene editing tools. It is hoped that germplasm collection resources along with the incorporation of the modern genomic breeding tools may provide ways to accelerate the use and exploitation of biodiversity. The productivity of millets can be enhanced through the consistent and integrated efforts of biotechnologists, breeders, agronomists, donors and policymakers at both institutional and individual level.

Appendices

Appendix I: Indian Research Institutes Relevant To Foxtail Breeding and Genomics

Institution	Specialization and research activities	Contact information and website
National Institute of Plant Genome Research (NIPGR) New Delhi-110067 India	Plant molecular genetics and genomics, stress biology	Dr. Manoj Prasad National Institute of Plant Genome Research (NIPGR), New Delhi Email:manoj_pds@yahoo.com Website:http://www.nipgr.res.in/research/dr_mprasad.php
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola -444104 (MS), India	Mutation breeding, in vitro culture, development of elite varieties, biofortification	Dr. M. P. Moharil Dr. Panjabrao Deshmukh Krishi Vidyapeeth,Biotechnology Centre, Department of Agricultural Botany, Akola (MS), India Email:mpmoharil@gmail.com Website:https://www.pdkv.ac.in/
Indian Institute of Millet Research, Hyderabad-506001, India	Hybrid breeding, molecular assisted breeding, genetic augmentation of grain yield in foxtail millet, biofortification	Dr. Hariprasanna K Indian Institute of Millet Research, Hyderabad Email:hari@millets.res.in Website:http://millets.res.in/
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502324, Telangana, India	Germplasm characterization, regeneration, conservation and documentation, plant breeding and genetics; plant genetic resources	Dr. M Vetriventhan International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502324, Telangana, India Email:vetriventhan@gmail.com Website:https://www.icrisat.org/

Appendix II: Foxtail Millet Genetic Resources in India And Their Salient Features 1989–2012

Cultivar	Pedigree	Important traits	Cultivation location
SiA 3085	Pure line from SiA 2644	Resistant to blast and downy mildew	Andhra Pradesh
HMT 100-1	RS 118 × PS 3	High tillering, suitable for early and late sowing	Karnataka
Suryanandi (SiA 3088)	Pure line fromSiA 1244	Non-lodging, Short duration, suitable for double cropping	Andhra Pradesh
TNAU 196	Co 5 × ISe 247	Resistant to rust	Tamil Nadu
Sri Lakshmi	Pure line Selection	High seed yield	Andhra Pradesh
PS 4	SiA 2616 (0.2% EMS induced mutant)	Wider adaptability	All States
TNAU 186	Co-5 × SiA 326	Tolerant to drought	Tamil Nadu
Krishnadevaraya (SiA 2593)	Selection from SiA326 × SiA 242	High seed yield	Tamil Nadu
AK 132–1	Pureline selection	Drought tolerance	Andhra Pradesh
K3	Selection from SiA 2567	Stay green character	Tamil Nadu
Meera (SR 16)	Pure line selection	Stay green character, resistant to downy mildew	Rajasthan

Source: http://millets.res.in/technologies/foxtail_millet.pdf

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Chapter 5
Breeding of Pearl Millet (*Pennisetum glaucum* (L.) R. Br.)

Ashita Bisht, Ashok Kumar, Rahul Dev Gautam, and R. K. Arya

Abstract Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a small-seeded cereal crop. Its protogynous nature renders it a highly cross-pollinated crop. Among cereal crops, pearl millet ranks sixth in importance based on world production, next to rice, wheat, maize, barley and sorghum; however, it is a more abundant source of nutrients than those cereal crops. Pearl millet covers 27 million ha worldwide and serves as a significant nutritional source supporting food security of more than 90 million inhabitants of arid and semiarid regions of India, South Asia and Sub-Saharan Africa. It is also a source of straw for grazing fodder, silage, hay and fuel. Due to its successful grain production even in harsh conditions, it has the potential to serve as an important cereal crop in extreme and erratic climate. Success in grain production is attributed to climate-smart vegetative, reproductive and physiological characteristics. Efforts have been made to understand its center of origin, taxonomical position, genetic resource diversity and conservation for effective utilization in breeding programs. Current circumstances demand new and improved varieties with enhanced productivity, improved quality and resilience to abiotic and biotic stresses. This requires continuous breeding based on conventional and molecular methodologies for further genetic improvement.

Keywords Composite · Cytoplasmic male sterility (CMS) · Hybrid · Pearl millet · Recurrent restricted phenotypic selection (RRPS) · Synthetic

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J. M. Al-Khayri et al. (eds.), *Advances in Plant Breeding Strategies: Cereals*,
https://doi.org/10.1007/978-3-030-23108-8_5

5.1 Introduction

Millets, in general, denote a group of small-seeded cereal crops including pearl millet, finger millet, little millet, foxtail millet and other minor crops. Millets are among the six most important cereals in the world (Kamal et al. 2013). Pearl millet [*Pennisetum glaucum* (L.) R. Br.] (Fig. 5.1) grain constitutes protein and oil content comparable to that of wheat, corn, sorghum and rice. Although, deficient in protein it has an excellent amino acids profile. Starch properties are similar in sorghum, corn and pearl millet. As compared to corn oil, pearl millet oil contains more palmitic, stearic and linolenic fatty acids (Burton et al. 1974).

Pearl millet provides staple food in the dry and semiarid regions even under the most difficult farming conditions (Rachie and Majumdar 1980). Its successful growth even in harsh conditions accounts for it being having climate-smart vegetative, reproductive and physiological attributes. It reliably produces grain in regions where the average annual rainfall is low (250 mm) while under similar condition maize, rice, sorghum and wheat would fail (Bidinger and Hash 2004; Vadez et al. 2012; Varshney et al. 2017).

It is also a good source of straw for grazing fodder, silage, hay and fuel (Burton and Forston 1966; Gemenet et al. 2015; Jauhar and Hanna 1998; Lata 2015; Newman et al. 2010; Passot et al. 2016; Sehgal et al. 2012; Serba et al. 2017; Shivhare and Lata 2016, 2017). The high dependency of people, mainly in arid and semiarid regions, on pearl millet is due to grain production capability even in the harshest conditions including low soil fertility, high soil pH, high soil Al^{3+} saturation, low soil moisture, high temperature, high soil salinity and scanty rainfall (Varshney et al. 2017).

Pearl millet tolerates air temperatures over 42 °C even during the reproductive phase that makes cultivation possible in the scorching heat of Northwestern India using irrigation (Gupta et al. 2015). It is a more abundant source of nutrients than rice, wheat, maize or sorghum (Kajuna 2001; Maman et al. 2006; Muthamilarasan et al. 2016; Serba et al. 2017; Tako et al. 2015). Its nutritional superiority comes

Fig. 5.1 Pearl millet crop showing grain filled ear heads

from higher levels of protein (8–19%), lower starch, higher fiber (1.2 g/100 g) (Lee et al. 2012; Nambiar et al. 2012) and elevated levels of iron and zinc (Agte et al. 1999; Serba et al. 2017; Serraj et al. 2005; Tako et al. 2015). Its popularity is increasing in the expanding health food market as a gluten-free food product (Gulia et al. 2007). Pearl millet designations in various languages (Burton and Powell 1968) across the world are given in Table 5.1.

Table 5.1 Vernacular names of pearl millet in different languages

Language	Country	Common name
African	Africa	Souna, sanio, gerohatsii, babala, mahangu, nyoloti, dukkin, mexoeira, mashela, mhunga, lebelebele, zembwe, botswana maiwa, nyoloti, cumbu and raa
Mbukushu, Oshiwambo	Angola, Zambia, Botswana	Mahangu
Arabic	Arab League	Dukhn
Australian	Australia	Bulrush millet
English	England	Bulrush, cattail, candle, dark or spiked millet
Tigrinya	Eritrea, Ethiopia	Biltug
French	France	Mil de chandelles, millet perlé, mil penicillaire, penicillaire, petit mil
German	Germany, Austria, Switzerland, Italy	Perlhirse, rohrkolbenhirse, pinselgras, negerhirse
Dutch	Holland	Parelgierst
Hindi, Urdu, Punjabi	India	Bajra
Kannada	India	Bajri, sajje
Tamil	India	Kambu
Malayalam	India	Kambam
Rajasthani, Marathi, Gujrati	India	Bajri
Telugu	India	Sajjalu
Italian	Italy	Miglio perlato
Bambara	Mali	Sano
Hausa	Niger, Nigeria	Gero
Borno Kanuri	Nigeria	Arum
Urdu, Kashmiri, Balochi, Pashto, Punjabi	Pakistan	Ba'ajra
Russian	Russia, Ukraine, Belarus	Proso
Spanish	Spain	Mijo perla and mijo negro
Kiswahili	Tanzania	Uwele
Yoruba	Togo, Benin	Oka
Turkish	Turkey	Dari
Shona	Zimbabwe, Mozambique	Mhunga

This chapter highlights the importance of pearl millet as a climate- resilient crop serving as an alternative to the other cereals. It describes the center of origin, taxonomic position, genetic resource characterization and conservation. It also assesses the present status of pearl millet with emphasis on its breeding approaches including conventional and advanced molecular techniques.

5.1.1 Botanical Classification and Distribution

The pearl millet plant is a diploid ($2n = 2x = 14$), short-day, photoperiod sensitive and allogamous species. Taxonomically, it belongs to the family Poaceae, subfamily Panicoideae, tribe Paniceae, subtribe Panicinae, section Penicillaria, and genus *Pennisetum* (Dwivedi et al. 2012). It is an annual robust bunchgrass, which received its descriptive genus name, *Pennisetum,* from an aggregation of two Latin words, *penna* signifying feather and *seta* bristle. It is among the most drought-resistant crops but cannot survive standing water (Lee et al. 2012). Its growth is most favorable in the light-textured and well-drained soil. It is a C4 grass, and has superior photosynthetic efficiency and biomass production potential. The plant is 2–3 m tall and can reach up to 5 m; culms vary in branching habit as simple or branched, girth as slender or stout and surface as smooth or hairy. Leaf sheaths, collars and blades may also be smooth or hairy. The length of the inflorescence (head) ranges from 5–150 cm, which represents false spikes. The spikelets have two kinds of flowers; one is sessile staminate floret, and has single lemma and 3 stamens and is borne below shortly pedicelled bisexual ones. Bisexual floret comprises 1 pistil, 2 feathery style and 3 anthers enclosed between the lemma and palea. Bisexual flowers appear 2–3 days earlier than staminate flowers. It exhibits a protogynous flowering habit (Fig. 5.2); the anthers emerge after the styles dry up. Cross-pollination is as high as

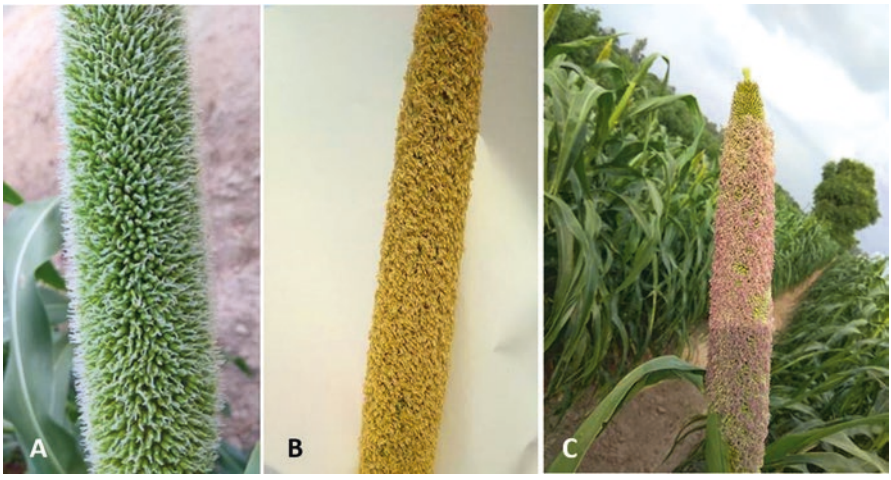

Fig. 5.2 Flowering in Pearl millet showing: (**a**) Protogyny, (**b**) Anthesis, (**c**) Pink-colored anthers

Fig. 5.3 Countries producing pearl millet worldwide

80% due to protogyny (Burton 1983; Burton and Powell 1968; Poehlman 1995; Rachie and Majumdar 1980).

Phylogeny is the evolution of a species or group especially in reference to lines of ancestry and interactions among expansive group of organisms. Phylogenetic relationship using 27 microsatellite markers on 84 wild and 355 cultivated accessions distributed among Asia and Africa revealed significantly higher diversity in the wild pearl millet group (Oumar et al. 2008). *Pennisetum* (excluding *P. lanatum*) is paraphyletic concerning the closely related genus *Cenchrus*, while sections *Pennisetum* and *Gymnothrix* are polyphyletic (Martel et al. 2004).

Pearl millet occurs worldwide (Fig. 5.3) and occupies an estimated 27 million ha (Varshney et al. 2017). It serves as a significant crop in the semiarid portions of northwestern India. It occurs in Africa from north to south, encompassing Senegal to Ethiopia; rarely being found in the southern portions of the Arabian Peninsula, Spain, and the Southeastern United States (Brunken et al. 1977). The Sahel zone of West Africa exhibits the most significant share of genetic diversity whereas the arid sections of India exhibit the highest hectarage.

5.1.2 Domestication, Selection and Early Improvements

The process of plant domestication derives from substantial alterations of plant phenotype due to the effects of demography and selection of diversity. Pearl millet domestication began in Sub-Saharan Africa around 4000–5000 years ago (Munson 1975). Single (Marchais and Tostain 1992; Oumar et al. 2008) and multiple (Harlan 1975; Porteres 1976) domestications in various geographical origins are proposed. Based on morphological diversity, Koernicke and Werner (1885) concluded that pearl millet was originally native to Africa. Vavilov (1949) placed pearl millet in the

Ethiopian center, Murdock (1959) in Niger, Clark (1964) in the highlands of the Sahara and Harlan (1971) in the Sahelian zone of domestication. The ultimate center of origin of its wild progenitors, *Pennisetum mollissimum* and *P. violaceum*, and ecotypes of *P. glaucum* ssp. *monodii,* is in the Saharan Desert (Brunken et al. 1977; Burton and Powell 1968; D'Andrea and Casey 2002; D'Andrea et al. 2001; Rachie and Majumdar 1980). Cytogenetic evidence also indicates the presence of accessory chromosomes, but only in regions of Africa, frequently observed in primitive rather than commercial varieties (Muntzing 1958; Pantulu 1960; Powell and Burton 1966).

5.2 Current Cultivation Practices and Challenges

Pearl millet is grown directly from seed. For grain purposes, the seeding rate of 4–5 kg/ha using a plow, while in a nursery, the seed rate decreases to 3.75 kg/ha. The seed rate varies for fodder purpose, which is 6 kg/ha if drilled and 16 kg/ha if broadcast (Sheahan 2014). The optimum temperature for seed germination is 12 °C or above, and seedling emergence occurs within 2–4 days. Temperatures of 25–30 °C to 32–35 °C are best suited for its growth (Hannaway and Larson 2004; Newman et al. 2010). Pearly millet grows in all types of soil but performs best in black cotton and sandy loam soils with good drainage. It can be cultivated in lands having low pH. Pearl millet, in general, requires lower input of nutrients, from manure or crop rotations with legumes (Myers 2002). Plant spacing is typically 40–45 × 10–15 cm. Development of hybrids have increased production, but many landraces like Jakhran, Alwar and Babapuri and composites are under cultivation in many areas of India.

Pearl millet is a largely neglected and underutilized crop. It is primarily grown in dryland areas, which are marginal production environments having poor inherent soil fertility and water-holding capacity. Traditional management practices include below optimum levels of tillage with minimal use of irrigation, fertilizer and pesticide. Other problems are reduction in arable area, unavailability of seeds of improved cultivars, incidence of diseases such as downy mildew (*Sclerospora graminicola*), ergot (*Clavisceps fusiformis*), smut (*Moeszimyoces penicillariae*) and *Bipolaris setariae*), and parasitic weeds such as striga (*Striga hermonthica*) (Dwivedi et al. 2012). Although pearl millet is a hardy crop, post-flowering drought stress can result in substantial losses in grain and fodder yield (Mahalakshmi et al. 1987).

Traditional breeding methodologies in combination with genomics era technologies are used to achieve solutions to the previously mentioned challenges. There is the need for diversification of the genetic base of hybrids by developing novel sources of male sterility and apomictic sources to fix heterozygosity. Development of new varieties with a high degree of buffering capacity to extreme and erratic climate is the next step in pearl millet improvement. Many research organizations (Appendix I) are working on pearl millet to overcome these challenges.

5.3 Germplasm Biodiversity and Conservation

Germplasm refers to the complete set of the gene pool collection, consisting of primitive cultivars, natural hybrids, wild and weedy relatives, wild species, traditional landraces, obsolete varieties, elite lines, breeding lines, mutants and polyploids, and interspecific and intergeneric hybrids developed systematically (Haussmann et al. 2004).

Pearl millet has tremendous genetic diversity, which serves as a potential resource for allele mining for almost all qualitative and quantitative traits. Systematic evaluation and screening of germplasm have led to the characterization of specific sources of resistance to biotic stress, tolerance to abiotic stress, photoperiod sensitivity, phenology, shelf life and specific grain characteristics in accordance with market demand (Dwivedi et al. 2012). Indian landraces of pearl millet contribute earliness, high tillering, high harvest index and local adaptation, early flowering and maturity, better tillering and shorter stature. African landraces, on the other hand, have contribute disease resistance, more prominent panicles, larger seed size, larger head volume, higher resistance to diseases, and better seed quality (Manga 2015; Yadav and Bidinger 2008; Yadav et al. 2017). These landraces evolved under natural selection in unfavorable climatic conditions and in cultivation give better results under stress conditions (Ceccarelli and Grando 2002).

The genus *Pennisetum* consists of 80–140 species (Brunken 1977; Clayton and Renvoize 1986). The basic chromosome numbers vary in multiples of $x = 5, 7, 8$, or 9 (Jauhar 1981a, b). The ploidy exhibit variation from diploid to octoploid levels, B chromosomes are frequently reported (Vari et al. 1999), while the occurrence of aneuploids is reportedly somewhat common (Schmelzer 1997). This genus includes species with both sexual and apomictic reproductive behavior, with lifeforms, which vary from annual to perennial types. *Pennisetum glaucum* ssp. *monodii, P. violaceum, P. mollissimum* and *P. ramosum* are diploid with 14 chromosomes and sexual reproductive behavior, all these species have annual life cycle. *Pennisetum mezianum* is also a diploid having 16 chromosomes with sexual and perennial behavior. *Pennisetum purpureum* is tetraploid specie with 28 chromosomes, sexual reproductive behavior and perennial life cycle. Other species with higher ploidy level are *P. setaceum* can occur in either a triploid form with 27 chromosomes or hexaploid form with 54 chromosomes; it is apomictic and perennial in nature. *Pennisetum villosum, P. pedicellatum* and *P. orientale* are tetraploids with 36 chromosomes, *P. villosum* is apomictic but *P. pedicellatum* and *P. orientale* are sexual and all are perennial. *Pennisetum squamulatum* is a hexaploid with 54 chromosomes and apomictic reproductive behavior and a perennial life form (Dwivedi et al. 2012; Martel et al. 1997). Pearl millet has a wide range of genetic variability for drought and heat tolerance (Arya et al. 2014).

The genetic relationship between species is the basis for gene pool classification of pearl millet and its wild relatives (Harlan and de Wet 1971). It was later extended to the concept of *complex of species* (Pernes 1984) based on crossing possibility, cross fertility and gene transfer complexity to cultivated *Pennisetum glaucum*. This classification includes primary, secondary and tertiary gene pools (Table 5.2).

Table 5.2 Classification of pearl millet based on the gene pool

Gene Pool	Ploidy	Species	Crossability	Hybrid Behavior	References
Primary gene pool (GP1)	Diploid ($2n = 2x = 14$, AA)	*Pennisetum glaucum* ssp. *glaucum*	Easily crossed	Fertile hybrids with normal chromosome pairing	Dujardin and Hanna (1989), Robert et al. (2011)
		P. glaucum ssp. *monodii*: 2 ecotypes, *P. violaceum, P. mollissimum*			
Secondary gene pool (GP2)	Tetraploid ($2n = 4x = 28$, A9A9BB)	*P. purpureum* Schum.	Easily crossed	Highly sterile hybrids	Dujardin and Hanna (1989)
	Octoploid ($2n = 8x = 56$)	*P. squamulatum*	Easily crossed	Highly sterile hybrids	Dujardin and Hanna (1989), Kaushal et al. (2008)
Tertiary gene pool (GP3)	Various ploidy levels	*P. flaccidum*, *P. mezianum, P. setaceum, P. squamulatum*, *P. polystachyon*, *P. pedicellatum*, *P. orientale*	Difficult (*in vitro* or complex hybrid bridges)		Dujardin and Hanna (1989)

Based on morphological variations, pearl millet (*Pennisetum glaucum*) is classified into five different sections (Stapf and Hubbard 1934). These are *Penicillaria* (tropical Africa and India)*, Brevivulvula* (pan-tropical), *Gymnothrix* (pan-tropical), *Heterostachya* (Northeast Africa), and *Eupennisetum* (tropical and subtropical Africa and India), which covers various species. Pearl millet belongs to the *Penicillaria* section. Section *Penicillaria* represents species with seven basic chromosome numbers and comprises cultivated pearl millet (*P. glaucum*) and napier grass (*P. purpureum*). Other sections comprise 22 species in *Gymnothrix*, 5 in *Pennisetum,* and 3 each in *Heterostachya* and *Brevivulvula* (Pattanashetti et al. 2016). The cultivated pearl millet is also classified based on characteristic seed shapes into 4 races: *typhoides, nigritarum, globosum* and *leonis* (Brunken et al. 1977) (Table 5.3). These racial differences detected in crops are the results of various independent domestication events (Porteres 1976). The current pattern of variation in seed morphology of the crop is a result of independent domestications at very early migration events, followed by a combination of geographic and ethnographic isolation (Pattanashetti et al. 2016; Rao and de Wet 1999).

Table 5.3 Differences in seed morphology of cultivated races of pearl millet

Race	Distribution	Characteristic feature		
		Caryopses	Grain	Inflorescence shape
typhoides	India, northern to southern Africa (Senegal to Ethiopia)	Obovate; an obtuse and terete appearance from frontal and cross-section views respectively	Shorter and enclosed by floral bracts	Cylindrical rarely elliptical
nigritarum	From western Sudan to northern Nigeria	Angular in cross-section with three and six facets	Grains apex is usually truncate with a purple tinge, and mature grain is elongated and protrudes beyond the floral bracts	Candle
globosum	Central Burkina Faso to western Sudan (Nigeria, Niger, Ghana, Togo and Benin)	Spherical with an equal cross-section, otherwise terete and obtuse	Large-seeded grain and depth always exceeds 2.4 mm	Candle
leonis	Sierra Leone, infrequently observed in Senegal, southern Mauritania, and the hilly areas of southern India	Oblanceolate and terete with acute apex due to remnants of the stylar base	Elongated and nearly oblanceolate from all lateral perspectives. One-third of the grain protrudes beyond the floral bracts at the time of maturity	Candle

5.3.1 Germplasm Characterization

Characterization of germplasm diversity utilizes phenotypic (morphological) screening in conjugation with biochemical (isozyme) and molecular markers. An array of molecular markers are being used for fingerprinting such as restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD), microsatellites, amplified fragment length polymorphism (AFLP), sequence tagged site (STS), expression sequence tags (EST), single nucleotide polymorphism (SNP), insertion and deletion (InDel), conserved-intron scanning primer (CISP) and diversity array technology (DArT). Molecular markers are more efficient, precise and reliable in the characterization of genetic diversity at the DNA level.

5.3.1.1 Pearl Millet Inbred Germplasm Association Panel (PMiGAP)

ICRISAT created the Pearl Millet Inbred Germplasm Association Panel (PMiGAP) for diversity analysis, cultivar characterization and association mapping for essential traits. It constitutes 250 inbred lines, representative germplasm cultivated from Africa and Asia to capture extensive genetic diversity. Study of this global collection revealed six subpopulations within PMiGAP associated, primarily with lineage or similar agronomic traits (Sehgal et al. 2015). To analyze diversity and to genotype genetic structures, SSR and CISP markers were employed. To study yield and yield components, and morpho-physiological traits, SNPs and InDels were used for phenotyping and genotyping. Extensive characterization of selected germplasm traits was achieved, as discussed below.

5.3.1.2 Agronomic and Morphological Traits

Characterization of accessions for different morpho-agronomic traits using the descriptors for pearl millet developed by IBPGR and ICRISAT (1993) showed sizeable phenotypic diversity for quantitative traits. Diversity for qualitative traits was assessed for traits such as days to flowering, photoperiod response, plant height, number of tillers, panicle shape and length, seed shape, seed color, seed texture, seed and green fodder yield (Manga 2015; Pattanashetti et al. 2016; Upadhyaya et al. 2007). Characterization of *Pennisetum* spp. is common for different purposes, such as *P. schweinfurthii* for bold grain size and waxy coating, *P. purpureuma* and *P. clandestinum* for forage and pasture, *P. setaceum* and *P. villosum* for ornamental utility and *P. hohenackeri* for thatching and rope making (Manga 2015).

5.3.1.3 Abiotic Stress Tolerance

A multifarious array of germplasm collection consisting of traditional landraces, breeding lines and commercial varieties represent a potential reservoir for drought, salinity and heat tolerance. Phenotypic screening can identify tolerant germplasm. Traditional landraces are highly adapted to drought stress, as they reportedly produce significantly higher yields of biomass, grain and stover than commercial cultivars (Yadav 2008). Reports of landraces with plant types having profuse tillering and small panicles produced higher grain yield than the plant type having low tillering and large panicles, under severe moisture stress (Van Oosterom et al. 2006). A changing climate is making the agriculture sector vulnerable and there is an immediate need to develop genotypes better adapted to the changes predicted to occur.

Drought The key factor for decreasing productivity is drought. Under severe drought conditions, grain yields decrease by about 50% in pearl millet (Bidinger et al. (1987). During a drought, there is a time reduction in the grain-filling period

resulting in reduced grain size and decreased grain numbers due to grain abortion (Arya et al. 2010). Early drought can result in total crop failure. Various lines tolerant to drought are CZMS 44A (landrace 3072), IP 8210 landraces 220, 184, 235, 238, CZP 9802, 863B, ICMP 83720, ICMV 9413, ICMV 94472 and PRLT 2/89-33 (Dwivedi et al. 2010; Kusaka et al. 2005; Manga and Yadav 1997; Yadav 2004).

Heat Stress Heat stress occurs when the temperature is temporarily high enough to cause irreversible damage to the functioning and development of the plant. Soil temperatures >60 °C have occasionally been recorded in India and Africa (Peacock et al. 1993; Singh 1993; Soman et al. 1981; Yadav et al. 2006, 2013). Heat stress causes biochemical and physiological changes in plants hindering their growth and ultimate loss in production. The observed and most marked effect of heat stress on crop establishment and yield is mostly at the seedling and reproductive stages. Field screening techniques for heat tolerance include the seedling thermo-tolerance index (STI) (Peacock et al. 1993) and seed to seedling thermo-tolerance index (SSTI) (Singh 1993; Yadav et al. 2006, 2011, 2013). Yadav et al. (2009a, b, 2013) suggested the membrane thermo-stability test (MTS), a laboratory technique to screen large numbers of genotypes or germplasm accessions. Yadav et al. (2013) also suggested field-based STI and SSTI screening indices to evaluate varieties before release. Accessions IP 3201 (Howarth et al. 1997) and IP 19877 (Gupta et al. 2015) are characterized as heat tolerant at seedling stage. Yadav et al. (2009a, b, 2013, 2014a, b) screened 46 genotypes of pearl millet for supra-optimal temperature tolerance at early seedling stage. The genotype H77/833-2 showed the highest heat tolerance followed by 99HS-18, CVJ-2-5-3-1-3, 96AC-93, Togo-II, G73-107 and (77/371 × BSECT CP-1).

Yadav et al. (2014a) observed partial dominance for STI and ear length. Epistasis was found to be absent for STI and SSTI. The additive component of variance (D) had a significant presence for STI and SSTI stress environments. The ratio denotes the number of gene groups exhibiting dominance. The value of h2/H2 indicated one gene group responsible for STI and SSTI (Yadav et al. 2014a). Yadav et al. (2011) demonstrated that the non-additive (dominance) component was more prominent for heat tolerance and suggested heterosis breeding could be successful. Whereas, narrow sense heritability and additive genetic variance for STI and SSTI also indicated good chances of effective selection (Yadav et al. 2012). Genotypes CVJ 2-5-3-1-3 and (77/371 × BSECT CP 1) were identified as the best general combiners for both STI and SSTI, the heat tolerance indices.

Salinity It is the condition when excessive salts in the soil or water stops or limits the plant development or growth. Salinity affects metabolic processes like protein synthesis and photosynthesis. It causes osmotic stress and ionic toxicity that decreases the plant productivity by inhibiting seed germination, plant growth and yield (Hasanuzzaman et al. 2013). Substantial diversity for salinity tolerance among pearl millet germplasm have been reported in accessions 93613, KAT/PM-2, Kitui, Kitui local, MH 333, PNBH-1, GHB 100, MBH 142, MBH 131, MBH 139, ICH

451, 93612; 10876, 10878, 18406, 18570; IP 3757, 3732, Birjand pearl millet, ICMV93753, ICMV 94474; 863-B, CZI 98-11, CZI 9621, HTP 94/54, ICMB 02111, ICMB 94555, ICMB 95333, ICMB 00888, ICMB 01222, ICMP 451, IP 3732, IP 3757, IP8210, PRLT 2/89-33, IP 6112; IP 3616, 6104, 6112 and ZZ ecotype by various researchers (Ali et al. 2004; Ashraf and McNeilly 1992; Chopra and Chopra 1997; Dua and Bhattacharya 1988; Dwivedi et al. 2010; Esechie and Al-Farsi 2009; Kafi et al. 2009; Krishnamurthy et al. 2007; Nadaf et al. 2010; Radhouane 2013).

5.3.1.4 Biotic Stress Tolerance

Most often, germplasm collections are evaluated for material resistant to major diseases. Phenotypic screening helps identify breeding lines, varieties and landraces which possess genes for abiotic resistance. Major prevailing abiotic stresses in pearl millet are downy mildew, smut, ergot and rust. Certain germplasm is also reportedly a source of resistance to multiple diseases (Dwivedi et al. 2012). Described below are sources of resistance to major diseases.

Downy mildew *(Sclerospora graminicol)* Downy mildew is systemic in nature and infects young plants. During severe cases infected plants become chlorotic and do not produce tillers. The most striking symptom of downy mildew is green ear in which floral parts are converted into leafy structures. A light drizzle and cool weather are favorable to downy mildew infestation accompanied by a temperature range of 15–25 °C and relative humidity above 85%. Oospores are highly viable and remain viable for up to 10 years in soil causing primary infection in host plants. Resistant cultivars for downy mildew include: several accessions of *Pennisetum schweinfurthii*, IP 2696; P 310, 472, 1564, 700516, D322/1/-2-2, P 1449-3, P 8695-1, 8896-3, 3281; DMRP 292 (IP 18292); ICML 12, 13, 14, 15, 16 and 22; IP 16438 and 16762; P 310-17 and P 1449-3; IP18292; IP18293; 700651, ICMP 312, 423 and 85410; 7042S; 841A; IP 9, 55, 104, 253, 262, 336, 346, 498, 545 and 558; Gwagwa, PS 202 and landraces such as Ardi-Beniya Ka Bas, Dhodsar local and Desi Bajri-Chomu (Khairwal and Yadav 2005; Sharma et al. 2007, 2015; Singh and Navi 2000; Singh and Talukdar 1998; Singh et al. 1988, Singh 1990; Thakur et al. 2006; Wilson et al. 2004, 2008).

Ergot *(Claviceps fusiformis)* The first ergot epidemic was reported in 1957. Estimated losses due to ergot are 58–70% in hybrids. Infested grain when consumed by humans causes nausea, vomiting and may prove fatal in extreme cases. The causal fungal organism develops in the ovaries after they have infected the florets, and produce a pink or red colored sweet liquid called honeydew. Pollen and anther sacs often adhere to honeydew. Sclerotia are dark-colored hard structures that develop from infected florets initially dark at the tip and then turning completely black. Varieties resistant to ergot are: ICML 1, 2, 3, 4, 5, 6, 7, 8, 9, 10; ICMA 92666 and ICMB 92666, ICMA 91333, 91444 and 91555; ICMPE 13-6-30, 134-6-9, 134-

6-34, 13-6-27, 37 and 71 (Khairwal and Yadav 2005; Rai et al. 1998a; Thakur and King 1988a; Thakur et al. 1982, 1992; Willingale et al. 1986).

Rust *(Puccinia substriata)* Initially, the distal surface of leaf is commonly infected, then the fungal bodies (sori) spread to both sides of the leaf. There is pre-mature drying of leaves and substantial reduction in production when there is rust attack during seedling stage. Its negative effect on yield depends on its severity and crop growth stage. Resistant varieties to rust are: 2696-1-4; landrace 192; ICML 5, 6, 7, 8, 9 10, 17, 18, 19, 20, 21, Tift 3 (PI 547035), Tift 4 (PI 547036), Tift 89D2, Tift 65 (*monodii*), Tifleaf 37042-1-4-4, IP 8695-4, 700481-27-5 (Andrews et al. 1985; Bourland 1987; Burton and Wilson 1995; Hanna and Wells 1993; Hanna et al. 1985, 1997; Pannu et al. 1996; Singh 1990; Thakur and King 1988a; Wilson and Burton 1991; Wilson et al. 1989a). GP1 wild species, *Pennisetum glaucum* ssp. *stenostachyum* and GP2/3 *P. schweinfurthii* are a good source for rust resistance (Pattanashetti et al. 2016; Singh and Navi 2000).

Blast *(Pyricularia grisea)* Blast appears as lesions on foliage; they are diamond or elliptical in shape. These lesions are gray when fresh and turn brown on drying. Blast is a serious disease in pearl millet, affecting both forage and grain production. Various varieties resistant to blast are: Tift 85D2B1 (*monodii*); landrace 122, 162, 192; accessions 36, 41, 46, 71, IP 7846, 11036, 21187 (resistant to 4 pathotypes) (Hanna et al. 1987; Sharma et al. 2013; Wilson et al. 1989b). *Pennisetum glaucum* ssp. *stenostachyum* also is a source of blast resistance. (Pattanashetti et al. 2016; Singh and Navi 2000).

Smut *(Moesziomyces penicillariae)* Smut is transmitted by seed in pearl millet during maturity, turning brown, and releasing brown teliospores. Smut is reported from India, Pakistan and several countries in Africa. Commercial cultivation of hybrids is the main reason for increased severity in smut. Using resistant cultivars is an easy way to control smut. Various genotypes resistant to smut disease are *Pennisetum glaucum* ssp. *monodii,* SSC 46-2-2-1, SC 77-7-2-3-1, SSC 18-7-3-1; ICMV 8282, ICMV8283; ICMA 88006A and ICMA 88006B; landrace 133, 224, 192; SSC 46-2-2-1, SC 77-7-2-3-1, SC 18-7-3-1, ICMA 91333, 91444 and 91555; ICML 5, 6, 7, 8, 9,10; ICMPS 100-5-1, 700-1-5-4, 900-1-4-1, 900-3-1, 900-9-3, 1300-2-1-2, 1400-1-6-2, 1600-2-4, 1500-7-3-2, 1800-3-1-2, 2000-5-2; SSC FS 252-S-4, ICI 7517-S-1, ExB 132-2-S-5-2-DM-1, ExB 46-1-2-S-2, ExB 112-1-S-1-1 and P-489-S-3 (Khairwal and Yadav 2005; Rai et al. 1998b; Thakur and King 1988b; Thakur et al. 1986; Wilson et al. 1989a; Yadav and Duhan 1996).

Striga (*Striga hermonthica***)** Striga is a root parasite, which draws out nutrients from pearl millet plants. There are many species of striga. Striga found in India often has white flowers, but pink and purple-colored striga are also found. Striga can decrease the yield by 30–60%. Varieties with resistance include: Serere 2A9, 80S224, P2671, P2950; uduma-Chad; PS 202, 637, 639, 727 (Gworgwor 2001; Roger and Ramaiah 1983; Wilson et al. 2004). Shibras (*Pennisetum stenostachyum*)

mimics cultivated plants in floral and vegetative morphologies and shows resistance to *Striga* under field condition (Parker and Wilson 1983).

5.3.2 Research Initiatives to Combat Global Climate Change

Global climate change requires breeding to make pearl millet more resilient and adaptive to a new changing environment. Research needs to focus on ensuring sustainability in food grain production and to provide nutrition security. This change also introduces new pests, diseases and change in cropping patterns. Pearl millet is a crop for the future due to its ability to tolerate heat and drought conditions. India's mean surface temperature may increase by 3–5 °C by the end of this century, especially affecting northern parts of the country (Rupa et al. 2006). Rao and Poonia (2011) revealed that in arid regions the annual water requirement for pearl millet is 308–411 mm.

Research should be focused on a future when there will be a temperature surge. Varshney et al. (2017) studied the pearl millet genome by using the whole genome shotgun and BAC sequencing. The latest sequencing techniques such as Bionano Genomics optical mapping are available to obtain chromosome level assembly. Pucher et al. (2018) generated a linkage map based on single nucleotide polymorphisms using mapping population and genotyping by sequencing. Mohammed and Hamza (2018) evaluated the genetic variation in different accessions using morphological descriptors and random amplification of polymorphic DNA.

Sustainable food grain production requires the development of cultivars that are photo-insensitive, extra early for a shortened moisture availability period, tolerant to high temperature, salinity and water stress.

5.3.3 Genetic Resources Conservation Approaches

Germplasm conservation employs two basic approaches: in situ and ex situ conservation. In situ conservation involves growing and conserving the germplasm in its natural habitat allowing the evolutionary process to continue, while ex situ conservation includes gene banks (seed, plant or field, shoot-tips, cells, organs and DNA) (Singh 2017).

5.3.3.1 In Situ Conservation

In situ conservation includes farmers' fields, community seed banks and living gene banks. Farmers' fields generally represent marginal areas characterized by a complex combination of stresses and therefore serve as an important source of genes for these traits. Community seed banks refer to seed collection, maintenance, and

administration by the local communities. NGOs promote living gene banks by maintaining local germplasm collections, such as that of the Swaminathan Foundation in India, CONSERVE in Mindanao and SEARICE in Bohol (Manga 2015). Various landraces from India like Jakhrana, Alwar, Kanpur local, Babapuri are maintained at ICRISAT. Under harsh conditions, improved cultivar performance has not been superior, so the attitude toward landraces has become more favorable (Weltzien and Fischbeck 1990).

5.3.3.2 Ex-Situ Conservation

Several gene banks are conserving and maintain cultivated and wild pearl millet germplasm. A worldwide collection of 56,580 accessions in 70 gene banks of 46 countries are available; some are listed in Table 5.4 The largest gene bank collection is held by ICRISAT in India, comprising 23,841 pearl millet accessions from 51 countries (http://genebank.icrisat.org/). The most considerable part of pearl millet germplasm is comprised of landraces, followed by breeding material and wild relatives.

Table 5.4 List of some significant institutes having more than 1000 pearl millet accessions, in descending order

Institute	Website
International Crop Research Institute for the Semi-Arid Tropics (India)	www.icrisat.org/
National Bureau of Plant Genetic Resources (India)	www.nbpgr.ernet.in/
Embrapa Milho e Sorgo (Brazil)	https://www.embrapa.br/milho-e-sorgo
Institut Francais de Recherche Development en Cooperation	https://en.ird.fr/
Plant Gene Resources of Canada (Canada)	http://pgrc3.agr.gc.ca/index_e.html
International Crops Research Institute for Semi-Arid Tropics (Niger)	http://www.icrisat.org/tag/niger/
Serere Agriculture and Animal Production Research Institute (Uganda)	https://www.genesys-pgr.org/wiews/UGA001
Plant Genetic Resources Conservation (USA)	https://www.ars.usda.gov/southeast-area/griffin-ga/pgrcu/
Institut national de la recherche agronomique du Niger (Niger)	www.gfar.net/organizations/institut-national-de-recherche-agronomique-du-niger
National Plant Genetic Resources Centre (Namibia)	http://www.nbri.org.na/sections/npgrc
Plant Genetic Resources Program (Pakistan)	http://www.parc.gov.pk/index.php/en/pgrp-home

5.4 Cytogenetics

Cytogenetics is the branch of genetics concerned with the study of inheritance in relation to the structure and function of chromosomes. Cytogenetics studies chromosome numbers, location of genes on chromosome and behavior of chromosomes in various processes. Rau (1929) first determined the somatic chromosome number of pearl millet as 2n = 14, while Reader et al. (1996) first used fluorescence in situ hybridization (FISH) to characterize the somatic complement of pearl millet. Pearl milled has seven large chromosomes (Jauhar and Hanna 1998; Rau 1929). Karytope measurements of an inbred I-55 of pearl millet recorded by Tyagi (1975) revealed that the seven pairs of chromosomes were numbered 1–7 according to their descending total length, chromosome 1 being the longest (5.51 μ) and chromosome 7 being the shortest (3.24 μ). The shortest chromosome was the SAT chromosome (Pantulu 1960). Furthermore, the seven chromosomes of the haploid set were classified as median (chromosomes 1, 2, 3 and 5), sub-median (chromosomes 4 and 6) and sub-terminal (chromosome 7). Most lines of pearl millet have one pair of nucleolus-organizing chromosomes (Pantulu 1960). The somatic karyotype of pearl millet (2n = 14) consists of metacentric, sub-metacentric and acrocentric pairs of chromosome which are 2 or 3, 2 or 3 and 1 or 2 in number respectively (Pantulu 1961). Some African origin varieties have 1 or 2 of the longer pairs that reveal secondary constrictions in their long arms. The shortest chromosome pair of the complement carries the nucleolar organizer in the short arm. B-chromosomes occur only in pearl millet populations of African origin. These B-chromosomes exhibited nucleolus-organizing activity (Burton and Powell 1968; Pantulu 1960; Powell and Burton 1966).

Several investigators recognized haploid plants in pearl millet (Gill et al. 1973; Jauhar 1970; Manga and Pantulu 1971; Pantulu and Manga 1969; Powell et al. 1975). However, occasional haploid plants are observed among twin seedlings produced by polyembryonic caryopses (Manga and Pantulu 1971; Pantulu and Manga 1969; Powell et al. 1975). Triploidy in pearl millet is induced using mutagens viz. X-ray (Krishnaswamy and Ayyangar 1941), gamma ray (Pantulu 1968) and colchicine treatment (Manga 1972). Several researchers reported induction of tetraploidy by colchicine treatment (Gill et al. 1966; Jauhar 1970; Minocha et al. 1972; Krishnaswamy et al. 1950) and gamma ray (Singh et al. 1977). Spontaneous origin of tetraploids also occurs (Hanna et al. 1976; Koduru and Rao 1978; Powell and Burton 1968). Primary monosomic (Gill et al. 1970a; Jauhar 1970; Manga 1972; Virmani and Gill 1971), secondary (monotelodisomic – the plant lacking one arm of a chromosome, i.e. monosomic for one arm) (Pantulu et al. 1976) and tertiary (Koduru et al. 1980) trisomics have been reported in pearl millet. In addition to the primary trisomics, double trisomics (2n + l + l) (Gill and Virmani 1971a; Gill et al. 1970b; Manga 1972; Pantulu and Rao 1977b) triple trisomics, tertiary trisomics (Gill and Virmani 1971b; Minocha et al. 1974; Pantulu and Rao 1977a; Tyagi 1975; Venkateswarlu and Mani 1978) and interchange trisomics (Laxmi et al. 1975;

Manga 1977; Minocha and Brar 1976; Narasinga Rao and Narayana Rao 1977) were also recorded.

There are many reported investigations of wide interspecific hybridization in pearl millet. Recently, intergeneric wide hybridization between oats (*Avena sativa* L.) and pearl millet (*Pennisetum glaucum* L.) showed retention of all seven pearl millet chromosomes besides the genome of oat during embryogenesis. In general, such distant relative species show uniparental chromosome elimination after successful fertilization. However, hybrid seedlings developed but showed necrosis after light irradiation (Ishii 2017).

5.5 Conventional Breeding

Introduced breeding stock may be used in several ways which includes (1) growing it en mass directly as a new variety, (2) selection from the introduced material to produce a new variety and (3) using introductions as parents to transfer desirable genes for resistance to disease, drought, lodging or quality and other desirable agronomic characters into selected lines. From several African introductions, selections among them led to the development of Jamnagar Giant, Improved Ghana and Pusa Moti (Joshi et al. 1961; Krishnaswamy 1962; Murty 1977). The variety S 530 known from its African parentage for bristling, which is a defense mechanism against birds (Athwal and Luthra 1964). The introductions of cytoplasmic male sterile lines Tift 23A and 18A from the USA (Burton 1983) led to the development of pearl millet hybrids in India.

5.5.1 Mass Selection

The mass selection procedure is one of the oldest and simplest selection methods. It is a useful technique of improving characters with high heritability and utilized to improve such characters. This method only involves selection for the female source of gametes, as there is no control over the male gametes due to random pollination. Selection for the male gamete is also feasible for pre-flowering traits like selection for tillering ability, plant type and downy mildew resistance. In this case, before flowering occurs unwanted plants are culled in order to pollinate the selected plants by selected male gametes (Gill 1991). The population PCB 101 is an example of mass selection subjected to improvement for resistance to ergot and better yield, at Ludhiana and Faridkot, India, during 1983; other examples are T 55, Co 1 and AKP 1 from India (Rachie and Majumdar 1980). Increases in pearl millet grain and forage yields per cycle of selection have been small and progress slow.

5.5.2 Population Improvement

5.5.2.1 Recurrent Restricted Phenotypic Selection (RRPS)

RRPS is a modification in mass selection to increase its efficiency, proposed by Burton (1974), which is less resource consuming than the other procedures, which demand extensive progeny testing. Similar to mass selection, the phenotype is the basis of screening and therefore it is only valid when plants are space planted for the complete expression of their phenotype. Only females are selected, as males are expected to contribute no genetic advance. The rate of advance can be doubled by restricting the source of pollen. Cultural practices are restricted for the provision of a consistent environment for each space-planted phenotype. To adjust soil heterogeneity effects, selection within grids is practiced which further accentuates the efficiency of RRPS, which is commonly known as the grid method of mass selection.

5.5.2.2 Composite Varieties

Composite varieties are created by pooling germplasm having one or more desirable characters in common. To develop these varieties GCA testing is not done and the population cannot be re-synthesized. Some examples of composites are: WC-C75 which is developed by crossing seven full-sib progenies of World Composite similarly HC 4 was developed by eight component lines of World Composite. Various varieties developed through this breeding method are shown in Appendix II.

5.5.2.3 Synthetic Varieties

Synthetic varieties result from pooling several sources of germplasm that are tested for their general combining ability (GCA) and have one or more desirable characters in common. Except for the desired traits, much diversity among component lines is preferred to widen the genetic base, maximize heterozygosity and yield. Crossing continues until each germplasm enters into the final cross in equal frequency. Synthetics offer a rapid procedure for developing improved populations that maintained by open-pollination and reconstituted as needed. In highly cross-pollinated crops like pearl millet, the first synthetic generation developed from inbred lines carries a high proportion of single crosses, which exhibit considerable heterosis, so they usually yield more than stabilized synthetics, achieved after several generations of increase in isolation. Therefore, only stabilized synthetic should be tested before release to the public. Synthetics may be more desirable than hybrids in low-income areas as it would not be necessary for the farmer to purchase new seed every year. Starr is a popular synthetic variety of pearl millet, which was developed for forage production in Georgia (Hein 1953), Burton and Powell (1968)

developed Tiflate and ICMS 7703 is a result of a crossing between seven inbred lines of India × African crosses.

5.5.2.4 Recurrent Selection

This method helps increase the frequency of desirable genes through the recombination and selection cycles. When the ultimate goal is the improvement of the variety or population itself, only one population is subjected to recurrent selection. However, when the principal objective is the improvement of hybrids, it is necessary to develop complementary pairs of populations for reciprocal recurrent selection. In pearl millet, the complementary populations are often a B population of maintainer lines and an R population of restorer lines.

ICRISAT released ICMV 1 in 1982 which was bred from a World Composite from Nigeria by full sib recurrent selection; 441 full-sib families were tested and 7 full-sib families were selected for downy mildew resisrance and selfed at screening nursery. This variety is tall medium in stature and matures in 85–90 days producing bold grains having a slate gray color and with resistance to downy mildew.

ICMV-88908 developed by ICRISAT is under cultivation in Namibia as Okashana-1. Two varieties developed from Togo germplasm (ICTP 8203 and ICTP 8202) and two varieties closely related to them were chosen as parents. After production of C3 cycle by intermating, selfing was done in the half-sib and S_1 progeny test was performed. Out of progenies obtained 65 were selected for recombination. In the final generation mass selection done, harvest is bulked to form the variety. Maternal ancestry is maintained for the minimum inbreeding. This variety is obtained after eight generations of recurrent selection.

5.5.3 Inbred Development and Apomixis

Inbred development is an important step for hybrid production, achievable either by selfing of heterozygous lines or by doubling of haploids. The inbred lines developed to produce homozygous inbred lines in pearl millet; pollination is controlled by bagging (Fig. 5.4) spikes in successive generations. The vigor declines with selfing as defective genes are uncovered, and the segregants containing the defective genes eliminated. Evaluation of inbred lines is done for its performance in hybrid combination with other inbreds by two methods, as described below.

5.5.3.1 Top Cross Method

Davis (1927) invented the top cross method; it is a cross between inbred and OPV. This method tests the performance of an inbred line for its general combining ability (GCA) at multiple locations. The line exhibiting the best characters is

Fig. 5.4 Bagging in Pearl millet for conventional breeding

Fig. 5.5 Selfing in pearl millet for inbred development

selected. Testing for general combining ability is conducted from third, fourth and fifth selfed generations (Fig. 5.5).

5.5.3.2 Single Cross Method

The lines or inbreds selected from the top cross method are tested for their specific combining ability by this method. Selected lines are crossed in all possible combinations (Fig. 5.5) and crosses are evaluated in multi-location trials. After superior

lines of hybrid are identified they are multiplied for seed production. The best crosses are identified and released as varieties. Kanfany et al. (2018b) evaluated 99 inbred lines of 7042S and Sosat C88 as checks for downy mildew susceptibility and resistant checks. A total of 55 lines were resistant to downy mildew including Sosat C88 and 20 lines among the 55 different inbred lines were reported disease free.

The developed inbreds serve as parent lines in a hybrid millet-breeding program. After six or seven generations of inbreeding, male-sterile plants that will not produce seed during inbreeding will be lost, and reasonable genetic stability will be reached. Inbred lines are also helpful in providing various genetic background information for apomixis. Some inbreds of pearl millet with characters are listed in Table 5.5.

Table 5.5 Some inbreds of pearl millet developed for hybrid breeding

Inbred	Key character
81 A/B	Leaf and leaf sheath hairy, midrib dull white, nodal hair absent
843 A/B	Pigmented node, tip sterility present
842 A/B	Nodal hair absent, nodal color green, prominent tip sterility
ICMP 451	Bristled ear
ICMP 423	Leaf and leaf sheath non-hairy, anther color cream, tip sterility present
H90/4-5	Oblanceolate ear, anther color cream, no tip sterility, dwarf, stay-green, compact lanceolate ear head, good combiner
H77/833-2	Anther color yellow, no tip sterility, heat tolerant at early seedling stage
G73-107	Profuse tillering, narrow leaves, typical orange brown anthers and resistant to major diseases and pests, gray grain colored, heat tolerant at early seedling stage
CVJ-2-5-3-1-3	Resistant to downy mildew, heat tolerant at seedling stage
96AC-93	Supra optimal temperature tolerant at seedling stage
Togo-II	Tolerant to high temperature at seedling stage
99HS-18	Tolerant to heat at early stages of plant development
ICMA94222	White grain color
ICMA 89111	Gray grain color
HMS 36B	White grain color
CSSC-46-2	Medium late maturity, stay-green type, bold grain, compact ear head and resistant to downy mildew and blast
H77/29-2	Medium dwarf, nodal pigmentation – violet brown, resistant to downy mildew, shoot fly, gray weevil, chaffer beetle and leaf
	Roller
HTP-94/54	Nodal pigmentation – light violet, bold grains with whitish gray grain color, very tall, resistant to downy mildew and tolerant to moisture stress
ICMR-01004	Moderately susceptible to rust
ICMR-01007	Resistant to rust
1250	Stay-green type with resistant to downy mildew and blast

5.5.3.3 Apomixis

Apomixis is induced in pearl millet by mutation, as it does not occur naturally (Hanna et al. 1992). Apomixis can replace cytoplasmic male sterility (CMS) lines for producing hybrids. Apomixis is a naturally- occurring mode of reproduction resulting in embryo formation without the involvement of meiosis or fertilization of the egg. Seed-derived progeny of an apomictic plant are genetically identical to the maternal parent. A wild relative of pearl millet, *Pennisetum squamulatum*, an obligate aposporous species, is cross-compatible with pearl millet when used as a pollen donor in the interspecific cross (Ozias-Akins et al. 1998). There are interspecific hybrids produced between sexual pearl millet *P. americanum* and apomictic *P. squamulatum* for transferring apomixis to pearl millet (Dujardin and Hanna 1983). This polyhaploid obtained was morphologically shorter and smaller than its parent was. Polyhaploid obtained was female fertile and male sterile (Dujardin and Hanna 1986). Kaushal et al. (2007) hybridized pearl millet with a new cytotype having chromosome number 2n = 56 which is useful to study of traits like apomixis.

Apomictic hybrids can simplify the commercial hybrid production process. It is an efficient method for the production of new genotypes as transferring apomixes would limit the need of cytoplasmic male sterility. Apomictic hybrids would breed true of heterozygosity. Akiyama et al. (2011) reported that the apospory-specific genomic region is shared by all apomictic species but is absent from all sexual species or cytotypes.

5.5.4 Exploitation of Heterosis

Heterosis, the term coined by Shull (1948), is the phenomenon in which the progeny perform better than their parents are. Heterosis was observed before the discovery of male sterility in pearl millet. Dutt and Bainiwal (2000) evaluated heterosis in pearl millet populations and found the best heterosis from the cross HP8601 × ICMV95778.

Vetriventhan et al. (2008) reported negative heterosis for plant height in the cross ICMA 94111A × PT 5259 and positive heterosis for tillers in the cross ICMA 94111A × PT 5423. Maryam (2015) reported heterosis for yield and resistance to downy mildew in cross PEO5984 × P1449.

Chittora and Patel (2017) evaluated heterosis and obtained positive results in the cross JMSA-9904 × AIB-15 for traits of ear-head girth, grain yield and panicle harvest index. Kanfany et al. (2018a) crossed 17 inbreds with Sosat C88 and Souna 3 by the line × tester design and found positive GCA effect. Standard heterosis for grain yield was found to be maximum in IBL 206-1-1 × Souna 3.

Burton (1958) suggested producing chance hybrids by blending parental lines in 1:1 ratio and allowing them to mate randomly. The seed so produced would be a mixture of crossed, sibbed and selfed seed. These chance hybrids were mixtures of

40% hybrid and 60% parental seeds. However, hybrids thus produced could not become popular because of their narrow adaptability and difficulties of seed production. Hybrids U1US produced in India (Chavan et al. 1955) and Gahi l (Georgia Hybrid No.1) are examples of chance hybrids (Poehlman 1995).

This chance hybrid method allows farmers to harvest more fodder from the F_1 generation. Additional lines are developed to obtain high yield in all combinations. In India, the first attempt at chance hybrids was made by Burton and Powell (1968), using two inbreds; 10–25% more yield was obtained than in open-pollinated cultivars (Burton 1983). In 1965 the first hybrid HB 1 was released (Pray and Nagarajan 2009).

5.5.5 Cytoplasmic Male Sterility (CMS) System and Hybrids

Utilization of hybrid vigor in pearl millet breeding became possible after the discovery of cytoplasmic male-sterility and fertility-restoring genes. The prime use of CMS is its extensive exploitation in hybrid production for grain in India and forage in the United States. The male sterile line Tift 23A from Tifton, Georgia (Burton 1958) signaled the commercial exploitation of heterosis all over the world. Initially identified three sources were identified: Tifton 23A, L 66A and L 67A of cytoplasmic male-sterility available were designated as A_1, A_2, and A_3, respectively, and their fertility restorer genes as Ms_1, Ms_2, and Ms_3, respectively. After that, a few more sources of male sterility were discovered and are presented in chronological order in Table 5.6. The Indian Council of Agricultural Research-Central Arid Zone Research Institute, Jodhpur, India, developed numerous CMS lines. Some of these lines are CZMS 44A and CZMS 47A (Manga and Yadav 1997). A total of 16 more lines are named CZMS 0001A to CZMS 0016A, out of these CZMS $0001A_1$ to CZMS $0007A_1$, CZMS $0013A_1$ and CZMS $0014A_1$ had A_1 cytoplasm, while CZMS $0008A_4$ to CZMS $0012A_4$, CZMS $0015A_4$ and CZMS $0016A_4$ had A_4 cytoplasm (Manga and Kumar 2013). The hybrids are produced by crossing cytoplasmic-male-sterile inbred (A-line) with a male-fertile and fertility-restoring (R-line) to produce the hybrid pearl millet seed. The male-sterile A-line is maintained by pollination from the male-fertile counterpart (B-line). For forage purpose, fertility restoration is unnecessary and undesirable since sterile hybrids will continue vegetative growth for a longer period than hybrids producing seed. While for grain purpose, fertility restoration is essential.

The first pearl millet hybrid using cytoplasmic male sterility was developed in India, HB 1 (Hybrid Bajra No.1), which was a cross between Tift 23 A_1 × BIL 3B by Athwal (1965). Subsequently, four more hybrids were also developed in India, viz. HB 2 (Tift $23A_1$ × J 88), HB 3 (Tift $23A_1$ × J 104), HB4 (Tift 23A × K 560) and HB 5 (Tift 23A × K 559) in 1967, 1968 and 1972, respectively. These five hybrids were based on the Tift 23A line. One of the most popular and widely cultivated hybrids, HHB 67, released in 1989 by Haryana Agricultural University, succumbed to downy mildew. A superior new hybrid HHB 67 improved was developed and

Table 5.6 Various sources of cytoplasmic male sterility (CMS) in pearl millet

CMS system	Source material	Year discovered	Place of discovery	Reference
A_1	Inbred 556	1958	Tifton, Georgia, USA	Burton (1958)
A_2	IP 189	1965	Punjab Agricultural University, Ludhiana, India	Athwal (1965)
A_3	Amber grain stock	1965	Punjab Agricultural University, Ludhiana, India	Athwal (1965)
ex-Bornu	Gero pearl millet	1981	University of Ibadan, Ibadan, Nigeria	Aken'Ova and Chheda (1981)
PT 732A	Landrace	1982	Tamil Nadu Agricultural University, Coimbatore, India	Appadurai et al. (1982)
A_v	ssp. *monodii* × Tiotande	1985	Office de la Recherche Scientifique et Technique, Bondy, France, Gif sur Yvette, France	Marchais and Pernes (1985)
A_4 (A_m)	ssp. *monodii*	1989	Tifton, Georgia, USA	Hanna (1989)
A_{egp}	Gene pools	1994	ICRISAT, Patancheru, India	Sujata et al. (1994)
A_5	Large-seeded gene pools	1995	ICRISAT, Patancheru, India	Rai (1995)

released by the Haryana State Varietal Release Committee in 2005, which was resistant to downy mildew (Khairwal and Hash 2007). Previously, many hybrids have been developed through this method and are extensively cultivated (Appendix II). Today, many hybrid cultivars have been developed worldwide through conventional and modern breeding techniques with better traits like early maturity, higher yield, improved nutritional quality and improved grain quality. Resistant plants to various biotic stress like ergot, downy mildew and smut have been developed. Breeders around the world have developed varieties tolerant to abiotic stress like salinity, drought and heat stress. Many pearl millet hybrids have been developed using various breeding methods. A list of hybrids released in India is provided in Appendix II.

5.6 Role of Biotechnology

Traditional breeding methodologies mainly rely on selection and hybridization. Selection based on phenotype and cross-hybridization is a very time-consuming and tedious process. These methodologies have extensively focused either on grain or fodder yield, which are primarily polygenic traits and require many years for improvement, and hybrids may not combine the desirable characters. Commonly, only one in a few hundred to a thousand crosses exhibits the desired combination. These shortcomings cannot be overcome by traditional approaches alone. Therefore,

Fig. 5.6 Tissue culture of pearl millet showing in vitro seed germination

biotechnological approaches are necessary. Another limitation in pearl millet breeding is that, although being an out-breeding crop, it has only a few known sources of male sterility (Table 5.6). This leaves hybrids developed by the CMS method mainly susceptible to biotic stresses due to common ancestry, leading to a narrowing of the genetic base.

Conventional plant breeding has contributed immensely to improve the crop yield and nutritional quality of pearl millet. Still, neither the existing genetic resources possesses all the requisite traits, nor the traditional approaches are time efficient. Therefore, for target-specific breeding and to speed the breeding process, there is a need for cutting-edge genetic manipulation tools. Several biotechnological approaches such as in vitro tissue culture (Fig. 5.6), protoplast fusion, QTL mapping and genetic engineering have helped to introduce essential traits in pearl millet, like downy mildew resistance, rust resistance, heat tolerance and drought tolerance. A detailed discussion of each method is provided below.

5.6.1 Somatic Cell Hybridization

Somatic fusion, also known as protoplast fusion, is a type of genetic modification in plants. This method involves fusion of protoplasts of two distinct species together to form a new somatic hybrid plant with the characteristics of both the parents. Somatic hybrid cell lines with embryogenic capacity obtained by the fusion of protoplasts were isolated from an embryogenic cell line of pearl millet somatic hybrids. *Pennisetum glaucum*, resistant to S-2-amino-ethyl-L-cysteine (AEC), used for screening hybrids from non-hybrids, were fused with *Panicum maximum*

(Ozias-Akins et al. 1986) and sugarcane (*Saccharum officinarum*) (Tabaeizadeh et al. 1986). The somatic hybrid calli showed both parental homodimer bands plus an intermediate heterodimer band concerning their electrophoretic pattern in both cases. Somatic hybridity confirmed hybridization of a maize ribosomal DNA probe to XbaI (Ozias-Akins et al. 1986; Tabaeizadeh et al. 1986) and EcoRI (Ozias-Akins et al. 1986) digests. Robert et al. (1988) recovered somatic hybrid callus lines from the culture of *Triticum monococcum* with *Pennisetum americanum.* These lines were confirmed by electrophoretic patterns. Singh et al. (2010) reported a favorable increase in pollen viability in BC_8 generations and BC_7 generation were observed with more aposporous embryo sacs. Apospory in pearl millet was transferred from *P. squamulatum* through backcrossing.

5.6.2 Genetic Engineering

Genetic engineering is the process of modification or manipulation in the gene construct of an organism using biotechnological tools. It opens a vast horizon for the creation of a new plant type due to the availability of all the three gene pools, viz. primary, secondary and tertiary, to work with. Transgenic organisms are created by the addition of genetic material from different species to the host. However, cisgenic organisms are resultant from the addition of genetic material of the same species that can naturally breed with the host (Jacobsen and Karaba 2008). There are only a few documented works done in the area of genetic engineering in pearl millet, although O'Kennedy et al. (2011) described an example of biolistic-mediated gene transformation of pearl millet strain 842B. Transgenic plantlets thus produced via somatic embryogenesis, were bipolar structures that formed shoots and roots simultaneously, and rarely regenerated via organogenesis. The stages included propagation of immature zygotic embryos for biolistic-mediated transformation, induction and maintenance of transgenic embryogenic tissue on mannose-containing selection medium, maturation (both morphological and physiological) and germination of the somatic embryos. Jalaja (2011) standardized protocols for transformation via *Agrobacterium* and microprojectile methods in pearl millet. Gene-specific amplification of chitinase, osmotin and bar genes confirmed transformation of the plants. The pollen produced sterile transgenic plants.

5.6.3 Molecular Marker-Assisted Breeding and Functional Genomics

Agriculturally essential characters like yield, quality and some types of abiotic and biotic resistance are quantitative traits. The specific regions within a genome associated with these traits are known as quantitative trait loci (QTLs) (Collard et al.

2005). Discovery of molecular markers (RFLP, RAPD, AFLP and microsatellites, STS, EST, SNP, InDel, CISP and DArT) aided in the development of linkage maps. Linkage maps led to acceleration in the molecular/marker-assisted breeding by identification of chromosomal regions controlling simple and complex trait(s) by cutting down prolonged conventional phenotypic evaluation and selection. The DFID-JIC-ICRISAT project, conceptualized in 1991, aimed to develop molecular markers for advanced pearl millet breeding (Serba and Yadav 2016). A significant milestone in the molecular breeding of pearl millet was the construction of first genetic linkage map (Liu et al. in 1994) using restriction fragment-length polymorphisms (RFLPs) markers. The map contained 181 RFLP markers covering the seven linkage groups corresponding to the haploid chromosome number of pearl millet chromosomes spanning a genetic distance of 303 cM. High levels of polymorphism in the pearl millet genome led to consequent research in mapping and tagging of genes responsible for controlling essential traits.

Creation of a discrete population using a set of molecular markers is of utmost importance for gene mapping and is commonly called a *mapping population*. Primary mapping populations are based on biparental mapping populations developed by crossing two homozygous lines, usually having contrasting alleles for the traits of interest. These are of the following types: second filial generation populations (F_2), backcross populations (BC), backcross inbred lines (BILs), nearly isogenic lines (NILs), recombinant inbred lines (RILs), doubled haploids (DH), chromosomal segment substitution lines (CSSLs) and recurrent selection backcross (RSB) populations. Secondary mapping populations are created by hybridizing two lines selected from the primary mapping population. These populations employ multi-parent allelic diversity and are developed primarily for fine mapping of QTLs for the desirable trait of interest. These are advanced intercross lines (AIL) and are of following types: association mapping populations (AM), nested association mapping populations (NAM), multi-parent advanced generation intercrossing populations (MAGIC) (Singh and Singh 2015). Next-generation sequencing (NGS) ushered in advances in linkage mapping population from the biparental to the secondary multiparent populations (Bohra 2013). Secondary mapping populations come with the advantage of utilizing the resources of both linkage and association mapping in plant breeding (Serba and Yadav 2016). Several QTLs have been discovered in pearl millet while identification of some employed both primary and secondary mapping populations (Table 5.7). Genome-wide association study (GWAS), or whole genome association study (WGAS), is the recent advancement in analyzing genetic sequence. Recently, whole genome sequencing of reference genotype Tift $23D_2B_1$-P1-P5 along with re-sequencing of 994 pearl millet genotypes, including 963 inbred lines and 31 wild accessions revealed 1.79 Gb genome size of pearl millet with about 38,579 genes (Varshney et al. 2017).

Kumar et al. (2016) constructed a linkage map to identify QTLs for iron and zinc in grain using SSRs and DArT markers in RIL populations. Zala et al. (2017) used the transcriptome data from resistant and susceptible downy mildew pearl millet genotypes to develop expressed sequence tag-SSR markers, which can be used for the development of downy mildew resistant varieties. Kumar et al. (2017) mapped

Table 5.7 List of some important molecular markers in pearl millet

Molecular markers	Reference
Development of first BAC library of pearl millet using nuclear DNA	Allouis et al. (2001)
25 SSRs were derived from bacterial artificial chromosomes (BAC) library	Qi et al. (2001)
18 SSRs were derived from the genomic library out which 11 were used to assess genetic diversity	Budak et al. (2003)
Single strand conformational polymorphism (SSCP)-SNP primer pairs were developed by comparison of rice and pearl millet EST sequences	Bertin et al. 2005
Identification of 19 SSR loci	Yadav et al. (2007a, b)
Development of 16 EST-derived polymorphic SSRs	Mariac et al. (2006)
Development and mapping of 21 polymorphic EST-SSRs and 6 genomic SSRs	Senthilvel et al. (2008)
4 EST-SSRs and nine conserved sequence scanning primer (CISPs) were used in detecting polymorphism in one or more of 4 pearl millet biparental mapping populations	Yadav et al. (2008)
Generation of functional 19 ISSR primers for molecular characterization of downy mildew	Jogaiah et al. (2009)
250–280 DArT markers were analyzed for polymorphic in each of 3 pearl millet RIL populations	Senthilvel et al. (2010)
Identification of 258 DArT and 63 SSR marker loci to generate a linkage map	Supriya et al. (2011)
Development of 75 SNPs and CISP markers derived from ESTs using parents of two mapping populations for 18 genes	Sehgal et al. (2012)
More than 100 polymorphic EST-SSR markers developed and mapped using 4 pearl millet RIL populations	Rajaram et al. (2013)
Development of ISSR derived SCAR marker associated with downy mildew resistance linkage group with genetic linkage distance of 0.72 cM	Jogaiah et al. (2014)
92 unique EST were identified for heat stress	James et al. (2015)
37 SSRs and CSIP markers developed, representing all 7 linkage groups analyzed under both well-watered and drought conditions, 22 SNPs and 3 InDels for abiotic stresses	Sehgal et al. (2015)

QTLs in pearl millet for agronomic traits like flowering time, plant height and grain weight in the RIL population of a ICMB 841-P3 × 863B-P2 cross which can be used for genomic and marker-assisted selection.

5.6.4 Bioinformatics

Bioinformatics is the field of science that develops software tools and methods for computing biological data. Gene finding, protein structure prediction, protein structure alignment, genome assembly and prediction of gene expression are

predominate areas in bioinformatics. High throughput methods leading to a many-fold increase in data that must be processed with necessary bioinformatics tools to gather relevant biological information. Various important databases and repositories holding genomic information are available including, Genbank (http://www.ncbi.nlm.nih.gov/genbank), EMBL (http://www.ebi.ac.uk/ embl) and DDBJ (http://www.ddbj.nig.ac.jp) which acts as a general public sequence repository. UniProt (http://www.uniprot.org/) provides protein sequences and functional information. NCBI (http://www.ncbi.nlm.nih.gov/) provides genomical information, The Gene Index Project (http://compbio.dfci.harvard.edu/tgi/) provides information on transcriptome data and GOLD (http://genomesonline.org/cgi-bin/GOLD/bin/gold.cgi) functions as a repository of genome databases. Plantgdb (http://www.plantgdb.org), CropNet (http://ukcrop.net/), Gramene (http://www.gramene.org/) and Phytozome (http://www.phytozome.net/) are plant genomic database (Perez-de-Castro et al. 2012). In 2017, the transcriptomic signature of drought response in pearl millet was first reported using the web-based genomic resource (http://webtom.cabgrid.res.in/pmdtdb/) (Jaiswal et al. 2018). This tool helped in the discovery of candidate gene-based SNP and trait-based association studies. A web-based database https://www.ars-grin.gov/ and https://www.genesys-pgr.org/c/pearlmillet provides information on pearl millet germplasm.

5.7 Mutation Breeding

In conventional breeding, selection is the first step. However, when germplasm does not possess all the requisite variation, mutation serves as the basis for creating additional variation. An array of mutants can be created with novel characteristics, which could not be generated with normal genetic recombination. Therefore, induced mutation can assist in producing a spectrum of genotypes, especially for genetic studies and trait mapping in pearl millet. Mutation can be of two types, spontaneous or induced. Since pearl millet already possesses enormous natural genetic variation, the utility of induced mutations in its improvement is limited. Physical mutagens include both ionizing and non-ionizing radiations X-rays, gamma rays, ultraviolet, laser beams and thermal neutrons. Chemical mutagens reported to induce mutation in pearl millet are ethymethylsulfonate (EMS), ethidium bromide, streptomycin (Burton and Hanna 1976, 1982), mitomycin, N-nitroso-N-methyl urea (NMH) and diethyl sulfate.

5.7.1 Seed Mutagenesis

Seed mutagenesis creates mutants with desirable traits to breed with other lines by treating seeds chemically or exposing them to forms of radiation. Seeds of inbred lines, Tift 23B and Tift 239DB were treated with thermal neutrons, EMS and

diethylsulfate individually or in combination. Some early mutants were recovered in the M_2 generation exhibiting early flowering within 35–50 days after planting as compared with normal lines which bloom in 80–90 days (Hanna and Burton 1978). In Namibia, gamma ray treatment resulted in 11 early maturing advanced mutant lines with better yield, seed shape, large and different colored seed, and tolerance to drought (http://www-naweb.iaea.org/nafa/news/2016-plant-mutation-breeding-namibia.html).

Short-duration pearl millet produce grain with even less water and fit well in multiple-cropping systems. Treatment of 10 pearl millet inbreds with either thermal neutrons or EMS yielded plants with delayed seedling emergence and days to maturity, and reduced seedling height, plant height and leaves per culm (Burton and Powell 1966). That study could help to identify dwarfing genes to reduce plant stature. The reported negative effect of EMS were reduced percent emergence, delayed maturity and chlorophyll deficiency in seedlings (Burton and Powell 1966; Burton et al. 1974). CMS mutants were also recovered from the treatment of EMS (Burton and Hanna 1976), streptomycin and mitomycin (Burton and Hanna 1982). Resistance against downy mildew was induced in 159 MS 5071 B from Tift 23 B, and MS 5071 A, by irradiation of seeds in India for the production of a NHB hybrid series (Kumar et al. 2012). Triploidy and tetraploidy in pearl millet were induced using mutagens such as X-ray, gamma ray and colchicine (Gill et al. 1966; Jauhar 1970; Krishnaswamy and Ayyangar 1941; Krishnaswamy et al. 1950; Manga 1972; Minocha et al. 1972; Pantulu 1968; Singh et al. 1977) for cytological studies.

5.7.2 Molecular Approaches for Mutation Identification

Targeting induced local lesions in genomics (TILLING) is a tool in molecular biology used to identify mutations. McCallum et al. (2000) employed the model plant *Arabidopsis thaliana* for screening of chemically-induced mutations through TILLING which uses ethyl methanesulfonate (EMS) and denaturing high-performance liquid chromatography (DHPLC) for the detection of changes in a base pair by heteroduplex analysis. TILLING (Till et al. 2003) is a novel technology to screen induced mutations while its variant Ecotype TILLING (EcoTILLING) (Comai et al. 2004) identifies allelic variants in natural mutations. Discovery of induced-point mutations is easier and faster with TILLING within mutated individuals. This technique allows high throughout detection of single nucleotide polymorphisms in target genes.

These approaches are based on recognition and removal of mismatched base pairs by an endonuclease, present in the two strands of DNA (Till et al. 2004; Triques et al. 2008). For mutation discovery, a commercial denaturing HPLC apparatus is used in the original tilling method (Henikoff et al. 2004). A TILLING population in pearl millet has been developed to identify specific genes attributing to traits like resistance for downy mildew and rust.

5.7.3 Improved Traits Through Mutation

Improved morphological traits recovered from mutation breeding includes bold grains and reduced plant height, which imparts lodging resistance; earliness which helps in better survival as plants can mature before soil moisture depletion and permits a double-crop system.

Various morphological traits reportedly improve through mutation. Murty (1980) and Raut et al. (1974) treated seeds with gamma rays and induced resistance to downy mildew. Burton et al. (1980) reported increased forage yield of 21.7–38.2% after three cycles of recurrent mutagen treatment. Reports are also in favor of traits affecting reproductive behavior. Krishnaswamy and Ayyangar (1942) reported male sterility in progenies of X-rayed seeds of pearl millet. Hanna and Powell (1973, 1974) reported facultative apomixes and female sterility with aposporous apomictic development. Various qualitative traits that included chlorophyll-deficient mutants reported by Chandola et al. (1963) and dwarfness by Joshi (1968).

CMS has direct practical application in hybrid production in pearl millet breeding and resistance to downy mildew and tolerance to drought (Burton and Hanna 1976, 1982; Burton and Powell 1966; Burton et al. 1974; Kumar et al. 2012). Other agronomic traits are yield, seed shape and color (http://www-naweb.iaea.org/nafa/news/2016-plant-mutation-breeding-namibia.html).

5.8 Grain Quality and Biofortification

Because pearl millet is a rich source of protein and nutrients, efforts are being made to improve the nutritional quality of the grain. Gopalan et al. (2003) reported higher fat content in pearl millet (5–7 g/100 g) than other cereals like wheat, sorghum and rice with balanced amino acids and higher vitamin A (220 I.U./100 g). Khangura et al. (1980) reported 14–63 μg/100 g beta-carotene. ICRISAT (1997) reported high beta-carotene accessions from Burkina Faso, which can increase the beta-carotene content of new varieties.

Pearl millet grains may be grayish white, yellow, brown, cream, ivory, light blue, purple or gray in color (Jain and Bal 1997) and in shape varying from obovate, lanceolate, elliptical, and hexagonal to globular (Arya et al. 2013; IBPGR/ICRISAT 1993). Moreover, white and yellow grain pearl millet (Fig. 5.7) are rich in protein and carotene. White grain color in pearl millet is dominant and simply inherited. Therefore, breeding for grain color through simple selection, mass selection, pedigree selection and the backcross method will be effective (Arya and Yadav 2009). Arya et al. (2009a) concluded that high protein content is genetic property but is highly influenced by environmental conditions. Gray grain colored hybrids exhibited negative correlation for starch and fats, while white grain color hybrids resulted

Fig. 5.7 Pearl millet with gray and white grain color

in significant correlation for starch, in a study conducted by Arya et al. (2009b). Consuming pearl millet can limit the risk of diabetes and aid in weight control due to its low glycemic index (Martins-Dias et al. 2017). Verma et al. (2018) reported a high range of carbohydrates, protein, tryptophan and lysine in pearl millet.

5.8.1 Antinutritional Factors

Pearl millet has high nutrient content but the nutrient bioavailability is low, due to the presence of certain antinutritional factors. One of the antinutrients of pearl millet grains is phytate, with a range of 172–327 mg/100 g (Taylor 2004). Phytate binds multivalent metal ions such as calcium and iron, thereby interfering with their absorption in the gut. However, some fermentation and processing reduces these antinutrients, liberates the nutrients locked into the plant structure, and cells by indigestible materials. Akingbala (1991) reported the presence of the phenolic compounds, C-glycosyl flavones concentrated in the outer layers of the grains and they contribute to the gray grain color (Taylor 2004). Polyphenols limit protein and starch utilization either by binding with proteins or by inhibiting digestive enzymes, especially trypsine and amylase. Yadav et al. (2010) reported a lower concentration (50.87 mg/100 g) of polyphenol in cultivar HTP 94/54.

5.8.2 Rancidity in Flour

Rancidity is hydrolysis of fats and oils when exposed to air, light, moisture or bacterial activity, which results in unpleasant odor and taste. Rancidity reflects the storage ability of pearl millet grain flour and its products. Sharma and Saharan (2006) reported that the rancidity development in pearl millet flour is caused by higher phenol contents and higher peroxidase activity. There is a breakdown of lipid and production of fatty acids and glycerol in hydrolytic rancidity, while in oxidative rancidity, there is oxidation of fatty acid chains. Yadav et al. (2010) reported that fat acidity is not linked with rancidity and found that the total phenols, C-glycosylflavones and peroxidase activity were responsible for rancidity.

5.8.3 Biofortification

The process of improving the nutrient concentration in a crop is termed *biofortification*, achieved by conventional or modern breeding methods. Biofortification is an effective, cheap and long-term sustainable way of providing nutrients to people who cannot afford a fully balanced diet and malnutrition is a serious problem. It can provide nutritional security to dwellers of arid and semiarid regions where pearl millet is the daily diet (Pfeiffer et al. 2018).

ICRISAT is developing high-yielding iron and zinc-rich varieties under the Biofortification Platform Initiative of the Indian Council of Agricultural Research and Harvest Plus (Yadav and Rai 2013). It is a global effort to curb micronutrient deficiency by enriching staple food grains. Biofortified iron and zinc rich varieties are expected to reach 28 million people in India within a decade, which is its major target. ICRISAT released biofortified variety Dhanshakti along with Mahatma Phule Krishi Vidyapeeth in 2012 and in 2018 a biofortified pearl millet variety Chakti in Africa to help fight populations with anemia. Chakti has 20% more iron than average requirement. Chakti is popular among farmers in Africa as it matures earlier and yields 30% more than their local landraces. The crossing of pearl millet with its secondary gene pool napier grass (*Pennisetum purpureum*) has high forage potential. Similarly, *P. squamulatum,* an obligate apomict, offers a beneficial potential of fixing heterosis in the future.

5.9 Conclusions and Prospects

Pearl millet is an important crop for the future because of its ability to withstand high temperatures and drought conditions. It has a similar nutritional value to that of other cereal crops. Future research needs to focus on the use of proper breeding methods and modern tools. Development of new molecular markers and disclosure

of its genome has broadened the basis for future research. Advanced biotechnology tools will accelerate the breeding of pearl millet to achieve favorable objectives. Landraces are a rich source of favorable traits. The conservation of landraces in their natural habitat or in gene banks is a priority. The diversity of the crop worldwide will facilitate the work of breeders on various characters such as early maturity, grain and fodder yield, tolerance to abiotic stresses, resistance to diseases and pests, plant height, grain and fodder quality. Efforts are required to utilize the diversity for development of new hybrid varieties and to expand the cultivated gene pool. Farmers need to be encouraged to boost cultivation of pearl millet and create new opportunities in food and beverage industries.

Appendices

Appendix I: List of Some Major Institutes Relevant to Pearl Millet Research

Institution	Specialization and research activities	Contact information and website
Acharya N G Ranga Agricultural University, Guntur, Andhra Pradesh, India	Research	E-mail: vicechancellorangrau@gmail.com
		http://www.angrau.ac.in
All India Coordinated Research Project on Pearl Millet (Indian Council of Agricultural Research) Mandor, Jodhpur Rajasthan	Planning and execution of research	Phone:0291-2571408, +919414087558
	Strengthening of research facilities	Fax: 0291-2571909
	Developing efficient strategy for production	E-mail: aicrp.pearlmillet@icar.gov.in, aicpmip@gmail.com
	Distribution of germplasm to cooperating centers	http://www.aicpmip.res.in
CCS Haryana Agricultural University Hisar	Research	Phone: 1-800-1803001
		http://www.hau.ac.in
Central Arid Zone Research Institute, Jodhpur, Rajasthan	Research	Phone: +91 291 2786584
		Fax: +91 291 2788706
		http://www.cazri.res.in
Consultative Group for International Agricultural Research, France	Research to improve food security and nutrition, natural resources and ecosystem	Phone: +33 4 67 04 75 75
		Fax +33 4 67 04 75 83
		E-mail: contact@cgiar.org
		https://www.cgiar.org

(continued)

Institution	Specialization and research activities	Contact information and website
Indian Institute of Millets Research Rajendranagar, Hyderabad-Telangana	Developing improved production and crop protection technologies in millets	E-mail: millets.icar@nic.in
		director.millets@icar.gov.in
	DUS testing in pearl millet	Director: +91 – 040 – 2459 9301
		General No.: +91 – 040 – 2459 9300
	Distributing technologies	Fax: +91 – 040 – 2459 9304
		http://www.millets.res.in
Institute of Biological, Environmental and Rural Sciences Aberystwyth University, Penglais, Aberystwyth, Ceredigion SY23 3DA	Education	Phone: +44 (0)1970 621986
	Research	Fax: +44 (0)1970 622350
	Developed oat varieties	E-mail: ibers@aber.ac.uk
	Use of nondestructive imaging techniques for understanding plant under stress	http://www.aber.ac.uk
International Atomic Energy Agency, Vienna, Austria	Enhancing crop productivity by managing irrigation water in Sudan	Phone: (+43-1) 2600-0
		Fax: (+43-1) 2600-7
	Crop improvement by mutation breeding	E-mail: Official Mail
	Publication of protocol for mutation breeding in plants	Website: www.iaea.org
International Center for Agricultural Research in the Dry Areas Beirut, Lebanon	Focused identification of germplasm strategy (FIGS) that helped breeders to identify useful traits easily	Phone: + 961 1 843472/813303
		Office fax: + 961 1 804071/01-843473
	Developed heat tolerant varieties for Africa	http://www.icarda.org
	Provided rust-resistant varieties in Ethiopia	
International Center for Tropical Agriculture, Cali, Colombia	Building a center for preserving and deploying crop diversity	Phone: +57 2 4450000
		Fax: +57 2 4450073
	Developing new methods and toold for analyzing food systems	http://www.ciat.cgiar.org
International Crops Research Institute for the Semi-Arid Tropics Patancheru, Telangana, Hyderabad, India	Working on crop improvement and integrated crop management	E-mail: icrisat@cgiar.org
		Phone: +91 40 30713071
	Working on micro nutrients present in pearl millet	Fax: +91 40 30713074
	Developing high yielding hybrids for Sub-Saharan Africa	http://www.icrisat.org

(continued)

Institution	Specialization and research activities	Contact information and website
International Food Policy Research Institute Washington, DC 20005-3915 USA	Research in agricultural crops	Phone: +1 202-862-5600
		Fax: +1 202-862-5606
		E-mail to IFPRI
		Skype ifprihomeoffice
		http://www.ifpri.org
International Institute of Tropical Agriculture, Oyo State, Nigeria	Developing new varieties	Phone: +234 700800IITA, +1 201 6336094
	Improving food and nutrition security	Fax: +44 208 7113786
		E-mail: iita@cgiar.org
		http://www.iita.org
Junagadh Agricultural University, Junagadh, Gujarat	Released GHB-538, GHB-757, GHB-744, GHB-732 varieties of pearl millet	Phone: (Office) +91-285-2672080-90
		Telefax: +91-285-2672482
		E-mail: registrar@jau.in
		http://www.jau.in
Punjab Agricultural University Ludhiana	Developed first hybrid of pearl millet	Phone: 91-161- 2401960-79 Ext:- 213
	Enhancement of germplasm by developing transgenics	Fax: 91-161- 2400945
		Email: registrar@pau.edu
		http://www.pau.edu
SKN Agriculture University Jobner, Jaipur	Research	Telefax-01425-254980
		E-mail: info@sknau.ac.in
		http://www.sknau.ac.in
Swami Keshwanand Rajasthan Agricultural University	Developed RHB-121, RHB-154, RHB-173, RHB-177 varieties of pearl millet	Tel. No. 0151-2251083
		E-mail: cimca@raubikaner.org
		http://www.raubikaner.org

Appendix II: Pearl Millet Genetic Resources

Variety	Important traits	Cultivation location
Chakti	Biofortified with iron	Nigeria
Co 10	Medium, gray-brown bold grains, resistant to downy mildew	Tamil Nadu
MPMH 21 (MH 1777)	Early, gray-brown hexagonal grains, resistant to downy mildew, blast, smut	Rajasthan, Gujarat, Haryana

(continued)

Variety	Important traits	Cultivation location
86M01 (MH 1760)	82 days, purple anther color, medium plant height, conical ear heads, obovate gray colored grains	Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu, Rajasthan, Uttar Pradesh, Punjab, Haryana, Gujarat, Madhya Pradesh, Delhi
PHB 2884	Late maturing, gray seeds	Punjab
Proagro Tejas	Early, Resistant to downy mildew	Rajasthan
Pusa Composite 701 (MP 535)	Dual purpose variety, resistant to downy mildew and blast	Rajasthan, Haryana, Western Gujarat
86M88 (MH 1816)	Mature in 86 days, a rainy hybrid with wider adaptability and excellent potential. It has a high tolerance to leaf blast and rust	Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu,
NBH 5767	Mature in 83 days, purple anther, medium plant height, compact lanceolate ear heads, deep gray-colored grains	Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu,
KBH 108 (MH 1737)	Late maturing, tall plant height, purple anther color, cylindrical very compact ear heads, obovate gray seed, resistant to downy mildew, blast, and smut	Rajasthan, Gujarat, Haryana, Punjab, Delhi, Uttar Pradesh and Madhya Pradesh
HGM 686	Early maturing	USA
NPH-5423	Maturity in 80–85 days, 220–225 cm, well exerted compact non- bristled ear heads, shiny ash gray bold grains, highly tolerant to downy mildew, stay green quality fodder	All India
NPH-5286	Maturity in 80–85 days, 210–220 cm, compact, well exerted bristled ear heads, shiny ash gray bold grains, stay green fodder quality, highly tolerant to downy mildew	All India
HHB 234 (MH 1561)	Early maturing, candle shaped ear heads with small bristles, medium seed size and tolerant to downy mildew	Western Rajasthan and drier part of Gujarat and Haryana
MRB-2240	Suitable for all well drained, light to medium soil types, compact ear heads	All India
Tower (MRB-2210)	Suitable for all well drained, light to medium soil types, long ear head with bold and brown colored grains.	All India
CO 9	Medium maturing, medium height, candle shaped compact ear heads, grayish yellow seed color	Tamil Nadu
ABPC-4-3 (MP 484)	Late maturing, medium plant height, lanceolate ear heads, globular gray seeds	Maharashtra
86M66 (MH 1617)	Late maturing, medium height, conical compact ear heads, brownish yellow anthers, broad leaves, purple node color, gray seed color, resistant to downy mildew	Rajasthan, Gujarat, Haryana, Punjab, Delhi, Uttar Pradesh and Madhya Pradesh

(continued)

Variety	Important traits	Cultivation location
PAC 909 (MH 1435)	Medium maturing, medium height, medium thick compact cylindrical ear heads, light yellow colored anthers, gray seed color, resistant to downy mildew	Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu
HHB-226 (MH 1479)	Medium maturing medium height, dark green leaves, candle shaped bristled ear heads, resistant to downy mildew	Western Rajasthan and drier part of Gujarat and Haryana
Mandor Bajra Composite2 (MBC 2) (MP 489)	Early maturing, medium height, medium long semi compact cylindrical ear heads, obvate gray colored seed	Rajasthan, Haryana, Gujarat
Pusa Composite 612 (MP480)	Medium maturity, medium to tall plant height, compact cylindrical ear heads	Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu
RHRBH 9808	Medium maturing, medium tall plant height, dark green leaves, cylindrical ear heads, yellow anthers	Maharashtra
86M11	Medium maturity, long and compact ear heads	All India
86M35	Early maturity, compact ear head, slate green color	All India
86M74	Tolerant to heat stress	All India
HHB 223 (MH 1468)	Medium maturing, conical ear heads with long purple bristled, resistant to downy mildew, tolerant to drought	Rajasthan, Gujarat, Haryana, Punjab, Delhi, Uttar Pradesh and Madhya Pradesh
HHB 216 (MH 1421)	Medium maturing, resistant to downy mildew, candle shaped medium long to ear heads with brownish long bristled	Western Rajasthan and drier part of Gujarat and Haryana
NPH-1651	Maturity in 85–90 days, 250–260 cm, tall and late maturing, non-bristled compact, long ear heads, shiny ash gray bold grains, highly tolerant to downy mildew	Rajasthan, Gujarat, Uttar Pradesh, Haryana, Madhya Pradesh, Punjab, Delhi
NPH-9	Mature in 79–75 days, 200–210 cm height, non-bristled compact ear heads, shiny ash gray bold grains, highly tolerant to downy mildew	Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu
Pusa Composite443 (MP 443)	Early maturity, medium tall, rod-shaped ear heads with a bold grain	Rajasthan, Haryana, Gujarat
HHB 197	Early maturity, medium tall, dark green leaves, cylindrical medium Togo ear heads with long bristles, highly resistant to downy mildew disease	Rajasthan, Gujarat, Haryana, Punjab, Delhi, Uttar Pradesh and Madhya Pradesh
GHB 757	Early maturity, medium tall, compact cylindrical shaped ear heads with purple anthers, globular gray, brown grains	Western Rajasthan and drier part of Gujarat and Haryana
PCB 164	Mature in 85–88 days, a dual-purpose variety with broad leaves, thick stalks, cylindrical ear heads, bred from seven elite population and 27 diverse inbred lines	Punjab

(continued)

Variety	Important traits	Cultivation location
GHB 538	Early maturity, highly resistant to moisture stresses, resistance to downy mildew and lodging	Western Rajasthan and drier part of Gujarat and Haryana
HHB 67 Improved	Extra early maturity, highly resistant to moisture stresses, resistant to downy mildew. The first commercial cultivars developed using marker-assisted selection in India	Western Rajasthan and drier part of Gujarat and Haryana
GHB 577	Mature in 80–85 days, tall, medium thick stem with basal pigmentation, semi-compact cylindrical ear heads with slightly incomplete exertion, globular grains	Rajasthan, Haryana, Madhya Pradesh, Gujarat, Uttar Pradesh, Delhi
PB 180	Mature in 85 days, tall, high tillering, pubescent green nodes, compact candle shaped ear heads, yellow anthers, obovate dark gray grains	Summer cultivation areas of Rajasthan, Gujarat and other states
HHB 117	Mature in 74–78 days, medium tall, yellow anthers, compact candle- shaped medium thick ear heads, obovate gray grains, possesses the stay-green character	Haryana
HHB 146	Mature in 75–80 days, tall, violet nodes, hairy leaf sheath, long well- filled compact ear heads, obovate gray grains, slow senescence	Rajasthan, Haryana, Madhya Pradesh, Gujarat, Uttar Pradesh, Delhi
GHB 558	Mature in 75–80 days, medium tall, broad leaves, long thick compact candle shaped ear heads, yellow anthers, obovate, dark gray bold grains	All India
GHB 526	Mature in 85 days, medium tall, good tillering, narrow leaves with greenish-white mid-rib, good exertion, yellow anthers, compact conical ear heads, obovate gray, brown grains	Summer cultivation areas across India
PB 172	Mature in 84 days, medium tall, good nodes, compact candle shaped ear heads, yellow anthers, globular gray grains	Summer cultivation areas of Gujarat
CZP 9802	Mature in 70–72 days, medium tall, good tillering, thin stem, narrow leaves, thin candle-shaped ear heads, yellowish grains of medium size, drought tolerant, very high stover of good quality	Dry areas of Rajasthan, Gujarat, and Haryana (Zone A1)
		Dry areas of Rajasthan, Gujarat, and Haryana
CZP 9802	Mature in 70–72 days, medium tall, good tillering, thin stem, narrow leaves, thin candle-shaped ear heads, yellowish grains of medium size, drought tolerant, very high stover of good quality	Dry areas of Rajasthan, Gujarat, and Haryana
TifGrain 102	Shorter, earlier maturing, slightly larger grain, short stature, earlier maturing, slightly larger grain, resistance to southern and peanut root knot nematodes, rust resistance	USA

(continued)

Variety	Important traits	Cultivation location
HC 20	Mature in 80–83 days, tall, compact, thick cylindrical ear heads, yellow anthers, obovate gray grains, developed from S1 progenies derived from a gene pool selected for drought tolerance	Haryana
RHB 121	Mature in 85 days, medium tall, compact, thick conical ear heads, yellow anthers, long purple bristles, globular gray, brown grains	Rajasthan, Haryana, Madhya Pradesh, Gujarat, Uttar Pradesh, Delhi
JBV 3 (GICKV 96752)	Mature in 82 days, tall, long compact cylindrical ear heads, yellow anthers, obovate gray grains, developed from cycle 3 of SRC II	Rajasthan, Haryana, Madhya Pradesh, Gujarat, Uttar Pradesh, New Delhi
Pusa Composite 383	Mature in 82 days, tall, thick stems and panicles, resistant to lodging and downy mildew	Rajasthan, Haryana, Madhya Pradesh, Gujarat, Uttar Pradesh, New Delhi
HHB 94	Mature in 73–76 days, medium tall, synchronous tillering, semi-compact cylindrical ear heads, yellow anthers, obovate gray grains	Haryana
RHB 90	Mature in 85 days, medium tall, compact cylindrical ear heads, yellow anthers, purple bristles, obovate, yellow, brown grains	Rajasthan
HC 10	Mature in 75–80 days, tall, medium thick semi- compact ear heads, purple anthers, obovate gray, brown grains, a dual-purpose variety developed from high yielding, early flowering and drought tolerant	Haryana
Pusa 605	Mature in 74–80 days, medium tall, compact cylindrical ear heads, yellow anthers, obovate gray grains	Rajasthan, Haryana, Gujarat, Madhya Pradesh, Uttar Pradesh, Delhi
Pusa 415	Mature in 78 days, medium tall, compact, thick lanceolate ear heads, yellow anthers, obovate, yellow-brown grains	Rajasthan, Haryana, Gujarat, Madhya Pradesh, Uttar Pradesh, Delhi
Pusa Composite 334	Mature in 75–80 days, tall, thick semi compact cylindrical ear heads, obovate gray, brown grains, developed from three selected lines and eight elite inbreds	Dry areas of Rajasthan, Gujarat, and Haryana
Pusa Bajri 266	Mature in 80 days, tall, thick, semi-compact, cylindrical ear head, yellow anthers, obovate gray, brown grains, developed from nine lines	Dry areas of Rajasthan, Gujarat, and Haryana
PABH 3	Mature in 85 days, medium tall, resistant to downy mildew	Marathwada area of Maharashtra
Pusa 444	Mature in 85 days, medium tall, thick semi-compact cylindrical ear heads, purple anthers, globular yellow, brown grains	All India

(continued)

Variety	Important traits	Cultivation location
RHB 58	Mature in 85 days, medium tall, compact conical ear heads, purple anthers, obovate, yellow-brown grains	All India
GHB 235	Mature in 80 days, medium tall, compact conical ear heads, purple anthers, globular gray grains	Gujarat
MBH 160	Mature in 80 days, medium tall, high tillering, semi-compact bristled ear heads, bold grains	All India
HHB 68	Mature in 60–62 days, compact conical ear heads, yellow anthers, globular brown grains, resistant to drought, fits well in inter-and multiple-cropping	Haryana
Pusa 322 (MH 322)	Mature in 80 days, medium tall, thick cylindrical semi- compact ear heads with sterile tip, globular gray, brown grains	All India
ICMH 312	Mature in 85 days, medium tall, long semi- compact conical ear heads, yellow to purple anthers, globular gray grains	Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu
ICMH 356	Mature in 75–80 days, medium tall, semi-compact thick conical ear heads, yellow anthers, obovate bold yellow, brown grains	All India
ICMV 221	Mature in 75–80 days, medium tall, thick lanceolate semi-compact ear heads, purple anthers, globular dark gray grains, bred from the C3 cycle of the bold seeded early composite (BSEC)	All India
Raj 171	Mature in 82–85 days, tall, medium thick stem, long cylindrical semi-compact to compact ear heads, obovate gray, brown grains, resistance to downy mildew, bred from inter varietal composite	All India
Eknath 301	Mature in 80–85 days, medium tall, non- pubescent internodes, good tillering, ligules light red colored, yellow anthers, long bristles, creamy gray grains	All India
MLBH 104	Mature in 80 days, medium tall, pubescent ligules, fully exerted ear heads, anthers light pink, globular smooth gray grains	All India
HGM 100	Used as grain	USA
HHB 67	Mature in 60–62 days, medium tall, thin stem, medium narrow leaves, semi-compact spindle shape ear heads, yellow anthers, gray globular grains, escape drought and tolerates salt stress, fits well in inter- and multiple-cropping	All India

(continued)

Variety	Important traits	Cultivation location
VBH 4	Mature in 80 days, medium tall, good tillering, compact non-bristled ear heads, medium bold grains	All India
Raj Bajra Chari-2	Mature in 85–90 days, tall, broad and succulent leaves, good quality green fodder, suitable for saline soils	All India
MBH 136	Mature in 85 days, medium tall, high tillering, compact conical very thick ear heads, yellow anthers, pink medium long bristles, gray seeds	Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu
Pusa Safed	Mature in 83–87 days, medium tall, semi-compact ear heads, yellow anther, yellowish white grains of medium size	Dry areas of Rajasthan, Gujarat, and Haryana
ICMH 423	Mature in 75–80 days, medium tall, hairy nodes, glabrous leaves, compact cylindrical to conical ear heads, purple glumes, yellow anthers, gray hexagonal grains	All India
HHB 50	Mature in 76–80 days, medium and thick stem, long, dense pointed ear heads, medium bold grains	Haryana, Tamil Nadu, Gujarat
HHB 60	Mature in 74–76 days, tall, high tillering, medium thin stems, compact candle ear head, obovate brown grains, resistant to drought and salt stress, good quality fodder	Haryana
TifLeaf 2	Dwarf leafy variety for fodder, rust resistant	USA
Pusa 23 (MH 169)	Mature in 82 days, medium tall, glabrous leaves, yellow anthers, compact cylindrical ear heads, light gray obovate grains	All India
HHB 45	Mature in 82–85 days, Medium tall, remains green till harvest, suitable for both early and late maturing sown conditions	Haryana
PHB 47	Mature in 85 days, tall, thick, sturdy stem, long thick ear heads with profuse gristles, remains green till harvest	Punjab and Tamil Nadu
HC 4	Mature in 82–86 days, tall, medium broad leaves, medium thick stem, resistant to downy mildew, high fodder yield, developed by eight component lines of World Composite	All India
ICMS 7703	Mature in 85–90 days, medium tall with 3–5 tillers, compact cylindrical ear heads, short and straw-colored glumes, bred by crossing inbred lines derived from 7 crosses	All India
RCB 2	Mature in 80–85 days, tall plants with 4–6 tillers, broad and glabrous leaves, long compact and cylindrical ear heads, yellowish gray grains, developed from 20 inbreds of diverse origin	Rajasthan

(continued)

Variety	Important traits	Cultivation location
Pusa 46 (CM 46)	Mature in 75–78 days, tall, thin stem, 4–6 tillers, narrow, hairy leaves, spindle-shaped ear heads	All India
MBH 110	Mature in 85–87 days, medium tall, loose and thick conical ear heads with long bristles, bold gray shining grains	All India
GHB 27	Mature in 80–85 days, medium tall, nodal pigmentation, long and cylindrical ear heads, bold grains	Gujarat
Co 5 (Kullan Cumbu)	Mature in 80 days, medium tall, cylindrical to spindle-shaped compact ear heads, suitable for both rainfed and irrigated areas, a selection from Kullan Cumbu cultivar	Tamil Nadu
X 4	Mature in 80–85 days, tall, purple pigmentation at the base of the stem, high tillering, dark green leaves, yellow and purple anthers, short to medium bristles, medium to bold grains	Tamil Nadu
KBH 1	Mature in 85–90 days, tall, white midrib, short bristles, gray grains	Tamil Nadu
Pusa 763 (BD 763)	Mature in 90–95 days, tall, thick stem, long, broad leaves, rod shape ear heads	All India
PSB 8	Mature in 82–85 days, tall, long, none-bristles ear heads tapering towards the tip, light slate color grains, developed by chain crossing among early to medium maturity and tall inbred lines	Punjab
WC-C75	Mature in 82–85 days tall, thick and dense panicles, developed crossing seven full-sib progenies of World Composite	All India except Rajasthan and Punjab
AMP 2	Mature in 85 days, tall, medium thick stems, dual propose variety	Marathwada area of Maharashtra
BD 111	Mature in 85–90 days, tall, good tillering, small bristles, resistant to lodging	Andhra Pradesh, New Delhi, Haryana
BJ 104	Mature in 75–82 days, medium tall, good basal tillering, nodal joints red, medium long cylindrical ear heads tapering towards tips, conical grains	All India
BK 560	Mature in 85–90 days, medium tall, good tillering, sturdy plants, green, yellowish stems, rod-shaped ear heads, resistant to lodging	All India
MBH 104	Mature in 85–90 days, medium tall, dark green leaves, compact long cylindrical ear heads, long tan bristles, medium bold grains	All India (rainfed)
CJ 104	Mature in 75 days, medium tall, good basal tillering, pigmented nodes, non-bristles cylindrical ear heads	Drought-prone areas of Gujarat

(continued)

Variety	Important traits	Cultivation location
HS 1	Mature in 75–80 days, tall, solid and juicy stem, compact medium long bristled ear heads, tolerant to lodging and drought	Haryana
K 2	Mature in 85–90 days, dwarf, good tillering, juicy and solid stem, cylindrical compact and non-bristled ear heads	Tamil Nadu
Nagarjuna	Mature in 75–80 days, tall, good tillering, semi-compact ear heads, developed from 20 elite lines of African and Indian origin	Low rainfall areas of Andhra Pradesh
Visaka	Mature in 75–80 days, tall, dark green foliage, cylindrical and compact ear heads, light gray grains	Andhra Pradesh
Balaji	Mature in 75–80 days, medium tall, dark green foliage, long cylindrical ear heads, bold light gray grains	Andhra Pradesh
Gahi 3	Tall, leafspot resistant	USA
Tifleaf 3	Semi-dwarf, leafy, high-quality forage hybrid resistance to rust 6 feet	USA
Tift 8593	Used to produce TifLeaf 3, long panicle	USA
NHB 5	Mature in 85 days, medium tall, thick stems, cylindrical ear heads, medium bold light gray grains	Rajasthan, Uttar Pradesh, Tamil Nadu, Andhra Pradesh, Maharashtra, Karnataka, Haryana
S 530	Mature in 85 days, tall, semi-compact medium long ear heads, a derivative of African × Indian cross	Punjab
Pusa Moti	Mature in 90–95 days, medium tall, bold grains, downy mildew tolerant	Maharashtra, Punjab, Haryana
RSJ	Mature in 80–90 days, tall, poor tillering, long cylindrical compact ear heads, a selection from local material	Alwar, Sikar, Jhunjhunu areas of Rajasthan
RSK	Mature in 90–95 days, medium tall, medium thick long ear heads, developed from local material	Jaipur, Ajmer, Bharatpur and Swai Madhopur areas of Rajasthan
Co 4	Mature in 80 days, medium tall, medium long cylindrical ear heads, suitable for both rainfed and irrigated areas, selected from Bombay cultivar	Tamil Nadu
T 55	Mature in 85–90 days, medium tall, thin stems, compact ear heads, gray grains	Drought-prone areas of Haryana and Punjab
Jakharana	Mature in 80–85 days, tall, exceptionally long ear heads slightly curved at the tip, a dual-purpose variety	Central and eastern part of Rajasthan
NHB 3	Mature in 70–75 days, medium tall, light green foliage, cylindrical ear heads with tapering tips	Gujarat

(continued)

Variety	Important traits	Cultivation location
GHB 1399	Mature in 85–90 days, dwarf, good tillering, dark green foliage, long cylindrical ear heads	Gujarat
NHB 4	Mature in 85 days, medium tall, cylindrical ear heads with tapering tips	All India
HB 3	Mature in 80–85 days, medium tall, profuse tillering, pigmented nodes, glabrous leaves, conical ear heads	Dry areas of Maharashtra, Gujarat, Rajasthan, and Haryana
HB 1	Mature in 80–85 days, medium tall, profuse tillering, succulent stem, green ligules, long ear heads, slow senescence	All India
HB 4	Mature in 85–90 days medium tall, profuse tillering, rod-shape ear heads, greenish yellow grains	All India (except Punjab)
HB 5	Mature in 78–85 days, medium tall, cylindrical ear heads with tapering towards tips	All India
HB 2	Mature in 80–85 days, medium tall, good tillering, pink pigmented at stem base, long compact ear heads	Dry areas of Madhya Pradesh, Gujarat, and Rajasthan

Source: Website of Indian Institute of Millets Research (IIMR) and various institutes and private seed companies

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Chapter 6
Proso Millet (*Panicum miliaceum* L.) Breeding: Progress, Challenges and Opportunities

Dipak K. Santra, Rituraj Khound, and Saurav Das

Abstract Proso millet (*Panicum miliaceum* L.) is an annual cereal crop domesticated approximately 10,000 years ago in the semiarid regions of China. It is primarily grown in India, Nigeria, Niger, and China. Proso millet is used in Europe and North America as fodder and birdseed despite its highly nutritive and health-promoting benefits. Recently, the high content of different minerals and amino acids along with a low glycemic index and gluten-free property of the grains have attracted the industry and scientific communities. Proso millet has been used as a rotational crop in the winter wheat-fallow cropping system in the western Great Plains of the USA owing to its high water-use efficiency. This practice not only prevents the loss of organic matter from the no-till soil but also reduces weed and disease pressure. Regardless of the impeccable environmental and health benefits of proso millet, it remains as an under researched and underutilized crop. Plant breeders across the globe are trying to develop superior varieties using both classical and advanced breeding procedures. However, the lack of a genetic map and adequate genomic resources has slowed the crop improvement process. Proso millet germplasm representing a wide genetic diversity is conserved in gene banks maintained by several countries. The rapid growth in genomic research in the form of a linkage map development, novel molecular marker identification and availability of next-generation sequencing, together with high-throughput phenotyping promise to accelerate proso millet breeding. The development of proso millet cultivars which are high yielding, lodging and seed-shattering tolerant, direct combine-ready and nutrient enriched, would promote its increased cultivation, and use in the food industry.

Keywords Gluten-free · Glycemic index · Molecular marker · Semiarid · Small millet · Rotational crop · Water-use efficiency

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J. M. Al-Khayri et al. (eds.), *Advances in Plant Breeding Strategies: Cereals*,
https://doi.org/10.1007/978-3-030-23108-8_6

6.1 Introduction

Millets are small-grained, annual cereal grasses comprising several species including pearl millet (*Pennisetum glaucum* (L.) R. Br.), and a number of minor or small millets, which include finger millet *(Eleusine coracana* Gaertn.), proso (also known as broomcorn or common) millet (*Panicum miliaceum*), foxtail millet (*Setaria italica* (L.) P. Beauv.), little millet (*Panicum sumatrense* Roth ex Roem. & Schult.) and kodo millet (*Paspalum scrobiculatum* L.) (Dwivedi et al. 2011; Saha et al. 2016). Millets are well-adapted to marginal lands owing to their ability to withstand various types of stress thereby contributing to sustainable low-input food production (Saha et al. 2016). Although all the millets belong to the Poaceae family, they show tremendous diversity at species, genus, tribe or subfamily levels. They further show a great deal of diversity in terms of genome size, ploidy levels and breeding systems (Dwivedi et al. 2011).

The agricultural origins of the millets are primarily in Africa and Asia. Phylogenetic studies suggest a monophyletic origin of domesticated pearl millet in Western Africa (Dussert et al. 2015; Oumar et al. 2008; Ozainne et al. 2014). Northern China is considered as the agricultural origin for proso millet with the record of cultivation dating back to 10,000 B.C. (Hunt et al. 2014). Finger millet is believed to be of African origin (Fuller 2014). There is no substantial evidence for the center of origin for foxtail millet cultivation. The green foxtail (*Setaria viridis* (L.) P. Beauv.), the ancestor of the current Eurasian foxtail millet, purportedly originated in Africa, which later gave rise to other species of *Setaria* grasses (Diao and Jia 2017). Radiocarbon dating studies also indicate the possible origin of foxtail millet in Northern China (Hunt et al. 2008). Kodo millet was domesticated in India approximately 3000 years ago (De Wet et al. 1983). The wide representation of little millet among the cereals across the majority of the agricultural zones in India suggests its ancient domestication by independent agrarian communities in India (De Wet et al. 1983).

Millets are cultivated globally with major contributions from India, Nigeria, Niger, China, Mali and Burkina Faso (FAOSTAT 2018). As of 2016, the total global acreage of millet was 31.7 million ha with the average yield of 8944 hg/ha. The acreage has decreased approximately 12% since 2006. However, the global millet yield has increased by ~1.2% in the last decade. The total production of millet all over the world was 28.3 million mt in 2016, a decrease of 11% as compared to 2006 (32 million mt), possibly due to a decrease in hectarage (FAOSTAT 2018) (Fig. 6.1).

Millets have high nutritive value being rich in proteins, dietary fibers, vitamins and minerals such as iron, zinc, calcium, potassium, and phosphorus (Vinoth and Ravindhran 2017). Millet proteins are reported to be good sources of essential amino acids (Saleh et al. 2013). Pearl millet constitutes the major portion of the diet in Western India and the Sahel Region of Africa (Tako et al. 2015). It contains significantly high amounts of starch, fibers, minerals and antioxidants (Saleh et al. 2013).

Finger millet is extensively cultivated in Africa and South Asia and contributes 12% to the total millet production worldwide (Vetriventhan et al. 2016). It is a rich

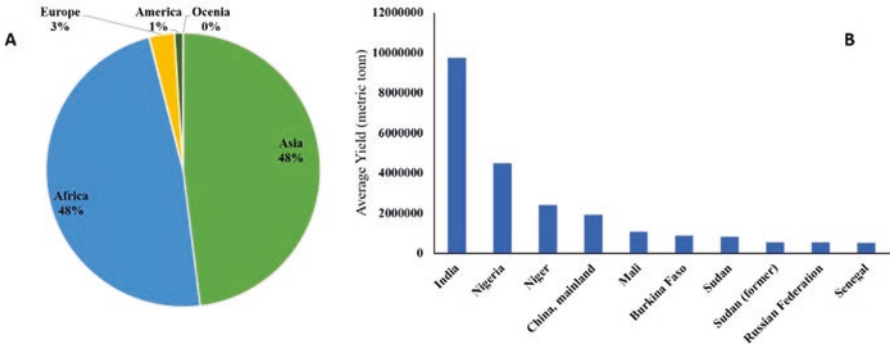

Fig. 6.1 Average millet production from 1994 to 2016. (**a**) Continent-wise contribution to millet production, (**b**) Top ten millet producing countries in the world. (Source: FAOSTAT 2018)

source of calcium, dietary fiber, phytates, proteins, minerals, phenolic compounds, thiamine and riboflavin (Chandra et al. 2016). Foxtail millet is extensively cultivated for grains and fodder in Asia, Europe, North America, Australia, and North Africa. Foxtail millet seed is an excellent source of protein, fat, crude fiber, minerals like iron and vitamins (Vetriventhan et al. 2016). Kodo and little millets are cultivated extensively by tribal people in India (Upadhyaya et al. 2016). Both kodo and little millets contain 37–38% dietary fiber, which is the highest among the cereals, and high polyunsaturated fatty acid proportion in total fat content (Saleh et al. 2013). Millet protein has considerably low proportions of glutenin and gliadin content, which are known to trigger celiac disease, compared to wheat, rye, barley and oats, making it ideal for gluten-free food preparation (Jnawali et al. 2016). Millets, in general, are rich in several phenolic compounds which reportedly confer health-promoting properties on these cereals, such as amelioration of oxidative stress, anti-tumorigenic, anti-diabetic, anti-inflammation, anti-hypertensive and low glycemic index (Taylor et al. 2014; Upadhyaya et al. 2016).

This chapter focuses on proso millet germplasm, genetics, genomics and breeding based on currently available literature. Objectives are to summarize proso millet in terms of (1) origin and world production, (2) unique nutritional value and food use, (3) germplasm characteristics, (4) breeding and (5) future prospects of genetic research.

6.1.1 Origin

Proso millet is one of the oldest cereal crops, the agricultural origin of which dates back to 10,000 BC in the semiarid parts of China (Hunt et al. 2014; Lu et al. 2009a). It is known by different names depending on the geographic location. Proso, the pan-Slavic name for millet, is known as common millet and hog millet in the USA, broomcorn millet in China, common millet in Japan, Korea and other counties in the Asia Pacific, hersey millet in Germany and French white in France (Rajput et al.

2014). This shallow-rooted, short-season annual crop exhibits high water-use efficiency which makes it well-suited for growing in hot and dry environments (Graybosch and Baltensperger 2009; Henry et al. 2008; Lyon and Baltensperger 1993). Proso has the shortest growing season (60–90 days) among the cereals which contributes to its drought tolerance (Goron and Raizada 2015; Hunt et al. 2014). This small millet either possesses tolerance to drought and intense heat or avoids these extreme conditions owing to its short maturity time (Henry et al. 2008). Proso millet is cultivated in the west-central Great Plains of the USA as a rotational crop in the winter wheat-based cropping system (Agdag et al. 2006; Graybosch and Baltensperger 2009). It is presently cultivated in Asia, Australia, North America, Europe and Africa (Rajput et al. 2014).

Proso millet, belonging to the Panicoideae subfamily of the Poaceae family, is considered to be one of the earliest domesticated cereals in human history (Lu et al. 2009a). There are different theories about the origin of proso millet. Investigations of the charred seed remains recovered in Dadiwan in Northwestern China suggested the possible period of proso domestication to be approximately 5900 BC (Miller et al. 2016). In order to estimate the time of the domestication of proso millet, Lu et al. (2009b) studied phytoliths discovered in ancient storage pits at the archeological site of Cishan situated near the boundary between the Loess Plateau and the North China Plain. They opined that the earliest proso millet farming began in the semiarid regions of China by 10,000 years BC based on the carbon-dating results of the 47 archeological samples investigated in the study. Relatively drier environments during the early Holocene probably encouraged the domestication of proso millet over other cereals. Lu and coworkers also suggested that proso was domesticated independently as a staple food in Northern China approximately 10,000 years ago, and later spread to other neighboring areas in Russia, India, the Middle East and Europe (Lu et al. 2009a). Zhao (2011), however, claimed that the actual age of the samples recovered from an excavation in Cishan is ca. 7600 to 8100 years old. He expressed doubts over the estimations by Lu et al. (2009a) as the samples were already in a decayed state. The samples used for dating were possibly a mixture of different grains, instead of actual millet grains (Zhao 2011). Miller et al. (2016) also questioned the estimate of proso millet origin as it was based on the analysis of *problematic* phytolith identification. Nevertheless, all the evidence indicates that the domestication of proso millet occurred in the semiarid region of the Northeast China. Miller et al. (2016) suggested that proso millet spread to Europe and West Asia towards the end of the first millennium BC because of the changes in agricultural practices in those areas (Miller et al. 2016).

6.1.2 Production

In the USA, proso millet is grown under dryland/rainfed conditions in primarily three states: Colorado, Nebraska and South Dakota (Santra 2013). It is highly adapted to this semiarid High Plains region of the country (Fig. 6.2). As of 2017,

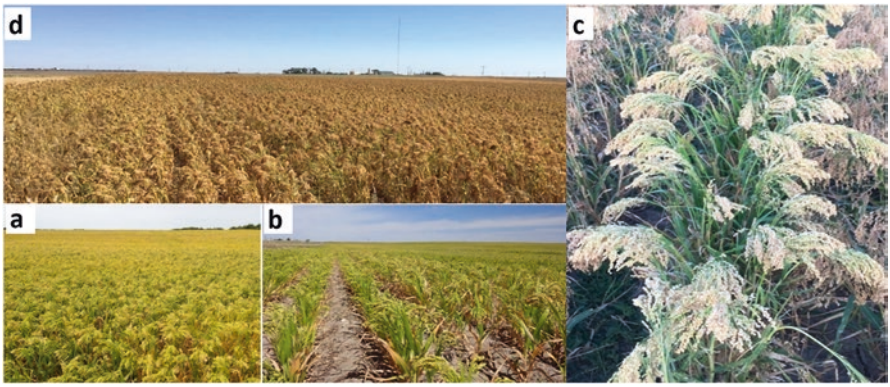

Fig. 6.2 Proso millet field in Nebraska, USA. (**a**–**c**) Physiological maturity, (**d**) Ready for direct harvesting. (Photos by: R. Khound, D.K. Santra and S.G. Rajput, unpublished)

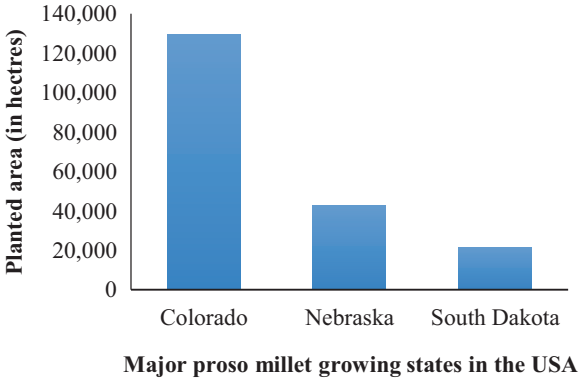

Fig. 6.3 Proso millet production area in three major States of the USA in 2017. (Source: NASS 2017 https://www.nass.usda.gov/)

Colorado ranked first in proso millet hectarage with an estimated planting area of 129,500 ha, followed by Nebraska (42,500 ha) and South Dakota (21,500 ha). The total area under proso millet planting and harvest in 2017 were 193,440 and 163,493 ha, respectively (Fig. 6.3). The total production was 330,370 mt in 2017, which increased ~16% compared to the data from 2016 (284,810 mt). The national yield of proso millet was recorded to be 2.02 mt/ha in 2017 with a ~19% improvement over 2016 (USDA 2018). Total market value of proso millet in the High Plains of USA is ~USD 100 million with wide range depending on market price. However, the value of this crop is significantly higher when considering its economic impact on wheat production in the region since proso millet provides multiple production benefits when used as a rotational crop. The economy of proso millet in other countries is not reported but it can be generally assumed that it is very significant crop in Russia, Ukraine and China.

6.1.3 Botanical and Genetic Features

Proso millet is regarded as a short-day plant and is usually an erect, 30–100 cm-tall plant (Baltensperger 2002). Proso can be classified into cultivated types and wild types. Unlike the wild types, which have lax panicles, jointed spikelet stalks and narrow lemmas, the cultivated proso millet types are characterized by either lax or compressed panicles, spikelet stalks without joints and wider lemmas. The plants possess seminal roots at the young stage, which are later replaced by the adventitious root system. The stem is usually hollow and cylindrical known as a *culm*. Tillers and lateral branches appear from the axillary buds at the lower internodes. Proso millet plants produce drooping panicles which may be open or compact and are usually 10–45 cm long (Gomashe 2017). The proso flower consists of a lower and upper glume, a lower and upper lemma, and a single palea (Lu et al. 2009b). The seeds, botanically a *caryopsis*, are small with approximately 3 mm length and 2 mm width and are oval in shape. The proso seed shows variation in color and shades, varying from white, cream, yellow, orange, red, black, to brown (Baltensperger 2002).

Proso millet is predominantly self-pollinating, however, more than 10% natural cross-pollination is also observed (Baltensperger 2002). Despite the lack of adequate genetic characterization, proso millet is generally regarded as an allotetraploid with 36 chromosomes (2n = 4x = 36). The ancestors of proso are not yet clearly known. However, Hunt et al. (2014) tried to identify possible candidates as parental donors of the proso genome. They used nuclear and chloroplast DNA sequences from proso millet (*Panicum miliaceum)* and several diploid and tetraploid relatives for in situ hybridization study. In situ hybridization revealed that half of the chromosomes of *P. miliaceum* hybridized strongly with genomic DNA from witchgrass, *P. capillare* L., and the other half with that of torpedo grass, *P. repens* L., a tetraploid. The genomic probe also differentiated two sets of 18 chromosomes in *P. repens*. The authors concluded that the study supports the tetraploid origin of proso millet with contributions from the maternal ancestor *P. capillare* (or a close relative) and the other ancestor *P. repens*. Hunt and co-workers also acknowledged the importance of further studies encompassing more *Panicum* species from the Old World for more conclusive evidence for the origin of the tetraploid proso genome (Hunt et al. 2014).

6.1.4 Nutritional Quality and Uses

Proso millet is primarily used as birdseed and pet food in the USA and Europe (Graybosch and Baltensperger 2009; Kalinova and Moudry 2006). However, proso millet serves as a major source of human food in many countries including China, India, in Africa and in the former USSR (Carpenter and Hopen 1983). It is a good source of carbohydrates (70–74 g/100 g), protein

(12–14 g/100 g), fat (3.5 g/100 g) and crude fiber (5.2 g/100 g) (Motta Romero et al. 2017; Saleh et al. 2013). In fact, proso millet caryopsis has higher protein, fat and crude fiber content than other popular cereals such as brown rice, wheat, maize and sorghum (Saleh et al. 2013). Proso seeds are also rich in minerals and vitamins such as B1 (thiamin) and B3 (niacin) (Kalinová 2007). Moreover, proso millet grain has higher protein quality compared to wheat by virtue of a significantly higher portion of the essential amino acids leucine, isoleucine and methionine. Millet protein has been found to have beneficial effects on cholesterol metabolism and liver injury. Furthermore, the specific prolamin fraction in proso protein is below the permitted level, which makes proso a suitable choice as a gluten-free diet for patients suffering from celiac disease (Kalinova and Moudry 2006). Proso millet also contains phenolic compounds including tannins and phenolic acids such as ferulic acid and p-coumaric acid. Besides tannins, proso seeds contain other anti-nutritional factors such as phytates and oxalates, which reduce the nutritional quality of the seeds. However, processing methods such as dehulling significantly reduce the amount of these compounds. Dehulling and decortication result in a 12–65% loss of phenolics in proso millet (Taylor et al. 2014). Some phenolic compounds, nevertheless, reportedly protect against cancer and heart diseases (Kalinová 2007). Low glycemic index and high amounts of protein, lipids, fiber and phenolic compounds in proso millet-based food products make proso millet seed a good candidate for the production of gluten-free food for people suffering from type-2 diabetes mellitus, cardiovascular disease and celiac disease (McSweeney et al. 2017).

The uses of proso millet as food date back to prehistoric times, 8000–10,000 years ago in Russian, German and Chinese cuisines (Santra and Rose 2013). In early times, Russians prepared proso millet as a sweet dish especially mixing it with milk and sugar or sometimes as a savory dish with meat or vegetable stews. The Chinese used to consume proso mixing it with beans, sweet potato and squash. Germans ate it as a sweet mixed with honey and apple. Proso millet may not be a consumed as food in western countries, but it is an important source of energy for people in Asian and African countries. Recent research on the nutritional profile of proso millet and its complementation to wheat and other primary cereal crop primary nutrients has drawn attention from the food industries for commercialization. Several food companies around the globe have started incorporating proso millet in their gluten-free recipes for individuals with celiac disease. Proso millet alone or in combination with other grains are now used for making bread, flour, pasta and fermented beverages. Millet is also a traditionally important grain in some cultures. In Nepal, millet is used to brew indigenous alcoholic drinks such as *tongba* and *rakshi*. Millet is also used by the Tao people of Orchid Island in Taiwan to brew beer. The gluten-free characteristics of millet has also attracted some brewing companies especially in the USA Great Plains: Colorado Malting Co., New Planet Beer, Eddyline Restaurant and Brewing Co. and Pagosa Brewing Co., all Colorado enterprises. Also, the Modern Monks Brewery in Lincoln, Nebraska, is using proso millet to make beer (Santra and Rose 2013).

In European countries, proso millet is mainly used for livestock feed. The comparable nutrition profile and high content of amino acids like methionine and cysteine make it a perfect for livestock. It is used to feed calves, growing and finishing cattle, dairy cows, sheep, swine and poultry. Recent research has found that methionine can improve the pregnancy rate in dairy cattle by improving the lipid droplets inside the preimplantation embryo (Acosta et al. 2016).

Biofuel is another exciting prospect for proso millet uses. Similar starch content as in corn makes it an alternative to corn for ethanol production. Research at the University of Nebraska-Lincoln found that fermentation efficiencies of proso millet vary from 84% to 91%, comparable to the 97% fermentability of corn hybrids (Rose and Santra 2013).

6.2 Sustainable Rainfed Agricultural System

In the semiarid Great Plains of the USA, where average annual rainfall is 356–406 mm, soil conservation with minimum water loss and maintaining high organic matter have always been the primary focus. Therefore, an efficient strategy for producing a crop under water-deficit condition or a plant adapted to drought stress is favorable for the summer fallow (Seghatoleslami et al. 2008). The Great Plains has undergone a vast change in the selection of rotation crops keeping in view no-tillage for the conservation of soil organic matter (Anderson 2011). Though there are a wide variety of crops like corn, teff, millets, soybean, sunflower and pea as a rotation crop for summer fallow, proso millet would be the best-adapted no-tillage drought-resistant alternative crop for the plains (Anderson et al. 1999). According to Rasmussen (1988) including alternative crops in annual cropping system as rotation and planting in early spring can maintain the soil organic matter (Rasmussen 1988). The cropping system with summer fallow depletes the soil organic matter due to high biological oxidation and absence of carbon input in the fallow period (Nielsen and Calderón 2011; Rasmussen et al. 1998). But warm-season crops like proso millet could replace the summer fallow in winter wheat cropping system. Growing proso millet in the summer fallow to cover the surface and minimize the biological oxidation of the topsoil surface is beneficial. In addition, as a C4 crop, proso millet has a low transpiration ratio and can efficiently fix carbon under conditions of drought, high temperatures and limited nitrogen (Habiyaremye et al. 2017a). The shallow root system of proso millet (~92 cm) also helps in the conservation of deep soil moisture to be used by winter wheat. The water use efficiency of proso millet works in a synergistic way with the wheat and increases the yield capacity and produces more grain (Anderson 2011; Habiyaremye et al. 2017b). Synergism with proso millet also reduces weeds, especially the grassy weeds, summer annual broadleaf weeds and perennial broadleaf weeds. The seed bank density of summer annual weeds was found to decline by nearly 90% if proso millet is followed by two winter crops (Anderson et al. 1999). Proso millet also helps to manage the disease and insect pressure on the wheat (Habiyaremye et al. 2017a). In dryland or rainfed farming, the effect of rotational crops, especially proso millet, is prominent due to

stresses such as drought (Anderson 2011; Habiyaremye et al. 2017b). The short growing season allows proso millet to avoid the drought sensitivity by reaching maturity rapidly (Baltensperger et al. 1995a). In addition, when the temperature reaches above 30 °C, proso millet stops vegetative growth and ceases to flower, maintaining its height shorter to better resist the drought condition (Changmei and Dorothy 2014; Sateesh 2010).

In a comparative study of conventional tillage, reduced tillage, and no-tillage production systems, it was found that wheat-corn-proso millet and wheat-millet rotations produced almost double the total grain yield compared to the conventional wheat-fallow rotation (Anderson et al. 1999). A continuous cropping system with wheat-corn-proso millet rotation with no-tillage increases the concentration of glomalin in the soil, which is a glycoprotein produced by arbuscular mycorrhiza and is important in soil aggregation (Comis 2002; Habiyaremye et al. 2017a). A continuous cropping system with no-till or reduced till also maintains the water content of the soil layer because of reduced evaporation from the surface due to the development of continuous crop residues (Nielsen et al. 2005). Moreover, winter wheat sown into proso millet stubble under no-till systems are less prone to damage from blowing soil compared to those planted into summer fallow. Increased soil residue also improves the snow capture, providing the much-needed moisture for the crops (Habiyaremye et al. 2017a; Nickel 2015). Proso millet can also be used in rotation with corn or sorghum as it can tolerate atrazine, a primary chemical used in the corn and sorghum production systems. Furthermore, the warmer soil temperatures in corn or sorghum stubble fields allow proso millet to be planted earlier (Habiyaremye et al. 2017a). Therefore, growing crops such as proso millet in place of summer fallow would provide more surface cover and help farmers meet conservation practice requirements. As an added benefit, proso millet can be an additional cash crop in the wheat-proso-fallow rotation.

6.3 Germplasm and Genetic Diversity

6.3.1 Germplasm Resources

Global agriculture has witnessed a drastic decline in the diversity of cultivated crops due to overreliance on a few major species (Haussmann et al. 2004; Nass et al. 2012). The lack of genetic variability may lead to disease and pest epidemics, increased vulnerability to abiotic stresses, such as drought, salinity or temperature and minimal variation in quality traits such as grain component quality (Haussmann et al. 2004). In addition to the reduction in interspecific diversity among cultivated crops, improvement via plant breeding has resulted in diminished intraspecific diversity by developing and promoting genetically uniform cultivars (Haussmann et al. 2004). For instance, all the six commonly grown proso millet cultivars developed by selection from landraces and through conventional breeding in the USA

were developed using few parents, thereby resulting in a narrow genetic base (Rajput et al. 2014). Therefore, development and maintenance of a repository of the germplasm or plant genetic resources of all the cultivated crops is of utmost importance, especially for underutilized and under-researched crops like millets (Upadhyaya et al. 2014). Germplasm resources can complement crop improvement programs by functioning as a source of inter- and intraspecific diversity for exploitation in plant breeding efforts (Haussmann et al. 2004; Jia et al. 2017). Improvement of agricultural production in the future may depend ever more on the efficient use of these genetic resources (Byrne et al. 2018). The gene banks storing the majority of proso millet germplasm accessions are located in the Russian Federation, China, Ukraine, India and the USA (Table 6.1). The N.I. Vavilov All-Russian Scientific Research institutes of Plant Industry (VIR) in the Russian Federation maintains 8778 proso millet accessions. China has the second largest number with accessions of 8451 conserved in the Institute of Crop Science, Chinese Academy of Agricultural Sciences (ICS-CAAS). In Ukraine, two gene banks conserve 5022 proso millet accessions. In India, three organizations namely, the All India Coordinated Millet Improvement Project (AICMIP), International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) and the National Bureau of Plant Genomic Resources (NBPGR) conserve 2767 accessions (Upadhyaya et al. 2016). In the USA, 721 proso millet accessions are conserved at the North Central Regional Plant Introduction Station, USDA-ARS in Ames, Iowa.

Even though a large number of accessions are now available worldwide (Table 6.1), plant breeders often struggle to find the appropriate parents for use in crop improvement programs (Jia et al. 2017). The success of a breeding program relies immeasurably on the availability of trait-specific germplasm and its effective use (Upadhyaya

Table 6.1 Major gene banks conserving germplasms of proso millet worldwide

Country	Institute	Germplasm accessions		
		Cultivated	Wild	Total
Russia Federation	N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry (VIR)	8878	–	8778
China	Institute of Crop Science, Chinese Academy of Agricultural Sciences (ICS-CAAS)	8451	–	8451
Ukraine	Plant Production Institute nd. a. V. Ya. Yuryev of NAAS (IR)	1046	–	5022
	Ustymivka Experimental Station of Plant Production (UDS)	3975	1	
India	AICRP on Small Millets (AICRP- Small Millets)	920	–	2767
	International Crop Research Institute for the Semi-Arid Tropics (ICRISAT)	849	–	
	National Bureau of Plant Genetic Resources (NBPGR)	994	4	
USA	United State Department of Agriculture-Agricultural Research Service, North Central Regional Plant Introduction Station, (USDA-ARS, NCRPIS)	717	4	721

Source: Upadhyaya et al. (2016)

et al. 2013). The necessity to effectively utilize the large germplasm resources led to the concept of the *core collection* which involves identification of subsets of all the available accessions which can represent most of the diversity within the full set of germplasm collections (Jia et al. 2017). Core collections can facilitate cost-effective identification of accessions with desired traits for effective exploitation of germplasm resources in plant breeding (Upadhyaya et al. 2013, 2014). Additionally, core collection can support judicious management of a large collection. For example, it can be used to sort out redundancy in the large set of collections (Williams and Nyle 1991). Core collections cover approximately 5% of the full set, however, capture 90% of the full genetic spectrum. An even smaller subset, designated as a *mini core*, containing less than 1% of the full germplasm set, covers approximately 70% of the total range of genetic diversity (Jia et al. 2017). A core collection was generated for proso millet by Upadhyaya and his colleagues at ICRISAT, Hyderabad, India, based on geographic information and 20 qualitative and quantitative traits of the whole germplasm collection. All the 833 accessions were arranged into five groups on the basis of races and 20 morpho-agronomic traits were used to create clusters. A core collection comprising 106 accessions was constructed by random selection of approximately 10% accessions (or at least one) from each of the 101 clusters (Upadhyaya et al. 2011). More advancement in the development of core and mini core collections would confer robustness to plant breeding capabilities to achieve the goal of increased proso millet productivity.

6.3.2 Conservation of Genetic Resources

Conservation of proso millet genetic resources is very important. In vitro conservation is a method of indoor conservation of already-available germplasm for future uses. By contrast, in situ conservation allows nature to create new genotypes over time under a natural environment through natural evolutionary processes. There are a number of non-tissue culture based efforts for seed-based germplasm conservation of proso millet germplasm across the world. Unfortunately, there is no report on an in situ conservation effort, which is extremely critical for creating noble genetics adapted to future climatic conditions. The following section describes available plant germplasm institutes which conserve proso millet genetic resources in seed form under cold storage.

6.3.2.1 Core and Mini-core Collections

The scope of the global scientific community and research institutes working on various aspect of proso millet genetic improvement is very limited, unlike major the cereal crops like rice, wheat and corn (Appendix I). The majority of the institutes focus on germplasm collection, conservation and characterization. Only two and three institutes worldwide are actually working on proso millet variety development through hybridization-based plant breeding.

Proso millet germplasm collections are now conserved at a number of gene banks situated in several countries. They consist of more than 25,000 genotypes and there is tremendous diversity of various phenotypic characteristics including maturity (Fig. 6.4a), panicle morphology (Fig. 6.4b) and seed color (Fig. 6.5). Future proso millet genetic improvement depends on efficient utilization of this genetic diversity in breeding. A core collection should consist of genotypes from diverse environments and locations so that all the genetic variations can be captured. Trait-specific germplasm can be assembled to develop core collection in such a way as to cover maximum diversity with a minimum number of accessions. Core and mini core collections in proso millet can be developed based on important morpho-

Fig. 6.4 Proso millet germplasm diversity. (**a**) Maturity, (**b**) Panicle morphology variations. (Photos by: R. Khound, D.K. Santra, S.G. Rajput, unpublished)

Fig. 6.5 Proso millet germplasm diversity reflected in seed color variations. (Photos by: R. Khound, D.K. Santra, S.G. Rajput, unpublished)

agronomic and nutritional traits, such as drought tolerance, seed shattering and lodging tolerance, pest resistance, protein content, and phenolic compound levels. Such core sets would enable selection of parents with a great deal of diversity for the desired traits to obtain superior cultivars.

6.3.3 Germplasm Molecular Characterization

Information on genetic diversity is critical for conservation and the use of germplasm resources in crop improvement (Habiyaremye et al. 2017a). However, the genetic diversity in proso millet has been explored to only a limited extent (Goron and Raizada 2015). Genetic diversity study in proso millet is challenging due to its tetraploid genome and unavailability of sequence information (Hunt et al. 2011). Genomic resources such as molecular markers and genomic sequences can contribute significantly to genetic studies. Molecular markers have been playing a pivotal role in studies pertaining to genetic diversity assessment, taxonomic relationship

and population structure across diverse species (Karam et al. 2004; Rajput and Santra 2016). The number of markers available in proso millet, however, are very few (Rajput et al. 2014). Nevertheless, molecular markers, such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers have been employed in genetic diversity studies in proso millet (Habiyaremye et al. 2017a).

RAPD molecular markers were used to evaluate diversity among four species of *Panicum* and within proso millet accessions. They observed the distinction among the four species in terms of RAPD patterns and low correlation values. The proso millet accessions were separated into groups based on geographical origins. Their study demonstrated that molecular markers can be used as an effective tool in genetic diversity studies (M'Ribu and Hilu 1994). A little over a decade later, amplified fragment length polymorphism (AFLP) markers were utilized to investigate the genetic variation among three domestic and nine wild proso millet biotypes from the USA and Canada. An unweighted pair group method with arithmetic mean (UPGMA) cluster analysis on 39 polymorphic DNA fragments formed two separate groups irrespective of the geographic location. One group comprised six weed biotypes with cultivated types while the rest of the weed biotypes were placed in the other group. That suggested possible gene flow between weed and cultivated proso biotypes (Karam et al. 2004). Lágler et al. (2005) used inter simple sequence repeat (ISSR), simple sequence repeat (SSR) and cleaved amplified polymorphic DNA (CAP) markers to draw comparisons between medieval millet landrace to 20 common proso millet cultivars.

SSR markers or microsatellites are favored in genetic diversity assessment studies as they are abundant, evenly distributed in the genome, multi-allelic, codominant, highly polymorphic, easy to score and highly reproducible (Habiyaremye et al. 2017a; Rajput and Santra 2016; Rajput et al. 2014). SSR markers for proso millet have been extracted from the genomic sequences from other plants due to unavailability of sufficient proso genomic resources (Habiyaremye et al. 2017a). Hu et al. (2009) assessed the genetic diversity of 118 Chinese proso millet germplasm accessions collected from various ecological areas using 46 SSR markers from rice, wheat, oat and barley. The accessions showed moderate to high genetic similarity (GS) coefficients. UPGMA clustering analysis grouped the accessions into five distinct groups which were consistent with their ecological origins (Hu et al. 2009). Rajput et al. (2014) screened 8 proso millet genotypes with 548 switchgrass SSR markers as switchgrass is genetically the closest to proso millet. Out of the 548 SSR markers tested, 339 were amplified in proso, suggesting that 62% of the switchgrass SSR markers could be transferred to proso millet; 254 of these 339 SSR markers were found to be highly polymorphic among the 8 genera studied. Moreover, 984 alleles ranging from 50 to 1300 bp were amplified by the 254 SSR markers (Rajput et al. 2014).

The first SSR markers from the proso genome were developed by Cho et al. (2010). They developed and characterized 25 polymorphic SSR markers by constructing an SSR-enriched library from proso millet genomic DNA. A total of 110 alleles with an average of 4.4 alleles per locus were detected. A mean genetic similarity coefficient of 0.3711 indicated a wide genetic variation among the 50 proso

accessions used in the study. Hunt et al. (2011) investigated genetic diversity among 98 landrace germplasm from across Eurasia with the help of 16 proso millet-specific SSR markers. The accessions were split into two distinct genetic clusters, eastern and western clusters, consistent with their geographic origin. There was moderate genetic differentiation between the two clusters. They also put forth several postulations regarding the dissemination of proso millet from its centers of domestication (Hunt et al. 2011). Rajput and Santra identified 100 polymorphic markers by screening 709 SSR markers on 8 diverse genotypes. Out of the 100 polymorphic markers, 80 were from switchgrass (Rajput et al. 2014), 6 were from proso millet SSR markers developed by Cho et al. (2010) and 14 were from other species, namely, rice, wheat and oats (Hu et al. 2009). The selected polymorphic markers were successively used on 90 genotypes from 25 different countries, which included 15 North American cultivars (14 from the USA and 1 from Canada) and 75 landraces maintained by USDA. The authors observed a wide array of diversity among the 90 proso millet genotypes, which could be considered as a core collection of germplasm in the USA. However, all the cultivars from the USA were placed in the same group, possibly due to a narrow genetic base (Rajput and Santra 2016). Liu et al. (2016) developed 500 proso millet specific-SSR primer pairs through high-throughput sequencing. They screened the SSR primers on 8 accessions selected randomly from 73 non-repeated germplasm collections. Of all the SSR primers tested, 162 primers produced reproducible polymorphic fragments. A total of 67 SSR primers were used to investigate the genetic diversity in 88 accessions collected from different provinces in China. The study showed moderate diversity levels among the accessions studied (Liu et al. 2016).

Besides DNA markers, other genomic resources have also been utilized in genetic diversity studies in proso millet. Hu et al. (2008) used polymerase chain reaction (PCR) with 6 intron splice junctions (ISJ) and long random primers to assess the genetic diversity among 32 germplasms from China. The primers produced 42 polymorphic and reproducible fragments out of a total 56 fragments synthesized. The clustering of the accessions was linked to their geographic origins. An association between the glutinous/non-glutinous trait and the clustering was also established in the study (Hu et al. 2008). Hunt et al. (2010) sequenced the granule-bound starch synthase I (*GBSSI*) gene (*waxy* gene), which catalyzes amylose synthesis in the grain endosperm. The *waxy* gene was found to have two homologs, L and S forms, which are present in all proso millet accessions. They also identified three polymorphisms in the exon sequence coding for the *GBSSI* gene, one 15-bp deletion in the S type, and two sequence polymorphisms in the L type. The loss of function of the proteins resulting from deletion of the *GBSSI-S* gene was found to have a 100% correlation with the waxy phenotype (Hunt et al. 2010). Phenotyping evaluation of *GBSSI* genotypes showed that *GBSSI-S* locus the major locus responsible for the amylose content in the endosperm (Hunt et al. 2013). Genotyping of 178 proso millet individuals representing 147 landrace accessions from Eurasia with 2 *GBSSI* and 16 SSR markers (identified by Cho et al. 2010) revealed that *GBSSI* alleles exhibit distinct spatial distributions. The two *GBSSI* alleles were also found to have strong associations with phylogeographic clusters (Hunt et al. 2013).

Araki et al. (2012) theorized a new hypothesis on the origin and spread of waxy Japanese landraces with the use of PCR-based markers they developed for the *waxy* gene. Based on the analyses, they proposed two independently dispersing lineages of proso millet in Japan (Araki et al. 2012). Hou et al. (2017) used Illumina sequencing technology and de novo transcriptome assemblies to develop short-read sequences and de novo assembly of proso millet, respectively. They found 25,341 unique gene sequences and identified 4724 genic SSR markers from the sequence data of the proso millet transcriptome. A total of 229 SSR markers were used on 56 germplasms collected from 14 geographic regions in China. Upon validation, 14 polymorphic SSR markers with a polymorphic marker ratio of 6.1% (14/229) and 43 alleles were identified. The two clusters obtained from the Bayesian population analyses of the 56 accessions showed a high genetic similarity. There was no correlation between genetic diversity and the geographic location of the proso millet germplasm (Hou et al. 2017).

6.4 Conventional Breeding Approaches

Plant breeding encompasses methods for the creation, selection and establishment of superior phenotypes with the goal to obtain improved cultivars with enhanced yield, enriched nutritional qualities, abiotic and biotic stress tolerance and other commercially-important traits. Prehistoric selection of improved crop varieties based on visual cues led to the domestication of first crop varieties thereby laying the foundation of modern-day plant breeding (Moose and Mumm 2008). Breeding efforts for proso millet improvement have been carried out in different parts of the world, and some success has been achieved despite the lack of sufficient genetic characterization. The countries with major contributions to proso millet breeding are China, India, the USA, Russia and Kenya (Gomashe 2017).

China pioneered the breeding work in proso millet by initiating an improvement program in the 1940s. The primary research work in China on proso millet involves germplasm management, which includes germplasm collection, sorting and evaluation and breeding (Diao and Jia 2017). The earliest work was started by the Agricultural Experiment Farms of the former province of Suyuan (currently town of Langshan, Linhe District, Inner Mongolia) and the Guanghua Farm (presently known as the Yan'an Research Institute of Agricultural Sciences) of the Shan-Gan-Ning Region, which involved screening of large numbers of germplasm and landraces, selection and recommendation of well-performing cultivars for farming. Langshan 462 and Micang 155 were the two earliest proso millet cultivars screened by the Agricultural Experiment Farm. The major nationwide proso germplasm management effort began in 1957 with several prominent agricultural institutes initiating germplasm collection, characterization and evaluation. The Crop Breeding Institute of Heilongjiang Academy of Agricultural Sciences initiated the use of a hybridization technique in proso millet breeding. This led to the development of 10 cultivars including Longshu 16, the first Chinese proso millet variety

bred by the intraspecific hybridization technique (Gomashe 2017). The primary methods employed in proso millet breeding in China are pure line selection and cross-based pedigree selection. Due to the difficulties in hybridization, attributable chiefly to the self-pollinating nature of proso, only a few developed cultivars have been registered and released for cultivation. The most notable cultivars are Neimi 5, Ningmi 9, Longmi 4, Ningmi 10, Longshu 16 and Jinshu 4. Approximately 162 cultivars have been released in China since the 1950s. (Diao and Jia 2017).

In India, a small millet improvement effort is led by All India Coordinated Small Millets Improvement Project (AICSMIP) headquartered in Bangalore, centers located in state agricultural universities (SAUs), ICAR institutes and cooperating centers. Germplasm from various sources has been collected in order to pool available germplasm and make it available to the breeders in India. AICSMIP maintains 939 accessions of proso millet. Sixteen proso millet cultivars have been released since the inception of the project. The improved cultivars have been developed using pure-line selection, pedigree breeding and backcrossing (Gomashe 2017). Ram Cheena, with an improved yield of 2000–2500 kg/ha, was the first proso millet cultivar developed using pure-line selection, in 1960. Tamil Nadu Agricultural University (TNAU) has been the leader in variety development with 10 cultivar releases from 1980 to 2011. The K2 was the first proso cultivar developed at TNAU by screening and selection from local lines. The cultivar offered resistance to major pests and diseases known in proso millet. Cultivars TNAU 145 and 151, with resistance to rust and shoot fly, were released in 2009 (Gomashe 2017).

The only proso millet breeding program in the USA is located at the University of Nebraska-Lincoln, Panhandle Research and Extension Center (UNL-PHREC), Scottsbluff, Nebraska. It was established in 1972 by Lenis Nelson with the aim to improve proso millet productivity. Hybridization in proso millet is difficult due to its floral morphology and the pattern of anthesis (Upadhyaya et al. 2016). Nelson described emasculation and crossing techniques applicable to proso millet hybridization (Nelson 1984). He released four cultivars (Sunup, Dawn, Cerise, Rise) with enhanced productivity. Sunup showed excellent yield potential, producing white seeds on compact panicles (Nelson 1990). Sunup exhibited lodging tolerance and less susceptibility to grain shattering compared to the common proso cultivars of that time; it became the most commonly growing proso millet cultivar upon its release. Dawn is a very short, early-maturing cultivar with a compact panicle and white seed. Dawn may be direct-harvested owing to its short height and early maturity. Rise is a taller, better-yielding cultivar than Dawn. However, both showed lower yield compared to Sunup. Cerise is an early-maturing cultivar with small and red-colored seed. Its seed is of low value as feed owing to a high tannin content (Baltensperger et al. 1995a). Lenis's successor, David Baltensperger, took over the responsibility of the breeding program in 1988. He released several popular proso millet cultivars namely, Earlybird (Baltensperger et al. 1995b), Huntsman (Baltensperger et al. 1995c), Sunrise (Baltensperger et al. 1997), and Horizon (Baltensperger et al. 2004). Dawn was used as a common parent in the crosses made to develop these cultivars. Early bird is a short, medium-maturity variety with large, white seeds and good straw strength. Huntsman is a moderately-maturing white-

seeded variety with yield performance similar to Dawn. Sunrise is a moderately-maturing, short stature, a large and white-seeded cultivar with slightly better yield performance than Sunup. All these cultivars were developed from multiple crosses, whereas Dawn was a common parent in all the cultivars. Horizon was developed by the single-plant F_4 selection made on a bulk population created by hot water emasculation and random pollination for two generations, and subsequent single-seed descent selection. Horizon is mid-early maturity, a short cultivar with large, white seeds. All these cultivars are grown extensively in Colorado, Nebraska and South Dakota (Gomashe 2017; Santra 2013). Waxy type (amylose-free starch) of proso millet is of great importance in human food market due to its glutinous property and excellent adhesion quality after cooking. Six waxy accessions were identified from 650 accessions (USDA-ARS North Central Regional Plant Introduction Station, Ames, Iowa) (Graybosch and Baltensperger 2009). One of the identified waxy accessions, PI 436626 (Lung Shu 18), a Chinese accession, was crossed with Huntsman to obtain a new cultivar, Plateau (Santra et al. 2015). The focus of the current breeding program led by Santra at UNL-PHREC is to devise strategies to develop and exploit genomic resources and use genomic tools to hasten the proso millet variety development process.

The primary focuses of proso millet breeding programs in Russia are to increase productivity, disease resistance and grain quality (Gomashe 2017). Around 50 cultivars of proso were bred using intraspecific hybridization, the most notable of which were Bistroye and Krupnoskoroe. The latter cultivar is known for the large and uniform grains it produces. In 2006, two cvs. Sputnik (spp. *coccineum*) and Slavjanskoe (spp. *subflavum*), endowed with the high yielding capability (7 mt/ha) were developed. Another cv. Sojuz with resistance to smut and a high yield of 6 mt/ha was developed. In 2011, a cultivar Regent developed via anther-culture technique exhibited high yield, medium-maturity and lodging and shattering tolerance (Gomashe 2017).

6.5 Breeding Challenges and Opportunities

Although predominantly self-pollinating, natural cross-pollination in proso millet may exceed 10% (Baltensperger 2002; Gomashe 2017). This presents an opportunity to make artificial crosses to generate intervarietal hybrids. However, plant breeding for proso millet improvement is challenging in several ways. The major pitfalls and challenges of the current millet breeding strategies are discussed below.

6.5.1 Disease and Pest Tolerance

No significant insects and diseases are reported in most proso millet production regions in the world. This is not a significant issue of proso millet germplasm development except for a few specific production regions with high humidity in Russia,

Belarus and China where smut is a common disease. Smut is a fungal disease caused by fungal pathogen with multiple races (Sp_1, Sp_2, Sp_3, Sp_4). A few smut resistant cvs. (Sputnik, Slavjanskoe, Quartet) are available at the All-Russia Research Institute of Legume and Groat Crops (GNU VNIIZBK) in Orël (Zotikov et al. 2012). Sputnik and Slavjanskoe are resistant to races Sp_2, Sp_3, and Sp_4, whereas Quartet is resistant to all four races.

6.5.2 Flowering Time and Floral Morphology

A primary challenge in proso millet improvement via intraspecies hybrid generation is the brief and unsynchronized period of flowering and the complex flower morphology. Proso flowers usually open between 1000 and 1200 h and there is a narrow window of roughly 7 min between the opening and closing of flowers. The floral morphology with small florets with tightly held lemma and palea makes emasculation before the advent of anthesis reasonably difficult (Gomashe 2017). Successful crossing in proso millet requires exceptional skills and experience.

6.5.3 Grain Shattering and Lodging

Seed ripening in proso millet is usually ununiform, starting from the top of the panicle and progressing downward. This unsynchronized ripening results in immature, green seeds in the lower part of the panicle. There is a risk of considerable loss of yield from seed shattering if harvesting is delayed allowing maturity of the complete panicle (Gomashe 2017). Besides, significant yield loss is also attributable to lodging. Furthermore, proso millet cannot be direct-combined due to its high tendencies of seed shattering and lodging (Rajput et al. 2016). Proso millet is commonly swathed once it attains physiological maturity to prevent yield loss due to lodging and seed shattering of standing plants from high wind, rain and hail damage (Henry et al. 2008; Rajput et al. 2016). However, this method results in considerable loss of yield and quality. These difficulties call for the development of proso millet cultivars which can tolerate the brunt of seed shattering and lodging on yield better than the available cultivars and are suitable for the direct combine (Rajput et al. 2016).

Seed shattering, lodging and high moisture content in the straw in proso millet are the major stumble blocks in the mechanization of the harvesting procedure. Development of direct harvesting capabilities would prevent the yield loss incurred from the usual windrowing or swathing method of harvesting. Thus, there is the need to develop cultivars with erect stature, optimal height, branching and panicle morphology suitable for combine harvest (Gomashe 2017).

Seed shattering and lodging are two primary constraints in developing directly-harvestable proso millet cultivars. Therefore, it is important to develop cultivars with tolerance to lodging and shattering. Rajput et al. (2016) detected QTLs and flanking markers for seed shattering and lodging on the linkage map they developed. These can be used as molecular markers in future studies for understanding the genetics of these high-value traits and developing resilient proso millet cultivars.

6.5.4 Phenotyping and High-Throughput Phenotyping

The manifestation of important agronomic traits in crops is determined by quantitative or qualitative evaluations of phenotypes, which relies on the interactions of genotypes with the environment (GxE). The traditional phenotyping procedures employed are time-consuming, labor-intensive, expensive, and mostly destructive, resulting in a bottleneck in proso millet improvement efforts (Fahlgren et al. 2015). Therefore, it is imperative to develop and incorporate the capacity for rapid and precise high-volume phenotyping systems in millet breeding programs to take advantage of the advances in DNA sequencing technologies.

Significant advances have been made in high-throughput genotyping wherein genome-wide genetic markers are routinely used (Chen et al. 2014). However, plant phenotyping still relies on long, expensive and laborious traditional procedures resulting in a bottleneck in crop improvement (Fahlgren et al. 2015). The recent development of high-throughput phenotyping techniques involving high-resolution imaging, spectroscopy, robotics and high-performance computing models has revolutionized the field of plant phenomics (Chen et al. 2014). High-throughput phenotyping has been used to study various plant phenotypic characteristics such as plant growth (Ge et al. 2016), biomass (Golzarian et al. 2011), leaf morphology (Ma et al. 2013; Yang et al. 2013) and nutrient status (Pandey et al. 2017), to name the few. Aerial imaging-based high-throughput phenotyping in proso millet was recently initiated for limited phenotypic traits (heading date, maturity, panicle morphology) at the University of Nebraska-Lincoln (Schnable et al. 2018). Advances in plant phenomics could play a pivotal role in the advancement of conventional, molecular and transgenic plant breeding for improvements of important crops (Araus and Cairns 2014). Therefore, future proso millet breeding programs could involve both high-throughput genotyping and high-throughput phenotyping methods to make crop improvement faster and more efficient.

6.5.5 Wide Hybridization

Proso millet cultivars usually have a narrow genetic base as they originated from intraspecific crosses involving only a few selected parents. As a result, the plants may progressively lose effectiveness in resistance to persistent biotic and abiotic

pressures. Moreover, inbreeding depression may also lead to significant reductions in yield. Therefore, it is imperative to explore the wild relatives in search of novel genes for introgression to make new combinations of genes (Gomashe 2017). Hunt et al. (2014) identified *Panicum capillare* and *P. repens* as the probable diploid parents of the tetraploid proso millet. Such relatives can be exploited to expand the genetic base to create cultivars with improved biotic and abiotic stress responses.

6.5.6 Genome Editing

Rapid developments in genome editing technology has opened up new dimensions in basic plant biology research, as well as genetic improvement of economically-important crops (Bortesi and Fischer 2015). Concerns about the presence of foreign DNA in the host genome in genetic engineering has been mitigated to a large extent by using sequence specific nucleases (SSNs) in the genome editing approaches (Soda et al. 2018). A widely used genome editing tool is CRISPRs-Cas technology, which uses clustered regularly interspaced short palindromic repeats (CRISPRs) and CRISPR-associated nucleases (Cas), transcription activator-like effector nucleases (TALENs) and zinc-finger nucleases (ZFNs) (Zhu et al. 2017a, b).

CRISPR-Cas 9 has been used to induce mutation and knock-in or knock-out in genes of various functions in different plant species. Targeted mutation of diverse genes has been achieved in *Arabidopsis*, soybean, rice, tomato, *Populus* spp., potato, and maize. Knock-out of the *ZmIPK* gene in maize, *potPDS* and *4LC2* genes in *Populus trichocarpa*, and knock-in of *DD43* region in soybean and *ANT1* gene have been done using CRISPR-Cas9 gene editing (Soda et al. 2018). ZFNs are designed restriction enzymes with sequence-specific DNA binding domains, which mediate doubled strand breaks and error-prone non-homologous end joining (NHEJ) repair resulting in mutations. ZFN-mediated heritable mutations have been reported in *Arabidopsis* (*ABI4*, *ADH1* and *TT4* genes) and soybean (*DCL4a* and *DCL4b* genes) (Petolino 2015). Similar to ZFNs, TALENs introduce precise genome modifications by mediating DSB locations and subsequent repair mechanisms. TALENs have been successfully applied in both monocot and dicot plant species including tobacco, *Arabidopsis*, rice, maize, barley and a grass (*Brachypodium* sp.) (Wright et al. 2014). CRISPR-Cas9-mediated genome editing in wild foxtail millet (*Setaria viridis* (L.) P. Beauv.) (Chuanmei Zhu et al. 2017a, b). Although the application of genome editing in proso millet is in its nascent phase, the development of first linkage map (Rajput et al. 2016) and several promising techniques (Varshney et al. 2010) for genetic modification of this crop has opened a new dimension to this dryland crop's research. Availability of proso millet genome sequences, genome databases and transformation method will encourage the future research.

6.5.7 Mutation Breeding

Mutation breeding is the process of purposely inducing mutation via artificial mutagenesis and breeding for variations not present in the genetic pool of a plant species. The mutagenic agents used for mutagenesis can be classified as physical, such as ionizing (X-rays and ɣ-rays) and non-ionizing radiations (ultraviolet rays), and chemical agents such as ethyl methane sulfonate (EMS), methyl methane sulfonate (MMS), diethylsulfate (DES), and nitrosoguanidine (NS). Among the chemical mutagens, ethyl methanesulfonate (EMS) is widely used for its effectiveness and easy handling procedure (Pathirana 2011). There are only a few available reports on mutation breeding in millets. A combination of ɣ-rays and EMS was used to produce high yielding progeny in finger millet (Muduli and Misra 2007). In another study, dry seed of two finger millet cultivars were exposed to four doses of ɣ-rays. The resulting M2 progeny exhibited significant changes in flowering, maturity and plant height (Ambavane et al. 2015). Nirmalakumari and coworkers (Nirmalakumari et al. 2007) observed high variability for plant height and number of tillers in two little millet cultivars irradiated by ɣ-rays. In proso millet, treatment with 0.2% EMS produced M2 progeny with relatively improved panicle filling, shattering tolerance and grain yield (Singode et al. 2018). Mutation breeding may have increased applications in future proso millet breeding programs as a means to overcome the challenges of poor combining ability and narrow genetic base in introducing economically significant genetic variations to cultivated germplasm.

6.6 Role of Biotechnology in Genetic Improvement

There is little reported biotechnology research based on tissue culture (except a couple of anther culture protocols) and genetic engineering primarily because proso millet is considered a minor crop. Nevertheless, both these methods are extremely important for development of new genotypes for future use. A genetic transformation technique would be necessary for future application of genome editing technology in specific areas such as biofortification and disease resistance.

6.6.1 Genomic Selection

Implementation of biotechnology tools such as genomic selection (GS) and double-haploid technology has tremendous potential to increase proso millet breeding efficiency. GS requires a large number (several thousand to millions) of DNA markers distributed throughout the genome, which is very limited in proso millet. Single nucleotide polymorphism (SNP) markers are ideal for this purpose. This requires proso millet genome sequencing and which was recently completed (Zou et al.

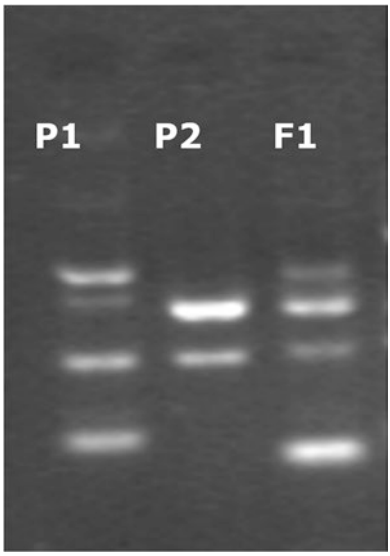

Fig. 6.6 DNA (SSR) marker-based identification of proso millet hybrid. (Photo by S.G. Rajput and D.K. Santra, unpublished)

2018). Once the genome sequence is publicly available, millions of SNP markers can easily be developed for utilization in GS-based molecular breeding. Besides, the SNPs will be highly useful in true F_1 identification. False F_1 hybrids, due to small flowers and short crossing time, is one cause of inefficiency in proso millet breeding. SSR-based DNA markers are used in the detection of true F_1 but their usefulness is limited due to scarce polymorphism of SSR markers in proso millet (Fig. 6.6). Therefore, SNP-based detection of true hybrids will be a normal practice in proso millet breeding with availability of thousands of SNPs.

6.6.2 Double Haploids

Double-haploid technology for developing 100% homozygous plants is another powerful biotechnology tools in modern plant breeding. The essence of plant breeding is to generate genetically superior crops by making intraspecific hybrids and pure-line selection for the desired traits. Pure-line selection often involves 5–6 generations of inbreeding and selection to attain 98% homozygosity. For this reason, it often takes 11–13 years to release a new crop cultivar (Khound et al. 2013; Yan et al. 2017). The coveted homozygosity can be reached in a much shorter time by applying doubled haploid techniques. For instance, microspore culture can produce 100% homozygous lines in one generation, thereby reducing the time, space and labor required for cultivar development (Khound et al. 2013). Heyser and Nabors (1982) developed proso millet calli from a variety of explants using Linsmaier and Skoog (L and S) medium supplemented with different levels of auxins. They reported that 32% of the embryogenic calli showed shoot regeneration. Bobkov and Suvorova

(2012) used anther culture technique to study the efficiency of embryogenic callus induction and regeneration in proso millet. They observed successful callus formation (3.3–9.2% average frequency) and regeneration after applying heat (32 °C) and cold (4 °C) stresses to the anthers (Bobkov and Suvorova 2012). More studies need to be done on improving the haploid plant generation from anther and microspore culture techniques to assist in proso millet breeding activities. When both genomic selection and double-haploid technology are used together in breeding, selection efficiency is improved significantly and cultivars with multiple desirable traits can be developed in a short time, which is not possible in conventional plant breeding.

6.6.3 Genomics and Phenomics

Significant advances have been made in understanding the basic structural and functional aspects of plant genomes. Robust crop improvement strategies may be devised by admixing the basic knowledge from genomic research with the conventional plant breeding techniques (Varshney et al. 2005). Plant genomics provides the tools essential for meeting the challenge of enhancing sustainable grain yield, quality and production stability in crop breeding programs (Kole et al. 2015; Varshney et al. 2014). Incorporation of genomics approaches in advanced plant breeding strategies may allow a better understanding of genetic diversity at the species and gene levels, germplasm enrichment and faster crop improvement via application of marker-assisted selection (MAS) in crossbreeding (Kole et al. 2015). More efficient breeding strategies are being developed by combining genomic tools and high-throughput phenotyping techniques. One approach adopted in advanced crop breeding is to develop genetic maps and identify gene functions through association studies and quantitative trait locus (QTL) mapping. With the knowledge of the location of the gene of interest and QTLs, effective parental selection and MAS can be performed. Another approach is to take advantage of the tremendous advances made in next-generation sequencing (NGS) technology. Sequencing of large populations using NGS has significantly improved the resolution of gene and QTL discovery. The amalgamation of genotypic and phenotypic information can assist in genomic selection (GS) by developing prediction models (Varshney et al. 2014). Application of high-throughput genotyping and phenotyping in plant breeding can enable assessment of large plant populations in a very short time (Varshney et al. 2018), thereby saving time, labor and monetary resources associated with crop improvement programs.

Scant research has been conducted in the fields of proso millet genetics, genomics and breeding largely due to its minor crop status, despite its potential implications in food diversification around the globe. No genetic linkage and QTLs map in proso millet was available until Rajput et al. (2016) published the first-ever linkage map. They developed the first genetic linkage map of proso millet based on 833 genotypes by sequencing single nucleotide polymorphism (GBS-

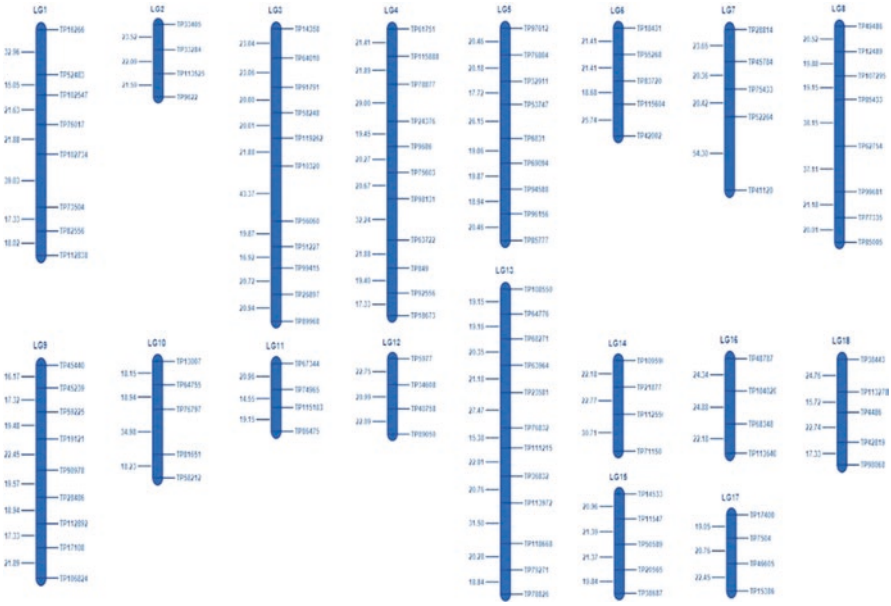

Fig. 6.7 Genetic linkage map of proso millet (18 major linkage groups) constructed using 93 recombinant inbred lines of cv. Huntsman X Minsum. GBS-SNP marker names are on right-hand side and genetic distances (cM) between the markers are on left-hand side. (Source: Rajput et al. 2016)

SNP) using markers of 93 recombinant inbred lines. The SNPs formed 18 major and 84 minor linkage groups (LG), of which the 18 major LGs were considered as 18 haploid chromosomes of proso millet as there was no prior report on proso linkage maps (Fig. 6.7). They also detected 18 QTLs for 8 morpho-agronomic traits such as plant height, heading date, peduncle length, lodging, grain shattering, panicle length, grains per panicle, and 100 seed weight (Fig. 6.8). The phenotypic variance associated with the QTLs was in the range of 13.2–35%. DNA markers flanking the QTLs were also detected, which can be used in MAS of high-value traits (Rajput et al. 2016).

Yue et al. (2016) used Illumina sequencing technology to sequence and assemble the proso millet transcriptome. The reads obtained after sequencing of the two genotypes, Yumi No. 2 and Yumi No. 3, were assembled into unigenes, 62,543 contigs of which were assigned to 315 gene ontology (GO) categories. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis mapped 15,514 unigenes to 202 KEGG pathways. Besides, 292 differentially-expressed genes were identified in the study. Additionally, Yue et al. (2016) identified 32,216 SSR markers and little more than 400,000 SNP loci which may function as molecular markers in genomics-based breeding techniques (Habiyaremye et al. 2017a; Yue et al. 2016).

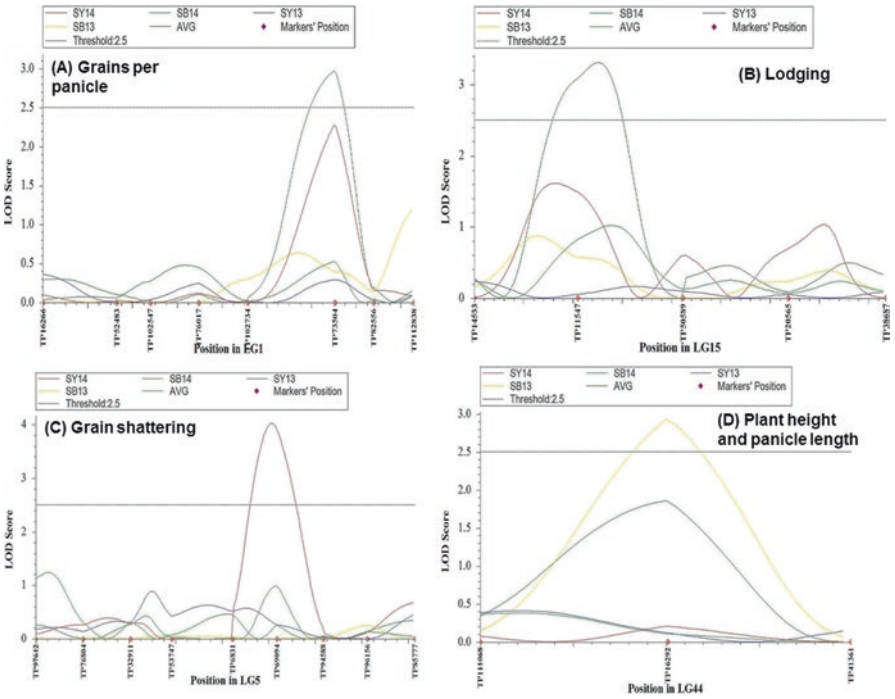

Fig. 6.8 QTLs detected following composite interval mapping CIM for: (**a**) Grains per panicle, (**b**) Lodging, (**c**) Grain shattering and (**d**) Plant height and panicle length based on phenotypic evaluation at Scottsbluff (SB) and Sidney (SY) locations in 2013 and 2014. Marker name are given on X-axis and LOD values are on Y-axis. (Source: Rajput et al. 2016)

6.6.4 Biofortification

Biofortification is an effective way to deal with malnutrition in developing countries resulting from micronutrient deficiency in basic foods (Boncompagni et al. 2018). Millets are highly nutritious owing to the presence of high levels of proteins, crude fiber, minerals like iron and zinc, vitamins and essential amino acids. However, the good health effects are marred by the presence of anti-nutrients, such as, phytic acid, polyphenols and tannins, which interfere with the bioavailability of multivalent cations, e.g. Fe^{2+}, Zn^{2+}, Ca^{2+}, Mg^{2+} and K^{+} (Vinoth and Ravindhran 2017). The presence of these antinutrients has slowed biofortification efforts in millets (Boncompagni et al. 2018). Vinoth and Ravindhran (2017) proposed two strategies to biofortify millets: (a) enriching millet grains with nutrients and (b) ameliorating the bioavailability of minerals by reducing antinutrient levels. Vetriventhan and Upadhyaya (2018) observed large variation in proso millet accessions in terms of grain content of Fe, Zn and Ca. They also identified 26 accessions with high levels of multiple grain nutrients (Vetriventhan and Upadhyaya 2018). Such accessions can be used as parental populations in breeding programs aimed at developing high-yielding, nutrient-rich varieties. Levels of the antinutrient phytic acid have been successfully

lowered in rice by producing low phytate (lpa) mutants and RNAi silencing of the gene coding for IPK1 (Inositol 1,3,4,5,6-pentakisphosphate 2-kinase) enzyme involved in phytic acid biosynthetic pathways (Vinoth and Ravindhran 2017). Similar strategy can be adopted for proso millet grain quality improvement by reducing the amount of the antinutrients.

6.7 Conclusions and Prospects

Proso millet is a neglected minor crop. Research funding is extremely scarce and only a small number of scientists worldwide are investigating the crop. Therefore, effective cooperation and collaboration in all aspects of genetic improvement (germplasm exchange, characterization, genetics, genomics- and phenomics-based breeding) is imperative. There has already been excellent progress in recent decades. The proso millet genome sequence is already available in a private seed company and the company is using the sequence in genomic selection in its breeding program. Public proso millet genome sequence will soon be available. Research in high-throughput phenomics and transcriptomics has recently started. Therefore, it is anticipated that the benefit of modern plant breeding tools will soon be applied in public proso millet breeding programs around the world. Therefore, the availability of improved proso millet cultivars with resistance to critical traits (lodging, seed shattering, fungal diseases) and unique grain quality characteristics for human food and other uses is now a reality. Genome editing techniques such as CRISPR-Cas9 can remove antinutritional factors in proso millet with the identification and characterization of the involved genes. Such progress will improve proso millet productivity, diversify its uses beyond bird feed especially in gluten-free cereal-based healthy food. In near future, proso millet may become an important common food crop and play significant role not only in global food security, but in human and climate health security as well.

Proso millet is a very suitable crop with respect to climate change mitigation. Proso millet is both climate-smart and climate-friendly as well as a healthy human food crop. Proso millet is drought and heat tolerant, requires less amounts of N-fertilizer, and has a short growing season. Proso millet is not affected by any major diseases and insects. This is a cereal with the highest water use efficiency (requiring the least amount of water to produce a kg of grain) among all cereal crops. Future climate change predicts warmer climate with serious scarcity of fresh water and new disease and insect outbreaks due to extreme climate variability. Proso and many other millets (pearl, finger, foxtail) can play critical roles in future global food and nutritional security because of their unique potential in future climate mitigation. Therefore, millets must receive more attention from public policymakers and the scientific community and greater progress in genetic improvement and related research.

Acknowledgments The authors acknowledge informal contributions on various aspects of proso millet genetic and breeding (often unpublished) by global proso millet scientists at various national and international scientific meetings.

Appendices

Appendix I: Research Institutes Relevant to Proso Millet

Institution	Specialization and research activities	Contact information and website
USDA-ARS North Central Regional Plant Introduction Station	Proso millet germplasm collection, conservation, and characterization	Plant Introduction Unit, G212 Agronomy Hall, Iowa State University, Ames, IA 50011, USA. E-mail: dbrenner@iastate.edu Website: https://www.ars.usda.gov/midwest-area/ames/plant-introduction-research/
University of Nebraska-Lincoln Panhandle Research and Extension Center	Proso millet germplasm characterization, utilization, breeding, genetics, genomics, and biotechnology	University of Nebraska-Lincoln 4502 Avenue, Scottsbluff, NE 69361, USA E-mail: dsantra2@unl.edu; schnable@unl.edu Website: https://www.unl.edu/ https://extension.unl.edu/statewide/panhandle/
Institute of Crop Science, Chinese Academy of Agricultural Sciences (ICS-CAAS)	Proso millet germplasm collection and characterization	ICS-CAAS, No.12 Zhongguancun South St., Haidian District, Beijing, China 100,081 Email:zuokesuo@caas.cn Website: http://ics.caas.cn/en/
Northwest A&F University	Proso millet breeding, end-use characterization	Northwest A&F University, No.3 Taicheng Road, Yangling, Shaanxi, China, 712,100 Email: web@nwafu.edu.cn, fengbaili@nwsuaf.edu.cn Website: http://en.nwsuaf.edu.cn/
Crop Research Institute, Gansu Academy of Agricultural Sciences	Proso millet breeding and germplasm evaluation	Nongkeyuan Xincun 1, Anning District, Lanzhou City, Gansu Province, China; E-mail: yangtianyu@gsagr.ac.cn Website: http://www.gsagr.ac.cn/
Shanghai Centre for Plant Stress Biology, Chinese Academy of Sciences	Proso millet genome sequencing and characteristics	No. 3888 Chenhua Road, Shanghai 201,602, China E-mail: zhangheng@sibs.ac.cn Website: http://www.psc.ac.cn
International Crop Research Institute for the Semi-Arid Tropics (ICRISAT)	Germplasm characterization and conservation	ICRISAT, Hyderabad, India, Email: ICRISAT@cgiar.org; m.vetriventhan@cgiar.org Website: https://www.icrisat.org/
Indian Institute of Millet Research (IIMR)	Breeding and biotechnology	Hyderabad-500,030, Telangana. India, E-mail: director.millets@icar.gov.in; avinash@millets.res.in Website: http://millets.res.in/

(continued)

Institution	Specialization and research activities	Contact information and website
Kangwon National University	Germplasm collection, conservation, and characterization	101–904, Ilsung Apt. Hyoja 3dong, Chuncheon, Korea. E-mail: chpark@kangwon.ac.kr; Website: https://www.kangwon.ac.kr/english/index.do
All-Russia Research Institute of Legume and Groat Crops (GNU VNIIZBK)	Breeding, genetics, biotechnology, tissue culture	All-Russia Research Institute of Legume and Groat Crops (GNU VNIIZBK), Russia, Orel, E-mail: office@vniizbk.orel.ru; svbobkov@gmail.com, Website: http://www.vniizbk.ru/en/structure/selection-center/laboratory-of-breeding-of-groat-crops.html

Appendix II: Proso Millet Genetic Resources

Cultivar	Important traits	Cultivation location
Dawn	Very early maturing; uniform ripening; large seed	USA
Rise	Stable under wide range of production environments; small seed. Tight panicle	USA
Sunup	Stable under a wide range of production environments	USA
Earlybird	Good straw strength, tight panicle; very large seed size, Early maturing	USA
Huntsman	Closed type panicle, large seed size, late maturity	USA
Sunrise	Large seed size, compact panicle	USA
Horizon	Large seed size; closed type panicle	USA
CO-3	Shining golden yellow grain drought tolerant, leaf pubescent	India
Nagarjuna	Early maturity	India
Sagar	High seed yield	India
Bhawna	Stout, dwarf	India
CO(PV)5	Resistant to brown spot and tolerant to rust and grain smut	India
TNAU151	Tolerant to rust and shoot fly	India
TNAU164	Resistant to rust and grain smut	India
TNAU202	Drought resistant	India
Bistroye	High yielding early maturing	Russia
Kruppnoskoroe	High yield, early maturing, large grain	Russia
Sputnik, Slavjanskoe	High yield, mid-early maturing, resistant to smut, excellent groat quality	Russia
Alba	High yield, mid-early maturing, vigorous ripening, resistant to lodging, resistant to shattering, easy to hull, high output of groat	Russia

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Chapter 7
Quinoa (*Chenopodium quinoa* Willd.) Breeding

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Abstract Quinoa is native to the Andean Region, with recognized nutritional value and the ability to thrive in marginal agricultural environments. It is a very important alternative crop to face the negative environmental changes that are reducing yield and quality, and causing food insecurity during recent decades. This species has been cultivated in the Andean Region for thousands of years in very marginal environments from sea level in Chile to more than 4000 m elevation in the Peruvian and Bolivian Altiplano. High genetic diversity of quinoa ecotypes made it possible to yield quality grains in soil pH values of 4.5–9.5> in diverse annual rainfall 200–2000 mm, and at very low temperatures in flowering and grain-filling periods, with diseases, insect epidemics and other negative management practices. The recognition of quinoa values since the 1980s has increased significantly the demand and interest from other countries to grow this plant in marginal lands. Cultivation has increased notably in the Andean Region, in North America, Europe, Asia and Africa, with very good agricultural and industrial results. Current wide distribution and planting in large-scale farms have shown limitations because growth conditions are different from those typical in the origin center. High susceptibility to biotic factors (diseases, pests and weeds), low heat tolerance, damage by long photoperiods, lack of appropriate culture technologies for different farming systems, and limitations in food elaboration and industrial uses, are major limitations. These can be overcome with new improved varieties using the highly diverse germplasm and appropriate breeding methodology; and employing appropriate agronomic practices for sustainable production to ensure food security in marginal lands and environments.

Keywords Abiotic factors · Adaptation · *Chenopodium* · Germplasm · Nutritious value · Quinoa · Tolerance

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J. M. Al-Khayri et al. (eds.), *Advances in Plant Breeding Strategies: Cereals*,
https://doi.org/10.1007/978-3-030-23108-8_7

7.1 Introduction

7.1.1 Botanical Classification

Quinoa (*Chenopodium quinoa* Willd.) belongs to the genus *Chenopodium* sensu stricto of the subfamily Chenopodioideae in the family Amaranthaceae in the APG II system (http://www.itis.gov). Using nuclear ITS and chloroplast trnL-F and matK/trnK sequences, Fuentes-Bazan et al. (2012a, b) and Jellen et al. (2015) provided evidence that the genus *Chenopodium* is divided into *Chenopodium* (*C. quinoa*, *C. album*); *Chenopodiastrum* (C. *murale*, *C. hybridum*); *Oxybasis* (*C. glaucum*, *C. rubrum*, *C. urbicum*); *Lipandra* (*C. polyspermum*); *Blitum* (*C. capitatum*, *C. bonus-henricus*); *Dysphania*, *Teloxys* (*C. aristatum*).

7.1.2 Distribution

Quinoa was a staple food in Pre-Columbian times. The descendants of the Inca Empire (millions of Quechua- and Aymara-speaking native peoples of the Andes from Colombia, Ecuador, Peru, Bolivia, Chile and Argentina) used quinoa as an important component to their daily diet. Its use, however, declined due to the Spanish introduction of crops such as wheat, barley, oats, broad beans and peas. Quinoa's lower yield, lower demand and higher production costs made it difficult to compete with these introduced crops. Over centuries, quinoa was cultivated at a range of elevations (0–4000 m) by small-scale farmers in traditional regions of the Andean countries of Bolivia, Peru and Ecuador. In Argentina, Chile and Colombia, quinoa cultivation was carried out in very small areas mainly in farm orchards, gardens or in a few rows in the field among other crops.

Worldwide recognition of quinoa's nutritive value opened a broad national and international market for quinoa producers and industry. This increased the cultivated area and yield in Bolivia, Ecuador and Peru by different magnitudes until 2014 (Bedoya-Perales et al. 2018). In the past 2 years, the area, yield and production showed a reduction in trend because national and international market prices decreased (Table 7.1). In other Andean countries like Chile, Argentina and Colombia the area and production are still small.

The most important activity promoting quinoa expansion worldwide was the American and European Test of Quinoa made by the FAO Project in 1996–1998 (Mujica et al. 2001) and actions developed during the International Year of Quinoa contributing to recognition of quinoa's nutritive value and adaptation to marginal lands.

Nowadays, according to Bazile et al. (2016b), there are 95 countries performing quinoa agricultural trials. Results are very promising and in some countries, such as Tibet, Morocco, France, India (Bhargava and Ohri 2015), China, United Kingdom, Sweden, Denmark, Netherlands, Italy (Pulvento et al. 2015), Kenya (Oyoo et al. 2015) and Mali (Coulibaly et al. 2015), farmers have started growing quinoa using organic and conventional practices.

Table 7.1 Historical Data Series: Area, yield and production of quinoa in Bolivia, Ecuador and Peru

Data type	Country	Year					
		2011	2012	2013	2014	2015	2016
Area (ha)	Bolivia	63,307	131,192	147,312	113,506	121,186	118,913
	Ecuador	2781	2889	3287	4122	7148	2214
	Peru	35,475	38,495	44,868	68,140	69,303	64,223
Yield (kg/ha)	Bolivia	647	388	428	597	623	551
	Ecuador	906	961	904	900	1778	1763
	Peru	1161	1149	1162	1684	1525	1234
Production (mt)	Bolivia	40,943	50,874	63,075	67,711	75,449	65,548
	Ecuador	2519	2775	2972	3711	12,707	3903
	Peru	41,182	44,213	52,129	114,725	105,666	79,269

Source: FAOSTAT (2018)

7.1.3 Importance

Quinoa has an exceptionally nutritious balance of protein, fats, minerals, vitamins, flavonoids and starch. The protein content of quinoa seeds is 14–22%, significantly higher than that of cereal grains (Cardozo and Tapia 1979; Fairbanks et al. 1990; Mota et al. 2016; Nowak et al. 2016). However, the most significant nutritional value is the amino-acid composition of the seed proteins. Quinoa seeds contain high levels of essential amino acids such as lysine, methionine and cysteine. Therefore, quinoa is an excellent complement to other cereal grains (deficient in lysine) and legumes (deficient in methionine and cysteine) (Mota et al. 2016; Nowak et al. 2016; Repo-Carrasco et al. 2003; Wright et al. 2002).

The sodium content in quinoa grains is low but calcium, phosphorous, magnesium, potassium, iron, copper, manganese and zinc levels are higher than in other cereals (Abugoch 2009; Pasko et al. 2010). Grains, leaves, tender stems and inflorescences can be consumed in the human diet and as animal feed. Studies of animal feeding have demonstrated beneficial effects during lactation resulting in higher milk yields (Barros-Rodriguez et al. 2018; Groot 2004; Liu et al. 1999; Zhang et al. 1995). In addition, quinoa leaves are rich in phenolic compounds (e.g. ferulic, sinapinic and gallic acids; kaempferol, isorhamnetin and rutin) that have antioxidant and anticancer properties (Gawlik-Dziki et al. 2013). Other helth-promoting bioactive compounds of quinoa are isoflavones such as genistein and daidzen (Lutz et al. 2013a, b). The content of phytochemical components of quinoa varies among genotypes and is affected by environmental conditions. Lutz and Bascuñan-Godoy (2017) point out that certain amino acids and other compounds increase in stress conditions.

The quinoa seed coat (pericarp) is usually covered with bitter saponin compounds that must be removed before consumption (Ahumada et al. 2016; Gomez-Caravaca et al. 2012; Mastebroek et al. 2000; Reichert et al. 1986; Varriano-Marston and De Francisco 1984; Vega-Gaávez et al. 2010; Woldemichael and Wink 2001).

Although the toxicity of saponins to mammals has not been proven, their presence may affect efforts to expand quinoa markets.

Quinoa has been grown for thousands of years in the arid and semiarid areas of the Andean Region of South America, in adverse environments with low levels of soil fertility. Quinoa development in these marginal lands has turned it into a rustic species. The ability of quinoa to thrive in marginal conditions is related to its genetic tolerance to drought, low temperatures and salinity (Hariadi et al. 2011; Jacobsen et al. 2007; Martinez et al. 2009; Razzaghi et al. 2012; Rosa et al. 2009; Ruiz et al. 2014) and its high efficiency in the use of natural and agricultural resources available in poor soils (Aguilar and Jacobsen 2003; Bois et al. 2006).

Due to its high nutritional value, genetic diversity, stress tolerances and wide adaptation to different environments, quinoa has been considered as an exceptional crop with potential to contribute to food security worldwide (FAO 2011).

7.1.4 Domestication, Selection and Early Improvements

It is not known exactly where and when *Chenopodium quinoa* was domesticated in South America. Archaeological data provide chronological information about when it was probably used, domesticated and/or introduced and incorporated into farming systems. The macro remains recovered were seeds, inflorescence branches and flowering stems and stems. The recovered materials with other domestic and wild plant species are evidence of the importance of quinoa in the diet of the ancient populations of South America. On the other hand, stems recovered from archeological sites with signs of cuttings could be an indication of early domestication. Humans in the Americas began domesticating fauna and flora about 8000 years ago (Planella et al. 2015).

In Peru, the earliest archeological evidence indicates that quinoa domestication started around 5000 BC in Ayacucho, located in the central highland (Lumbreras et al. 2008; Uhle 1919). In the Nanchoc Valley in Cajamarca (northwestern highlands), specimens are placed chronologically at 5500–6000 BC. (Dillehay et al. 2007). In the sites of Cuevas de Pachamachay, and Panaulauca and Pancan in Junin (central highlands), seeds have been dated to between 3000 and 700 BC. The larger size of seeds found, suggests they belonged to domesticated quinoa; a process that may have started around 3000 BC. (Pearsall 1980, 1989, 2008). Considering Formative/Pre-Classic Chronology; in the Lake Titicaca Basin in Peru and Bolivia, quinoa was a part of an agricultural complex that developed during the regional Formative Period, from 1500/1800 BC to 500 AD (Bruno and Whitehead 2003).

In Bolivia, in the northern Altiplano from the Taraco Peninsula to the Tiwanaku Valley, the macro remains corresponded to the Formative Period (1500/1800 BC–300 AD with high densities of several species of *Chenopodium* used as food and in ritual ceremonies (Bruno 2008; Planella et al. 2015; Whitehead 2007). In the central Bolivian Altiplano, seeds recovered at the La Barca site at Oruro were quite different from seeds observed in the Lake Titicaca Region and

belonged to the Formative Period (1500/1800 BC–400 AD) (Langlie et al. 2011). In the southern Bolivian Altiplano, quinoa recovered at numerous archeological sites was chronologically dated to 900–1550 AD. Carbonized seeds indicate they were used after saponin extraction, and eaten as whole seeds and in soups. These seeds resembled modern quinoas such as cvs. White or Yuraj Real Cashlala, Pasankalla, Pink or Puca, Orange and Black (Lopez and Nielsen 2012; Lopez 2012).

In Chile, remnants of *Chenopodium* sp. grains were found in Chinchorro Complex-Chile dated to 3000 BC. Grains were also recovered from Indian graves at Tarapaca, Calma, Colchaqui-Diaguita, Tiltil and Quilagua (Bollaert 1860; Latchman 1936; Nuñez 1974; Tapia et al. 1979).

In Argentina, seeds and fruits of *Chenopodium* were identified from botanical remnants recovered at different archaeological sites. In the Puna of Catamarca, the oldest evidence of quinoa use comes from hunter-gatherer groups, approximately 3500 years ago (Andrade et al. 2015). Rodriguez et al. (2006) reported that quinoa crops were present at least 1440 AD, at Sierra, Catamarca, Southern Argentinean Puna and Cueva Salamanca 1, El Aprendiz, Punta de la Peña 9 and Punta de la Peña4 and in Pampa Grande (Salta) corresponding to Punta de la Peña 4 – layer 3, with botanical remnants dated to ca. 760–560 BP.

Domestication syndrome traits were related to plant morphology and physiological changes that created the differences between wild relatives and domesticated quinoa. Seed coat thickness, margin configuration, surface patterns and increased diameter were the most important changes. In wild populations, the seed coat tends to be thick, hard and dark to prevent penetration of external elements that accelerate the development and growth of the embryo before full maturity, but also to protect embryos from possible dehydration and insect attack. The selection of grains with thinner skin influenced the fruit margins. The grains of wild species are characterized by round to biconvex forms, while the grains of the cultivated genotypes have truncated margins due to the flattened shape of their ventral and dorsal sides, the growth of cotyledons and the increased volume of the perisperm (reserve organ). The occurrence of thin husked seeds and truncated margins among the domesticated chenopod grains reflects an adaptive response to selective pressures of human manipulation for both reduced germination dormancy and seedling viability (Murray 2005; Smith 1992). Other differences in the fruits of the wild and domesticated forms were the change of seed-coat surface patterns. In the cultivated forms the grains had a smooth texture without protuberances, whereas in the wild forms a reticulated testa structure was observed. (Bruno 2006; Smith 1992). Another feature was that the domesticated types present lighter colors due to a lower lignification of the epidermis and selection for white color types (Tapia 1979; Wilson 1981).

Changes in the plant include inflorescence compaction, loss of natural shatter mechanisms, grains, reduction of bitterness; reduction of plant height, life cycle, reduction of branching habit and other characters adapted to crop management by the farmers and the food uses (Mujica et al. 2001). From natural and human selection, composite populations were developed as varieties and some are still grown. The variability of these varieties was in traits such as length of growth cycle, dormancy (time elapsed between physiological maturity and sprouting), disease resis-

tance; frost, salt and excessed rainfall tolerance, that give yield stability and sharing common characteristic such as plant height, color of grains and quality related to the end-use. Besides consumption criteria, farmers also paid attention to the reduction of saponin content, aptness for preparation of traditional dishes or drinks, and secondary uses, such as quinoa leaves as vegetables or animal feed (Gomez 2015).

7.2 Crop Production and Agricultural Challenges

7.2.1 Crop Production in the Andean Region

Up to the 1980s, quinoa was grown almost exclusively under traditional production system in small plots, but mechanization of the crop and some improved cultural practices were introduced in recent decades; therefore, the traditional cultivation practices of quinoa have been modified in various ways to increase yields and to improve the quality of the harvested grains. Currently the technology goes from improved traditional to high technology to produce organic-ecological and conventional quinoa (Fig. 7.1).

The cultivation of quinoa has a spatial and temporal distribution in the different crop systems of the ecological zones of the Andes at elevations of 0–4200 m elevation. Quinoa in the Andean highlands is cultivated in rainfed systems and in the Peruvian coast land in irrigation systems. The most commonly used rotations are potato-quinoa-cereals (maize, barley, wheat or oats-fodder) legumes (broad bean, peas or lupine); considering the elevation and the adaptation of the other species. In general, they are cultivated in association with crops used in the rotation systems; however, in recent decades monoculture has become widespread (Gomez and Aguilar 2016).

Varieties The commercial cultivars of quinoa are composites (mixture of genotypes) with some common agronomic and morphologic characteristics to facilitate agronomic management and grain uniform color for trade. Under unfavorable seasonal conditions, they show different growth rates and yields contributing to stable production and reducing the risks losses. In Peru, there are more than 50 varieties cultivated in the highland, most of them with local names. The major improved commercial cultivars used lately are the Altiplano ecotypes: Salcedo INIA, INIA 431-Altiplano, INIA 427-Amarilla Sacaca, INIA 415-Pasankalla, INIA 420 Negra Collana, Illpa INIA, Blanca de July, Kancolla, Cheweca; and the valley ecotype: Amarilla de Marangani, Blanca de Junin, Quinua Hualhuas, Quinua Huancayo (Apaza et al. 2013; Gomez-Pando et al. 2015). In Bolivia, the varieties cultivated are Sayaña, Ratuqui, Robura, Santamaria, Surumi, Jilata, Jumataqui, Patacamaya, Real Blanca, Toledo, Utusaya, Rosa Blanca, Kellu, Pandela, Chillpi, Achachino, Manzana, Toledo Amarilla, Real Ela, Rosada and Lipeña, Jacha Grano, Kurmi, Blanquita, Qusuña, Aynoqa and Horizontes. It is important to explain that nearly 50 local varieties fall under the generic name of Quinoa Real (Bonifacio 2003;

Fig. 7.1 Cultural practices of quinoa cultivation in Peru. (**a**) Row seeding, (**b**) Mechanical weed control, (**c**) Hand weed control, (**d**) Hilling, (**e**) Commercial field of Rosada Huancayo variety, (**f**) Commercial field of Blanca Hualhuas variety, (**g**) Hand plant cutting, (**h**) Mechanical harvesting

Bonifacio et al. 2012; Gandarillas et al. 2015; Winkel et al. 2015). In Ecuador, some commercial cultivars are reported such as INIAP-Tunkahuan, INIAP-Ingapirca, INIAP-Cochasqui, Imbaya, Chaucha, Tanlahua, Piartal, Porotoc, Chimborazo Bitter, Imbabura Bitter, Purple and INIAP-Pata de Venado (Peralta and Nelson 2015). In Colombia, two cultivars have been obtained: Nariño and Sweet of Quitopampa (Gomez and Aguilar 2016). In Chile two ecotypes, Salares and Coastal (lowlands) are grown. There are some varieties developed: Baer, Lito, Faro and Picchaman (Gomez and Aguilar 2016). *Regalona* quinoa variety was developed for the southern part of Chile (Bazile et al. 2016b).

Seed and Density In the Andean countries, certified seeds are available, mainly produced by public institutions. However, farmers use their own seeds, generally of low quality, which spread certain seed-borne diseases such as downy mildew. Optimum sowing densities vary according to the soil type, the variety, the environments and the sort of technology applied for sowing. In the marginal highland areas, the amount of seeds recommended is 15–20 kg/ha, while in the Peruvian coast areas 10–12 kg/ha. Using hand-sowing horticultural equipment, 4–5 kg/ha; with seeder-fertilizer machines pulled by tractors 10–12 kg/ha and for transplanting 1 kg/ha of seeds (Aguilar and Jacobsen 2003; Gomez and Aguilar 2016).

Land Preparation Quinoa can grow in soils with a pH from 4.5 (Cajamarca, northern Peru) to 9.5 (Uyuni, southern Bolivia), but good drainage is required to avoid root diseases.

Land preparation in small traditional areas is still made by hand using especial agricultural implements (*chaquitacclla, llaukana, taquiza* or *tanccana*) and animal traction due to the high slope steepness and/or low economic level of farmers. In some flat extensive areas of the highland and coast, land preparations are mechanized (Gomez and Aguilar 2016; Mujica 1992).

Fertilization Quinoa responds well to the application of organic or synthetic fertilizers. In the Peruvian highland, grain yield of 3406 kg/ha has been reported with 800 kg of guano (bird manure) which provide about 80-80-16 of NPK. In Bolivia, yield increases were reported with sheep and llama manures. In the coastland, to achieve a target quinoa yield of 6–7 mt/ha, it is recommended to apply 20–30 mt of organic fertilizer, 300 kg/ha of nitrogen, 120 kg/ha of phosphorus, 300 kg/ha of potassium and some other macro and micro minerals. Usually nitrogen applied is split; in the highland, during sowing and hilling time and on the coast at sowing, hilling and flowering time (Gomez and Aguilar 2016). Rojas et al. (2004) recommended the application of 80 kg/ha of nitrogen, 50% during the topping of the flower primordium and 50% during the pre-flowering stage.

Sowing Quinoa seeds are sown in an optimal bed and covered with a 1–2 cm layer of soil. Sowing is generally in rows (0.50–1.2 m between rows). This system of row sowing facilitates subsequent agricultural practices such as weeding, thinning and hilling. Other traditional sowing methodologies used in small scales are: broadcast

sowing (small terraces, *waru-waru* and embankments), transplanting (valley with irrigations systems) and hole-sowing (Bolivian salares) (Gomez and Aguilar 2016; Risi and Galwey 1984). The sowing time depends on the elevation and the rainy season; it is very important to have moisture to ensure the initial establishment of the crop (Gomez and Aguilar 2016).

Weed Management Weeds are one of the important problems in quinoa production. There are no herbicides to control broad leaf weeds. The control of weeds must start during land preparation which allows quinoa to germinate and grow free of weeds for the first 7–14 days. Farmers use an integrated approach for the management of weeds. This combines all options available such as hand weeding, mechanical methods of hoeing, harrowing and the application of some contact herbicide (Jacobsen et al. 2010). Special devices are used to protect quinoa plants from such herbicides (Gomez and Aguilar 2016).

Plant Thinning In general, the number of seedlings germinated is high, especially with quality seeds. Therefore, thinning is relevant to allow strong plants to growth in the appropriate space. It is important to cull weak seedlings with less than four to six true leaves. The highest yielding crops have an evenly distributed plant population across the whole field of approximately 400,000–500,000 plants/ha. It is important to note that at low density, plant branching and vigor increase, and the number of days to maturity can be longer; besides, there is more space for weed development. On the other hand, at high densities, plants are weak and susceptible to lodging, with lower yields and grain quality (Aguilar and Jacobsen 2003; Gomez and Aguilar 2016).

Hilling Hilling is done after weed control and thinning to give support to plants and reduce lodging problems, especially with some valley-type varieties that can reach more than 2 m in plant height (Gomez and Aguilar 2016).

Insect Pest Management In the traditional area of quinoa cultivation, the main problems are insects of the *Eurysacca* complex, whose larvae damage developing flowers and grains (*Eurysacca melanocampta, E. quinoae, E. media*) and the noctuid complex whose larvae feed on the plants leaves, bore into the stems at the panicle base and eat grains (*Helicoperva quinoa, Copitarsia incommoda, C. decolora, Agrotys ipsylon, Spodoptera eridania, S. frugiperda*, among others) (Saravia et al. 2014). In new cultivation areas of the Peruvian coast, the problem with pests has increased by a group of bug complexes (*Liorrhyssus hyalinus, Nysius simulans, Dagbertus* nr *fasciatus, Dagbertus* spp.) that cause grain damage (Gomez and Aguilar 2016). To control these pests, some cultural practices such as good land preparation, adequate date of seeding, good moisture management and chemical applications are conducted. In organic systems, farmers apply some organic insecticides; the best known are aqueous extracts of neem (*Azadirachta indica*), pyrethrum (*Chrysanthemum cinerariaefolium*), muña (*Satureja perviflora*), ñacathola (*Baccharis incarum*), umathola (*Parastrephia lucida*), ccamásayre (*Nicotiana taba-*

cum), pepper tree (*Schinus molle*) and chachacomo (*Polylepis incana*) (Gomez and Aguilar 2016).

Disease Management Downy mildew (*Perenospora variabilis*) is the main disease from the first stages of plant development, especially in areas with environments promoting the pathogen proliferation. Downy mildew causes significant losses, reaching 99% under cultivation in South America (Danielsen et al. 2001). In general, it is chemically controlled because there are not resistant commercial varieties. Other potential diseases reported are brown stalk rot (*Phoma exigua* var. *foevata*), seedling damping off (*Rhizoctonia* spp., *Fusarium* spp., *Pythium* spp.), foliar diseases (*Cladosporium* sp., *Phoma* spp., *Ascochyta hyalospora, Cersospora* spp.) and bacterial stain (*Pseudomonas* spp.). Implementation of good agronomic practices such as adequate varieties, appropriate nitrogen fertilization, and reduction of drainage problems have been recommended to reduce disease developments. For all these diseases, there are chemical control recommendations. In organic systems, it is recommended to use biocides based on *Trichoderma* spp. and *Bacillus subtilis,* and extracts of some plants with anti-fungal properties such as garlic (*Allium sativum*) and horsetail (*Equisetum arvense*) (Gomez and Aguilar 2016; Saravia et al. 2014).

Nematode Management In quinoa, the presence of three nematodes has been reported: *Thecavermiculata andinus* (nematode de la oca), *Nacobbus aberrans* (false root nematode) and *Globodera* spp. (potato cyst nematode) causing some levels of economic damage (Franco and Hidalgo 2003; Gomez and Aguilar 2016). These nematodes affect a wide range of crops like quinoa, potatoes, lupine, oca (*Oxalis tuberosa*) and ulluco (*Ullucus tuberosus*), limiting the numbers of alternative crops for rotations in the Andes. Nematode controls are mainly with cultural practices like selecting the appropriate crop for rotations, adequate time of sowing, nutrient management, plant density, phytosanitation and tillage practices and irrigation (when available) (Gomez and Aguilar 2016).

Bird Management Birds are important problems in quinoa, particularly early in the germination phase and from flowering to harvest time. Yield can be reduced by 30–40% per field; especially in isolated areas. The main natural control is conferred by a bitter-saponin present in the outer layer of the grains. Damages are severe in semi-sweet and sweet varieties. The use of nets and some devices to scare off birds have been developed (Gomez and Aguilar 2016).

Water Management The major quinoa cropping area is located at the highland under a rainfed system. However, in the Peruvian coastland, the crop is under irrigation. Moisture at planting must be enough to stimulate good germination and crop establishment. Subsequent irrigations can be applied every 10, 15 or 20 days, depending on the type of soil and climate of the area (Gomez and Aguilar 2016). In the southern Bolivian Altiplano, a 2-year fallow land system is used because of low annual precipitation of 150–300 mm. During the first year, the precipitation is accu-

mulated in the prepared soils and in the second year quinoa is planted with a full-year growing cycle (Michel 2008). Some experiments on quinoa irrigation in the Peruvian coast have been conducted and demonstrated the high water-use efficiency of quinoa. The effects on yield of three regimes of drip irrigation plus moisture plastic retainer were measured. The water layer was calculated using the CROPWAT 8.0 FAO program; the experiment was conducted in La Molina, with the mutant line LM 89-77. The amount of fertilizer applied was 40-60-0 kg/ha of NPK at planting. Irrigation protocols were 3235 m^3/ha (T0 + without plastic layer), 3235 m^3/ha (T1, with plastic layer), 2470 m^3/ha (T2 + with plastic layer) and 1623 m^3/ha (T3 + with plastic layer) applied during the total life cycle. Grain yields recorded for T0, T1, T2 and T3 treatments were 3163 kg/ha, 3333 kg/ha, 3039 kg/ha and 2234 kg/ha, respectively. Results indicated that the decrease in irrigation regimes has no significant effect on grain yield. Furthermore, the mutant line La Molina 89-77 showed high water-use efficiency in water-stress conditions, reaching a maximum value of 1.68 kg/m^3, higher than T1 and T0 with values of 1.21 kg/m^3 and 1.15 kg/m^3, respectively (Leon 2014).

Harvest Quinoa can be harvested by hand, with a stationary or a combine thresher. After threshing, quinoa must be winnowed to separate the light chaff from the heavier quinoa grains (Dominguez 2003; FAO 2011; Gomez and Aguilar 2016).

Selection and Storage The grains are cleaned using a series of sieves before storage. Quinoa grains need to be free from dust, dirt, stubble, insects and cracked grains for storage, and consequently they maintain good quality for trade or consumption. Grains must be conserved in cool environments to preserve germination capacity (Dominguez 2003; Gomez and Aguilar 2016).

7.2.2 Current Agricultural Challenges

The worldwide recognition of its nutritional value and higher resistance to adverse abiotic factors make quinoa an important species for crop diversification in marginal lands or areas more affected by climate change, in different parts of the world, outside its genetic origin. The introduction of quinoa to new environments has been relatively fast, only around 30–50 years. In the past, introduced crops took as long as 200 years to attain acceptance and popularity on a broad scale (Jacobsen 2015).

In the Andean Region, from the 2000s to the 2010s, the areas cultivated with quinoa have increased in the traditional highland and in new areas (Peruvian yunga and coastland), because of the high demand of national and international markets, and good prices at the farm gate. Large-scale cultivation, introduction to new areas and the effects of climatic change have shown the real magnitude of quinoa agricultural limiting factors. Increase of biotic stresses (diseases, weeds, pests); susceptibility to high temperatures during flowering and grain-filling stages; lack of harvest and postharvesting technologies, including grain storage; and the unsustainable

management of fragile large extensions of environments in the agriculture systems of the Andes are the most relevant limiting factors (Gandarillas et al. 2015; Gomez-Pando et al. 2015; Peralta and Nelson 2015).

Outside the Andean regions, efforts to grow quinoa were reported as early as the 1900s in North America (Caldwell 2013). It was first introduced in Brazil in the early 1990s with the objective of selecting new options for savanna agricultural systems (Spehar et al. 2015). The United Kingdom was the first country where the quinoa crop was introduced in Europe in the 1970s, before spreading to other countries (Jacobsen 2015; Risi and Galwey 1984). The crop has dispersed to other continents such as Asia and Africa, where it has shown that it can perform well (Bazile et al. 2016a; Bojanic 2011; Jacobsen 2003, 2015).

Quinoa is recognized as a new option available to agriculture in marginal lands and environments, because it is capable of tolerating drought, frost and high soil salinity, while ensuring acceptable yields (Jacobsen 2012). On the basis of market value, the high prices of quinoa and its by-products have favored its insertion into the agriculture of many countries (Bazile et al. 2015b, 2016b).

Many problems have been identified in the new countries growing quinoa at commercial and experimental levels that can be resolved with research and appropriate use of germplasm.

7.2.2.1 Biotic Problems

Downy mildew may be a significant problem for quinoa yields in locations with humid environments. On the other hand, the rapid movement of quinoa crop throughout the world in recent years could facilitate the spread of pathogens causing high losses in yield and grain quality. One way could be by the introduction of seeds from other countries or the use of food and feed grains as seeds for planting, that could bring new strains of seed borne quinoa diseases, such as downy mildew, which is present as oospores in the seed pericarp (Danielsen 2004). *Perenospora variabilis* is heterothallic and has the potential for sexual reproduction if compatible mating types are present (Danielsen 2001). According to Peterson et al. (2015b), the inadvertent introduction of new downy mildew strains could result in the establishment of mating populations in North America and the formation of new biotypes; additionally, other new pathogens have been identified affecting quinoa such as *Passoloria dubia* and *Asochita* spp.

Quinoa is likely to attract novel pests, particularly those that prey on related *Chenopodium* spp. which are major weeds in some countries. This has been seen in the Peruvian coast areas with *Liorrhyssus hyalinus, Nysius simulans, Dagbertus* nr *fasciatus, Dagbertus* spp. (Gomez and Aguilar 2016) and in Europe with *Cassida nebulosa* and *Scrobipalpa atriplicella* (Sigsgaard et al. 2008). Lygus bugs and aphids were identified as common pests in North America (Peterson et al. 2015a, b).

Weeds are the major challenges for quinoa cultivation. Closely-related *Chenopodium* spp. are an important constraint when they are the major weed problem. Quinoa and *Chenopodium* have similar growing habits and appearance, particularly during crop establishment (Peterson et al. 2015b; Piva et al. 2015; Spehar et al. 2003).

7.2.2.2 Environmental Problems

Temperatures above 32 °C, long days and low relative humidity during anthesis restrict pollen viability, reducing significantly quinoa seed production, as reported by Jacobsen et al. (1994), Iliadis et al. (1997) and Bois et al. (2006).

Preharvest sprouting is a potential problem for areas with rainfall when seeds have begun to mature. This could be overcome with tolerant varieties (Mastebroek and Limburg 1997; Peterson et al. 2015b).

7.2.2.3 Seed Quality

Maintaining varietal genetic purity, especially in genetic and foundation seed categories, is difficult due to the outcrossing of quinoa. Cross-pollination of 5.78–17% has been reported in different environments depending on the floral biology and pollinators present (Lescano 1980; Rea 1969; Spehar 2001). In Brazil, data on cross-pollination in the savannahs indicate an average of 15% hybrid seeds between varieties grown adjacently and simultaneously (Spehar et al. 2015). Moreover, outcrossings between quinoa and related species, such as *Chenopodium berlandieri,* have been observed in the American states of Colorado, Oregon and Washington, and hybrids can be fertile (Wilson and Manhart 1993). Outcrossing among quinoa and other species of *Chenopodium* could bring undesirable traits such as black seeds, but also, could provide genes related to local environmental adaptation, such as increased heat tolerance or disease resistance (Peterson et al. 2015b).

Quinoa seeds lose germination viability rapidly when stored at room temperature; Souza (2013) reported germination power losses when seeds are stored at high temperatures. The germination power of seeds, independent of moisture levels, is maintained at temperatures around 4 °C and is lost at 25 °C. The difficulty of maintaining seeds with good germination capacity under farm conditions without cold storage systems could negatively influence the production of high quality seeds and their conservation periods (Spehar et al. 2015).

7.3 Improvement Strategies

Genetic improvement, using the high diversity available, is relevant to preserve the special quinoa organic production with denomination of origin, to make the difference between Andean quinoa and those quinoas produced in other countries of the world, and maintain a safe market.

Research and development must continue to fill the existing agronomic gaps, such as how to operate large commercial fields in extremely marginal environments with limited resources. It is important to improve soil fertility, plant nutrition, pest management and mechanization.

Research should be oriented toward the development of varieties adapted to different environments with high-yield potential, tolerance to abiotic stresses, resistance to biotic stresses and quality. The cropping technology for different areas still remains to be perfected.

However, the increase and maintenance of quinoa cultivation will depend also on marketing opportunities for the farmers growing quinoa, still a new crop in many countries. Farmers are accustomed to move towards the cultivation of other well-known crops when prices are attractive, rather than investing in quinoa, which has a risky market.

Nonetheless, it is very important to move towards environmentally sustainable, technically feasible, and economically and socially viable quinoa production around the world.

7.4 Germplasm Conservation and Biodiversity

7.4.1 Quinoa Ecotypes

Quinoa has been grown for thousands of years in diverse climates, soils, topography and has been selected and used by different civilizations established in the Andean Region, with a long agronomic history and ancestral knowledge. Quinoa spread gradually from its center of origin around Lake Titicaca between Peru and Bolivia, north to Ecuador and Colombia and south to Chile and Argentina, and also from the highlands to the valleys and coastal regions over millennia (Bertero et al. 2004). These diverse environmental conditions promoted the development of many landraces, traditional agricultural practices and uses for quinoa.

The results of natural and human selections are five quinoa ecotypes, each adapted to a particular growing environment (Tapia et al. 1980):

(a) Sea level quinoas: They are represented by plants with a height of 1.0–1.4 m, with a branching habit. Its seeds have transparent cream color or *chullpi type*. Originated in Chile (Linares and Concepción at 36°S Lat.). Their morphological characteristics have great similarities with *Chenopodium nuttalliae* (Huahzontle) cultivated in Mexico at 20°N Lat.

(b) Valley quinoas: Adapted to grow between elevations of 2500–3500 m. They show plant heights up to 2.5 m or more, a long life cycle and are greatly branched with lax inflorescences. Some of the Valley quinoas have quantitative resistance to mildew (*Perenospora variabilis* = *Peronospora farinosa*) but are generally associated with a long life cycle (Fig. 7.2a).

(c) Altiplano quinoas: These quinoas have evolved in the broad plains of the Bolivian-Peruvian high plateau, surrounding Lake Titicaca, in areas mostly 3600–4000 m in elevation. It is in this area where the greatest variability of quinoa is found. This group includes most of the traditional varieties and commercial varieties that are characterized by the predominance of plants without

Fig. 7.2 Varieties of different ecotypes of quinoa from the Andean Region of Bolivia and Peru. (**a**) Intervalley ecotype (Junín, Peru), (**b**) Altiplano ecotype (Puno, Peru), (**c**) Salares ecotype (Bolivia)

branching or a simple stem with a compact terminal inflorescence, with plant height of 0.5–1.5 m and a high susceptibility to mildew disease (Fig. 7.2b).

(d) Salt flat quinoas: This group evolved in the high salt flat (*salare*) areas of southern Bolivia with 300 mm of annual precipitation. These quinoas have a morphology similar to those of the altiplano. They are characterized mainly by a large grain size, larger than 2.2 mm in diameter, and some of their varieties are known as Royal Quinoa. In general the grains have a thick pericarp and high saponin content (Fig. 7.2c).

(e) Quinoas of the Yungas: A small group of quinoas that has adapted to the Bolivian Yungas, 1500–2000 m elevation, and show branched development to a certain extent. They are green plants growing up to 2.2 m and, in the blooming stage, the whole plant turns orange.

7.4.2 Ex Situ Conservation

In Bolivia, more than 6721 quinoa accessions are conserved in 6 seed banks. The National Institute of Agricultural and Forestry Innovation (INIAF) has the national collection of quinoa germplasm, followed by the Oruro Technical University (UTO) and the Universidad Mayor de San Andres (UMSA) (Rojas et al. 2015). Accessions of the national collection are well characterized and evaluated agro-morphologically, including yield, resistance or tolerance to biotic (downy mildew) and abiotic factors (frost, drought, hail), phenologic stages and duration of the crop cycle. Moreover, some quality traits have been recorded such as grain size (diameter, thickness), saponin and protein contents, and grain color.

In Ecuador, the National Institute of Agricultural Research (INIAP), in collaboration with IBPGR (now Bioversity International), led the collection of quinoa in the early 1980s. Nowadays, the Ecuadorian national collection has about 673 accessions conserved by INIAP (4-FAO WIEWS 2013). Most of the quinoa collection has been characterized using descriptors recommended by Bioversity International. Additionally, INIAP and universities have conducted some studies of genetic diversity (Peralta and Nelson et al. 2015).

In Peru, the numbers of accessions preserved in germplasm banks of different organizations are around 6302. INIA (National Agriculture Research Institute) manages 1029 accessions in Puno, Cuzco, Ayacucho, Cajamarca and Junin. Other germplasm banks are in the Universidad Nacional del Altiplano in Puno with 1910 accessions, Universidad Nacional Agraria with 2089 accessions, and Universidad San Antonio Abad del Cuzco with 400 accessions. Small collections are maintained by other universities (FAO-WIEWS 2013; Rojas et al. 2015). Almost all accessions have been evaluated considering grain yield, plant height, lodging resistance, maturity, resistance to diseases (downy mildew) and insect pests. Quality traits; such as grain protein content, grain saponine contents, grain size, 1000 grain weight and colors; have also been recorded (Gómez and Eguiluz 2011).

Chilean germplasm was selected by Andean farmers (Aymaras) on the arid coastline of the central zone, and Mapuches in the Southern areas. Andean and coastal Chilean populations of quinoa were found to be distinctive groups (Wilson 1988a, b). Studies of the wild relatives have shown that there is self-pollination and cross-pollination evident in the genetic material of the coastal ecotype and a very active interaction with the cultivated types and the weeds of the same family that could indicate the existence of a genetic flow. In Chile, quinoa ex situ conservation is carried out by INIA (Institute for Agricultural Research). The germplasm is composed by approximately 441 accessions and 9 wild relatives (Bazile et al. 2015a).

In Argentina, the national collection is represented by nearly 90 accessions from the Northwest region. The germplasm shows special features apparently associated to the collection environment. On the other hand, some wild relatives are also conserved. There have been many evaluation studies of quinoa to know more about this crop, that was abandoned in the past and currently is been re-introduced as exotic varieties (Andrade et al. 2015).

Quinoa was introduced to Brazil in the early 1990s from a USA germplasm collection in Iowa. Other ecotypes from different collections were also introduced. A high cross-pollination rate in the savannas allows hybrid development and is expanding the existing collection (Spehar et al. 2015).

In the USA, the North Central Regional Plant Introduction Center at Ames, Iowa, preserves a collection of 164 publicly-available quinoa accessions and a wide range of *Chenopodium* spp. The collection is formed by accessions of different origins and was used in programs in the USA and internationally (Christensen et al. 2007; Peterson et al. 2015b).

Rojas et al. 2015, reported that at world level there are approximately 16, 422 quinoa accessions and related species, including *Chenopodium quinoa, C. album, C. berlandieri, C. hircinum, C. petiolare, C. murale* and *Chenopodium* sp. They are preserved in 59 germplasm banks in 30 countries. More than 80% of quinoa accessions are preserved in the Andean Region.

7.4.3 In Situ Conservation

Cultivated quinoa and wild relatives are grown together in systems known by different names such as *mandas*, *laymes, aynoqas*, *sayañas*, *huyus* and *jochiirana* in Bolivia and Peru (Bravo and Catacora 2010; Ichuta and Artiaga 1986; Mujica and Jacobsen 2000; Rojas et al. 2010). In some areas of Bolivia, considered microcenters of diversity, farmers apply cultural practices, perform rituals and festivals observing a series of biological indicators that occur during the growth cycle of the crop. The organized peasant communities choose leading families (*yapu campus*) that will be responsible for the care of the fields, protecting them from hail, frost and flood damage (Rojas et al. 2010). In Chile Mapuche women kept seeds and cultivated quinoa for several generations preserving the diversity in their gardens for medicinal purpose (von Baer et al. 2009).

These different ancient management practices are maintained for several different purposes such as conservation of genetic diversity in situ, rational use of plant diversity, food security, management of soils and pests.

In the inter-Andean valleys and in the Bolivian-Peruvian Altiplano, fields of cultivated quinoa have as common and frequent weeds related wild species such as: *Chenopodium carnosolum*, *C. petiolare, C. ambrosioides, C. hircinum* and *Swaeda foliosa* (Bonifacio 2003, 2004; Mujica and Jacobsen 2005). Wild species can be found within the fields, on the edges, along roads and in some locations considered sacred (*gentilwasi o phiru*). The wild forms have been preserved in the areas of origin because they are used as human food and animal feed, for medicinal purposes and for religious or social rituals. Use of wild forms is more frequent in periods of climatic perturbations which are frequent in the Andean highlands of Peru and Bolivia (Mujica and Jacobsen 2005).

7.4.4 Genetic Erosion

Genetic erosion of quinoa in the Andean region can be attributed to the extreme climatic problems that have been exacerbated over time, to the significant decrease in quinoa cultivation areas, which were replaced by new species introduced during colonization, especially in the inter-Andean valleys, and the replacement of quinoa in the daily diet. In Bolivian and Peruvian quinoa collections, Altiplano and Salares ecotype accessions are predominant, along with some valley type ecotypes; possibly the yunga ecotypes of quinoa are already lost.

Another important factor, mentioned by Bertero (2003), is the foundation effect that could have occurred during the process of dispersion of quinoa from the highlands to the inter-Andean valleys and other more distant areas.

7.4.5 Germplasm Diversity Characterization

Gandarillas (1968) made one of the first descriptions of quinoa germplasm of Bolivia, Peru and Ecuador using morphologic descriptors such as plant habit, type of inflorescence, leaf shape, seed and leaf dent number (Fig. 7.3). Using these data he described four races in the North of Cusco (Pichincha, Ancash, Cajamarca, Junin), three in Cusco (Cusco, Sicuani, Puca), four around the basin of Lake Titicaca (Copacabana, Dulce, Achacachi, Puno), four in the Andean valley located at the Southeast of Lake Titicaca (Potosi, Sucre, La Glorieta, Cochabamba) and two races around the Lake Poopó Basin (Real, Challapata). The region with the highest quinoa diversity is located between Cuzco (Peru) and Lake Poopó (Bolivia) (Bonifacio et al. 2015; Christensen et al. 2007; Wilson 1988a).

Fig. 7.3 Inflorescence and seed variations of quinoa. (**a**) Amaranthiphorme type inflorescence, (**b**) Glomerulate type inflorescence, (**c**) Variation of quinoa grains (aquenio)

Several studies have been conducted on quinoa germplasm collection in recent decades. De Santis et al. (2016) *observed s*ignificant variation for seed yield, and morphological and quality traits in 25 accessions of *Chenopodium quinoa*, 4 of *C. giganteum* and 1 of *C. berlandieri* ssp. nuttalliae. Additionally all the morphological traits studied (excepting data related to life cycle) and quality traits showed significant positive and negative associations with seed yield, respectively.

The variability reported usually shows the differences in plant morphology, branching, color, height duration of the growth cycle, seed dormancy, yield and yield components, tolerance to frost, salt and drought, grain color, grain protein and saponine contents. Disease and pest resistances and responses to environmental stresses have also been studied. In the last decade, evaluation has also been focused on the nutritional value of quinoa, as well as agro-industrial variables.

7.5 Uses of Germplasm

7.5.1 Varieties Development

Land-tradition varieties derived by mass or individual selection gave rise to some of the varieties still used at present. In Bolivia, Sajama, Samaranti, Huaranga, Kamiri, Chucapaca, Sayaña, Ratuqui, Robura, Jiskitu, Amilda, Santa María, Intinarira, Surumi, Jilata, Jumataqui, Patacamaya, Jacha Grano, Kosias, Kurmi, Horizontes, Aynoq'a and Blanquita (Bonifacio et al. 2006; Espindola and Bonifacio 1996; Rojas-Beltran et al. 2010). In Ecuador, from the germplasm available, the following varieties were selected and released: INIAP-Cochasquí, INIAP-Imbaya in 1986, INIAP-Ingapirca, INIAP-Tunkahuan in 1992 and INIAP-Pata de Venado in 2005 (Peralta and Nelson 2015). In Peru, in the same way, the evaluation of germplasm permitted the selection of more than 100 elite genotypes. Small-scale farmers are growing these varieties to different extents. Twenty genotypes have been officially released as commercial varieties by INIA and other organizations: INIA 431-Altiplano, INIA 427-Amarilla Sacaca, INIA 420-Negra Collana, INIA 415 Pasankalla, Illpa INIA, Salcedo INIA, Qillahuaman INIA, Ayacuchana INIA, Amarilla Marangani, Blanca de July, Blanca de Junin, Cheweca, Huacariz, Hualhuas, Huancayo, Kancolla, Mantaro, Rosada de Junin, Rosada Taraco, Rosada de Yanamango (Gomez and Aguilar 2016).

During the last decade, these improved varieties, derived from landrace germplasm, used in conjunction with efficient agricultural practices in their appropriate niches, have contributed to the significant increase of productivity and production of quinoa in the Andean region.

7.5.2 *Identification of Genotypes with Tolerance to Abiotic Stresses*

7.5.2.1 Hail and Frost

In the highlands of the Andean region, tolerance to hail and frost are increasingly important traits due to the effects of climate change. Hail affects quinoa cultivation causing leaf, stem and inflorescence damages, and mature grain dehiscence. On the other hand, the delay of the rains make plants susceptible to frost in the reproductive phase. Therefore, genetic improvements of these characters have been initiated (Bonifacio et al. 2015).

7.5.2.2 High Moisture

In the humid Ecuadorian temperate valleys of the highlands, quinoa was long grown without drought stress. However, due to climatic change, water shortages are more frequent, and the National Program of Andean Legumes and Grains (PRONALEG-GA) of Santa Catalina Experimental Station started a breeding program in 2000 to face this drought problem. Some studies resulted in an early variety named INIAP-Pata de Venado with improved cold tolerance, low saponine content and adapted to high and cold areas 3000–3600 m in elevation (Mazon et al. 2007).

7.5.2.3 Salinity

Quinoa is a species of the subfamily Chenopodioeae and the family Amaranthaceae. The highest number of halophytic genera belong to this family. Quinoa grows in the Peruvian and Bolivian Altiplano, surrounding Lake Titicaca, in soils with a pH of more than 8. The plant can also be cultivated on the salares (salt flats) of the Bolivian Altiplano and in the coastal zones of Chile with high soil salinity. To be able to grow in saline soils, quinoa has developed mechanism of salt tolerance.

Almost 2500 quinoa accessions available to date have shown differences in their response to salinity during seed germination, and later during the growth cycle (Biondi et al. 2015). *Chenopodium quinoa* had a higher tolerance to salinity than *Amaranth caudatus* (Moreno et al. 2017). Koyro and Eisa (2008) reported reduction of germination capacity of quinoa grains and other characters such as plant growth, seed yield, yield components by salinity.

Kancolla, a commercial variety of the Altiplano ecotype, showed remarkable tolerance in the germination phase achieving 75% of emergence at 57 mS/cm (Jacobsen et al. 2003).

Cultivar Andean Hybrid was submitted to different treatments (3, 7, 11–19 dS/m) with $MgSO_4$, Na_2SO_4, NaCl and $CaCl_2$ in greenhouse conditions. There was no statistically significant reduction in plant height, leaf area or fresh weight until 11 dS/m (Wilson et al. 2002).

Delatorre-Herrera and Pinto (2009) studied the response to salt stress of four different Chilean genotypes. Using 200 mM NaCl, there was observed a 50% reduction of germination ability in the Hueque selection and 6% reduction in Amarilla selection, indicating quinoa salinity tolerance is dependent on genotype. At 400 mM NaCl, the germination rate was lower in all genotypes, suggesting that salinity not only reduces germination percentage but also delays the process.

A total of 182 Altiplano ecotypes were evaluated in Peru under different concentrations of NaCl at germination and during the life cycle of the plants. All accessions showed more than 60% germination at 25 dS/cm, and less than 60% with 30 dS/cm. During the life cycle, the same salt concentration affected root dry weight per plant, but not the life cycle duration. Some accessions increased plant height, leaf dry weight and grain yield in the salinity experiment. Four accessions were identified as tolerant to salt stress (Gomez-Pando et al. 2010).

Responses to salty soils of four Chilean coastal genotypes (PRJ, PRP, UdeC9, BO78) were evaluated considering in vitro germination, growth and short-term physiology. BO78, originating from humid areas, was the least salt tolerant, suggesting a link between drought and salinity tolerances (Ruiz-Carrasco et al. 2011).

Varietal differences and mechanisms involved in quinoa's response to salt stress were evaluated in 14 accessions. Results identified three varieties as less affected in relative biomass production and plant height at maturity, from Bolivia: Pandela Rosada and Utusaya (Royal type), adapted to extreme marginal adverse climatic conditions of the southern Altiplano of Bolivia and from Peru: Amarilla de Marangani from the southern Andes of Peru (Adolf et al. 2012).

Tolerance to salinity was evaluated in quinoa cv. Titicaca, using irrigation with fresh and saline water (30, 20, 10 dSm^{-1}), combined with different levels of drought stress. Both type of stresses affected crop grain and biomass yields severely, but salinity stress alone did not affect them significantly because of natural tolerance of quinoa to salinity (Yazar et al. 2015).

Plants of cv. Titicaca from the six-leaf stage were irrigated with saline water having either 0 or 400 mM Na Cl; saline irrigation decreased the growth, physiology and yield of quinoa but saponin seed priming reduced the negative effects of salt stress (Yang et al. 2018).

The results of many experiments suggest that quinoa tolerates salt through different types of mechanisms. Accumulation of salt ions in some tissues, to avoid physiological damage, was observed in quinoa, in order to maintain cell turgor and adjust leaf-water potential and limit transpiration under saline conditions (Jacobsen et al. 2003). Carjuzaa et al. (2008) observed the presence of a dehydrin component in mature embryos of two quinoa cultivars, from high elevation and sea level locations. Dehydrin is one of the principal components present in the process of plant adaptation to severe environmental conditions (Burrieza et al 2012). Accumulation of some osmoprotectants increased in response to salt stress in two genotypes of the Salare ecotype (Chipaya and Ollague), and two genotypes of the valley ecotype (Peruvian CICA-17 and Chilean KU-2) in hydroponic conditions. It was found that betaine, trehalose and mainly trigonelline contents increased in response to salt (Morales et al. 2011). Karyotis et al. (2003) observed and increase of 13 to 33% in the protein content of quinoa grains under saline-sodium soil conditions with electrical conductivity (EC) of 6.5 dSm-1.

Decrease of quinoa stomatal size, density or both were considered as a morphological mechanism for controlling transpiration and water use efficiency, under saline conditions (Adolf et al. 2013; Orsini et al. 2011; Shabala et al. 2012). A reduction of stomatal length was reported for the Chilean genotype BO78 under saline conditions (Orsini et al. 2011). Salinity also decreased gas exchange and transpiration (Bosque Sánchez et al. 2003). The combined effect of abiotic stresses caused by salinity and drought increased concentrations of abscisic acid (ABA) in shoots and roots of quinoa. This could be related to the ABA signaling role in closing stomata and regulating stomatal conductance (Razzaghi et al. 2011).

Bolivian variety Achachino was evaluated in a high temperature and soil salinity combination in greenhouse conditions. Stress caused by salt and high temperature affected the number and size of stomata. Salt stress increased the number of stomata and reduced their size on both sides of the leaves. High temperature stress significantly increased the size of the stomata on the abaxial leaf surface. The two combined stresses determined the reduction of the size of the stomata only on the abaxial leaf surface and significantly increase of number of epidermal bladder cells, protecting the photosynthetic quantum performance (Becker et al. 2017).

7.5.2.4 Drought

Quinoa is exceptionally adapted to the different arid climates of the Andean Region (Mujica et al. 2001). This is one of the driest areas of the southern Altiplano, located near 3700 m elevation, with sandy or rocky soils, a cold and arid climate with more than 250 days of frost per year and annual rainfall of 150–300 mm (Aroni et al. 2009).

Drought tolerance of quinoa has been attributed to many mechanisms such as drought escape, tolerance and avoidance. Drought escape in quinoa is related to precocity or short life cycle (Geerts et al. 2008a, b, c; Jacobsen et al. 2003). In general, many accessions of Altiplano and Salare agroecological types show earliness (Gómez and Eguiluz 2011). In these ecological areas, quinoa grows with very irregular seasonal rainfall, in amount and distribution, which cause some periods of water deficit at any time of the plant life cycle. The most sensitive stages are crop establishment, flowering and grain milk phase (Geerts et al. 2008a).

Drought avoidance of quinoa has been attributed to some important mechanisms. One is the morphology of the root system, which is highly branched and long (1.0–1.5 m deep) (Alvarez-Flores 2012). Real varieties from the Salare region have developed vigorous root systems, with a strong main root axis that allows exploring deep soil layers more rapidly and efficiently. This is a critical feature in early stages of plant development, enabling plants to be efficient in the use of water and nutrient resources (Gandarillas et al. 2015). Other mechanisms reported in quinoa are:

(a) Capacity to reduce leaf area by leaf shedding. Leaves wilt under severe drought, thus decreasing leaf transpiration by reducing the leaf surface exposed to direct solar radiation. However, quinoa has a remarkable ability to rapidly resume leaf

formation and former photosynthetic level after a period of drought stress (Jacobsen et al. 2003, 2009; Jensen et al. 2000).

(b) Presence of leaf vesicles containing calcium oxalate, which could reduce transpiration (Jensen et al. 2000; Siener et al. 2006). Young leaves, stems and inflorescences are covered by multiple bladders containing calcium oxalate and silicic anhydride that are hygroscopic in nature and reduce transpiration, suggesting an indirect role in water economy and turgor maintenance (Canahua 1977; Jensen et al. 2000; Shabala and Mackay 2011).

(c) An anatomical feature consisting of stomata deeply sunken in the leaf epidermis (Dizes and Bonifacio 1992).

(d) Presence of small and thick-walled cells preserving turgor even after severe water losses; and stomatal regulation (Jensen et al. 2000).

Quinoa gas exchange is within the normal C_3 plant range, and water relations are characterized by low osmotic potential that can be a major trait associated to drought tolerance. Drought effects on stomatal conductance, photosynthesis and leaf water relationships at different phenological stages have been determined (Jacobsen et al. 2001; Razzaghi et al. 2011).

Quinoa tolerates drought mainly through tissue elasticity and low osmotic potential. Plants under drought stress and control treatment did not show statistically significant differences in levels of fructose, sucrose or starch. However, contents of total soluble sugars and glucose were recorded. Moreover, higher proline accumulation was also reported in drought-stressed quinoa plants (Gonzalez et al. 2011). Others mechanisms reported under increasing drought stress were osmotic adjustment to maintain turgor and anti-transpiration compounds, other than ABA, in the xylem sap (Hariadi et al. 2011; Jacobsen et al. 2009). Induction of ornithine and raffinose pathways was observed in water stress lowland ecotypes, Faro and BO78, which showed differences in the stress recovery response and changes in the activity of nitrogen assimilation-associated enzymes (Bascuñan-Godoy et al. 2016).

It is well known that quinoa possesses low water requirement and high water-use efficiency (Jacobsen et al. 2009). In central Chile, the response of nine quinoa genotypes to two watering regimes (dry and irrigated) were studied, observing grain yield reduction of less than 50% when irrigated at 44 and 80% with reference to evapotranspiration. Significant interactions were determined between genotypes and environments for yield, harvest index and grains/m^2 (Garrido et al. 2013).

7.5.2.5 Temperature

Quinoa, in the Andes region, grows at temperatures of 10–25 °C; 15–20 °C being adequate for optimum growth and production. Seed fill was affected by high temperatures above 30–35 °C, particularly when combined with the long photoperiod common to temperate latitudes. Pollen sterility, plant stunting and re-absorption of seed content have been reported as detrimental responses to high temperatures (Bertero et al. 1999; Bonifacio 1995). Heat stress during anthesis is one of the major

limiting factors in the new areas where quinoa has been introduced (Meehl et al. 2007). Studies of the effects of high temperature during the flowering-anthesis and initial grain-filling periods, determined average reduction in grain yield of 47–100% (Mendoza 2013).

Varieties from the highland-altiplano are more affected by global warming than those originated in warmer and more humid climates. In the highlands, varieties have short seed-filling periods to avoid late season drought and frost (Bertero 2003). Quinoa germplasm from southern and central Chile showed the highest heat tolerance in trials outside the Andean Region (Peterson et al. 2015b).

Some quinoa varieties can tolerate certain degrees of frost, but this depends on the phenological stage of the plant and the duration and intensity of frost (Christensen et al. 1999; Jacobsen et al. 2005). Quinoa exposed to frost in the vegetative period was damaged by −8 °C during 2–4 h. During flowering stage, exposure to −4 °C during 4 h caused seed reduction by 66% (Bois et al. 2006). The main survival mechanism against frost in quinoa is the high level of soluble sugars that may cause a reduction in freezing temperatures and the mean lethal temperature, called *supercooling* (Jacobsen et al. 2007).

7.5.2.6 Photoperiod

The duration of all phases of quinoa development is influenced by photoperiod. Varieties from Colombia to southern Chile were classified as facultative short-day plants in terms of duration from emergence to flowering (Bertero 2003). The most important effects of extended photoperiods over 12 h are observed after flowering, as the seed fill and maturation stages are disrupted, hindering continued vegetative growth and flowering. Based on the foregoing, quinoa can produce glomeruli-flower clusters in any photoperiod but the phase of reproduction (pollination and fecundation) is inhibited in long photoperiods compared to those of its center of origin (Bertero et al. 1999, 2004; Christiansen et al. 2010).

Varieties Achachino (short day) and Titicaca (day-length neutral) were studied under different photoperiod conditions. There were observed increases in soluble sugar concentrations and ABA in response to photoperiod increase in both varieties. Achachino under a long photoperiod continued flowering with stem elongation and disruption of seed formation, on the other hand, Titicaca in all the photoperiods was able to produce seeds (Bendevis et al. 2014).

7.5.3 Identification of Genotypes with Tolerance to Biotic Stresses

It is of utmost importance to improve resistance and/or tolerance to biotic factors in quinoa, considering that farmers in traditional production and export zones of the Andean Region have their production directed toward an organic market.

Downy mildew (*Peronospora variabilis*) is the more important disease of quinoa in the Andean Region, it can be found in all regions, and almost all cultivated varieties are susceptible. Downy mildew distribution is increasing with the movement of varieties from dry areas to humid locations where rainfalls are concentrated in short periods. It is important to improve quinoa resistance to downy mildew to reduce the level of damage and contamination with pesticides. Field studies of plant/pathogen interactions were conducted to determine the evolutionary capacity of the pathogen and the plant response in accessions preserved ex situ. Using a core collection from the INIAP- Ecuador germplasm bank, three resistance genes were identified, which in turn allowed recognizing four biotypes of the pathogen (Ochoa et al. 1999). In Peru, at the National Agrarian University La Molina, a screening for resistance to mildew has been completed for 2089 accessions and out of them, 120 accessions were identified with 30% maximum severity in leaves (Gómez and Eguiluz 2011).

On the other hand, insect damage reaches very high levels, especially kcona-kcona larvae (*Eurysacca quinoae*) when they feed on grains in the inflorescences. Resistance can be obtained by non-preferential mechanisms (non-feeding or ovipositional), antibiosis and tolerance. In addition, some types of inflorescence can reduce housing to larvae during the grain damage phase (Saravia et al. 2014).

7.5.4 Identification of Quality Traits

In Bolivia, 3178 accessions from the national collection of quinoa germplasm were evaluated by quality traits such as size (diameter and thickness), saponin content, weight of 1000 grains and grain color. The nutritional value of 555 accessions (protein, fat, fiber, ash, carbohydrates, caloric energy) and the agro-industrial variables of 266 accessions (diameter of starch granules, water filling, inverted sugar) have been evaluated in order to recommend the use of germplasm in the production of quality processed products based on quinoa (Rojas and Pinto 2015). Research on the starch type and the amylose/amylopectin ratio was carried out to determine the better use of varieties. Quinoas with more content of amylopectin could have better performance in desserts and instant custard mixes, while quinoas with more amylose could be adequate for snack foods and noodles (Gandarillas et al. 2015; Rojas and Pinto 2015).

On the other hand, in Peru, nearly 900 accessions were evaluated for the following quality traits: 1000-grain weight, protein content, saponin content, and grain color and size (Gómez and Eguiluz 2011). Additionally, 120 accessions were evaluated using near infrared transmittance spectroscopy (NIT) – Infratec 1241 (850–1048 nm), to predict the contents of fat and ash in grains and flour. Ranges between 4.79 and 9.46% fat, and between 2.51 and 4.62% ash were recorded (Pereda 2016).

The study of germplasm from the quality point of view in the past focused on the nutritive value of white-cream grains, and their responses to industrialization. In the last decade, some changes in the preferences of consumers and industry have occurred. Quinoa with colored seeds such as red and black have been demanded by consumers and markets; probably due to the high association of seed color with some pigments and antioxidant effects.

7.6 Breeding Objectives

Genetic improvement of quinoa in the Andean countries started in Bolivia in 1965 at the Patacamaya Experimental Station (Gandarillas 1979). In Peru, it started at almost the same time in the Technical University of the Altiplano in Puno (Bonifacio et al. 2015). In similar ways, in the 1980s, Ecuador and Chile initiated their quinoa breeding programs (Bazile et al. 2015b).

From the 1960s to the 1980s, quinoa genetic improvement objectives were high-yielding genotypes with large-white or cream grains and higher protein content (Gandarillas et al. 2015). Since the 1990s, objectives of breeding programs have been oriented to develop new varieties which are high yielding, resistant or tolerant to biotic and abiotic stresses, short life cycle, adapted to one specific environment, with large sweet and semi-sweet grains and high protein content (Gomez-Pando 2015).

Plant breeding in recent decades has made significant genetic improvements in quinoa crop productivity, resistance to diseases and pests, better adaptation to particular climates and soils, implementation of mechanized harvesting, uniformity of grains and other quality traits (Bhargava et al. 2006; Bonifacio et al. 2015; Mastebroek et al. 2002).

7.7 Conventional Breeding Methodologies

Various methods of conventional plant breeding are applied in quinoa genetic improvement such as selection (mass and individual), hybridization and mutation induction. Each method has advantages and disadvantages. However, it is very important to consider if they complement each other in achieving the goals outlined in a good breeding program.

Organic production of quinoa in the Andes Region is regulated by rules established at the level of the national public and private certifying organizations, and one of the requirements is that varieties used in this type of production must have been obtained by conventional breeding methods.

7.7.1 Selection

7.7.1.1 Mass Selection

This method consists of the selection of a large number of superior plants with a similar phenotype in traditional or landrace varieties. Their seeds are then harvested and mixed together to constitute the new variety. Mass selection is applied multiple times in the same population to improve the base population performance since land-traditional varieties contain considerable genetic variation. Varieties developed by mass selection have wide adaptability, a broad genetic base and yield stability over a long period. Varieties developed through mass selection are a mixture of different genotypes with different morphological and agronomic characteristics, yield potential and responses to abiotic and biotic stresses. However, they are uniform in plant height, maturity, seed characteristics (size, color), saponin content, and in other characters preferred by the general market and industry.

The more important varieties obtained using mass selection are: Real (Bolivia), Baer (Chile), Dulce de Quitopamba (Colombia), and Pasankalla, Chewecca, Blanca de Juli, Amarilla de Marangani, Blanca de Junín, Rosada de Junin and Blanca de Hualhuas (Peru) (Gomez-Pando 2014).

Colorado State University performed a selection on a Chilean landrace and released the cultivar Colorado 407 in 1987 (Johnson 1990). Stratified mass selection was used to preserve the commercially-established varieties originated from the Real (Royal) landrace, to keep the composition of established commercial varieties, in Bolivia.

7.7.1.2 Individual Selection

Individual selection is of single plants with superior characteristics, in the original genetically-variable population (landraces). The general recommendation is to select a large number of plants with adequate characteristics to achieve the improvement objectives, based on time, money and space. The selected plants are harvested individually. In the following growing season the seeds produced by each selected plant are sown in separate rows, in a planting called plant/furrow, with adequate spaces, for the observation of their offspring and the corresponding evaluation (resistance to diseases, height, components of the plant, yield, etc.). A modification to the method was established for quinoa, considering the percentage of cross pollination of the quinoa, called an *inflorescence-row*, in which the plants selected within the lines are self-fertilized (all or part of the inflorescence), during the entire selection process, enough to achieve homogeneity, the harvested seeds of each plant are called lines (Bonifacio 2003; Gandarillas 1979).

Using the information collected in the field and laboratory, more selection and cultivation is continued, eliminating the inconvenient lines until the number is greatly reduced and statistically comparing the initial material and other commer-

cial varieties. The number of campaigns depends on the breeder. The line selected as an improved variety can be evaluated in farmers' fields, during the selection process, and then proceeds to seed multiplication and distribution. Using this method, cultivar Sajama Amarantiforme was obtained.

7.7.2 Hybridization

Hybridization has been used to improve quinoa in the Andes Region (Bonifacio 1990, 1995; Gandarillas 1979). Other breeding programs outside the center of origin have reported the use of hybridization as a methodology to study the genetic inheritance of important traits and to develop new quinoa varieties adapted to North America (Peterson et al. 2015a), to Europe (Jacobsen 2015) and to Brazil (Spehar et al. 2015).

7.7.2.1 Polyploidy

The base chromosome number of the genus *Chenopodium* is n = x = 9 chromosomes, quinoa has 2n = 36 chromosomes and it is therefore considered an allotetraploid (Kolano et al. 2012, 2016; Maugham et al. 2007). In quinoa, allelic segregation analysis has shown disomic inheritance, with an independent assortment at homoeologous loci. At least for qualitative traits, disomic inheritance has been reported in several studies, where the character segregations in F_2 have been consistent with the classic Mendelian proportions of 3:1 and 9:3:3:1 for one or two gene pairs, respectively (Bonifacio 1990, 1991; Simmonds 1971).

7.7.2.2 Floral Polymorphism

Quinoa flowers are sessile or pedicelled and are grouped in glomeruli. The position of the glomerulus in the inflorescence and the position of the flowers within the glomerulus determine the size and number of grains or fruits. It is a gynomonoecious plant because it has two types of flowers in the same plant; hermaphrodite and pistillate. The hermaphrodite flowers are found at the apex of the glomerulus and are larger than the pistillate, with a diameter of 3–5 mm; they have 5 tepals, 5 anthers and 1 superior ovary with 2 or 3 stigmatic ramifications. The pistillate flowers are found around and beneath the hermaphrodite flowers, they are formed of 5 tepals, 1 superior ovary and 2 or 3 stigmatic branches and have a diameter of 2–3 mm. The proportion of hermaphrodite and pistillate flowers is variable; the range found varies from 2–98% (Bhargava et al. 2007; Gandarillas 1979; Rea 1969; Simmonds 1965). This proportion is important if the crop is sown in isolation since it influences the amount of fruits formed.

In addition, some varieties of quinoa are male sterile because in some genotypes there are sterile androecious flowers or perfect flowers that produce non-functional pollen (Gandarillas 1979; Lescano 1994; Saravia 1991; Ward and Johnson 1993). Another mechanism favoring cross-pollination reported in quinoa is dichogamy of the protandry type (Lescano 1994).

Quinoa is considered autogamous by Gandarillas (1979) and Wilson (1988a, b, c) with a variable outcrossing percentage depending of the space among plants: 1.5–9.9% (Gandarillas 1986), 5.78–94.22% self-pollination (Lescano 1994). According to the results for quinoa's center of origin, the outbreeding percentage exceeds 10% of natural crossing. A study by Silvestri and Gil (2000) in Mendoza, Argentina, showed an outbreeding rate of 17.36%, confirmed by Gandarillas et al. (2015).

7.7.2.3 Male Sterility

Quinoa shows cytoplasmic and genic male sterility. Gandarillas (1969) reported a recessive nuclear gene controlling male sterility in Bolivian quinoa germplasm lines. Cytoplasmatic male sterility was reported, in Peruvian line UNTA 292, with fertility restored by the pollen of the Bolivian sweet Sajama variety (Aguilar 1980).

Apelawa and Amachuma genotypes were identified as sources of male sterility. The first carried normal and male sterile cytoplasms while the second had a single nuclear recessive gene that in a homozygous state produces normal anthers devoid of pollen grains (Ward and Johnson 1993, 1994).

Another source of male sterility was identified in accession PI 510536 in the USDA-ARS collection, which had normal hermaphrodite and male sterile quinoa plants. Male sterility was classified as cytoplasmic, and that a dominant nuclear allele interacted with this male sterile cytoplasm to restore male fertility. It was recognized by the presence of small shrunken anthers and the absence of pollen (Ward 1998).

7.7.2.4 Selection of Parents

The selection of the varieties or lines that will be used as parents, in the crosses programmed to achieve new varieties, is carried out considering the objectives of the improvement plan in such a way that the recombinant products of the artificial crosses combine the valuable characters in the derived progeny of the crosses. Preferably, it is recommended to combine improved commercial varieties and accessions with valuable complementary characters for the characteristics to be improved. It is recommended to use as the first parent the most popular variety of the work region because it already has a valuable gene combination adapted to the area, and as a second parent accessions that complement the characteristics of the first parent.

The valuable characteristics are available throughout the germplasm, however, the characterization of the same has allowed identification to a greater frequency in certain ecotypes. Genotypes with downy mildew resistance (*Peronospora variabilis*) are more frequent in the Valley ecotype (Gómez and Eguiluz 2011) and coastal quinoa (Fuentes et al. 2009). The genotypes with shorter plant height, short life cycle and no stem ramifications are more frequent in the genotypes of the Altiplano and Salares ecotypes (Carmen 1984). The semi-sweet genotypes with low saponin content can be selected preferably in valley ecotypes and the sweet or very bitter grains in the ecotypes of the Salares. The source for size of grains are genotypes denominated Royal quinoa from the Salares ecotype. Genotypes with greater tolerance to drought and salt can be found more in the ecotypes of the Altiplano and the Salares. Genotypes appropriate to temperate temperatures and long photoperiod are found in the Coastal ecotype of the south-central zone of Chile (Risi and Galwey 1989).

7.7.2.5 Emasculation and Pollination

The flowering of quinoa is asynchronous, it starts in any part of the inflorescence with the opening of hermaphrodite flowers at the apex of the flower groups called the glomeruli. Hermaphrodite and female flowers, in general, open at the same time and remain open for 5–13 days from morning to evening. The largest number of open flowers and a high in pollen production occurs at midday. In an experiment using 8 land races and 5 commercial varieties, an anthesis and dehiscence average duration were determined equal to 14.5 days and 18.2 days, respectively, and with an average of floral aberrations equal to 2.5% (Gandarillas 1979; Ignacio and Vera 1976; Lescano 1980).

Emasculation techniques and artificial pollination are often simple procedures in many other crops, but are quite laborious in quinoa due to inflorescence characteristics (clustering of numerous small-sized flowers) making hybridization by manual emasculation very difficult. Procedures for artificial hybridization of quinoa have been described by Gandarillas (1979), Bonifacio (1990, 1995) and Peterson et al. (2015a, b). The presence of a high number of female flowers in the inflorescence is an advantage. Moreover, male sterility can also be utilized in crossing quinoa (Ward 1998; Ward and Johnson 1993).

The plots destined for crosses can be installed in the field, using appropriate sowing dates to ensure the synchronized flowering of the genotypes that will be used in the crosses. The obtaining of 41% and 63% of seed set is reported in the cross between cvs. Pasankalla x Salcedo INIA, and Pasankalla x Choclo, respectively, in field conditions (Leon 2004–2005). Plants can be cultivated in the greenhouse to carry out the programmed crossings, the latter is very advisable in areas with adverse climates. In both locations cultural management and climatic environment must be optimal and favorable to ensure good quality pollen and the formation of a large number of seeds in the female parents.

To distinguish F_1 plants, it is important to use some morphological markers, such as seed color, inflorescence color, axillary pigmentation and plant color with well-

known inheritance. Female parents must have the selected marker character in the recessive genotype (Peterson et al. 2015a).

7.7.2.6 Management of Segregating Generations (F_2 to F_{6-7})

The F_2 population should be as large as possible to ensure that all desired recombinants are represented in the population at a high frequency so that they can be easily selected. The recommended methods for the management of the segregating population are bulk selection, pedigree selection and the single-seed descent.

Bulk or Mass Selection Bulk selection is a preferred breeding method due to its simplicity and low labor cost. It is recommended to have more than 30,000 plants in the F_2 generation for mass selection. Plants selected from F_2–F_6 are harvested in bulk. A sample of the bulk in each generation is planted in a plot to allow natural selection (drought, frost, pest epidemics) increasing the frequency of valuable well-adapted types in the population. In this method natural selection plays a very important role, influencing the genetic frequencies in the population, depending on the selection factor and the environmental conditions present. At generation F_6 selection can begin for valuable plants and their future management as independent lines and begin the evaluation of their yield and quality potential, using appropriate statistical tests in the following generations until identifying the new variety.

Using this method, Gandarillas (1979), obtained the variety Sajama, with sweet grains or without saponin in Bolivia. The plants with bitter fruits or with high content of saponin were eliminated in the F_2 generation. In the succeeding generations, valuable sweet plants were selected with good inflorescence morphology and agronomic performance. Bonifacio (2003), reports that varieties Chucapaca, Huaranga, Kamiri Robura, were also obtained with this method of selection.

Individual or Pedigree Selection This method is quite simple and efficient to select highly heritable characters (controlled by few genes). In this method the selection of valuable individual plants begins in the F_2 generation and their progeny are sown in the F_3 generation *plant-inflorescence/furrow* and so on for as many generations decided by the plant breeder. The plants within the furrow are progenies of an individual plant and are considered a family. In each generation, the best plants present in the families should be selected based on the evaluation of their behavior. This means that the breeder is evaluating the behavior of individual plants and families and lines derived from them from early generations. In F_2 to F_4, the selection characters such as plant vigor, date of maturation, resistance to diseases or some insects, characters of the fruits etc. are used for selection. In more advanced generations, the selections must be related especially to yield and quality. Once the lines reach homozygosity, yield trials are made in different years and locations.

PROINPA, PREDUZA and McKnight projects, in Bolivia, made crossings with 25 varieties obtaining 36 true F_1 hybrids. Two segregating generations per year were managed from F_2, F_3 and F_4, using a greenhouse typical of the area called *waliplini*

(semi-subterranean greenhouse). The selection criteria used were resistance to downy mildew, seed size, precocity, seed color and plant vigor. Performance experiments were conducted in advanced generations in five locations. This improvement work resulted in Line L-26 (85) with good yield, precocity and seed size. This line was released as a new variety named Jach'a Grano which in the native language means *large seed* (http://www.proinpa.org).

The climatic conditions of the Brazilian savannas promotes natural high rates of cross-pollination in quinoa to create segregating plants which are identified and recovered as individual plants and managed and evaluated for nutritional quality following the same procedure of segregating plants obtained by artificial crosses. These efforts resulted in cultivars BRS Piabiru and BRS Syetetuba in 2000 (Spehar et al. 2015).

Hybridization between highland (0654) and coastal (NL-6) germplasm groups gave origin to 72 F2:6 recombinant-inbred lines with vast amounts of genetic variation for quantitative and qualitative traits. Observations made in 21 agro-morpho-phenological traits permitted identify transgressive segregation for seed yield (22.42 g/plant) and positive correlation of yield with panicle length and biomass (Benlhabib et al. 2016).

Combination of Individual and Mass Selection The combination of mass and individual selection can be used to manage the segregating material from F_2 to more advanced generations, depending on the objectives, environmental conditions and availability of resources. This combination allows the use of natural selection and the selection of the breeder and could improve the selection criteria and broaden the genetic variability for future breeding work through other crosses occurring during the breeding process. Bonifacio (2003), indicates that the Bolivian varieties Sayaña, Jilata, Patacamaya, Ratuqui, Jumataki, Intinaira, Surumi and Santamaria were obtained following this combined procedure.

Heterosis in Quinoa Wilson (1990) developed groups of quinoa crosses with parents of very diverse origin using sources of male sterility. In this genetic material the percentage of heterosis was studied within the groups and between the groups, the results showed the existence of heterosis in a range of 201–491% for the crosses between the groups and the absence of heterosis in the crosses within the groups. The discovery of hybrid vigor in quinoa was very important because of the possibility of producing commercial hybrid varieties with better yield than their progenitors (Ward and Johnson 1993).

Interspecific and Intergeneric Hybridization Interspecific crosses for the improvement of quinoa were made by Gandarillas (1986); Wilson and Manhart (1993) and Ward and Johnson (1993) and Bonifacio (1995). *Chenopodium quinoa* crosses with *C. nuttalliae*, *C. berlandierii*, *C. petiolare* and *C. carnosolum* were found viable. On the other hand, crosses of *C. quinoa* (tetraploid and with saponin or bitter grains) and *C. pallidicuale* (diploid without saponin or sweet grains) were made to obtain genetic material with sweet grains or without saponin and better

wide adaptation to the high elevations of the Andean region; however, no results were achieved due to the difference in the level of ploidy of the species and the size and fragility of the floral structures (Lescano 1994).

Crosses were also made between *Chenopodium* and *Atriplex* genera, obtaining hybrids between *C. quinoa* and *Atriplex hortensis* (high protein content) and *A. joaquiniana*, however the hybrid plants showed male sterility (Bonifacio 1995).

It would be very important to develop protocols to improve breeding strategies for interspecific or intergeneric crosses (bridging crosses, colchicine-mediated chromosome doubling or embryo rescue) to obtain new varieties; especially for organic quinoa production. Wild species of *Chenopodium* genus and others from the Chenopodioeae subfamily display considerable genetic resources that could be incorporated into cultivated quinoa (e.g. pest resistance, hardiness, frost, heat and drought tolerance). Other modern biotechnology tools could be used for marker-assisted gene pyramiding and alien gene introgression of the wild pool of genes.

7.7.3 Backcross Method

This method is used to improve valuable agronomic commercial varieties that have one or more negative characters; generally related to susceptibility to disease or crop quality. To ensure favorable results, the characters must be qualitative and dominant and easy to identify. An example of the use of this method is the improvement of the size of grains of the variety Patacamaya, a plant green in color and with sweet small grains, using as donor parent the line 1638, an accession of the Royal race type, pandela (pink), with large and bitter grains. This method is also being used in the improvement of cultivated varieties with genes donated from wild varieties (Bonifacio 2003).

7.7.4 Induced Mutations

Many mutant plant varieties from several crops have been released officially, directly as new varieties or as parents to create varieties, with improved yield, quality (oil, protein, starch), enhanced uptake of specific minerals, deeper rooting system, resistance to biotic and abiotic stresses (https://mvd.iaea.org/). The potential of induced mutagenesis has been enhanced by the use of tools that reduce space and time to evaluate each generation and molecular markers to advance in the process of selection.

Induced mutation is a plant-breeding methodology recommended in quinoa genetic improvement when necessary to modify one or a few traits in a traditional and or commercial variety used by farmers and industry. The use of good traditional or commercial varieties provides a chance for releasing directly and rapidly one

variety, if an appropriate mutant type is found, because adoption by farmers and industry is ensured by the slightly alteration of the basic genotype of their varieties. Traditional varieties of quinoa have valuable combinations of genes regarding adaptation to marginal environments and soils, and high nutrition values. However, some have agronomically undesirable traits that reduce yield and make their use difficult in modern agriculture of high inputs and extensive areas. Among these traits are plant heights above 2 m, excessive branching, long life cycle and susceptibility to diseases. In the same way, some negative quality traits can be observed, such as high saponin content, small grains and thick fruit layers.

7.7.4.1 Dosimetry

Doses used for mutation induction must be determined for each variety. Somatic (M_1) and genetic effects (M_2) after the treatments should be studied to identify the best dose for mutation induction (Gomez-Pando 2014; Maluszynski et al. 2009; Shu et al. 2012).

7.7.4.2 Management of M_1 Population

Quinoa has some percentage of outcrossing, so isolation is very important. The M_1 population should have normal commercial cultural practices, including irrigation, hand or mechanical weed control and pesticide application. In the same condition, a control or genotype without irradiation, should be included for further comparative use in succeeding generations. The harvests of seeds could be made plant by plant, single seed selection (one seed per plant) or bulk (Gomez-Pando 2014; Maluszynski et al. 2009; Shu et al. 2012).

7.7.4.3 Management of M_2 Population

In field conditions, all seeds from the plant or M_1-inflorescence or bulk are grown in different plot size or a single row. Thin planting or low density is recommended because it facilitates visual selection of mutants. At the seedling-cotyledonal stage, an evaluation, of chlorophyll or other pigments and morphological characteristics changes should be made. Each row or plant progeny with deviants should be marked for a further screening process during the growing season. The following plant traits should be evaluated in all the plant progenies: growing period, plant height, color and shape of leaves, inflorescence and grains, branching pattern, inflorescence density, response to disease and environment and other specific objectives of the improvement program (Gomez-Pando 2014; Maluszynski et al. 2009; Shu et al. 2012).

Plants with divergent characters and the group of normal-looking plants but more vigorous should be harvested for further selection of quantitative traits in M_3 or M_4.

The efficiency of mutation breeding is highly dependent on the effectiveness of genetic variant identification in M_2 or M_3.

7.7.4.4 Management of M_3 Population

In the M_3 generation, a progeny and a homozygosis test should be made for all the deviants or *putative* mutant plants selected in M_2. Depending on the generation in which the selection was performed, the homozygosis test must be done in the following generation. Only one of the (two or more) alleles of a locus is affected, inheritance is almost always recessive, therefore homozygosis is normally required for proper expression (Gomez-Pando 2015; Maluszynski et al. 2009; Shu et al. 2012).

7.7.4.5 Management of Further Generations

All the following generations are managed in a similar way to other breeding methodologies. The preliminary evaluation of agronomic traits could be done with selected mutant lines in the M_4 generation and to start the evaluation in the regional or national trials.

Seeds of cvs. Pasankalla and LM 89 were used as genetic materials to be improved using 150, 250 and 350 Gy gamma radiation. In M_1 generation, germination was delayed with increasing radiation dose, while seedling height, root length and leaf development were mostly reduced at 250 Gy; and plants did not survive when treated with 350 Gy. In M_2, the maximum spectrum and frequency of mutations increased with the higher doses. Chlorophyll mutations were observed, e.g. chlorine and xantha type mutations and variations of other pigments such as betalains. Doses of 150 and 250 Gy, produced mutations in the morphology of the plant (color of the stem and leaves, leaves morphology, branching grade, height of the plant and length of the floral pedicels), the duration of the life cycle, type of plant and improvement of the vigor of the plant and yield. Changes of seed color were also achieved (Gomez-Pando 2015; Gomez-Pando and Eguiluz-de la Barra 2013; Maluszynski et al. 2009).

Mutations were observed in grain yield, protein, color and saponin content in advanced generations of Pasankalla variety, treated with 150 and 250 Gy. Seven lines excelled in yield potential from 3220.7 to 4133.5 kg/ha; and differed significantly with the control or parent materials that reached 2227.5 kg/ha. Three mutants—MQPas-302, MQPas-378, MQPas-348—had higher protein content (13.2, 12.8 and 12.7%, respectively) than control materials (11.3%). Additionally, eight mutant lines, including MQPas-302, MQPas-378 and MQPas-254 showed 13.2, 12.8 and 12.0% proteins and similar yields to the parental materials. They also presented mutations of grain color such as cream and black. Modifications in saponin content were observed among the mutant lines (Quispe 2015).

Similar types of mutations were observed in LM 89 quinoa variety. The following changes were recorded: in grain yield 3358.57–4258.6 kg/ha, days to maturity 93–108, plant height 136.7–181.7 cm, downy mildew severity 10–16.7%, thousand kernel weight 2.7–3.5 g, grain protein content 10.6–13.4%, grain saponin content 0.45–1.1% and nitrogen use efficiency (NUE) of 34.23–53.06% (Sanchez 2015) and improved water use efficiency (WUE) with a value of 1.68 kg/m^3 (Gomez-Pando et al. 2017).

7.8 Modern Biotechnology: Techniques and Tools Applied to Quinoa Breeding

Some techniques and tools of modern biotechnology have been applied in quinoa genetic improvement such as in vitro cell and tissue culture. On the other hand, DNA-based genetic markers and genomic resources have increased knowledge and capacity to characterize genetic diversity in the germplasm and to improve conventional breeding.

7.8.1 Use in Genetic Stocks Increments and Seed Production

In vitro callus production of quinoa was conducted to develop male sterile lines for hybrid production (Tamulonis 1989). In vitro vegetative propagation of Peruvian quinoas has been carried out to increase populations of some accessions with very low germination capacities (Ruiz 2002). A protocol to clone quinoa and increase stocks of hybrid seeds to avoid cross-pollination was reported in Brazil (Rocha 2011).

7.8.2 Double Haploid Breeding

Preliminary studies have been carried out on quinoa to obtain doubled haploids from in vitro cultivation of anthers (microspores) of varieties Rosada de Huancayo and Blanca de Hualhuas (Soplin 2009). These tools could permit an acceleration of the development of new quinoa cultivars.

7.8.3 Phylogenetic Relationships Between the Chenopodium Genera

The use of biochemical and molecular markers in quinoa selection began with the use of Chenopodiaceae isozymes to attempt establishing the phylogenetic relationships between the *Chenopodium* genera (Wilson 1988a).

Maughan (2006), using fluorescence in situ hybridization (FISH), mapped the first tandem repetitive sequence of an rRNA gene loci. The 35S RNA gene loci were localized in the terminal region of two chromosomes. It was used to quantify the number of 45s RNA and 5s rRNA loci in quinoa (*Chenopodium berlandieri* var. *zschackei* and *C. berlandieri* ssp. *nuttalliae*). Results indicated quinoa and *C. berlandieri* share a common diploid ancestor.

Sederberg (2008) used FISH to examine the 5 s and 45 s RNA gene loci in New World *Chenopodium* spp. No definitive ancestors to quinoa were located, but a few potential ancestral species were determined. Kolano et al. (2011) used FISH for the DNA clone 18-24 J on *C. quinoa* and a range of other *Chenopodium* spp. Results indicated *C. quinoa*, *C. berlandieri* and hexaploid *C. album* share a common ancestor.

Matanguihan et al. (2015) indicated that one or more pairs of the 35S rRNA genes loci were deleted during quinoa evolution because the locus number was lower than the expected additive values of any known diploids. Kolano et al. (2012) reported similar results for other polyploid *Chenopodium* species.

Complete chloroplast (cp) genomes of *Chenopodium quinoa* and *C. album* were obtained by next generation sequencing. The cp genome of *C. quinoa* and of *C. album* are 152,099 bp and 152,167 bp, respectively. In total, 119 genes (78 protein-coding, 37 tRNA, and 4 rRNA) were identified. Additionally, 14 (*C. quinoa*) and 15 (*C. album*) tandem repeats (TRs); 14 TRs were present in both species and *C. album* and *C. quinoa* each had 1 species-specific TR. The trnI-GAU intron sequences contained 1 (*C. quinoa*) or 2 (*C. album*) copies of TRs (66 bp). Variation in the TR copy number in 4 species, *C. hybridum*, *C. pumilio*, *C. ficifolium* and *C. koraiense*, but not in *C. glaucum* was observed using InDel markers (Hong et al. 2017).

7.8.4 Molecular Marker Development

Molecular markers are important in modern plant breeding. Their use has improved conventional breeding by providing genomic tools, useful for the incorporation of genes that are highly influenced by the environment and others of difficult study such as those that confer resistance to diseases and to accumulate multiple genes for resistance to specific pathogens and pests within the same cultivar (gene pyramidation). It has also allowed the plant breeder the fastest generational advance, since through the use of PCR it can be detected if the gene is present in the lines evaluated in earlier generations during the breeding process. It is important for the identification of useful characters of tolerance or resistance to the main types of biotic and abiotic stress in the germplasm of a species, and their application in breeding making interspecific crosses and select germplasm with respect to these characters and preserving a core collection (Eathington et al. 2007).

RAPD markers were developed and used to screen for successful hybrids of interspecific and intergeneric crosses with *Chenopodium berlandieri*, *C. berlandieri* ssp. *nutalliae* and *Atriplex* spp. (Bonifacio 1995, 2004). RAPD were also used to identify the hybrid state of progeny and the genetic relationship between wild and cultivated quinoa. Ruas et al. (1999) and Del Castillo et al. (2007) used RAPD to

study the genetics and relationships between various accessions of *C. quinoa* and related species, as well as to evaluate the genetic structure of quinoa populations in the Bolivian Altiplano.

Wilson (1988b) and Christensen et al. (2007) found a strong genetic similarity between Coastal ecotypes and the Andean ecotypes in the characterization of germplasm of both origins. Fuentes et al. (2009) reported that quinoa varieties from the Andean region and the Chilean coast have 21.3% common alleles, while quinoa from the Andean region has a single allele frequency of 28.6% while Coastal ecotypes reach 50%.

AFLP (amplified fragment length polymorphism) markers were used to characterize the genetic diversity of quinoa accessions in the USDA germplasm database, and in the CIP-FAO International Nursery. Maughan et al. (2004) developed genetic linkage map for quinoa, which covered an estimated 60% of the genome. It was based primarily on AFLPs. Swenson (2006) examined the genetic diversity of Bolivian strains of downy mildew (*Peronospora variabilis*) collected across Bolivia in 2005 and 2006. Using AFLP markers, a high level of genetic diversity was found within the species. Quinoa resistance to the pathogen was found to be generally dominantly inherited. Kitz (2008) developed an inoculation method for downy mildew and characterized its infection pat- 555 tern using scanning electron microscopy. Nolaso et al. (2013) evaluated the polymorphism of six peruvian and Bolivian commercial varieties using AFLP technology and discriminate the Mantaro variety from the other varieties Quillahuman INIA, Hualhuas, Real Boliviana, Salcedo INIA and Illpa INIA.

Microsatellite markers are the most variable type of DNA sequences in eukaryotic genomes (Weber and May 1989); microsatellites have been extremely useful for determining taxonomic relationships among closely related individuals and assessing diversity within a species. Coles et al. (2005) developed SNP markers for quinoa from an EST library. Mason et al. (2005) developed the first set of 208 polymorphic microsatellites in quinoa, also known as short tandem repeats (STRs) or simple sequence repeats (SSRs). Fuentes et al. (2006) studied the genetic diversity of quinoa Chilean germplasm using microsatellites. Jarvis et al. (2008) characterized more than 400 microsatellite markers (SSR markers), and later reported an additional set of 2106 new SSR markers and a linkage map with 275 molecular markers, including 200 SSR markers.

Quinoa microsatellite markers clearly show that quinoa accessions can be broadly clustered in two main groups: one including accessions from the lowlands of Chile (Coastal ecotype), and the other comprising accessions from the Andean highlands (Altiplano ecotype) with origins in Peru, Bolivia, Ecuador, Argentina and extreme northeastern Chile. Quinoas of the Chilean Altiplano were genetically less diverse than the Chilean Coastal accessions, suggesting a potential loss of genetic diversity in the commercial growing zones of Chile (Christensen et al. 2007).

Costa-Tartara et al. (2012) studied the genetic structure of 90 cultivated quinoa cultivars from Northwest Argentina using 22 microsatellites. Aside from being underrepresented, Northwest Argentina's germplasm is also the southernmost point of quinoa distribution within the Central Andes. Accessions showed a high level of genetic diversity, which could be grouped into four regional ecogeographical clusters, depending on the collection sites: the transition region characterized by high elevations; puna, the highland plateau; eastern humid valleys; and dry val-

leys. Fernandez (2015) described the genetic diversity of peruvian germplasm from valley and altiplano ecotypes using 23 microsatellite markers with a PCR-Multiplex system, identifying exclusive alleles for the valley ecotypes. Morillo-Coronado et al. (2017) using seven microsatellite markers RAMs characterized 55 Colombian quinoa accessions, the Nei-Li coefficient with a level of similarity of 0.65 divided the accessions in four groups according to the site of origen.

The high frequency of SNPs offers the possibility to construct extremely dense genetic maps that are particularly valuable for map-based gene cloning efforts and for haplotype-based association studies.

Maughan et al. (2012) sequenced a genomic reduction quinoa library to identify 14,178 putative SNPs in 5 bi-parental quinoa populations. Out of 14,178 SNPs identified, 511 were successfully converted into functional SNP assays using KBioscience's competitive allele-specific PCR genotyping chemistry (KASPar™). A diversity screen of 113 quinoa accessions using these 511 SNPs clearly revealed the two major quinoa subgroups. Zhang et al. (2017) using 11 quinoa accessions evaluated genomic variation, population structure, and genetic diversity with insertion/deletion (INDel) markers and founded two major groups of quina one from the highland and the other from Chilean coastal area.

7.8.5 An EST Library

Coles et al. (2005) generated an EST (expressed sequence tag) library using immature seed and flower tissue. It described the first set of 424 ESTs from developing seed and floral tissue (GenBank dbEST ID #GI47561370–GI47561793). Out of these sequences, 349 had significant homology to protein-encoding genes from other plant species. Putative functions related to metabolism, protein synthesis, development and so forth, have been assigned to many of these EST sequences.

Balzotti et al. (2008) cloned and described the two *11S* genes, globulin seed storage proteins, representing the two subgenomes of allotetraploid quinoa. Identification and characterization of these genes provide important clues to understanding the high protein content and excellent balance of amino acids in quinoa grain.

Later, Reynolds (2009) constructed an EST library from seed tissue of a bitter saponin-producing quinoa variety. A custom microarray was developed and used to measure transcriptional differences between sweet and bitter quinoa varieties. Candidate genes potentially responsible for saponin biosynthesis in quinoa were also identified. Maughan et al. (2009) used this library to extensively characterize *SOS1* homologs in quinoa.

7.8.6 Development of a BAC

A BAC (bacterial artificial chromosome) library was constructed by Stevens et al. (2006) and was probed for seed protein storage genes. The library was constructed using two restriction endonucleases, *BamHI* (26,880 clones) and *EcoRI* (48,000

clones), with average clone insert sizes of 113 kb and 130 kb per insert, respectively; recognized as 9X bacterial artificial chromosome library. It consists of 74,880 clones.

7.8.7 Gene Expression

Quantitative expression of genes of interest has been investigated. The gene coding for 11S globulin seed storage protein, thought to contribute to the amino acid balance of quinoa protein, was investigated by Balzotti et al. (2008) using RT-PCR. Accumulation of the 11S globulin seed protein was measured using SDS-PAGE.

Maughan et al. (2009) cloned and characterized two homoeologous *SOS1* loci (*cqSOS1A* and *cqSOS1B*) from quinoa (salt overly sensitive), including full-length cDNA sequences, genomic sequences, relative expression levels, fluorescent in situ hybridization (FISH) analysis and a phylogenetic analysis of *SOS1* genes from 13 plant taxa. Genomic sequence analysis of two BAC clones (98,357 bp and 132,770 bp) containing the homoeologous *SOS1* genes suggests possible conservation of synteny across the quinoa subgenomes. The salt overly sensitive 1 *(SOS1)* gene encodes a plasma membrane Na^+/H^+ antiporter that play an important role in germination and growth of plants in saline environments.

Morales et al. (2011) also reported the development of primer information used for real-time expression of several additional salt tolerance genes, including *NHX1*, *TIP2* and *BADH*. Quantified expression of *SOS1*, *NHX1* and *TIP2* genes thought to potentially mediate salt tolerance, using RT-PCR on root and leaf tissue of different quinoa ecotypes.

Raney et al. (2014) reported the results of an RNA-seq transcriptome analysis of two quinoa accessions using four water treatments from field capacity to drought. cDNA libraries from root tissue samples for each variety × treatment combination were sequenced using Illumina Hi-Seq technology generating a de novo assembly of the quinoa root transcriptome consisting of 20,337 unique transcripts. These transcripts are publicly available from NCBI GenBank (SRA #SRR799899 and SRR799901). Gene expression analysis of the RNAseq data identified 462 putative gene products that showed differential expression based on treatments. Moreover, 27 putative gene products differentially expressed were identified and bioinformatic methods were used to implicate specific pathways putatively associated to water stress in quinoa.

7.8.8 Genome Sequencing

Yasui et al. (2016), using an inbred line of quinoa (Kd), reported an incomplete genome sequence. This novo genome assembly contained 25 K scaffolds consisting of 1 Gbp with N50 length of 86 kbp. The methodology used included sequencing on the Ilumina Hiseq 2500 and PacBio RS II sequencers.

A more complete sequence of the quinoa genome was made by Jarvis et al. (2017), using a coastal Chilean quinoa accession. It was observed an assembly of

439 sequence scaffolds that together covered 90% of the genome and aligned into 18 linkage groups. The total assembly size was 1.39 Gb. The methodology applied used single-molecule real time (SMRT) sequencing technology from Pacific Biosciences (PacBio), and optical chromosome-contact maps from BioNano Genomics and Dovetail Genomics, that include the SNP-based linkage map (Maughan et al. 2012) with two new linkage maps. Results of this research is a map with 6403 unique markers which span a total length of 2034 centimorgans (cM). This information suggests that quinoa genome could contains 44,776 genes.

A small genomic region encoding two transcription factors involved in saponin biosynthesis was also identified. Saponins confer a bitter taste to quinoa grains and are anti-nutritional compounds contained in the outer layer of the seeds. The amount of saponin present in the quinoa seeds depends on the genotype; some sweet accessions have been recognized. Jarvis et al. (2017) identified a mutation that causes alternative splicing, and a premature stop codon produces a truncated protein that results in reduced saponin production.

The identification of transmembrane proteins involved in salt tolerance were made by Schmockel et al. (2017), using quinoa genome sequence assembled by Jarvis et al. (2017), combined with RNA-seq analysis, comparative genomics and topology prediction. They identified 219 candidate genes, which were reduced to 15 genes by looking at single nucleotide polymorphisms. Additionally, large variation in salinity tolerance were observed among 21 *Chenopodium* accessions (14 *C. quinoa*, 5 *C. berlandieri* and 2 *C. hircimum*), with *C. hircimum* having the highest tolerance to salinity.

ABA-independent expression patterns were observed by Morales et al. (2017) studying the transcriptional responses of R49, the most drought tolerant genotype of three Chilean quinoas under drought conditions. The transcriptome of R49 was sequenced by the Illumina pair-ends method, using total RNA extracted from quinoa plants under drought stress and from total RNA extracted from control plants. Results showed that 1579 genes were over-represented under drought conditions, and 877 were underrepresented. Of the genes that were overrepresented, 19% were unknown genes, indicating that the drought response in quinoa might have several paths that are still unknown.

Liu et al. (2018) analyzed the *Chenoposium quinoa* genome and identified 16 *Hsp70* members of heat-shock proteins with important roles in response to biotic and abiotic stress. Phylogenetic analysis revealed the independent origination of those *Hsp70* members, with 8 paralogous pairs comprising the *Hsp70* family in quinoa. Additionally gene expression analyses revealed significant variations of *Cqhsp70s* in response to drought stress.

7.9 Conclusions and Prospects

7.9.1 An Overview of Status

Quinoa (*Chenopodium quinoa* Willd.) is a native crop of the Andean Region recognized for its nutritional quality, ability to thrive in marginal conditions, and economic value. It has been cultivated in the Andean region for thousands of years.

Quinoa, especially in the Andean highlands (3000–4200 elevation), along with potatoes, tubers, root crops and maize were the basic food for the ancient populations. With the introduction of cereals and legumes during the Spanish Conquest, quinoa cultivation decreased significantly, being restricted mainly to the agricultural areas of the Peruvian-Bolivian Altiplano, where only a few crop species can be cultivated. The decrease of cultivated area and the low demand of quinoa determined the loss of germplasm and knowledge related to its cultivation and use, especially in the low valleys of the Andean zone.

In 1980s, a process of revaluation of subutilized or marginalized crops began, highlighting quinoa, which was recognized as an important source of high-value proteins due to its balance of essential amino acids, its good amount and quality of fat, minerals, vitamins and carbohydrates. Afterwards, the problems caused by climatic change in agriculture, made it necessary to identify new species adapted to salinized soils, extreme temperatures, drought and flooding. With this background, the agronomic revaluation of quinoa became relevant as an important alternative.

Recognition of quinoa's nutritional value and changes of food habits in some populations determined the increase in demand for quinoa in the Andean region. To meet the great demand of quinoa with a good price of grain, farmers cultivated larger areas, especially in Peru and Bolivia. In the Andean Region, replacement of organic quinoa production by conventional practices has increased the environment contamination and reduced farmer income and food quality. Smallholder farmers are losing their traditional landraces because commercial interest is focused on a small group of quinoa demanded by the industry.

On the other hand, market requirements for high nutritional quality, tolerance to adverse climatic conditions, salinized and dry soils, has prompted many countries to initiate the introduction of quinoa into different farming systems and levels of technology (e.g. USA, Canada, Europe, Asia and Africa). Quinoa is cultivated or been tested in almost 95 countries of the world (Bazile et al. 2015b, 2016a, b). Studies of quinoa adaptation in many countries have demonstrated its great plasticity far from the quinoa center of origin. In each site studied, one or more genotypes with high agricultural performances have been identified (Bazile et al. 2016b).

However, the cultivation in extensive areas, the introduction to new areas and the effect of climatic change have shown the real limiting factors of quinoa production: biotic stresses (diseases, weeds, pests), abiotic stresses (heat waves, photoperiod duration), lack of appropriate technology or mechanization for production, harvest and postharvest in large scale farms.

Outside the Andean Region, researchers are mostly using only a few improved varieties (Puno, Regalona, Titicaca), registered through Plant Variety Protection (PVP) (Jacobsen 2015), and in general a narrow base germplasm for experimentation an adaptation to new environments. Despite the high levels of quinoa diversity conserved in the Andean Region, this is not currently exchanged globally due to highly-political issues of national sovereignty (Bazile et al. 2015b, 2016a).

The limiting factors of agronomic and environmental nature that reduce the yield and quality of quinoa, and its expansion worldwide, can be solved through the improvement of cultivation technologies and the development of new varieties to

allow sustainable production. There are available the knowledge and many conventional and modern biotechnological tools for genetic improvement of quinoa.

7.9.2 Current Research Initiatives to Face the Global Climate Change

Adverse climatic conditions make it necessary to expand the food base with other species; especially those with great nutritional potential and adapted to diverse marginal environments.

Quinoa is an important alternative crop because of its high nutritive value associated with a combination of traits conferring tolerance to extreme temperatures, water deficits and salinity, which make this plant important to combat global climate change. Hardiness and adaptability are major advantages in the context of climate change and salinization of agricultural lands worldwide.

There are many research studies identifying sources and mechanisms of quinoa tolerance to abiotic and biotic stresses that are nearly constant globally (Azurita-Silva et al. 2015; Biondi et al. 2015; Bonifacio et al. 2015; Bosque-Sanchez et al. 2003; Gandarillas et al. 2015; Gomez-Pando et al. 2010; Jacobsen et al. 1999; Nguyen 2016).

7.9.3 Recommendations for Future Research and Utilization

The objective of quinoa improvement is to develop new varieties of quinoa adapted to different environments and production systems (conventional or organic) using germplasm, knowledge and modern biotechnology tools. Quinoa varieties must have high yield potentials, adaptations, resistance to biotic stresses (diseases, pests, highly competitive with weeds) and tolerance to abiotic stress (salinity, drought, heat, flooding, cold). On the other hand, high efficiency in the use of nutrients, water, and other additional traits required in specific environments, as well as good physical grain traits and nutritive quality for food and industry are desirable.

Considering the expansion of cultivation areas, quinoa varieties must have changes in plant morphology to make mechanized agriculture possible in high population densities and with inputs. Therefore, it is important to reduce plant height and life cycle, select plants without branching, with one terminal inflorescence, and uniform maturity.

Another approach for quinoa improvement is the use of new sources of heat tolerance or disease resistance genes from wild relatives. Possible traits of interest include the free-threshing (utriculate) pericarp character of many of these taxa, including *Chenopodium standleyanum*; and extreme sodium tolerance in *C. nevadense*; drought tolerance in *C. desiccatum, C. hians, C. incanum, C. leptophyl-*

lum, C. petiolare and *C. pratericola*; and heat tolerance in several members of these taxa from the Mojave and Sonoran deserts in North America (Jellen et al. 2015; Matanguihan et al. 2015;).

On the other hand, growing new varieties must be complemented with good agronomic practices to gain the highest productivity in terms of quantity and quality under different environmental conditions (including climate and soil types). It is necessary to conduct research on irrigation systems, management practices (e.g. fertilization and pest control) for intensive and organic growth. Moreover, better harvest and postharvest technologies should be implemented to expand and improve the consumption and product uses of quinoa.

Appendices

Appendix I: Research Institutes Relevant to Quinoa

Institution	Specialization and research activities	Contact information and website
Instituto Nacional de Innovación Agraria (INIA) PERU	Plant breeding and agronomy	rsanchez@inia.gob.pe
Universidad Nacional Agraria La Molina	Plant breeding and agronomy	pcereal@lamolina.edu.pe
Universidad San Antonio Abad del Cusco	Plant breeding and agronomy	aalvarezcaceres@yahoo.es
Universidad Nacional del Altiplano	Agronomy, agroindustry	amhmujica@yahoo.com
Universidad de Buenos Aires – Argentina	Agronomy and physiology	Daniel Bertero <bertero@agro.uba.ar>
Instituto de Ecología, Fundación Miguel Lillo, Tucuman, Argentina	Agronomy and quality	Juan Antonio González <juanantoniogonzlez@gmail.com>
Instituto Nacional de Investigaciones Agropecuarias	Agronomy, plant breeding	Jose Ochoa <jbjochoa@gmail.com>
PROINPA Bolivia (Foundation for the Promotion & Investigation of Andean Produce	Agronomy, plant breeding	Wilfredo Rojas <w.rojas@proinpa.org>
Pontificia Universidad Católica de Chile	Agronomy, plant breeding	Franciso Fuentes <frfuentesc@uc.cl>
Brigham Young University	Molecular biotechnology	Jeff Maughan <jeff_maughan@byu.edu>
Washington State University	Agronomy and plant breeding	Kevin Murphy <kmurphy2@wsu.edu>

Appendix II: Quinoa Genetic Resources

Institution	Specialization and research activities	Contact and website
Instituto Nacional de Innovación Agraria (INIA) PERU	Plant breeding and agronomy	rsanchez@inia.gob.pe
Universidad Nacional Agraria La Molina	Plant breeding and agronomy	pcereal@lamolina.edu.pe
Universidad San Antonio Abad del Cusco	Plant breeding and agronomy	aalvarezcaceres@yahoo.es
Universidad Nacional del Altiplano	Agronomy, agroindustry	amhmujica@yahoo.com
Universidad de Buenos Aires – Argentina	Agronomy and physiology	Daniel Bertero bertero@agro.uba.ar
Instituto de Ecología, Fundación Miguel Lillo, Tucuman, Argentina	Agronomy and quality	Juan Antonio González juanantoniogonzlez@gmail.com
Instituto Nacional de Investigaciones Agropecuarias	Agronomy, plant breeding	Jose Ochoa jbjochoa@gmail.com
PROINPA Bolivia (Foundation for the Promotion & Investigation of Andean Produce	Agronomy, plant breeding	Wilfredo Rojas w.rojas@proinpa.org
Pontificia Universidad Católica de Chile	Agronomy, plant breeding	Franciso Fuentes frfuentesc@uc.cl
Brigham Young University	Molecular biotechnology	Jeff Maughan jeff_maughan@byu.edu
Washington State University	Agronomy and plant breeding	Kevin Murphy kmurphy2@wsu.edu

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Chapter 8
Molecular Breeding Strategies for Genetic Improvement in Rice (*Oryza sativa* L.)

Ritu Mahajan and Nisha Kapoor

Abstract The current progress in crop research has provided a useful benchmark to evaluate crop-breeding improvement using genomics and molecular breeding techniques. The generation of huge amounts of molecular-genetic data has provided several ways to utilize the available genetic resources and to find solutions to the demanding goals of plant breeding. Rice being a staple food is consumed as an essential part of the dietary requirement by most of the developing countries. With the increase in population growth, traditional breeding methods cannot find a viable solution for sustainable crop production and food security. Since genetics and breeding are closely associated, combining these two has resulted in remarkable progress in rice-breeding programs. The presence of genetic diversity within cultivated crops and their wild relatives provides a platform for gene discovery of the agronomical important traits yet to be sufficiently discovered and utilized. This progress of developing new rice varieties with specific agronomic characters was made by using marker-assisted selection that opened new avenues for basic plant research. Combining conventional methods with molecular genetics will help in understanding the inheritance pattern of targeted traits in plant breeding and thus will lead to crop improvement in the future. This in turn can open new ways of improving the efficiency of breeding programs. Next-generation sequencing is the largest advancement and a boon for gene identification and variations in the genome. Recent techniques like CRISPR/Cas9 system are creating a major revolution in genome editing by adding or removing the genetic material at particular locations in the genome. Hence, molecular techniques are influencing the breeding process from selection to introgression of known genes/traits and thus sustaining the world's food productivity.

Keywords CRISPR · Crop improvement · Introgression · Microarrays · QTLs

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J. M. Al-Khayri et al. (eds.), *Advances in Plant Breeding Strategies: Cereals*,
https://doi.org/10.1007/978-3-030-23108-8_8

8.1 Introduction

Rice (*Oryza sativa* L.) is the staple food in many developing countries of the world, especially in Asia. It is a model cereal crop due to its small genome size, complete genome sequencing and high transformation frequency (Li et al. 2018a, b). Increase in global population up to 9 billion by 2050, along with water scarcity, diminishing arable land, adverse impacts of climate change, biotic and abiotic stresses present great challenges for rice breeders and agriculturists (Collard et al. 2008; Phillips et al. 2017). The crop yield per unit area needs to be increased by 50% by 2030 (Cheng and Hu 2008) to assure global food security. Hence, breeders are under increasing pressure to use new breeding strategies and enhance food production.

However, significant progress in recent years has been made by plant breeders to cope up with food shortages by combining breeding and molecular approaches. DNA-based markers have been developed for the construction of genetic maps and the accessibility to complete genome sequences have eased the work of breeders to introduce new crop varieties in a short time (Ashkani et al. 2015).

With the completion of whole-genome sequencing in rice, and low genotyping costs, various functional genomics platforms that include collection of germplasm resources, generation of mutant libraries, specific gene markers, QTLs, full-length cDNA libraries, gene expression microarrays and RNA-sequencing techniques for expression analysis have been developed in rice (Li et al. 2018a, b; Sun et al. 2015, 2018). Both genetics and genomics have contributed to crop breeding strategies for quality and quantity improvement (Fig. 8.1). Nowadays, nutritionally-rich rice varieties with favorable traits are available for human consumption (Gande et al. 2014; Hiwasa-Tanase and Ezura 2016).

8.2 Genetic Diversity

Large number of rice varieties are released every year to meet the increasing demands of enhanced productivity. All rice varieties have a different genetic composition, which is highly influenced by environmental conditions to which the plants are adapted. This variation in genetic diversity helps the plant to survive in nature. The advent of PCR (polymerase chain reaction) based molecular markers has increased the potential of discovering and tagging new genes in plants with diverse genetic makeups.

Wunna et al. (2016) observed high genetic diversity in landraces and rice varieties from upland and lowland ecosystems of Myanmar and, based on the extent of variations, the rice accessions were divided into two cluster groups, I and II, related to *indica* and *japonica* groups. Reig-Valiente et al. (2016) elucidated the genetic relationship in rice varieties consisting of modern elite and old cultivars and traditional landraces, cultivated in temperate regions. Whole genome sequencing and SNP (single nucleotide polymorphism) results revealed a strong substructure in

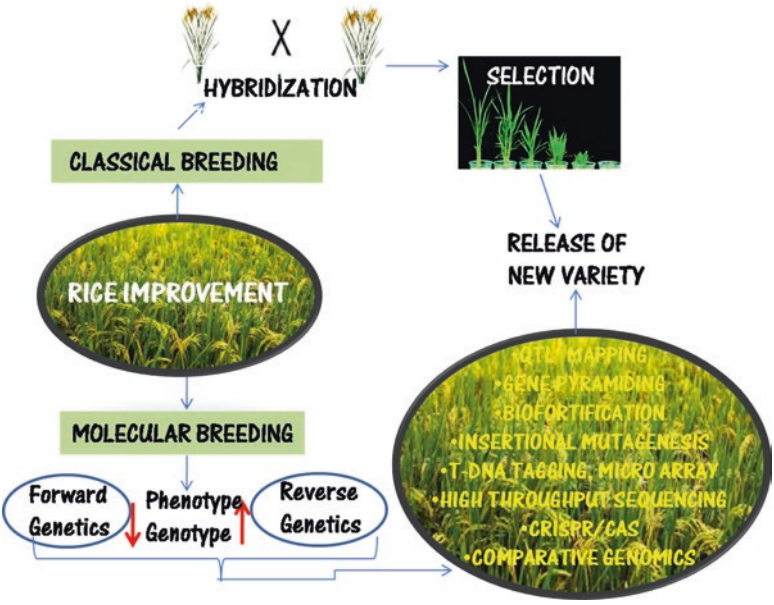

Fig. 8.1 Classical and molecular breeding approaches in rice: Continued improvement in rice involves the integration of different breeding approaches. Classical approaches involve hybridization followed by selection, while molecular approaches exploit rice genetics powered by functional genomics to meet the breeding challenges and safeguard food production

temperate rice populations which was based on grain type and the origin of the cultivars. A dendrogram also supported the population structure results. Aljumaili et al. (2018) studied the genetic diversity among aromatic rice accessions collected from Peninsular Malaysia, Sabah and Sarawak using SSR (simple sequence repeat) markers to quantify their genetic divergence. The presence of high genetic diversity could help in recognition of those accessions that can be used for introgression into the existing rice-breeding programs.

Indian rice varieties harbor huge amounts of genetic diversity but the trait-based improvement programs in recent decades have forced the breeders to rely on a few parents for crossing. As a result, there was a huge loss of gene diversity. Singh et al. (2016) studied the genetic diversity in 729 Indian varieties using HvSSR markers and divided them into two populations on the basis of cluster analysis. Anupam et al. (2017) genotyped germplasm of Tripura's local landraces, for genetic diversity and QTLs related to drought and blast resistance genes. However, a low level of genetic diversity was observed in contrast to high levels of genetic diversity among rice varieties in northeast India.

Genetic diversity is necessary for the survival of plant species under extreme climatic conditions or natural disasters. It acts as a natural defense system as it includes all the beneficial alleles for the plant species to thrive. With changing environmental conditions, only the best alleles are able to cope and adapt to new environments thereby playing an important role in evolution.

8.3 QTL Mapping

Molecular breeding in rice has played an important role in improving breeding efficiency. Many agronomic traits in rice are controlled by minor-effect genetic loci called quantitative traits, controlled by quantitative trait loci (QTLs) (McCouch and Doerge 1995). Hu et al. (2008) established a candidate gene strategy for the isolation of QTLs against bacterial blight and fungal blast. They observed that by combining different approaches like integrated linkage maps, expression profile and functional complementation analysis, a single minor QTL can be used in rice improvement. Zhou et al. (2014) studied in rice the inheritance of resistance to white tip disease in a segregating population derived from across between highly-resistant and susceptible cultivars. Six QTLs were detected after plotting on a genetic map which can be used for breeding resistant cultivars against white tip disease in rice.

Zeng et al. (2015) studied QTLs against sheath blight (SB) disease in a doubled haploid population from a cross between a *japonica* variety CJ06 and *indica* variety TN1, under 3 different environments. Based on SB resistance field data, a genetic map was constructed with 214 markers and it lead to the identification of 8 QTLs each against lesion height and disease rating, under 3 different environmental conditions. In addition, they also detected 12 QTLs for plant height, but observed that none of the plant height QTLs were co-located with the sheath blight QTL. Fiyaz et al. (2016) developed a population of 168 recombinant inbred lines from a highly resistant and a highly susceptible *indica* rice cultivar and mapped 3 QTLs against bakanae disease. Yao et al. (2016) identified QTLs associated with African rice gall mildew resistance in 3 independent bi-parental rice populations; 28 QTLs associated with pest incidence and pest severity were uncovered using composite interval mapping. A range of 1.3–34.1% of the phenotypic variance was observed with each of the individual QTLs.

High-throughput SNP genotyping has been used for various QTL mapping studies in rice (Chen et al. 2016). They performed high-resolution QTL mapping for grain appearance traits in *indica* rice. Duan et al. (2013) used high-throughput sequencing in the QTL mapping of a giant panicle rice accession, R1128, which has multiple major genes for good traits and detected 49 QTLs for 5 yield traits. For multiplexed samples, restriction-site associated DNA sequencing (RAD-seq) is a useful and cost-effective tool for genetic mapping that focuses only on short fragments of DNA adjacent to a particular restriction enzyme in the genome and allows efficient genotyping and high-density SNP discovery. Zhu et al. (2017) made a cross between *japonica* inbred Francis and *indica* restorer R998 and constructed a set of recombinant inbred lines so as to understand the genetic basis of rice yield traits in an elite restorer. A high density bin map was generated for QTL mapping of 6 yield-related traits.

Liu et al. (2006) analyzed QTLs against rice biomass yield, straw yield and grain yield in a population of 125 double-haploid lines from an inter-sub-specific cross of IR64/Azucena. A total of 12 QTLs were detected with additive main

effects, 27 involved in dysgenics interaction and 18 affected by the environmental conditions. Singh et al. (2017) mapped 38 and 46 QTLs on rice chromosomes 3 and 5 associated with tolerance to stagnant and irrigated conditions using a F7 mapping population. Gichuhi et al. (2016) identified 36 QTLs for yield-related traits from wild rice relative *Oryza longistaminata* for improving agronomic traits. Solis et al. (2018) identified QTLs associated with drought resistant traits by constructing a linkage map between two *japonica* cultivars with 213 markers. Composite interval mapping identified 6 QTLS associated with grain yield during drought conditions. Annotating candidate genes within QTLs suggested the role of genes and transcription factors that are involved in drought tolerance mechanism thus contributing to yield.

8.4 Assembling Multiple Desirable Genes by Pyramiding

Gene pyramiding has led to the development of genetic stocks and resulted in the improvement of plant breeding. Pyramiding involves stacking of multiple genes to develop durable resistance expression which further depends upon the number of genes to be transferred and the distance between the target genes and flanking markers (Joshi and Nayak 2010). Since traditional breeding is a time-consuming process, parent plants with desirable genes are crossed and the recombinants are selected from the progeny. As a result all the desirable genes are combined in a single breeding cycle, resulting in production of near recombinant lines (NILs) that contain homozygous alleles for the gene (s) of interest using fewer breeding cycles at lower cost. However, to track the presence or absence of a particular gene is slow. Although conventional breeding methods allow the transfer of desired genes between related species, genetic engineering balances and accelerates plant breeding programs by introducing genes from diverse sources. A large number of transgenic crops with durable resistance against biotic and abiotic stresses have been developed. Thus, it is possible to presume that genetic engineering is another useful method to pyramid novel genes into crop plants. However, efficient transformation and regeneration protocols need to be developed as a single gene transformation results in narrow spectrum disease resistance.

Huang et al. (1997) transferred 4 bacterial blight resistance genes (*Xa-4, xa-5, xa-13, Xa-21*) in rice and the pyramid lines showed a wider spectrum of resistance than lines with a single gene. They also developed PCR markers against 2 recessive genes (*xa-5, xa-13*) to screen a range of rice germplasm for the selection of parents in breeding programs. Pradhan et al. (2015) developed resistant cultivars in Jalmagna (an elite deepwater rice variety), against bacterial blight by transferring 3 resistance genes (*xa5 + xa13 + Xa21*) from the Swarna cultivar to develop a BB-susceptible cultivar, Jalmagna.

Fukuoka et al. (2015) developed near-isogenic experimental lines with 4 different QTL alleles and the lines were environmentally stable and resistant to blast disease. Similarly, Arunakumari et al. (2016) introgressed two bacterial blight

resistance genes (*Xa21*, *xa13*) and a major blast resistance (*Pi54*) gene into an Indian rice variety, MTU1010 through marker-assisted backcross breeding. Donor parents used were Samba Mahsuri (possessing *Xa21*, *xa13*) and NLR145 (possessing *Pi54*). The resultant lines had a high level of resistance against BB and blast, along with good yield, grain quality and plant type. Ji et al. (2016) developed 10 restorer lines by pyramiding genes resistant to blast, bacterial blight and brown plant hopper.

Gene pyramiding has played an important role in conferring strong and stable integration of alleles against resistance to various pests and pathogens. Yet, assembling large numbers of favorable alleles to provide stable agronomic performance is still time-consuming due to lack of molecular-marker information in certain plant systems. However, progress made in genome analysis by the use of high-throughput genotyping has contributed substantially to global food security.

8.5 Double Haploidy

Production of doubled haploids (DH) using anther or microspore culture under in vitro conditions is a successful and rapid approach to develop new rice cultivars, which otherwise requires at least 6–7 generations through conventional methods. The technique maintains the homozygosity that has wide uses in genetic studies, including gene and QTL mapping. In this method, the immature pollen grains or anthers (which are haploid) are cultivated in solid medium and induced to divide, to double their chromosome number so that the plants regenerated from them have two sets of chromosomes and hence double haploids.

Reiffers and Freire (1990) established a relation between the morphology of the panicle and the microspore stage. They observed that a cold-pretreatment of anthers at 4 °C for 8 days increased the regeneration frequency from 0 to 144.4%, while a few plants underwent spontaneous chromosome doubling. Chromium contamination in soil has become a severe threat to crop production and food safety. Qiu et al. (2011) detected 17 putative QTLs associated with Cr tolerance and Zn concentration using a rice DH population. Most of QTLs controlling Zn concentration had small genotypic variance and qSRZ4 related to Zn translocation showed growth condition-dependent expression. Fazaa et al. (2016) developed some DH lines through anther culture and evaluated them for yield and yield-related traits. Correlation analysis revealed grain yield to be positively correlated with panicle length. Grewal et al. (2011) produced double haploid lines through anther culture involving *indica* and *japonica* rice. They observed low anther culturability in the *indica* cultivars, as compared to *japonica* cultivars, and also variation regarding the Zn content in DH lines. A linkage map was constructed using SSR markers which revealed that the genes for anther culturability are partially dominant in *indica* cultivars and some DH lines had *indica* traits for high Zn content in polished rice.

Nguyen et al. (2016) created a DH population from the hybrid of *japonica* and *indica* rice, as earlier studies by Ikehashi and Araki (1986) indicated the presence of

a reproductive barrier between the two subspecies which was conditioned by the *S5* locus located on chromosome 6. Furthermore, it was observed that a neutral allele (*S5n*) can overcome this barrier because hybrids of either *indica* or *japonica* rice crossed to rice carrying *S5n* were fertile. Further genotyping of the *S5* locus, with allele-specific markers for *ORF3*, *ORF4* and *ORF5*, recorded a potential recombination hot spot in the *ORF3-ORF4* region. Haplotyping analysis revealed segregated distortion in the DH population, with a few lines having very low or very high *indica* alleles, with little effect of the *S5* allele. However, no effect of the $S5^n$ allele was observed on the agronomic traits studied.

Pauk et al. (2009) combined tissue-culture methods with conventional breeding to produce new rice varieties which are resilient than traditional ones. Risabell variety produced via anther culture was resistance to blast disease, high milling and cooking quality and long-grain type. Similarly for Janka variety, haploid cell cultures were developed and their vigorous regenerants were colchicine treated and the progeny has vigorous seedling growth, drought tolerance and good grain quality. However, for variety Ábel, the improvement was done through somatic tissue-culture regeneration followed by anther culture. Gueye and Ndir (2010) studied the response of anther culture of eight genotypes of *Oryza sativa* and *O. glaberrima* for callus induction and frequency of plant regeneration. They recorded more callus induction in *O. glaberrima* genotypes compared to *O. sativa* genotypes. Many albino plants were obtained while one *O. glaberrima* genotype (6202 Tog) produced green plants and thus it was concluded that the anther culture response is species and genotype dependent.

DH lines play an important role in inducing mutations thereby increasing selection efficiency. The technique is helpful to express popular recessive traits introduced through mutation or hybridization and hence enriching the germplasm. Even the double haploids govern major agronomic traits in cultivated rice that are influenced by QTLs. Also, double haploids overcome the problems of inbreeding by improving the selection efficiency in cross-pollinated species. The generation of DH lines plays an integral part in creating homozygosity as the purity of parental lines used in developing a hybrid/cultivar is reduced over the span of time due to gene drift, mutations, artificial breeding and exposure to various abiotic and biotic factors.

8.6 Increasing Nutritional Value with Biofortification

Rice is a major source of energy and micronutrients does not provide enough zinc to meet human nutritional requirements (Cakmak et al. 1999). This is due to the removal of large quantities of soil Zn by modern high-yielding varieties at each harvest, thereby lowering the residual concentration of soil Zn which further results in lower future grain Zn concentration (De Steur et al. 2014). Production of high-yielding rice varieties along with improved Zn concentration can be achieved when genetic and agronomic strategies are combined (Nakandalage et al. 2016). Screening

the germplasm, old landraces, traditional varieties and wild species using agronomic, breeding and genomic tools can increase Zn concentration in rice grain (Zaman et al. 2018). Further conventional plant breeding followed by marker-assisted selection has resulted in rice with enhanced level of micronutrients.

Several QTLs have been identified for high Zn in rice grain, which are used as a potential source for marker-assisted breeding (Chandel et al. 2011; Ishikawa et al. 2017). Identification of several candidate genes involved in Fe and Zn uptake and their accumulation in rice has led to their successful use in developing high Zn transgenic lines (Anuradha et al. 2012). Swamy et al. (2016) did a fine association of several gene specific markers with rice grain Zn. Similarly, several SSR markers and grain Zn trait associations identified in different populations and in a germplasm panel of rice can be further used in MAS (Brara et al. 2015; Khanin et al. 2016; Susanto 2008; Zhang et al. 2014).

Trijatmiko et al. (2016) reported Fe and Zn biofortification nutrition targets in rice under field conditions. The genes were introduced in IR64, an *indica* cultivar, and the trait was further bred into other popular rice cultivars deficient in Fe and Zn collected from South and Southeast Asia. An international initiative, The HarvestPlus program, aims to improve the micronutrient content of staple foods and reduce hunger. This program has increased Zn levels in brown and polished rice by 30 and 28 mg/kg, respectively (Johnson-Beebout et al. 2009). Trials are under way to identify and test certain sensitive biomarkers for Zn intake. However, experiments need to be carried out to evaluate the genotypes under Zn sufficient and deficient conditions at different stages of growth and development. Similarly, the interaction of environmental and genetic factors on Zn homeostasis needs to be established along with promoters and inhibitors of Zn bioavailability in rice grain (Nakandalage et al. 2016). Also, special attention should be given to the amount of antinutrients like phytate, as they significantly influence Zn bioavailability (Swamy et al. 2016).

Total Zn present in the aleurone layer, the outermost layer of the endosperm, is lost during processing, while that present in the inner endosperm (60–75%) is retained even after polishing (Hansen et al. 2009). This has been reported in large collections of rice germplasm at the International Rice Research Institute, Philippines (Boonchuay et al. 2013). The world's first Zn-enriched rice variety (BRRI dhan 62) was released in 2013 by the Bangladesh Rice Research Institute that contains 20–22 mg Zn per kg of brown rice.

Crops naturally lacking essential nutrients can use the transgenic plant breeding approaches to produce biofortified crops with desired nutrients and agronomic traits, and reduced antinurients (Bouis and Saltzman 2017). The expression of certain genes encoding enzymes involved in the synthesis or sequestration of IP6, have successfully reduced phytate concentrations in rice seed (White and Martin 2009). Golden Rice, rich in beta carotene, provides more than 50% increased vitamin A. However, the irony is that the Golden Rice has been available as a prototype since the early 2000s, but to date has been not introduced in any country due to the regulatory approval processes. Although transgenic varieties have great nutritional potential, their release to farmers will take several years, depending upon their approval through national biosafety and regulatory processes.

8.7 Induced Variations and TILLING

One of the efficient ways to study gene function is to create variations and then identify mutants that establish a link between genotype and phenotype. A potential approach to identify genes affecting trait variation is the use of induced mutants because they provide a clear understanding of molecular mechanisms involved in the plants (Sikora et al. 2011). Ethyl methyl sulfonate (EMS), is the most common mutagenic agent that induces chemical modification of nucleotides. The most common point mutations being GC to AT transitions. Once a gene structure is disrupted, the gene expression changes, resulting in changes in the phenotype which can be directly correlated to a gene responsible for it. However, knockout mutations are not always effective in functional analysis of the redundant genes because loss of function may not always lead to an obvious morphological variation, but also to the critical genes involved in plant growth and development (Wang et al. 2013). Induced mutants have been extensively used to identify gene(s) involved in various agronomically-important traits in rice. In India, six research institutes have collaboratively worked on an upland rice variety, Nagina 22, and identified mutants related to various important traits like plant growth and architecture, flowering, maturity, yield, grain number, shape and size, resistance to blast and bacterial leaf blight diseases and tolerance to drought and salinity (Mohapatra et al. 2014). The generated mutants, after registration, will be made available for various rice genetics and breeding programs.

Insertional mutagenesis, such as T-DNA insertions and transposon tagging, have been widely used in creating rice mutant libraries. Jeong et al. (2002) generated 13,450 T-DNA insertional lines using a new vector pGA2715 and suggested that the enhancer sequence present in the T-DNA improves the GUS-tagging efficiency. A reverse activation-tagging further identified the activation-tagged gene and enhancer effects.

Chen et al. (2003) characterized 1000 T-DNA tags in rice which were not spread randomly throughout the genome, but were inserted in gene rich areas. However, a few insertions, about 2.4%, were observed in repetitive regions. Good correlation was also observed in T-DNA insertions present in genic and intergenic regions with respect to size distribution. Krishnan et al. (2009) recorded the presence of insertion tags in 32,459 rice genes and also in 50% of predicted protein-coding genes with insertional mutagenesis. Chang et al. (2012) observed that out of a total of 372,346 mutant lines generated, 58,226 T-DNA or Tos17 flanking sequence tags have been isolated which have potential applications for more than 40 genes involved in stress responses, nutrient metabolism and plant architecture.

Characterization of the T-DNA insertion mutants resulted in identification of the biological functions of several genes. Wu et al. (2008) reported that there was no flowering in *rid1* mutant in rice while the *RID1* mutant was identified as a master switch that induces flowering. This was due to a regulatory gene *JMJ706* that resulted in H3K9 demethylation, a key step for development of rice floral tissues. Mutations in gene *JMJ706* carrying a T-DNA insertion resulted in altered floral

morphology and organ number due to increased di- and trimethylations (Sun and Zhou 2008). *ILA1*, a key factor for regulating the tissue formation at the rice leaf lamina, also showed abnormal mechanical tissue and cell wall composition in T-DNA mutant lines (Ning et al. 2011).

Targeting induced local lesions in genomes (TILLING) is a reverse genetic strategy developed to identify induced point mutations in a plant species, while the discovery and cataloguing of natural nucleotide variation present in populations is known as Ecotilling (Cooper et al. 2013). Till et al. (2007) developed two mutagenized rice populations on treatment with ethyl methane sulfonate (EMS), and sodium azide plus methyl-nitrosourea (Az-MNU) followed by further amplification of target regions of 0.7–1.5 kilobases using gene specific primers. They identified 27 nucleotide changes in the EMS-treated population and 30 in the Az-MNU population. Similarly, Cho et al. (2010) developed TILLING lines via the application of gamma-ray irradiation to rice seeds. The genetic diversity based on AFLP (amplified fragment length polymorphism) markers was assessed and changes in the coding regions of genes were observed with four loci exhibiting mis-sense mutations and two loci exhibiting silent mutations in the rice pseudomolecules.

8.8 Gene Discover by Next-Generation Sequencing

The dawn of next generation sequencing (NGS) has significantly revolutionized studies on rice functional genomics. With the release of genome sequences of *indica* and *japonica* rice, huge quantities of information and data are available and the variations in them can be identified by NGS efficiently and in a cost-effective manner. These variations are further used to identify the unique sequences for marker-assisted selection (MAS) in rice improvement programs (Spindel et al. 2016).

Rathinasabapathi et al. (2015) mapped the whole genome sequence of cultivar Swarna on the Nipponbare reference genome with high glycemic index (GI), and identified SNPs that could have a deleterious effect on protein functions. The changes in the position of SNPs in the granule bound starch synthase I gene and glucose-6-phosphate translocator gene contributed to a low GI. Similar variants were also observed in the genome of another *indica* rice variety collected from Columbia with low GI. Kharabian-Masouleh et al. (2011) observed SNPs and InDels in both coding and non-coding regions in candidate genes involved in starch synthesis. The *SSIIa* gene affected the starch quality and the amylopectin structure of starch present in the rice endosperm (Morell et al. 2003). The effect of this gene on cooking quality and starch content in the rice was studied by Umemoto and Aoki (2005) and further 31 SNPs and one InDel were detected in this gene.

NGS techniques could reveal the genetic basis of different phenotypes on the basis of DNA polymorphism under stress response conditions, even among closely-related cultivars. A contrasting response to drought and salinity stress in three cultivars of rice was studied by Jain et al. (2014). They observed that the distribution of SNPs and InDels was found to be uneven across and within the rice chromosomes.

These variations could be used as functional markers and to identify promising target genes for salinity and drought tolerance for molecular breeding programs.

NGS has enhanced the sensitivity of detecting mutations, thereby improving the screening efficiency by targeted gene amplification of pooled DNAs. Burkart-Waco et al. (2017) quantified and pooled DNA from a mutant rice population and amplified the target genes from these DNA pools. The amplicons were further combined to increase the probability of detecting mutations during sequencing. This approach can easily detect rare mutations. Ryohei et al. (2015) utilized the MutMap method, and its derivatives (MutMap+ and MutMap-Gap), to identify genes/QTLs of agronomic importance in rice (Abe et al. 2012; Fekih et al. 2013; Takagi et al. 2013).

8.9 Gene Introgression from Wild Relatives

Maintaining genetic integrity is essential for crops to sustain themselves in a changing environment. Alleles, introgressed for some beneficial traits, may result in genetic diversity. This changing diversity can satisfy the food needs of growing human population by delivering foods with high nutritional value and health benefits. All plants are domesticated from wild species, which are great reservoirs of genetic diversity due to stress and domestication traits, therefore the phylogenetic relationship between closely-related wild species is revealed by molecular analysis (Cheema et al. 2008; Dillon et al. 2007) that also gives an idea of their evolution under natural selection and their adaptation to the surrounding environment. Hence, conservation of wild relatives in the form of seed banks, or in situ and ex situ conservation is important for crop improvement (Brozynska et al. 2015).

Climate changes are harshly affecting food productivity, so it is of utmost importance to create crops which are genetically more diverse and resistant to biotic and abiotic stresses. Hence, there is an urgent need for the conservation of wild relatives from the available natural resources to ensure continuous food sustainability (Zhang et al. 2016). The consequences of 1.5 and 3.0 °C global temperature rise was studied for the coming years and then compared to the present climate. The results indicated an increase in taxa turnover and in the numbers of threatened taxa (Phillips et al. 2017). Thomas et al. (2017) studied the negative effect of climate on the distribution of genetic diversity in four wild relatives of rice and assessed a significant overlap between present and wild rice species. They observed that these species have a good opportunity to expand their distribution ranges in the near future to where rice is unlikely to be cultivated.

The distribution of genetic diversity within and among populations of the wild rice species *Oryza glumaepatula* in Costa Rica were observed by Fuchs et al. (2016). They evaluated that, how the incorporation of alleles from domesticated species may change the genetic makeup of wild species. A high level of genetic diversity was observed in *O. glumaepatula* populations in Costa Rica as compared to those present in South American populations, thus suggesting that the gene flow from cultivated *O. sativa* populations may have occurred in the recent past. This

could lead to the increase in likelihood of local extinction of pure *O. glumaepatula* populations, by the transfer of commercial traits from cultivated rice species.

Jin et al. (2018) determined crop-wild introgression from cultivated rice and its consequences in six *Oryza rufipogon* populations. Principal coordinates and cluster analyses indicated that the differentiation of wild rice populations that resulted in their altered genetic diversity is mainly associated with their spatial distances to cultivated rice fields. The level of overall genetic diversity detected with 34 SRRs and 34 In Dels recorded large wild-specific alleles in wild populations. Because crop-wild introgression can alter the genetic integrity of wild populations, appropriate measures need to be taken immediately for effective in situ conservation of pure wild relatives of crop cultivars.

8.10 Comparative Genomics

Comparative genomics is essential to study the minimal functions required by a plant for its normal growth, development and metabolism. It also explains the source of plant diversity and the molecular basis for its adaptation (Sasaki and Sederoff 2003). Rice has been used as a model crop for monocots to study its detailed structural and functional genomics due to the availability of its complete genome sequence, ESTs, transposons and production of transgenic plants (Shimamoto and Kyozuka 2002). However, previous studies revealed that the majority of rice genes are structurally and functionally homologous to major cereals, so the information obtained from rice genes can be easily utilized in studying their presence in other cereals (Hill and Li 2016; Wang et al. 2015). Comparative studies on the evolution of intergeneric regions in cereals has revealed that the large genome size in most crop plants is because of the presence of mobile elements rather than the functional genes.

Since *Arabidopsis* is a reference crop for dicots, the collinearity between the genomes of rice and *Arabidopsis* was studied by Liu et al. (2001) to compare and map rice BAC sequences with the *Arabidopsis* genome. Several regions were identified with preserved gene order but interrupted by non-collinear genes. Dodeweerd et al. (1999) investigated collinearity between rice and *Arabidopsis* by examining rice EST clones homologous to *Arabidopsis* genomic DNA sequences. A total of 24 homologous pairs, 5 with conserved order and a single inversion were observed. However, no conservation of gene order in rice and *Arabidopsis* across a 3-cM region in Chromosome 1 was identified by Devos et al. (1999).

Two genes *Hd1* and *Hd6* determining the flowering time were isolated by fine-scale, high-resolution mapping, corresponding to QTLs controlling the heading date of rice. It was observed that *Hd1* encodes a homolog of CONSTANS (CO) that functions in the photoperiodic control of flowering in *Arabidopsis* (Yano et al. 2000). Nelson et al. (2004) used sequence information from both the *indica* and *japonica* rice strains and identified 356 Cytochrome P450 genes and 99 related pseudogenes in the rice genome. When these rice genes were compared to P450

genes and pseudogenes in *Arabidopsis,* it was observed that many of the already-known plant P450 gene families existed before the divergence of monocot-dicot lineages took place. This study also highlighted the maintenance of certain lineage-specific families like Ranunculaceae and loss of lineage-specific families in *Arabidopsis* during the course of evolution.

Comparative genomics uses cross-genome comparisons of structure and function to estimate similarity of biological organization across species and genera (Wei et al. 2002). Sorrells et al. (2003) studied comparative sequence analysis of rice and wheat genomes and observed that wheat, a polyploid with a genome size 40 times larger than that of rice, has over 80% repeated DNA. Yan et al. (2003) studied microcollinearity in some regions between barley, wheat and rice. Analysis of the *Sh2/A1* orthologous region in rice, sorghum, maize and in some species of the Triticeae tribe revealed that this region was highly collinear with few differences (Bennetzen and Ramakrishna 2002; Li and Gill 2002)

Mayer et al. (2011) used the comparative genomics approach to unlock the genome of barley using a conserved synteny model with a model of grasses and assembled 21,766 barley genes in a putative linear order. It was observed that the barley genome exhibited a medley of structural similarity with hexaploid bread wheat. Thus the availability of the genomic resources for Triticeae plants after their genome sequencing have aided in the discovery of new genes using comparative genomics approach and in future this could further help in discovering alleles for adaptive traits to different agronomic environments (Mochida and Shinozaki 2013).

A centralized infrastructure, PLAZA (http://bioinformatics.psb.ugent.be/plaza/) is an online platform provides comprehensible and current research in the exploration of genome information (Proost et al. 2009). Here, all the data generated by different sequencing programs has been incorporated and combined and is further used for plant comparative genomics. This resource compiles structural and functional annotation of sequenced and published data and also maintains a large set of tools and softwares to study the function of genes and their evolution

8.11 Microarray and Gene Expression

High-throughput, genome-wide expression analysis in rice is facilitated by microarray technologies. The availability of complete genome sequences provides the necessary information required to design a microarray containing either all known or predicted gene models in the rice genome. A number of analytical tools have been developed to study gene relationships and functions from microarray data and the information obtained after analysis has been used in genetic dissection, drug discovery and disease diagnostics. Ma et al. (2005) analyzed the transcriptional activity of gene models and detected the expression of 41,754 known and predicted gene models. In addition, the expression patterns of best-matched homologous genes of rice and *Arabidopsis* indicated notable differences in the degree of conservation between

these two species. However, this lesser degree of conservation could be due to the diverged transposons and retrotransposons (Jiang et al. 2004).

Most of the genes, along with gibberellic acid (GA_3) and jasmonic acid (JA) play an important role in anther development and pollen fertility in rice. GA_3 controls the formation of pollen grains while JA signaling is required for pollen development and anther dehiscence. Wang et al. (2005) detected the expression level change of 2155 genes in anthers as compared to the seedlings using a cDNA microarray, with probes derived from meiotic anthers, mature anthers and treated suspension culture cells. A total of 314 genes responded to either GA_3 or JA treatment while 24 GA_3- and 82 JA-responsive genes were revealed. Furthermore, a significant difference was observed in the expression of GA_3 or JA treated genes at different developmental stages of anthers.

Microarray technologies facilitate high-throughput gene expression analysis but recent databases and softwares resulted in efficient expression analysis. There are different rice microarray platforms that can integrate microarray data for functional analysis and then can be effectively used in characterizing and differentiating the gene expression profiles from different rice tissues, organs, cell types, biotic and abiotic treatments, and miRNAs (De Abreu Neto and Frei 2016; Jung et al. 2015; Xue et al. 2009). The Rice Expression Profile Database (RiceXPro, http://ricexpro.dna.affrc.go.jp/), is a storehouse of different gene expression profiles of diverse organs and tissues at different developmental stages and environmental conditions (Sato et al. 2011). The Gene Chip rice genome array, designed by Affymetrix, contains 57,381 probe sets covering about 48,564 and 1260 transcripts from the *japonica* and *indica* cultivars, respectively (Cao et al. 2012), while the *Oryza sativa* Genome Oligo Set based on the draft *indica* and *japonica* sequences, was designed by the Beijing Genomics Institute and Yale University. Some other databases useful for expression pattern analysis of rice genes are OryzaExpress (Hamada et al. 2011), RicePLEX (Dash et al. 2012), Bio-Array Resource for Plant Biology (BAR) (Toufighi et al. 2005) and RiceArrayNet (Lee et al. 2009).

Another database Rice Oligonucleotide Array Database (ROAD, http://www.ricearray.org) is used for the exploration of gene expression based on rice microarray hybridizations. The database is user-friendly with a variety of tools that facilitate the study of gene expression profiles. ROAD supports analysis of genes expressed in different tissues, developmental stages and stress (both abiotic and biotic) conditions. Also, certain tools like Gene Ontology and KEGG (Kyoto Encyclopedia of Genes and Genomes) Orthology are fixed in the ROAD (Cao et al. 2012).

8.12 CRISPR for Targeted Genome Editing

Once sequencing of rice genome was completed, several tools became available for the functional characterization of genes. One of the most powerful and efficient tools which emerged recently for genome editing is CRISPR (clustered regularly interspaced short palindromic repeats). CRISPR has replaced the RNA interference

(RNAi) gene silencing technology for efficient and precise gene knock down by overcoming its limitations, like incomplete loss-of-function analysis and extensive off-target activities (Arora and Narula 2017). In rice, genes were modified for the traits related to biotic and abiotic stress, herbicidal resistance and yield, as well as genetic improvement of agricultural crops using CRISPR/Cas9 (Minkenberg et al. 2017; Xu et al. 2017) (Fig. 8.2). Efficient multiplex genome editing could be achieved by a synthetic gene that encodes for Cas9 protein with an intron containing polycistronic tRNA-gRNA in rice. Once a hybrid gene is formed, it could be expressed using one polymerase II promoter (Ding et al. 2018).

Rice seedlings are susceptible to low temperature, so a cold-tolerant transcription factor TIFY1b, involving genes was discovered in rice by Huang et al. (2017). They employed the CRISPR/Cas9 technique to edit this gene and also its homology gene in Nipponbare rice. High mutation rates due to insertion and deletion of one nucleotide were observed in transgenic lines. Thus CRISPR/Cas9 changed the DNA sequences at targeted sites and generated a variety of TIFY1 mutant lines in rice which were cold resistant.

Rice blast is one of the most destructive diseases affecting rice globally. Wang et al. (2016) reported the improvement of rice blast resistance by engineering a CRISPR/Cas9 and targeting the *OsERF922* gene for enhancing blast resistance. In addition, the number of blast lesions formed after pathogen infection was considerably decreased in all 6 mutant lines (identified from transgenic plants) both at the seedling and tillering stages. Also, no significant differences were observed between mutant lines and the wild-type plants with regard to the agronomic traits tested.

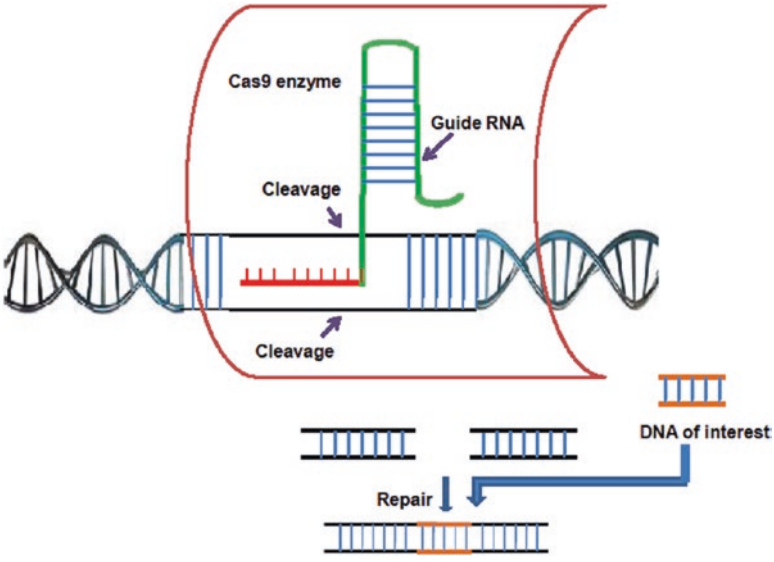

Fig. 8.2 CRISPR/Cas9, a RNA guided genome editing tool. CRISPR is a stretch of DNA while Cas9 is a molecular scissor that unwinds the DNA duplex and cleaves both the strands once the target sequence is recognized by the guide RNA

CRISPR/Cas system has been successful in rice protoplast cells with Cas9/sgRNA constructs targeting the promoter region of the bacterial blight susceptibility genes (Jiang et al. 2013). Genome editing generated the plants that were resistant to bacterial leaf blight, by down regulating the transcription of S-genes by the effector. Thus, the edited plants were resistant to certain bacterial strains because the effector was incapable of triggering the transcription of its target (Li et al. 2012). Similarly, multiple herbicide-resistant rice plants have been successfully achieved by CRISPR/Cas9-mediated in planta substitutions (Sun et al. 2016).

Cereals rich in amylose content offer potential health benefits. Previous studies using chemical mutagenesis have demonstrated that the fine structure and physical properties of starch is due to the starch branching enzyme (SBE) (Butardo et al. 2011). The targeted mutagenesis of rice *OsWaxy* gene by CRISPR/Cas9 resulted in high reduction in amylase content (14.6 to 2.6%) that resembles a natural glutinous rice variety (Ma et al. 2015). Sun et al. (2017) used CRISPR/Cas9 technology to generate targeted mutagenesis with In Dels in *SBEI* and *SBEIIb* in rice. Mutations were stably transmitted to the T1 generation. Wild type, sbeII mutants showed the presence of high amounts of long chains in debranched amylopectin in wild mutants that increased the amylase content by 25% in rice. The CRISPR/Cas9 technique has also been proved to be capable of editing and developing rice, photo-sensitive and thermo-sensitive male sterile lines to speed up breeding and exploit heterosis in rice (Li et al. 2016; Zhou et al. 2016).

Mutations resulting in complete knockouts and loss-of-function are very important to study gene functions, but their use becomes limited in many crop plants where gene expression is conferred due to point mutations. Multiple discrete point mutations in rice resulted from the introduction of the ALS gene using CRISPR/Cas9-mediated homologous recombination, which generated homozygous herbicide-resistant rice plants in one generation (Sun et al. 2016). Butt et al. (2017) applied CRISPR/Cas9 to generate targeted double-strand breaks and to deliver a RNA repair template for homology-directed repair in rice. For this, chimeric single-guide RNA molecules carrying sequences for target site specificity and repair template sequences flanked by regions of homology to the target were used. They concluded that this gene editing technology is very efficient in rice protoplasts to develop herbicide-resistant plants.

8.13 Conclusions and Prospects

This chapter emphasizes the recent advances and successful examples of molecular plant breeding that have led to significant improvement in rice. The adoption of molecular tools by breeders and researchers has helped them better understanding of the relationship between genotype and phenotype for complex traits, and the recent introduction of high-throughput genotyping platforms have increased the resources for plant breeding.

Similarly, the information extracted from genomics research has generated and added a wealth of information about gene structure and their functions, along with large numbers of molecular markers linked to QTLs. These generated resources will remain under exploited until breeding programs incorporate knowledge of pedigrees, phenotypes and marker genotypes during selection, and then combine them further with molecular approaches to discover new genes and their functions, which will further open innovative avenues for basic plant biology research.

In addition to the contributions made by breeders and researchers in adopting molecular approaches to meet certain plant breeding goals, the private sector should also participate by making investments to provide an appropriate training environment for agriculturists and scientists entering the molecular breeding workforce. This support can further bridge the gap between the latest techniques and the knowledge of plant molecular breeding research between the public and private sectors, and can help to meet the goals of sustainable increase in agricultural productivity.

Acknowledgement The authors are thankful to the School of Biotechnology, University of Jammu, Jammu, India.

Appendices

Appendix I: Research Institutes Relevant to Rice Genetic Improvement

Country	Institution	Specialization and research activities	Contact information and website
Africa	Africa Rice Centre	Conserving rice genetic resources, rice breeding, rice processing	01 BP 4029, Abidjan 01, Côte d'Ivoire Tel: +225 22 48 09 10 Email: AfricaRice@cgiar.org
America	University of California	Breeding and genetics	G. S. Khush 39399 Blackhawk Place, Davis, CA 95616, USA Tel: (+1-530) 750-2440 Email: gurdev@khush.org
Arizona	The Arizona Genomics Institute	Facilitate the high throughput movement of genomic resources	1657 E Helen St,Tucson, AZ 85705, USA Phone: +1 520-626-9596
Brazil	Agronomic Institute of Paraná (IAPAR)	Improvement of agronomic traits	Lutécia Beatriz Canalli InstitutoAgronômico do Paraná – IAPAR Rodovia Celso Garcia Cid, km 375. Londrina-PR 86047-902, Brazil. Tel: (+55) 42 3219 9712 Email: lutecia@iapar.br

(continued)

Country	Institution	Specialization and research activities	Contact information and website
China	China National Rice Research Institute	Identification of genetic resources, investigation of new genes, functional genomic research	359 Tiyuchang Road, Hangzhou City, Zhejiang Province310006, P.R. China Tel: +86-571-63370212 Email: icoffice_cnrri@126.com
	Huazhong Agricultural University	Plant protection	Chao-Xi Luo Huazhong Agricultural University, College of Plant Science and Technology, Shizishan, Hongshan District, Wuhan City, Hubei Province, China 430070 Tel: (27)-87281242 Email: cxluo@mail.hzau.edu.cn
Germany	University of Freiburg	Coordinator of Golden Rice – Project	Peter Beyer Institute of Biology II (Cell Biology), Fahnenbergplatz, 79085 Freiburg im Breisgau, Germany Tel: +49 761 203 2529 Email: peter.beyer@biologie.uni-freiburg.de
India	Indian Institute of Rice Research	Genetic diversity, better rice varieties	V. Ravindra Babu Rajendranagar, Hyderabad, Telangana 500030 Email: director.iirr@icar.gov.in Tel: +91-40-24591218; Fax: +91-40-24591217
	National Research Centre on Plant Biotechnology	Genome sequencing and annotation of crop plants	N. K. Singh Indian Council of Agricultural Research, Pusa Road, New Delhi Tel: 011-25860186 Email: nksingh@nrcpb.org
Nigeria	National Cereals Research Institute	Yield enhancement and grain quality	DanbabaNahemiahBadeggi, Nigeria Tel: +234 806 931 4862
Philippines	The International Rice Research Institute	Plant breeder, Project leader for Green Super Rice	Jauhar Ali International Rice Research Institute, Los Baños, Laguna, Philippines Tel: +63 2 580 5600 ext 2541 Email:j.ali@irri.org
Taiwan	Institute of Molecular Biology	Rice transformation	Su-May Yu Institute of Molecular Biology, Academia Sinica, Nankang, Taipei 115, Taiwan Tel: 886-2-2788-2695 Email: sumay@imb.sinica.edu.tw

Appendix II: Rice Genetic Resources

Cultivation location	Cultivar	Important traits
Thailand	Dinalaga	Drought resistant
Africa	IRAT106	Drought resistant
Australia	Doongara	High amylase content
	Kyeema	Long grain and fragrant
Bangladesh	IR64-Sub1	Submerged
	BRRI dhan69	Saline, irrigated
	BRRI Dhan72	High Zn content
Brazil	Tre Smeses	Drought resistant
China	Yunlu 99	Drought resistant
	Huhan3	Drought resistant
Ghana	CRI-Emopa	–
	CRI Aunty Jane	–
India	Pusa Sugandh 2	Lodging tolerance, resistant to BB
	Ambemohar	Fragrant variety
	Pusa Sugandh 2	Lodging and shattering tolerance
	DRR-Dhan 45	Drought resistant
	Sampada	Low glycemic index
	CR Dhan10	Protein rich
Kenya	Komboka	–
Nepal	Sookha dhan4	Rainfed, drought
	Sookha dhan1	Drought
	Sookha dhan2	Drought
Nigeria	IAC47	Drought resistant
	Ofada	Highlyaromatic
Nigeria	UPIA1	Irrigated, rainfed, tolerance to toxicity
Philippines	NSIC Rc25	Upland
	NSIC Rc352	Irrigated, inbred
	NSIC Rc390	Saline
Thailand	Dinalaga	Drought resistant
Tanzania	Tai	Rainfed, irrigated
Uganda	Okile	–
Vietnam	08Fan10	Rainfed, lowland

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Chapter 9
Hybrid Breeding in Rye (*Secale cereale* L.)

Thomas Miedaner and Friedrich Laidig

Abstract Rye is a robust and stress-tolerant cereal, grown on 4.4 million hectares, mainly in Northeastern Europe. Grain yields range, on average, from 2.0 to 5.8 mt ha^{-1} on farm level depending on the country, but reached >10 mt ha^{-1} in multi-locational official trials in Germany. Rye grain is used for bread making, distilling, homegrown feed and bioenergy production. Hybrid breeding has gained much attention caused by higher grain yields and a higher gain from selection compared to open-pollinated cultivars. Prerequisites are self-fertility, cytoplasmic-male sterility (CMS) with effective nuclear encoded genes to restore fertility (*Rf*) and distinct heterotic pools. Elaborated breeding plans are available. Commercial rye hybrids are crosses between a CMS single cross as seed parent and a restorer synthetic as pollinator. Molecular breeding was promoted in the last decade by the availability of PCR-based markers and the production of medium- to high-density single nucleotide polymorphism (SNP) assays. Markers are used for introgressing monogenic traits, developing landscapes of quantitative trait loci (QTL), and genomic selection. In the future, disease resistances to snow mold, stem rust, and Fusarium head blight, resilience to drought and heat stress, optimized feeding quality and yield improvement by broadening the genetic basis of hybrid breeding are important goals.

Keywords Breeding progress · Hybrid breeding · Molecular markers · QTL · *Secale* · SNP · Winter rye

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J. M. Al-Khayri et al. (eds.), *Advances in Plant Breeding Strategies: Cereals*,
https://doi.org/10.1007/978-3-030-23108-8_9

9.1 Introduction

Rye (*Secale cereale* L.) is a cereal with high winter hardiness, high tolerance to other abiotic and biotic stress factors, and especially suitable for nutrient-poor, sandy soils with low pH value. Under these conditions, rye outyields both wheat and triticale. Rye is grown almost exclusively in Northeastern Europe. Traditionally, dark, sour-dough bread rich in dietary fibers is produced from rye, but also bread mixed with wheat is common in some regions. Moreover, rye is used as grain feed for animals, for distilling spirits or for bioenergy production. In non-European countries, rye is often used as pasture, hay or as cover crop (Oelke et al. 1990). Rye has been used as a donor for disease resistances in wheat, e.g., against powdery mildew, stripe rust, stem rust (Crespo-Herrera et al. 2017). A 1BL/1RS chromosomal translocation resulted in high yielding wheat cultivars used worldwide (Villareal et al. 1998). Moreover, rye is a parent of triticale (×*Triticosecale* Wittm).

Rye is a diploid (2n = 2× = 14) species and the only cross-pollinating small-grain cereal with a highly effective gametophytic self-incompatibility system (Lundqvist 1956). Therefore, open-pollinated cultivars were traditionally bred. Hybrid breeding started in the early 1970s at the University of Hohenheim, Germany (Geiger and Miedaner 2009). Today, more than 70% of the rye acreage in Germany is grown by hybrid cultivars, ranging from 70% to 80% (Laidig et al. 2017). Hybrid cultivars are also listed in Austria, Poland, the Russian Federation, Denmark, Estonia, UK, Ireland, Canada, and USA. They provide a yield advantage of 15–20% over population cultivars (see Sect. 9.3), the basis for a targeted cultivar development for special purposes, and allow a systematic exploitation of molecular breeding techniques.

9.1.1 Origin and Distribution

Rye (*Secale cereale* L.) belongs to the tribe Triticeae in the grass family (Poaceae) and is evolutionarily related to barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.). Rye originated from Southwest Asia, particularly from Turkey, Lebanon, Syria, Iran, Iraq and Afghanistan (Sencer and Hawkes 1980). The genus includes a wide range of life cycles (annual/perennial), breeding status (wild/weedy/cultivated) and flowering biology (auto−/allogamous, Table 9.1). Confusion still exists on the systematics of the genus. There is a general agreement, that *S. silvestre* (or *S. sylvestre*) is the most ancient and distantly related species and *S. cereale* is the most recent species (Tang et al. 2011). The cultivated rye differs from all wild species by two translocations (Tang et al. 2011). *Secale strictum* ssp. *strictum* (syn. *S. montanum*) includes now all perennial *Secale* except ssp. *africanum.* Kobyljanskij (1983) determined four species (Table 9.1), but most authors accept only three species, placing *S. iranicum* into the wild *S. cereale* ssp. *ancestrale* group (Fredericksen

Table 9.1 Systematics, distribution and life style of the genus *Secale*

Botanical name	Distribution	Life style, status, flowering biology
Secale silvestre	Hungary to W-Siberia	Wild, annual, autogamous
S. iranicum	NW-Iran, Armenia	Wild, annual, autogamous
S. strictum		
ssp. *strictum*	Eastern Mediterranean, Iran, Russia	Wild, perennial, allogamous
ssp. *africanum*	South Africa	Wild, perennial, autogamous
S. cereale		
ssp. *cereale*	Worldwide	Cultivated, annual, allogamous
ssp. *ancestrale*	Central Asia	Weedy, annual, allogamous
ssp. *vavilovii*	Iran	Wild, annual, allogamous

Source: Fredericksen and Petersen (1998) and Miedaner (2014)

and Petersen 1998). The latter includes several partially domesticated forms that had been distinguished previously (former ssp. *dighoricum*, *segetale, afghanicum, turkestanicum, ancestrale*). They show a semi-brittle rachis, where only the upper part of the head disintegrates at harvest and are also known as primitive rye or weedy rye. This group represents a valuable source of germplasm, because they can be freely intercrossed with cultivated rye (ssp. *cereale*). The evolution of cultivated rye is still not clear. It might have arisen by direct selection from *Secale strictum*, by a cross of ssp. *vavilovii*, the only wild form within *S. cereale*, with *S. strictum* or by direct selection from ssp. *vavilovii*.

9.1.2 Economic Importance

Rye was cultivated worldwide on 4.4 million hectares in 2016 (FAO 2018). The six northeastern European countries shown in Table 9.2 produce 74% of the world harvest. Moreover, rye is an important cereal crop in the Baltic States and Finland.

Grain yields are highest in Germany and Denmark and lowest in Spain, Russia, and the USA (Table 9.2), where rye is cultivated only on poor soils with a low input of fertilizer and fungicides. Rye prevails as a winter crop; spring rye is found only in eastern Siberia and the mountains of Central Asia. In the European Union, rye grain is used as human food (41% of total harvest), as animal feed (32%), as substrate for bioethanol production (12%) and biogas production (10%) and for seed multiplication (5%) (EU 2017). In the USA, only one-half of the rye is used as grain, the remainder is used as pasture, hay, cover crop (Oelke et al. 1990) or for grazing in the southern US states (Newell and Butler 2013).

In the 1990s, rye growing rapidly declined in the three main producing countries (Fig. 9.1) due to higher grain yields in winter wheat, improvement of winter hardiness of other winter cereals, and changes of nutrition habits towards wheat bread. In Germany, remarkable gains in rye grain yields were achieved from 3.28 mt ha^{-1} in 1980 to 5.56 mt ha^{-1} in 2016 (FAO 2018). One cause is the stepwise substitution of

Table 9.2 The ten main rye producing countries in 2017

Country	Production (mt)	Hectarage (ha)	Grain yield (mt ha^{-1})
Germany[a]	3,173,800	570,900	5.56
Russian Federation[a]	2,541,239	1,249,782	2.03
Poland[a]	2,199,578	760,976	2.89
Belarus[a]	650,934	241,063	2.70
Denmark[a]	577,200	99,600	5.80
China	525,279	163,531	3.21
Ukraine[a]	391,560	143,600	2.73
Canada	382,000	131,000	2.92
USA	341,670	167,540	2.04
Spain	316,236	157,230	2.01
World	12,944,096	4,403,075	2.94

Source: FAO (2018)
[a]Countries producing together 74% of the world production

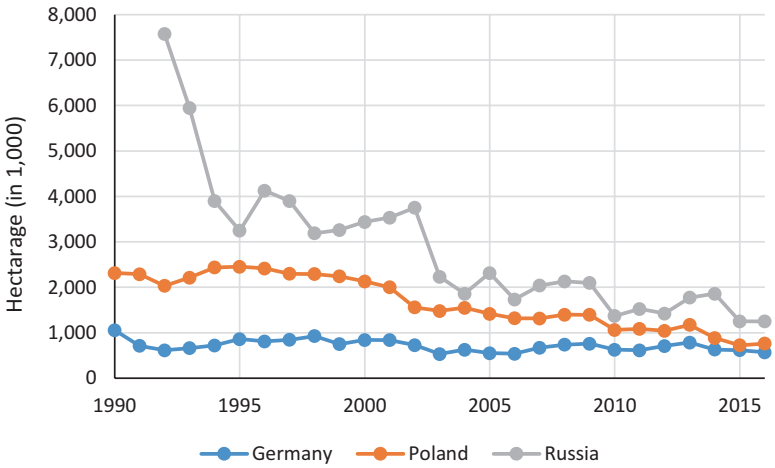

Fig. 9.1 Rye hectarage in the three main producing countries from 1990 and 1992 (Russia) to 2016. (Source: FAO 2018)

population by hybrid cultivars during this period. In the state of Thuringia on average 7.17 mt ha^{-1} were harvested in 2016 illustrating the high yield potential of rye (DESTATIS 2017).

In Northern Europe, rye is an important source of dietary fiber in human nutrition, e.g., in Finland and Denmark almost 40% of dietary fiber intake comes from rye (http://www.ryeandhealth.org/). The EU Commission (EFSA 2011) accepted a health claim for rye fiber to improve bowel function.

For the use as animal feed, rye has a similar nutrient content than triticale and wheat (Table 9.3). In detail, rye shows lower crude protein and crude fiber contents and a similar gross energy like triticale. The soluble β-glucans and arabinoxylans,

Table 9.3 Comparison of feeding value of rye, triticale and wheat

	Crude protein	Crude fiber	Starch	Gross energy	Sol. β-glucans	Sol. AX	Amino acids		
							Lys	Met	Thr
Cereal species	[% DM]			[MJ kg DM^{-1}]	[% DM]		[g $16gN^{-1}$]		
Rye (n = 22)	11.7[a]	1.79[a]	64.3[a]	18.4[a]	0.66[a]	3.09[a]	3.59[a]	1.52[ab]	3.23[a]
Triticale (n = 21)	12.4[b]	2.10[b]	69.9[b]	18.4[a]	0.09[b]	1.26[b]	3.23[b]	1.57[b]	3.05[b]
Wheat (n = 29)	13.7[c]	2.13[b]	71.3[c]	18.6[b]	0.20[c]	1.39[b]	2.72[c]	1.47[a]	2.86[c]

Source: Rodehutscord et al. (2016)
DM Dry matter, *MJ* megajoule, *N* nitrogen, *n* number of tested genotypes, *AX* arabinoxylans, *sol.* soluble
[a–c]Means within a column having a common superscript letter are not significantly different

however, are much higher than in any other cereal, most probably contributing to lower feeding value of rye (Antoniou et al. 1981) by induction of a higher viscosity (Fengler and Marquardt 1988; Rodehutscord et al. 2016). Correspondingly, the digestibility of the essential amino acids lysine, methionine and threonine in rye was substantially lower compared to wheat when studied in pigs (Eklund et al. 2016; Rosenfelder et al. 2015) and poultry (Zuber and Rodehutscord 2016; Zuber et al. 2016). In contrast, the content of essential amino acids in rye is higher than in wheat, thus rye would be a highly valuable feed when the content of non-starch polysaccharides (β-glucans, arabinoxylans) could be reduced and the amino acid digestibility enhanced by breeding. Because, however, the arabinoxylans (pentosans), are needed for superior baking quality, a special breeding scheme would be necessary for considerably improving the feeding value of rye for animals.

Rye is in practice largely used as homegrown feed in proportions of 25–50% depending on age and type of animals, piglets and calves should get lower percentages of rye (DLG 2006). Rye for feeding should contain <0.1% ergot sclerotia and not surpass the EU orientation values for deoxynivalenol (DON) and zearalenone (ZON) (EU 2007).

9.1.3 Domestication, Selection and Early Improvements

The main diversity center of rye comprises central and eastern Turkey and the adjacent regions of Northwestern Iran and Transcaucasia (Zohary 1971, cit. in Behre 1992). Small amounts of rye kernels have been found in the earliest excavations in the Euphrates Valley of Northern Syria from 8500 BC (Hirst 2017) and in several Neolithic sites in Turkey like Can Hasan III from 6600 BC (Hillman 1978). Rye reached Eurasia as a weed mixed in other cereals. A high percentage of rye remains in the human settlements occurred firstly during the pre-Roman Iron Age (ca. 800 BC–50 BC) in several sites, e.g., in Sachsen-Anhalt, Austria, Ukraine and Georgia. High winter hardiness and stress tolerance gave weedy rye an advantage in the harsh environments of eastern and northern part of Europe over wheat and barley. Rye was established in the Germanic and Slavonic regions as a crop of its own.

The cultivation increased further from the early Middle Ages (eighth–tenth centuries) onwards (Behre 1992), rye being always the staple food of peasants providing energy for hard work. People that were more prosperous preferred wheat bread. European settlers brought rye to the Americas in the 16th and 17th centuries. With increasing living standards, the use of rye declined. In 1930, for example, in Minnesota 2.9 million hectares of rye were grown, in 1989 only about 13,000 hectares remained (Oelke et al. 1990). In Germany, rye stayed the most important cereal until 1966, when wheat took over this role (FAO 2018).

As a cross-fertilizing species, rye landraces were grown as open-pollinated populations. They were adapted to specific environmental conditions and named after their region of origin. Rye breeding started with a mass-selection scheme by W. Rimpau in 1867 giving rise to the Schlanstedter Roggen (roggen = rye). Ferdinand von Lochow bred the first commercially successful cultivar in 1880 (Original F. v. Lochows Winterroggen cv.). He selected among the progenies of a cross between the two landraces Pirnauer Roggen and Probsteier Landroggen. His breeding station was in Petkus, a small village south of Berlin, on extremely sandy soils under dry conditions and in a harsh continental climate. Starting with mass selection he changed soon to family selection (ear to row). His first population cultivars were so successful that they served worldwide for the improvement of regional landraces. Most rye cultivars are still genetically related to the Petkus gene pool (Fischer et al. 2010; Parat et al. 2016). Rudolf Carsten started in 1908 in Bad Schwartau near Kiel an independent rye-breeding program with the landrace Heinrich Roggen. He kept his population separate from other rye germplasm and thus created the Carsten gene pool. It was adapted to sandy loam soils and maritime climate. In 1921, W. Laube created the method of saved seed (*Restsaatgutmethode*) in the F.v. Lochow-Petkus company (today KWS LOCHOW GMBH). In the 1970s, Geiger and Schnell developed the biological requirements of hybrid breeding in rye at the University of Hohenheim, Germany. The first three worldwide hybrids were listed in 1985.

9.2 Hybrid Rye Breeding

9.2.1 Breeding Goals

The use of hybrid rye is highly versatile. As young plant, rye can be used as livestock pasture or green manure. As substrate for biogas production rye can be harvested early at mid-shooting stage with a following crop like maize or at late milk ripening. Rye grain is used as flour for baking bread and other bakeries, as homegrown animal feed, and in alcohol distilling. In Germany, less than 20% of rye is used for baking, about 60% for feeding and 20% for bioenergy production (biogas, bioethanol). Breeding goals vary accordingly (Table 9.4).

Table 9.4 Rating of rye breeding goals according to the major usage

	Grain			Biogas substrate	
Breeding goals	Baking	Feeding	Ethanol	Early	Late
Grain yield	++	++	++		
Dry matter yield				++	++
Earliness	+	+	+	++	+
Plant height	−	−	−		++
Lodging resistance	++	++	++		++
Leaf-rust resistance	+	+	+	+	+
Ergot resistance	++	++	++		
TKW/test weight	++	++	++		
Falling number	++	+	+		
α-amylase activity	– –	−	++		
Viscosity/Pentosan content	++	– –	– –		
Raw protein content	+/−	++	– –		
Starch content	+	++	++		

++ very important, + important, +/− neutral importance and – negative effect, − − very negative effect. Empty = no importance

Productivity as grain yield or dry-matter yield is of highest priority in each breeding program. **Dry-matter (biomass) yield** can, at the moment, only be measured in special yield trials, because harvest time is already at milk ripening. Heritability is lower than for grain yield (Haffke et al. 2014). **Earliness** should be regarded during selection, because rye is an early crop in the rotation. **Lodging resistance** is important for all purposes, except for the early biogas substrate production. This can be achieved by using semi-dwarfing genes, like *Ddw1* (Korzun et al. 1996), but also by conventional selection for moderate height and straw stability. Given a high lodging resistance, **plant height** is not an important goal for grain use, but this trait is positively correlated to dry-matter yield at late harvest (=total biomass, Haffke et al. 2014). Concerning **disease resistances**, mainly leaf-rust and ergot resistance are important, the latter being of very high impact not only for feeding, but also for ethanol production, because the remaining DDGS (distillers' dried grains with solubles) is used as protein-rich feed. Resistances to stem rust and *Fusarium* head blight are under development (Miedaner 2019).

From the **quality traits**, alpha-amylase activity, starch, and pentosan contents are the major determinants of baking quality. Thousand-kernel and test weight are of prominent importance for milling. The Hagberg falling number is an internationally standardized method for measuring preharvest sprouting (Perten 1964) and should be in a range of 120–300 s. Genetic variation and heritability for this trait are large (h^2 = 0.8–0.9) and a high correlation between lines and their testcrosses prevails (Wehmann et al. 1991) leading to a very high gain from selection. The falling number correlates with α-amylase activity that should be low for grain use except for distillation where high activities are needed. For baking, high viscosity and high soluble and total pentosan contents are important (Buksa et al. 2010), for feeding and distilling, they should be low because pentosans, and more specifically the ara-

binoxylans, reduce nutrient digestibility for livestock (see Sect. 9.1.2). Raw protein content should be low for baking and ethanol production, because protein negatively affects ethanol recovery. For feeding and ethanol production, starch content is of high importance and should not be below 55%. Starch content and ethanol recovery are positively correlated (r = 0.6–0.8, Rode 2008). Low viscosity as a main target trait for feeding quality displayed a high genetic variation and heritability in breeding materials ($h^2 = 0.73$) (Boros 2007; Madej et al. 1990).

Global climate change will also affect winter rye. Climate projections are for higher temperatures and less rain during winter and spring for northeastern Europe. This might enhance the severity of pre-summer drought that is especially important on the light, sandy soils where rye is preferably grown. Although a relatively drought-tolerant crop, rye will suffer more from a rain deficit than wheat that is cultivated only on the best soils. The main challenge is that drought occurs in Central Europe at present episodically and unpredictable (Hübner et al. 2013). Since 2000, for example, rye suffered from drought in Germany in 2003, 2007, 2011 and 2018 resulting in reduced grain yields. Commercial rye cultivars should, therefore, perform superior under optimal, but also good under drought conditions.

In a large study under managed **drought stress**, we analyzed three segregating rye populations with 220 testcross progenies each, at three locations in each of 2 years (Haffke et al. 2015). Grain yield on the irrigated plots ranged from 5.1 to 10.1 mt ha^{-1}.Yield reduction in the rainfed regime ranged from 2 to 41% depending on the location × year combination. The ranking of the genotypes between irrigated and rainfed regimes was similar with genotypic correlations from 0.8 to 1.0. In addition, the coefficient of linear regression showed no significant deviation from 1. However, the mean square deviation from linear regression varied significantly illustrating that the entries showed differences in yield stability under drought conditions. In conclusion, it is recommendable to include locations with less rainfall in the selection environments, however, no special procedures with irrigated vs. non-irrigated regimes are necessary. Elite breeding populations obviously already combine high grain yield and yield stability under drought conditions, at least at the currently occurring drought level.

Another consequence of higher temperatures might be an increasing incidence of stem rust (*Puccinia graminis* f. sp. *secalis*) that is a thermophilic pathogen already producing regular epidemics in Europe an regions with a more continental climate (Miedaner 2019).

9.2.2 Prerequisites of Hybrid Breeding

Hybrid breeding allows the maximal exploitation of heterosis. As a cross-pollinating cereal, rye shows a similar amount of heterosis for grain yield as maize (Geiger and Miedaner 1999). Hybrid breeding in rye requires (1) self-fertility for developing inbred lines, (2) cytoplasmic-male sterility (CMS) for production of hybrid seed

Fig. 9.2 Cytoplasmic-male sterility in rye: Fully male fertile, partially male fertile, fully male sterile (from left to right)

(Fig. 9.2) and nuclear encoded, dominant *restorer to fertility* (*Rf*) genes, and (3) heterotic pools to maximize heterosis (Geiger and Miedaner 2009).

Self-fertility genes overcoming the self-incompatibility system of rye were detected in various European germplasm sources already in the 1930s (Ossent 1938). Inbred lines are produced by continued self-fertilization of single plants (Fig. 9.3), double-haploid (DH) technologies are not yet available. This resulted in considerable inbreeding depression, homozygous lines only yield 30–50% of non-inbred materials (Geiger and Miedaner 1999).

Several sources of CMS are available in rye (Łapiński and Stojałowski 2001). Yet practically all hybrids are produced by means of the Pampa (P) cytoplasm originating from an Argentinian landrace (Geiger and Schnell 1970). Restorer genes were found in European breeding populations (Geiger 1971). However, the efficacy of the European restorer genes strongly depends on the female genotype and the environment (Geiger et al. 1995; Miedaner et al. 2000). However, restorer genes detected more recently from rye germplasm from Argentina, Turkey and Iran are highly effective and stable across environments (Miedaner et al. 2005). However, these exotic *Rf* genes display negative side effects such as a lower grain yield, a lower 1000-grain weight, and a taller plant stature, even in testcrosses (Miedaner et al. 2017).

Fig. 9.3 Selfing of individual plants of rye by isolation bags

Petkus and Carsten gene pools were detected to exhibit the greatest hybrid performance in Central Europe (Hepting 1978). Both heterotic pools are still separated today as shown by molecular markers (Fischer et al. 2010; Miedaner et al. 2019; Parat et al. 2016).

9.2.3 Type of Hybrid and Breeding Plans

Commercial rye hybrids are crosses between a CMS single cross as seed parent and a synthetic composed of two restorer lines as pollinator (Geiger and Miedaner 2009):

$$\left(A_{CMS} \bullet B\right) \times Syn\left(C,D\right)_{Rf}$$

The single cross between unrelated homozygous lines secures seed production and delivers high quality seed due to the higher vigor of heterozygous rye. Two partially inbred lines constitute the restorer synthetic. This allows producing the hybrid seed in a mixed stand of seed and pollen parents that is economically more feasible than hybrid seed production in strips. A change of the hybrid type towards three-way or even single-cross hybrids like in maize would require considerably higher inbred line per se performances.

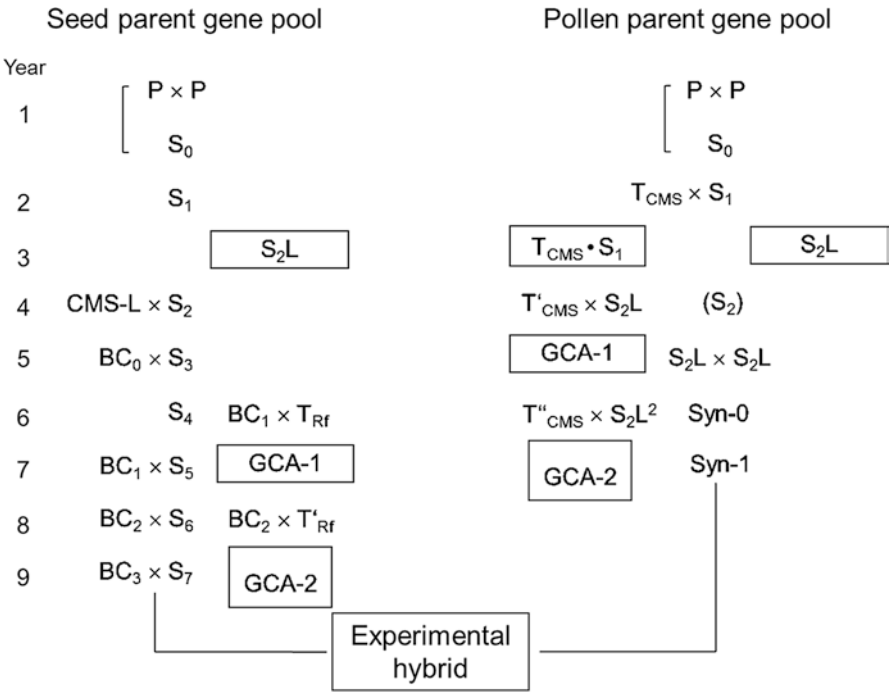

Fig. 9.4 Schematic representation of hybrid rye development starting with a single cross

Breeding cycles start with crossing of parental lines, followed by one stage of per se performance test of the S_2 lines and two stages of GCA (general combining ability) testing by using testers from the opposite heterotic pool (Fig. 9.4). Parental lines could be crossed in an off-season program, thus saving 1 year. S_2 lines are intensively selected in one machine-planted row each at two to three locations for flowering date, plant height, disease and lodging resistances, falling number and thousand-grain weight. These highly heritable traits have a reasonable correlation between the inbred lines per se and their testcrosses (Miedaner et al. 2014). Selected S_2 lines are crossed to a CMS donor (CMS-L) and subsequently backcrossed to transfer the seed-parent lines into the CMS-inducing (P) cytoplasm. These two backcrossing steps could also be done in an off-season program in the greenhouse. Intense check for male sterility (i.e., non-restorer ability) is necessary afterwards although most gametes in the European seed parent pool are lacking restorer genes for P cytoplasm. Backcrossing is continued throughout the complete breeding scheme to obtain near-isogenic lines in P and normal cytoplasm, respectively. The testcrossing phase comprises the BC_1 generation produced on S_4-line analogues (GCA-1). A topcross procedure is used with one tester as pollinator and the BC lines as seed parents. Strictly isolated field plots are needed to avoid contamination

by alien pollen. Testcrosses are evaluated on drilled plots of 5–6 m^2 size at several locations. The main focus is on grain yield (or dry matter yield for biomass production), but secondary traits like plant height, lodging resistance and quality traits are also recorded. BC_1 lines selected in the first stage of testcrossing are further tested as BC_2 generation produced on S_6-line analogues in a second stage (GCA-2) with more than one tester and a higher number of locations.

For pollen-parent lines, the principal breeding scheme is similar (Fig. 9.4). Often, the initial crosses are already produced with a CMS form of the restorer parent to directly select for restorer ability per se. When using European sources only, this and an *Rf* selection during the regular GCA-1 and GCA-2 tests are not sufficient. Selection for superior pollen fertility should then already start among S_1 lines based on testcrosses in parallel to the S_2 performance test. The testcrosses are produced plant wise with a CMS tester that is hard to restore. This allows a high selection intensity that is necessary for European sources due to the rare occurrence of effective restorer alleles (Geiger et al. 1995; Miedaner et al. 2005). This is dispensable when a highly effective exotic restorer gene is introgressed by backcrossing. Selected S_2 lines are testcrossed between isolation walls (Fig. 9.5) that should keep away from foreign pollen and multiplied in isolation cabins (Fig. 9.6). CMS single crosses of the seed parent gene pool are used as testers, because they produce more seed with higher quality than inbred lines. The testcross progenies are selected in GCA-1 and GCA-2 tests as described above and additionally for their restorer ability. In parallel, the best lines are intercrossed for producing new restorer synthetics that are forwarded to Syn-2. Inbreeding in the pollen parent gene pool will usually stop in generations S_2 or S_3, because the pollinator synthetic will be heterogeneous anyway. The expected GCA variance among S_2 or S_3 lines amounts to 75 and 87.5%, respectively, of that between fully inbred lines (Geiger and Miedaner 2009).

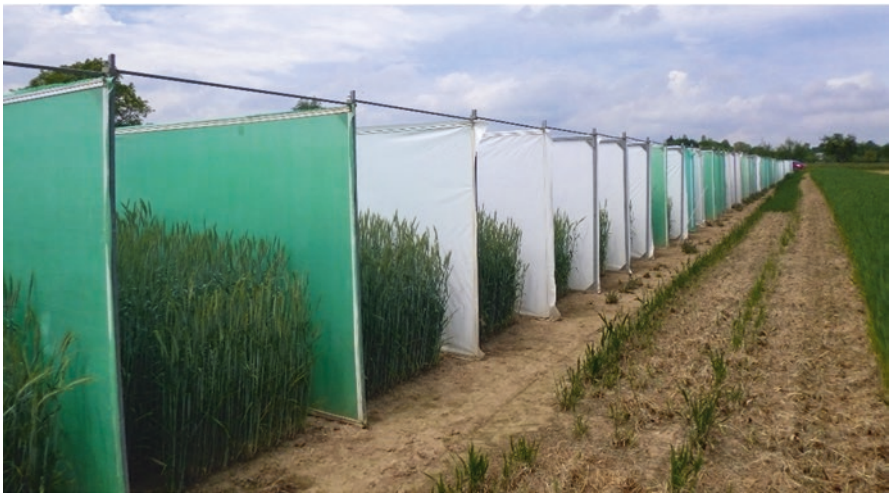

Fig. 9.5 Production of testcrosses with restorer lines between isolation walls; one outer row represents the pollinator, in the inner rows two CMS testers of the seed-parent pool are placed

Fig. 9.6 Multiplication of male-fertile materials (inbred lines, synthetics) in isolation cabins of two materials: plastic and pollen-dense fabric; the plastic cabins have a hole in the top to allow some air circulation

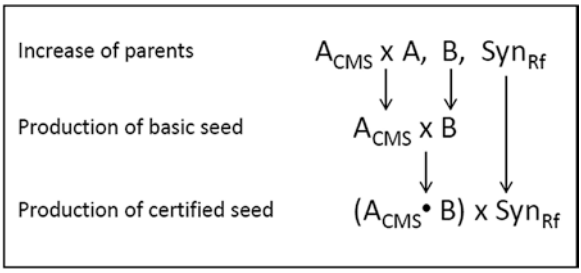

Fig. 9.7 Scheme for hybrid seed production

9.2.4 Hybrid Seed Production

Commercial hybrid seed production requires strict logistics because rye is a very efficient wind pollinator. All stands of seed production must be strictly isolated and carefully checked for off-types, especially for maintainer plants in CMS stands of seed-parent lines or crosses. All seed multiplication steps, except the production of certified seed, occur in regions without commercial rye growing, like in Northern Italy or major wheat regions in France.

In the first step, parental lines must be increased by multiplying A_{CMS} and its maintainer version A in strips (Fig. 9.7). This generally requires two to 3 years due to the low reproduction coefficients of inbred lines. In parallel, the other components of the hybrid (line B, Syn_{Rf}) are multiplied on isolation plots. Seed of the seed-parent cross $A_{CMS} \times B$ is also produced in strips that effectively allow controlling the stands for off-types. Line B should be removed mechanically after flowering to

avoid seed admixtures during harvest. No lodging may occur in all seed multiplication stands. The female:male ratio in basic seed production may be increased from 2:1 to 4:1 depending on the size and the shape of the field. Restorer synthetics are self-fertile and shed ample pollen.

Certified seed is produced in mixed stands with 90–95% CMS single cross and 5–10% restorer synthetic. This procedure substantially reduces the seed production costs, but must be permitted by national authorities. Because the synthetic is the only pollinator in the mixed stand, it will reproduce and, thus, be included in the certified seed for the farmer. In all seed production stands, higher percentages of ergot will occur than in commercial rye production, because there is generally less pollen available and flowering dates do not always perfectly match. Therefore, the use of color sorting machines is necessary that remove all seed lots from the dark ergot sclerotia to meet regulations.

9.3 Breeding Progress of Hybrid *vs.* Population Cultivars and On-farm Progress

9.3.1 Grain Yield and Yield-Related Traits

The first rye hybrids were entered in 1982 into official registration trials for testing their value for cultivation and use (VCU) in Germany. The first three hybrid cultivars were released for commercial use in 1985. At the beginning, they out-yielded the population cultivars by about 10%. The considerably higher yield potential of hybrids led to a fast introduction by farmers. Whereas in 1989 only 2% of the rye hectarage in Germany consisted of hybrid cultivars, by 1997 they occupied 50%. Today, the share of hybrids is 70–80% (Laidig et al. 2017).

The analysis of VCU trials between 1985 and 2016 across Germany demonstrated the enormous breeding progress achieved for hybrids as compared to population cultivars (Table 9.5). Results of 68 released hybrid and 23 population cultivars were analyzed separately. The overall yield trend was dissected into a genetic component due to new improved cultivars and a non-genetic trend (Piepho et al. 2014). The non-genetic trend can be influenced by many factors, like fertilizing, crop management or changing climate conditions. The on-farm results are national averages from the annual special harvest and quality survey (Besondere Ernte-und Qualitätsermittlung BEE 2017).

The overall yield trend of hybrids increased by 1.17% p.a., relative to 1985. However, the increase for population cultivars was only 0.82%, at a considerably lower level (Table 9.5). The gap between both types widened from about 7 dt ha^{-1} in 1985 to more than 16 dt ha^{-1} in 2016. The disparity between the yield performance of hybrid and population cultivars becomes even more apparent when looking at the genetic trends depicted in Fig. 9.8a. The genetic trend for hybrids (0.773 dt ha^{-1} a^{-1}) exceeded the trend for population cultivars (0.237 dt ha^{-1} a^{-1}) by about

Table 9.5 Estimates of trait levels and regression coefficients of grain- and quality-related traits. All percent trends (%) relative to 1985 trait levels

		Trait levels		Linear annual trends								
				Genetic			Non-genetic			Overall/On-farm		
Trait	Type	1985	2016	Abs.		%	Abs.		%	Abs.		%
Grain yield at 86% dry matter content [dt ha^{-1}]	Hyb	73.9	100.7	0.773	***	1.05	0.112		0.15	0.866	***	1.17
	Pop	67.0	84.1	0.237	***	0.35	0.242	*	0.36	0.551	***	0.82
	On-farm	43.8	55.7							0.383	**	0.87
Ear density [ears m^{-2}]	Hyb	475.9	593.1	2.494	***	0.52	1.510		0.32	3.783	*	0.80
	Pop	487.9	539.8	0.871	*	0.18	0.793		0.16	1.675		0.34
Single ear yield [g ear^{-1}]	Hyb	1.64	1.79	0.007	***	0.40	−0.002		−0.12	0.005		0.31
	Pop	1.46	1.64	0.002	*	0.17	0.002		0.15	0.006	*	0.41
Number of kernels per ear [kernels ear^{-1}]	Hyb	51.7	49.0	0.046		0.10	0.002		−0.26	0.038		−0.17
	Pop	45.7	43.4	0.037		0.22	−0.067		−0.50	0.033		−0.17
Thousand grain mass at 86% dry matter content [g (1,000 kernels)$^{-1}$]	Hyb	34.1	37.2	0.130	***	0.30	−0.041		−0.03	0.090		0.29
	Pop	34.2	38.3	0.018		−0.09	0.111	*	0.43	0.109	*	0.38
Falling number (Hagberg-Perten) [s]	Hyb	223.3	237.6	0.816		0.37	−0.197		−0.09	0.459		0.21
	Pop	213.8	214.0	0.814	*	0.38	−1.033		−0.49	0.009		0.00
	On-farm	186.2	219.4							1.07		0.57
Crude grain protein concentration (% of dry matter content) [%]	Hyb	11.0	9.4	−0.034	***	−0.31	−0.017		−0.16	−0.051	***	−0.46
	Pop	11.1	10.2	0.002		0.02	−0.032		−0.29	−0.030		−0.27
	On-farm	10.9	10.0							−0.03		−0.24
Maximum amylogram viscosity [AU]	Hyb	787.7	890.4	4.314		0.55	−0.867		−0.11	3.314		0.42
	Pop	749.2	688.3	0.906		0.12	−3.240		−0.43	−1.964		−0.26
	On-farm	565.5	881.5							10.19		1.80

(continued)

Table 9.5 (continued)

Trait	Type	Trait levels		Linear annual trends							
				Genetic			Non-genetic			Overall/On-farm	
		1985	2016	Abs.		%	Abs.		%	Abs.	%
Amylogram temperature at maximum viscosity [°C]	Hyb	69.6	69.5	0.086	***	0.12	−0.072		−0.10	−0.003	−0.01
	Pop	69.7	67.9	0.066	***	0.10	−0.131		−0.19	−0.059	−0.08
	On-farm	67.6	68.5							0.03	0.04

Hyb hybrid varieties, *Pop* population varieties, *Abs.* absolute values, *AU* Amylogram unit
*, **, *** Significant at 5, 1 and 0.1% levels, respectively

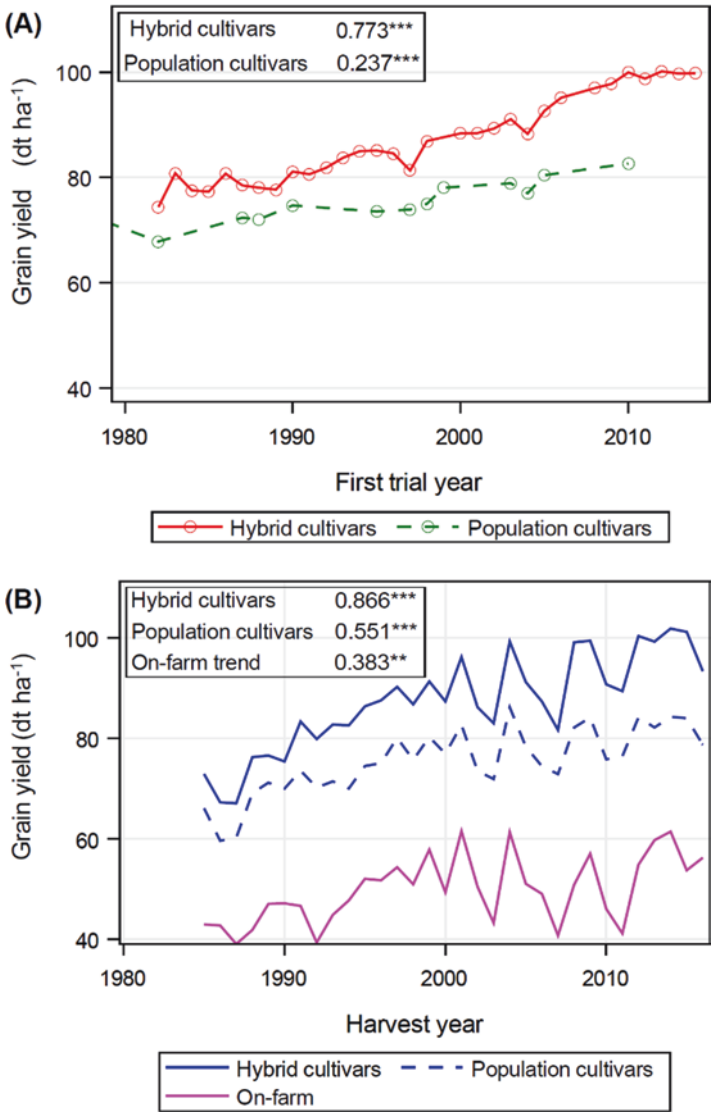

Fig. 9.8 Breeding progress for grain yield 1985–2016: (**a**) Genetic trends from VCU trials, (**b**) Overall trends separately for hybrid and population cultivars from VCU trials and on-farm trends

three times. This demonstrates that the yield progress for hybrids observed in trials is mainly due to new improved genotypes. The question arises, which yield component was the main cause of the strong progress of hybrids? Table 9.5 indicates that ear density of hybrids showed a significant overall increase of 0.80% p.a., mostly due to the genetic trend, whereas for population cultivars the increase was less than half with 0.34% p.a., and not significant. Number of kernels showed no significant

trends in both types. For population cultivars, overall trend for single ear yield and thousand grain mass increased significantly with 0.41 and 0.38% p.a. relative to 1985, respectively.

Despite of the rapid shift to the higher yielding hybrids in on-farm production, still a remarkable gap between trial and on-farm yields is apparent, as the yield trends in Fig. 9.8b demonstrate.

The absolute on-farm yield trend increased less than half (0.383 dt ha^{-1} a^{-1}) compared to that of hybrids (0.886 dt ha^{-1} a^{-1}), however, at a substantial lower level as compared to VCU trials (Fig. 9.8b). The main reasons for this notable gap are the decreasing growing area of winter rye connected with a shift to growing on less fertile sandy soils, and additionally, economic constraints in on-farm rye production, i.e. farmers do not realize the full yield potential, but rather the economical yield optimum.

9.3.2 Quality Traits

In rye, alpha-amylase activity, starch and pentosan concentrations are the major determinants of quality. This is in contrast to wheat, where protein concentration and protein quality play a key role. In rye, falling number, protein concentration, and amylogram values for viscosity and temperature were evaluated as the main quality-related traits in the VCU trials. As compared with other cereal crops, rye has only a low secondary dormancy. Thus, soon after physiological ripeness of the grain, germination processes may start when weather conditions are unfavorable. This causes the well-known large year-to-year fluctuation of rye baking quality, because grain germination is strongly affected by wetness, low temperature and radiation during harvest time (Laidig et al. 2017).

Table 9.5 indicates that there was no significant negative influence of increased grain yield on quality traits, except for protein concentration. For hybrids, a strong negative genetic and overall trend of −0.034% and −0.051% was observed, respectively. For population cultivars, the decrease was lower and not significant. For the other three quality-related traits no significant trends were found for both types of cultivars, due to the large year-to-year fluctuation. Laidig et al. (2017) also found no significant diverging genetic trends between hybrid and population cultivars for these three quality-related traits. Table 9.5 further indicates that the trait levels of falling number and amylogram values are lower for population than for hybrid cultivars.

The on-farm quality data are assessed on more than 800 representative harvest samples from the rye growing regions in Germany. The trend pattern for on-farm traits given in Table 9.5 was similar to that of VCU trials. For the on-farm traits no differentiation between hybrid and population cultivars is available. Thus, the respective trait levels in 2016 were just in between both.

In general, the enormous breeding progress achieved for grain yield of hybrids had no significant negative effects on rye quality, except for protein concentration,

that is, however, not important for rye baking quality. Additionally, Table 9.5 shows that the levels of falling number and amylogram values of hybrids in 2016 were clearly above the population cultivar levels.

In conclusion, the results confirmed, that hybrid cultivars accelerated yield progress in rye during the last 32 years, without loss in bread-making quality. A remarkable large yield gap, however, between VCU trials and on-farm yield still exists despite the introduction of high-yielding hybrids.

9.4 Germplasm Diversity and Conservation

Germplasm of rye consists of diverse types of collections such as wild species, wild and weedy relatives, landraces, foreign and obsolete population cultivars, elite lines and mutants (Haussmann et al. 2004). More than 22,200 rye accessions are documented from 94 gene banks (Schlegel 2014) where the largest are located in Russia (St. Petersburg), Poland (Warsaw), Germany (Gatersleben), and the USA (Beltsville), each with about 2–3000 rye entries. The searchable European *Secale* Database (http://bankgenow.edu.pl/en/baza-danych/europejska-baza-zyta/) maintained in Radzikow, Poland, includes passport information for ca. 13,000 accessions from 35 European institutions.

Nonadapted germplasm could be useful for introgression of special traits into elite rye (Table 9.6). They differ in terms of growth habit (winter, spring), population history (geographical origin, relatedness, dispersion) and level of improvement (none, low, elite) leading to different levels of genetic diversity (Parat et al. 2016). Wild and weedy rye were subjected to natural selection only, landraces were formed by natural selection and, most probably, some level of mass selection, forage rye is mainly from the Mediterranean Basin where rye was not used as a grain crop. In the evolutionary study by Parat et al. (2016), three Iranian and Turkish weedy ryes, two Russian populations and German, Polish and Belorussian populations each formed a distinct cluster. The latter illustrates a high gene flow between Central and Eastern European populations. The clusters of cultivated European rye landraces described

Table 9.6 Germplasm for improving elite rye (clusters of cultivated European rye according to Hagenblad et al. 2016)

Category, lineages	Origin	Interesting traits (examples)
Wild species	*Secale strictum*	Perenniality, rust resistances
Weedy rye	Iran, Turkey	Pollen-fertility restoration
Central European landraces	Germany, Austria, Poland, Belorus	Grain yield, lodging tolerance, baking quality
North-Eastern European landraces	Norway, Sweden, Finland, Estonia, Russia	Freezing tolerance, snow mold resistance
Iberian rye	Spain, Portugal, Morocco	Biomass yield, rust resistances
Balkan Peninsula rye	Serbia, Greece, Turkey	

by Hagenblad et al. (2016) indicate that they could have evolved in different regions and times and rather reflect past cultivation intensity than agro-climatic zones.

The main challenges for the use of such germplasm (genetic resources) in practical breeding are their low agronomic performance and poor adaptation to climate, photoperiod and/or modern cultivation conditions (Haussmann et al. 2004). Another problem is the tallness of genetic resources that may extend to 2 m and more. Further challenges for hybrid breeding are the low inbreeding tolerance of self-incompatible populations, the unknown heterotic pattern, and potentially a high linkage drag, i.e., the cotransfer of genomic regions with negative effects linked to the trait of interest.

9.5 Molecular Breeding

Effective molecular breeding started in rye with the advent of the first SNP arrays that made large-scale genotyping feasible. This is of special importance for speeding up practical breeding because no doubled-haploid technique has been successfully established in rye and genetic engineering is not practiced. The progress in genomics-based breeding has been recently reviewed (Miedaner et al. 2019).

9.5.1 Genomic Resources

Although rye was lagging behind in molecular methods caused by the low international interest, nowadays PCR-based marker techniques such as amplified fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs), diversity array technology (DArT) and next generation sequencing techniques are available (Rabanus-Wallace and Stein 2019). Comprehensive resources of expressed sequence tags (ESTs) were established and a 5 k, and later an extended custom 16 k Illumina BeadChip were produced based on single-nucleotide polymorphisms (SNP) (Bauer et al. 2017; Haseneyer et al. 2011). In the meantime, a high-density Affymetrix Axiom® Rye600k genotyping array was designed which enabled the construction of an 88 k high-density genetic map and anchoring of WGS contigs along the rye genome (Bauer et al. 2017).

Genome-wide high-density comparative analyses revealed the reticulate evolution of the rye genome and lead to a fine-scale definition of conserved syntenic linkage blocks in rye and barley (Martis et al. 2013). Despite its large genome of about eight Gigabase pairs and the high percentage of repetitive elements, a first draft genome sequence of rye was generated recently (Bauer et al. 2017).

From the plant side, segregating populations derived from biparental crosses for linkage mapping as well as large assortments of inbred lines for association mapping are available from private breeding companies. At the University of Hohenheim, two introgression libraries from the Iranian weedy rye Altevogt 14160 together with

78 inbred lines were developed (Falke et al. 2008) and intensively tested for line per se and testcross performance of various agronomic traits (Falke et al. 2009, 2010; Mahone et al. 2013).

9.5.2 Selection for Monogenic Traits

The most straightforward use of molecular markers in breeding is the selection of monogenic traits. Unfortunately, in rye there are not many commercially important monogenic traits that could be used for marker-assisted selection (MAS). First, pollen-fertility restoration from nonadapted sources (Iran, Turkey, Argentina) is a primary target of MAS. Stracke et al. (2003) reported for the first time the development of PCR-based markers for this exotic germplasm. Nowadays an array of markers is available (Hackauf et al. 2017a). They allow the effective selection of *Rf* loci in all exotic germplasm sources. All of them are, until now, located in the same region of chromosome 4RL (Korzun, pers comm). Other candidates for MAS are dwarfing genes, like the dominant *Ddw1* on chromosome 5R (Kalih et al. 2014), that was recently used in practical breeding of rye and triticale. Additionally, monogenic leaf rust resistance genes (Roux et al. 2004; Wehling et al. 2003) or stem rust resistance genes (Miedaner et al. 2016) could be a worthwhile target for MAS to rapidly introgress them from nonadapted sources or pyramid them to potentially prolong their durability. Further, PCR-based markers can identify CMS phenotypes (Stojałowski et al. 2004). They have also been used to test hybridity during seed multiplication.

9.5.3 Detection and Use Of Quantitative Trait Loci (QTL)

To enhance the gain from selection for quantitative traits, molecular markers have been proposed (Heffner et al. 2009). In rye, F_2-derived biparental populations must be used for QTL mapping because the production of doubled-haploid lines is not feasible. Early QTL mapping studies concentrated on single traits based on the line per se performance, e.g., α-amylase activity (Masojć and Milczarsk 2008). More recently, QTL for important traits of each of 220 testcross progenies from two biparental crosses within the Petkus gene pool were described (Miedaner et al. 2012). One to nine QTL were detected for eight agronomic and quality traits (grain yield, thousand-kernel weight, test weight, falling number, protein, total and soluble pentosan and starch contents). For all traits, a high genotype × environment interaction was observed. Recently, we reported on 22 QTL with significant effects for grain-related traits (grain yield, tiller number, thousand-grain weight) and heading date across 7 environments in an interpool testcross progeny of 258 entries (Hackauf et al. 2017b). All analyzed traits were quantitatively inherited with mainly low-effect QTL. However, even for complex traits, like grain yield (Hackauf et al.

2017b) or biomass yield (Miedaner et al. 2018), individual QTL with a high contribution and stable across a range of environments were found. They might become a target for MAS and candidate gene search in homoeologous genomes (Hackauf et al. 2017b). In future, the use of genome-wide association studies (GWAS) might be promising to sample more adequately the allelic diversity within the allogameous rye (Newell and Butler 2013).

9.5.4 Genomic Selection

While MAS is based on individual genes or large-effect QTL, genomic prediction or genomic selection (GS) uses marker information across the whole genome and can be used to predict the genetic value of non-phenotyped individuals (genomic estimated breeding value, GEBV, Heffner et al. 2009). This became feasible in rye by the development of medium- to high-density SNP chips (see Sect. 9.5.1). Selection includes then markers with such small effects that they cannot be detected in QTL studies, resulting in GEBVs with higher accuracy (Goddard and Hayes 2007). Consequently, a large number of small-effect QTL controlling the trait could be used for selection.

Genomic prediction was first analyzed in rye by studying two intrapool crosses (Wang et al. 2014). GS resulted in higher cross-validated prediction accuracies than MAS for grain yield, plant height, starch and pentosan contents. This was most obvious in scenarios where only a low number of QTL have been detected by mapping. Similarly, GS could predict phenotypic stability of grain yield and falling number more accurately than MAS (Wang et al. 2015). The study, however, also stressed the importance of genetic relationship between training and test population. Predicting GEBVs across selection cycles yielded considerably higher prediction accuracies when the data from multiple cycles were aggregated, again illustrating the importance of genetic connectivity between actual and predicted materials (Auinger et al. 2016).

For including GS into hybrid rye breeding schemes by a model study, the aim was to select five lines finally with a moderate prediction accuracy of 0.3 (Marulanda et al. 2016). As a result, a combination of phenotypic selection and GS, called *GSrapid*, yielded a 24% higher gain from selection compared to the standard phenotypic selection scheme assuming the same budget (Table 9.7). In *GSrapid*, all highly heritable traits are selected in the nursery and one stage of genomic selection for grain yield is followed by a one-stage phenotypic selection for GCA in the field. GS thus saves one stage of testcrossing comprising 2 years and reduces the necessary isolation plots accordingly.

Table 9.7 Optimal selection strategies for hybrid rye in two scenarios with phenotypic (PS) selection or including genomic selection (GS)

Year	Test resource	PS standard	*GS rapid*
		Number of entries	
1–3	Crossing and selfings		
4	Nursery selection	5820	7804
	Genomic selection	–	1951
5–6	GCA-1	1455 (L = 6, T = 1)	285 (L = 10, T = 4)
7–8	GCA-2	65 (L = 10, T = 9)	–

Source of data: Marulanda et al. (2016)
GCA general combining ability, *L, T*, numbers of locations and testers, respectively

9.6 Conclusion and Prospects

Considering the great economic significance of winter rye in Germany and some Eastern European countries, a high amount of research has already been carried out in these regions. Unfortunately, the research obtained in Russia is often not available for Western countries due to language barriers. Hybrid rye breeding is at present actively performed in Germany, Poland, Belarus and Russia. Hybrid cultivars are today further available in e.g., Austria, Denmark, Estonia, UK, Ireland, Canada and the USA. In future, rye will only get a larger market share with alternative uses, because the market for human nutrition is saturated. Possible applications are bioenergy production or feeding, either on an industrial scale or by using homegrown rye. Feeding quality could be optimized by reducing the contents of non-starch polysaccharides and increasing amino acid digestibility. Future research should concentrate on the improvement of the following areas.

Although rye is already stress tolerant further resilience to abiotic stress is needed. This is especially due for drought tolerance stimulated by global climate change and freezing tolerance in countries with a long, hard winter such as Russia or Canada. Disease resistances will gain a higher attention, mainly to snow mold in winter-cold areas, stem rust, and *Fusarium* head blight. Although less mycotoxin levels were detected in rye compared to wheat, still mycotoxins are of concern for food and feed security. Use of genetic resources might be necessary in future for introgressing special traits and high-effect QTL by using appropriate marker techniques. To ensure breeding progress in grain yield and other agronomic traits, broadening of the heterotic pools in practical hybrid rye breeding is of high priority. This is especially important when genomic selection will be used routinely in future, because it will accelerate breeding schemes and enhance short-term breeding progress, but also deplete long-term genetic variation. Therefore, new genetic variation must be fed into both heterotic pools on a regular basis.

Acknowledgement The authors wish to recognize the very significant contributions made to hybrid rye breeding by Prof. Dr. Dr.h.c. Hartwig H. Geiger, University of Hohenheim, Germany.

Appendices

Appendix I: Research Institutes Relevant to Rye

Institution	Specialization and research activities	Contact information and website
State Plant Breeding Institute, University of Hohenheim	Hybrid rye breeding, resistance genetics, genomics	Fruwirthstr. 21, 70599 Stuttgart, Germany https://www.uni-hohenheim.de/organisation/einrichtung/landessaatzuchtanstalt
Plant Breeding, Technical University of Munich	Construction of high-density SNP chips, genomic selection	Liesel-Beckmann Str. 2, 85354 Freising, Germany http://www.plantbreeding.wzw.tum.de
Federal Research Centre for Cultivated Plants (JKI)	Marker-based approaches	Rudolf-Schick-Platz 3a, 18190 Sanitz, Germany https://www.julius-kuehn.de/gross-luesewitz
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)	High-throughput sequencing, assembling rye genome	Corrensstr. 3, 06466 Gatersleben, Germanyhttp://www.ipk-gatersleben.de
Scientific and Practical Centre of Belarusian NAS for Arable Farming	Rye breeding	Timiryazeva st. 1, 222160 Zhodino, Belarus http://www.izis.by
Institute of Plant Genetics, Polish Academy of Science	Rye breeding research, molecular analysis of single traits	Ul. Strzeszyńska 34, 60–479 Poznań, Poland http://www.igr.poznan.pl
Institute of Plant Breeding and Acclimation – National Research Institute	Rye breeding research, Polish gene bank, data base on rye genetic resources	Radzikow, Poland http://www.ihar.edu.pl
N.I. Vavilov Research Institute of Plant Industry	Largest gene bank of rye, usage of genetic resources, resistance breeding	Bolshaya Morskaya st. 44, 190000 St.-Petersburg, Russiahttp://vir.nw.ru
Federal State Budgetary Scientific Institution Tatar Scientific Research Institute of Agriculture	Population rye breeding, resistance breeding, quality breeding	Orenburg tract, 48, 420059 Kazan, Tatarstan, Russia http://www.antat.ru/en/structure/agricultural
Moscow Nemchinovka Agricultural Research Institute	Rye breeding and research	Kalinia str. 1, Nemchinovka-1, 143026, Russia

Appendix II: Origin of Genetic Resources of Rye

Institution	Country	No. of accessions
Institute of Botany	Armenia	18
Laboratory of Plants Gene Pool and Breeding	Armenia	
Scientific Center of Agrobiotechnology	Armenia	
AGES Linz – Austrian Agency for Health and Food Safety/ Seed Collection	Austria	99
Genebank Tyrol/Tyrolean Government	Austria	
Institute of Special Crops, Agricultural Research Center Styria	Austria	
Arche Noah Association	Austria	
Research Institute of Agriculture	Azerbaijan	132
Genetic Resources Institute	Azerbaijan	
Institute for Plant Genetic Resources "K. Malkov"	Bulgaria	1248
Genetic Resources Institute, University of Banjaluka	Bosnia and Herzegovina	1
Station de recherche Agroscope Changins-Wädenswil	Switzerland	70
Sortengarten Erschmatt	Switzerland	
Genebank Department, Division of Genetics and Plant Breeding, Research Institute of Crop Production	Czech Republic	683
Genebank, Leibniz Institute of Plant Genetics and Crop Plant Research	Germany	2409
Comunidad de Madrid. Universidad Politécnica de Madrid. Escuela Técnica Superior de Ingenieros Agrónomos. Banco de Germoplasma	Spain	500
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria. Centro Nacional de Recursos Fitogenéticos	Spain	
Junta de Extremadura. Dirección General de Ciencia y Tecnología. Centro de Investigación Agraria Finca La Orden – Valdesequera	Spain	
Cabildo Insular de Tenerife. Centro de Conservación de la Biodiversidad Agrícola de Tenerife	Spain	
Jogeva Plant Breeding Institute	Estonia	6
Genetic Resources Unit, Institute of Biological, Environmental & Rural Sciences, Aberystwyth University	United Kingdom	1
Niko Ketskhoveli Institute of Botany	Georgia	4
Greek Genebank, Agricultural Research Center of Macedonia and Thrace, National Agricultural Research Foundation	Greece	11
Institute for Agrobotany	Hungary	360
Department of Agriculture, Fisheries and Food, National Crop Variety Testing Centre	Ireland	5
Lithuanian Institute of Agriculture	Lithuania	14
Latvian Forestry Research Institute "Silava"	Latvia	9
Faculty of Agriculture, University Ss. Cyril and Methodius	Macedonia	25

Institution	Country	No. of accessions
Institute of Agriculture	Montenegro	4
Nordic Genetic Resource Center	Sweden	335
Plant Breeding and Acclimatization Institute	Poland	2424
Polish Academy of Sciences Botanical Garden – Center for Biological Diversity Conservation in Powsin	Poland	2428
Banco de Germoplasma – Departamento de Recursos Genéticos e Melhoramento, Estaçao Agronómica Nacional, Instituto Nacional de Investigaçao Agrária	Portugal	567
Suceava Genebank	Romania	483
University of Agricultural Sciences and Veterinary Medicine Timisoara	Romania	
Agricultural Research Station Suceava	Romania	
N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry	Russian Federation	2928
Plant Production Research Center Piestany	Slovakia	161
Plant Genetic Resources Department	Turkey	425
Institute of Plant Production n.a. V.Y. Yurjev of UAAS	Ukraine	270
Sum		**15,620**
Not included:		
US National Plant Germplasm system https://npgsweb.ars-grin.gov/gringlobal/search.aspx	Diverse	1937

Sources: European Secale Database (https://bankgenow.edu.pl/en/baza-danych/europejska-baza-zyta/), US National Plant Germplasm system (see above)

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Chapter 10
Tef [*Eragrostis tef* (Zucc.) Trotter] Breeding

Solomon Chanyalew, Kebebew Assefa, and Zerihun Tadele

Abstract Tef or teff [*Eragrostis tef* (Zucc.) Trotter], a cereal crop which adapts to extreme climatic and soil conditions, is extensively cultivated in the Horn of Africa. It is also considered as nutritious and a life-style crop due to its richness in essential nutrients and health-related benefits. However, the productivity of the crop is extremely low due to little scientific improvement made globally. It is, therefore, in the category of *orphan crops*. Together with all cereal crops, tef belongs to the Grass or Poaceae family. The improvement of tef focuses on selection and hybridization techniques. However, recently, molecular and high-throughput techniques have also been implemented to a limited scale. Forty-two tef varieties were approved for release by the Ethiopian National Variety Release Committee in the past four decades. Due to the adoption of improved varieties and technologies, the national average yield of tef has more than doubled over the last 20 years. This review describes the progress in tef breeding and variety development as well as dissemination of the improved varieties to the farming community.

Keywords Accessions · Breeding · *Eragrostis tef* · Hybridization · Molecular breeding · Mutation breeding · Tef varieties

10.1 Introduction

Agriculture plays a key role in the economy of developing countries because a large number of their population engage in this sector. Smallholder farmers in these countries cultivate both major and minor crops such as cereals, legumes, root crops and vegetables. Major crops which include wheat, maize and rice are extensively

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J. M. Al-Khayri et al. (eds.), *Advances in Plant Breeding Strategies: Cereals*,
https://doi.org/10.1007/978-3-030-23108-8_10

cultivated globally and have been given emphasis by the global research community. However, minor crops which are also known as underutilized, orphan, or disadvantaged crops, play a key role in the livelihood of a large proportion of the population in the developing world as they are the main source of nutrition and income. The importance of orphan crops is also due to the high adaptation of these indigenous crops to the prevailing extreme environmental conditions and to preference by consumers (Tadele 2010, 2017).

Tef or teff [*Eragrostis tef* (Zucc.) Trotter], a cereal crop extensively cultivated in the Horn of Africa, is the focus of this chapter. Tef is the most preferred crop among farmers and consumers in Ethiopia. Farmers choose to grow tef due to its resilience to extreme environmental conditions and high quality of bread, *injera*, made from its grain. Tef is the primary crop in Ethiopia in terms of the area under cultivation. It is annually grown on over three million hectares, which is equivalent to about 30% of the country's total area allocated to cereals (CSA 2015) (Fig. 10.1a). About 6.5 million smallholder farmers grow the crop every year. The importance of tef to the economy of Ethiopia is witnessed by the continuous expansion in the area under cultivation and also to the simultaneous increase in productivity. In the last 20 years, the area under tef cultivation has increased by 55% (from 1.8 million to 2.9 million mt) while productivity rose by 123% (from 0.7 to 1.56 mt ha^{-1}) (CSA 2002, 2015) (Fig. 10.1c). This demonstrates that the increase in the area was less than that of the increase in the productivity. Due to this significant increase in productivity, about 4.8 million mt of tef grain was produced in 2015, which is a 50% increase in production over two decades ago. However, the expected productivity level has not yet reached the national level due to little dissemination and adoption of improved varieties and cultural practices. As a result, the national grain yield of tef is the lowest among major cereals cultivated in the country (Fig. 10.1b).

The extensive cultivation of tef in Ethiopia is related to the high price of the tef grain compared to other cereals. The market price of tef and other cereals in Addis Ababa market was USD 600 mt^{-1} in 2015 (Minten et al. 2016) although the price of tef grain has doubled in early 2018. In general, the price of tef is higher than maize, sorghum and wheat by 37, 41 and 53%, respectively (FEWS-NET 2017). Consumers in Ethiopia pay the highest price for tef grain due to the good quality bread called *injera* which is made from tef flour. *Injera*, a spongy, flat bread baked after 2–3 days of fermentation, is considered the national bread eaten with all types of stews.

Large-scale cultivation of tef is also related to the resilience of the plant to several abiotic and biotic stresses, which include both excess and scarce moisture, pests and diseases. For instance, tef is the crop of choice in the poorly drained soils, especially Vertisols, which occupy about 10% of the arable land in the country, as it tolerates the stress, especially at the early growth stage, which coincides with heavy seasonal rainfall.

Tef is a very nutritious cereal grain. Its nutritional content is generally comparable to that of the major world cereals like wheat, rice, barley and millets (Table 10.1) (USDA 2018). Tef is superior in many aspects particularly in minerals such as calcium, iron, magnesium, phosphorus and potassium. Tef grains are also rich in essential amino acids, particularly in alanine, methionine, threonine and

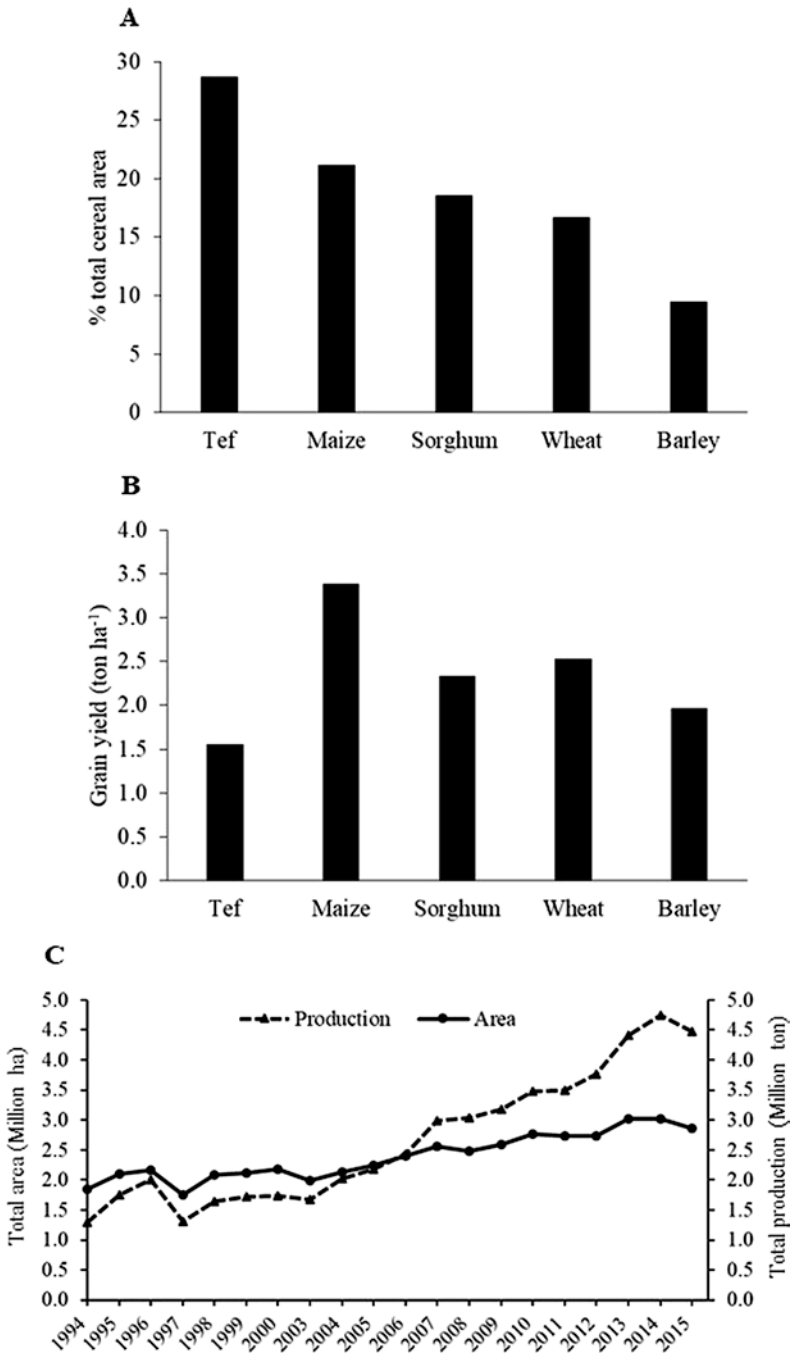

Fig. 10.1 The significance of tef in Ethiopia. (**a**) the proportion of area under tef and other major cereals in 2015. (**b**) The productivity of tef and other cereals in 2015. (**c**) The area under tef cultivation and total production from 1994 to 2015. (Source: Updated from CSA 2002, 2015)

Table 10.1 Nutritional composition of cooked tef grains (per 100 g) compared to wheat, rice, barley and millet

Item	Unit	Tef	Wheat	Rice	Barley	Millet
Proximates						
Energy	Kcal	101.00	132.00	97.00	123.00	119.00
Protein	g	3.87	5.71	2.02	2.26	3.51
Fat	g	0.69	0.83	0.19	0.44	1.00
Carbohydrate	g	19.86	27.60	21.09	28.22	23.67
Fiber	g	2.80	4.30	1.00	3.80	1.30
Minerals						
Calcium	mg	49.00	9.00	2.00	11.00	3.00
Iron	mg	2.00	1.76	0.14	1.33	0.63
Magnesium	mg	50.00	48.00	5.00	22.00	44.00
Phosphorus	mg	120.00	147.00	8.00	54.00	100.00
Potassium	mg	107.00	164.00	10.00	93.00	62.00
Sodium	mg	8.00	8.00	5.00	3.00	2.00
Amino acids						
Alanine	g	0.22	0.21	0.12	0.09	0.31
Arginine	g	0.15	0.27	0.17	0.11	0.12
Aspartic acid	g	0.24	0.31	0.19	0.14	0.23
Cystine	g	0.07	0.12	0.04	0.05	0.07
Glutamic acid	g	0.98	1.88	0.39	0.59	0.76
Glycine	g	0.14	0.23	0.09	0.08	0.09
Histidine	g	0.09	0.15	0.05	0.05	0.08
Isoleucine	g	0.15	0.22	0.09	0.08	0.15
Leucine	g	0.31	0.43	0.17	0.15	0.45
Lysine	g	0.11	0.16	0.07	0.08	0.07
Methionine	g	0.13	0.10	0.05	0.04	0.07
Phenylalanine	g	0.20	0.30	0.11	0.13	0.19
Proline	g	0.19	0.62	0.10	0.27	0.28
Serine	g	0.18	0.30	0.11	0.10	0.21
Threonine	g	0.15	0.17	0.07	0.08	0.11
Tryptophan	g	0.04	0.05	0.02	0.04	0.04
Tyrosine	g	0.13	0.14	0.07	0.07	0.11
Valine	g	0.20	0.27	0.12	0.11	0.18
Vitamins						
Thiamin	mg	0.18	0.10	0.02	0.08	0.11
Riboflavin	mg	0.03	0.03	0.01	0.06	0.08
Niacin	mg	0.91	2.31	0.29	2.06	1.33
Vitamin B6	mg	0.10	0.07	0.03	0.12	0.11

Source: Adapted from USDA (2018)

tyrosine (USDA 2018). In recent years, tef has become popular as a health and performance food in the global market. Since the grain is gluten-free, it is useful as food for humans suffering from the gluten protein allergy ailment known as celiac disease (Spaenij-Dekking et al. 2005). Several studies have been done to investigate the application of tef in gluten-free bakery products (Nascimento et al. 2018). In these cases, the type of tef products tested include bread, cookies, *injera*, muffins and pasta. The low glycemic index characterized by slow release type starches, also make tef suitable for diabetic patients (Baye 2014).

In addition to its grain, tef straw is the most palatable livestock feed and, therefore, fetches a high price compared to straw from other cereals (Yami 2013). In general, this shows the significance of tef in the livelihood of both farmers and consumers in Ethiopia.

With an ultimate objective of providing an overview of tef breeding in Ethiopia, this chapter emphasizes, first, the significance of the crop and subsequently the status of the achievements made with respect to the various methods of breeding thus far employed in its improvement. To this end, attempts have been made to summarize the cultivation and traditional breeding, germplasm biodiversity and conservation and conventional, mutation and molecular-breeding approaches, including genetic engineering employed in tef. Finally, conclusions and prospects are made on the basis of the foregoing highlights and expectations.

10.2 Botanical Classification, Domestication and Distribution

Tef belongs to the Grass or Poaceae family, subfamily Chloridoideae, tribe Eragrostideae and genus *Eragrostis*. Among the cultivated cereals, tef and finger millet (*Eleusine coracana* (L.) Gaertn.) belong to the subfamily Chloridoideae. The relationship between tef and other cultivated cereals were reported by Assefa et al. (2017) and Cannarozzi et al. (2018).

The botanical name of tef has undergone several changes until it settled on the current one. Synonymous names for tef were *Poa tef* Zuccagni in 1775, *Poa abyssinica* Jacquin in 1781, *Eragrostis abyssinica* (Jacq.) Link in 1827, *Eragrostis pilosa* ssp. *abyssinica* (Jacq.) Asch. and Graeben. in 1900, and finally *Eragrostis tef* (Zucc.) Trotter in 1918 (Ebba 1975).

Ethiopia is the center of the origin and diversity for tef (Vavilov 1951). However, the exact date and location for the domestication of tef is not known. There is no doubt that it is a very ancient crop in Ethiopia, where domestication took place before the birth of Christ (Ketema 1997). According to Ponti (1978), tef was introduced to Ethiopia well before the Semitic invasion of 1000–4000 BC. It was probably cultivated in Ethiopia even before the ancient introduction of emmer and barley. Tef has been introduced to different parts of the world through diverse institutions and individuals. The Royal Botanic Gardens, Kew, London, obtained tef seeds from Ethiopia in 1866 and 1886 and distributed them to some of the British Colonies (India, Australia, the USA, South Africa and Guyana). According to Ebba

(1975), Burt Davy in 1916 introduced tef to California (USA), Malawi, Zaire, India, Sri Lanka, Australia, New Zealand and Argentina; Skyes in 1911 introduced it to Zimbabwe, Mozambique, Kenya, Uganda, and Tanzania; and in 1940, Horuitz introduced tef to Palestine. Tef makes excellent hay in all these places.

Ethiopia is the center of both the origin and diversity for tef due to the existence of large diversity in the crop and the presence of wild progenitors. Scientific evidence from archeological remains show that tef was domesticated in Northern Ethiopia during the pre-Axumite period from 800 to 400 BC (D'Andrea 2008). Farmers in Ethiopia deserve high praise for maintaining this hardy crop over generations; except for being grown on a limited scale in Eritrea, formerly part of Ethiopia, no other country produces tef for human food.

Over the years, tef has been improved in Ethiopia through natural selection and by farmers' selection for desirable traits. As a result, greater diversity in terms of agronomic characteristics has enhanced the value of the tef genetic resources found in Ethiopia.

10.3 Cultivation and Traditional Breeding

Tef is mainly grown by smallholder farmers in Ethiopia. Most cultural practices show little improvement over the last several centuries. Plowing is in most places done with a pair of oxen using a traditional plow called a *maresha*.

Farmers sow tef by broadcasting the seed on top of the soil at a rate of 25–30 kg ha^{-1}, which is 2–3 times higher than recommended by researchers. Weed control is mainly done by hand weeding, but in recent years, the broadleaf herbicide 2,4-D is also widely used. Harvesting is also done manually using a sickle, while threshing uses oxen. Postharvest loss is high since tef seeds are extremely small, predisposing seed loss.

Tef provides a number of benefits to smallholder farmers in Ethiopia as the crop is resilient to a variety of biotic and abiotic stresses, which more seriously affect other cereal crops leading to greater losses from these environmental difficulties. Farmers also use tef for income generation due to the higher price of the grain, compared to other cereal grains (Minten et al. 2016). Despite these benefits, the cultivation of tef faces a number of constraints among which the major ones are indicated below:

(a) Inherent characteristics of the plant: Tef possess a tall and weak stem which renders it susceptible to lodging, the shoot falling over onto the ground (Assefa et al. 2011b). The roots of tef have also poor anchorage in the soil (Van Delden et al. 2009). Hence, tef plants suffer from lodging which is exacerbated by rain and wind, and when nitrogenous fertilizers are applied to enhance growth and yield of the crop.
(b) Extreme environmental conditions: Compared to other cereals, tef is more tolerant of extreme soil and climatic conditions. However, tef also suffers from

severe abiotic stresses prevalent in different parts of Ethiopia including drought, waterlogging, frost and soil acidity (Tadele 2016a).

(c) Being an orphan crop: Similar to other understudied crops, tef has received very little attention by the global scientific community, which classifies the crop as an orphan or neglected crop (Tadele 2014; Tadele and Assefa 2012). However, a few donor organizations including the McKnight Foundation and Syngenta Foundation for Sustainable Agriculture have been providing support for both research and development which have improved varieties harboring traits of interest that have been disseminated to farmers.

Ethiopian farmers have maintained tef over millennia by selecting and preserving types with traits of their interest which include high grain yield, nonshattering and resilience to biotic and abiotic stresses. Scientific research on tef started in 1950s at the then Debre Zeit Experimental Station. Selection from natural accessions was the only improvement method until 1974 when the first crosses were successfully made (Berhe 1975), following the discovery of the chasmogamous behavior of tef flowers. Consequently, the development of an artificial binocular-aided surgical hand emasculation and pollination technique paved the way for improving tef using hybridization. Details on tef breeding regarding techniques and achievements are provided in the next sections.

10.4 Germplasm Biodiversity and Conservation

10.4.1 Germplasm Diversity

Ethiopia is both the origin and center of diversity for tef, providing the country with a rich array of tef germplasm resources. Over 5000 tef germplasm accessions collected from diverse tef growing areas in the country are available at the Ethiopian Biodiversity Institute (EBI) (Tesema 2013). However, thus far only limited studies have been made to investigate the genetic diversity existing in these populations.

Diversity in tef has been studied for different traits, especially for important agronomic traits (Assefa et al. 2000; Chanyalew et al. 2009; Hundera et al. 1999; Jifar et al. 2015; Plaza-Wüthrich et al. 2013; Tefera et al. 1990). Although most diversity studies have been made on white-seeded tef germplasm, Jifar et al. (2015) reported diversity among 36 brown-seeded tef genotypes based on investigation at three tef growing areas in Central Ethiopia. This study of brown-seeded tef is important since food products from this type of grain are becoming popular due to its nutritional superiority over the white-seeded type. A recent review showed the extent of diversity in tef germplasm for diverse qualities, which included agronomic, nutritional and molecular traits (Assefa et al. 2015). The range of variability reported for selected traits is shown in Fig. 10.2. Traits with more than a six-fold higher value than the minimum include plant height, panicle length, peduncle length, kernel weight and harvest index. Traits with values 4–5 fold higher than the

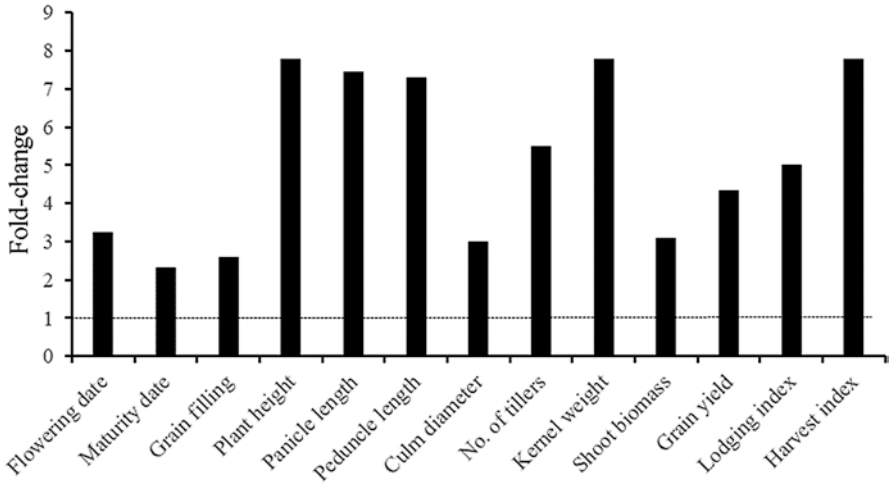

Fig. 10.2 Range of diversity for important agronomic traits in tef as shown in fold change between the minimum value (set to 1: dotted line) and maximum value shown in bar for each trait. (Source: Adapted from Assefa et al. 2000; Chanyalew et al. 2009; Hundera et al. 1999; Tefera et al. 1990)

minimum values were number of tillers, grain yield and lodging index. On the other hand, traits with modest diversity included days to flowering and maturity, and culm diameter. In general, this demonstrates that tef germplasm is rich in important agronomic and nutritional traits which can be exploited through selection and introgression.

10.4.2 Cultivar Characterization and Phylogeny

Ebba (1975) carried out the first scientific study of tef germplasm characterization and classification. It was based on morphological properties which include phenology, plant vigor, panicle form and ramification pattern, spikelet size, and lemma and caryopsis color. This detailed research identified 35 distinct tef cultivars or ecotypes which have been adopted in subsequent studies of germplasm characterization and groupings. A selection of 7 of the characters or traits used to characterize the 35 ecotypes are included in Table 10.2. These 35 ecotypes showed huge diversity in all of the characters studied. Among the ecotypes, the range for selected traits were plant height from 34 cm (Bunniye) to 95 cm (Murri), days to heading from 25 days (Gea-Lamie) to 60 days (Curati) and days to maturity from 60 (Gea-Lamie, Shewa Gemerra) to 120 days (Alba, Curati, Murri). These ecotypes also showed great diversity in the panicle form which ranged from very loose to very compact (Assefa et al. 2017). The variability among tef accessions for those indicated and other traits has been the focus of tef breeders in selecting genotypes of interest.

Table 10.2 Selected traits of 35 tef ecotypes representing tef germplasm

No.	Ecotype (cultivar) name	Plant height (cm)	Culm diameter (cm)	Panicle form[a]	Lemma color[b]	Seed color[c]	Days to	
							Heading	Mature
1	Ada	80	1.8	S-comp	PYG	yWh	45–50	95–115
2	Addisie	80	1.9	V-comp	PYG	yWh	45–50	95–110
3	Adoensis	70	1.6	V-loose	PYG	mBr	45–50	90–95
4	Alba	85	2.2	F-loose	PYG	yWh	45–50	95–120
5	Balami	88	1.8	V-loose	D-purple	yWh	40–45	90–110
6	Beten	70	1.6	V-loose	PYG	yWh	40–45	85–95
7	Bunniye	34	1.0	V-loose	PYG-green	mBr	35–40	75–85
8	Burssa	58	1.5	S-comp	G-green	yWh	45–50	85–90
9	Curati	88	2.3	S-comp	PYG	poW	50–60	95–120
10	Dabbi	70	1.6	V-loose	G-purple	mBr	40–45	80–95
11	Denkeye	60	1.5	S-comp	G-green	mBr	45–50	90–115
12	Dschanger	75	1.8	F-loose	G-green	mBr	40–65	90–110
13	Enatite	70	1.5	V-loose	PYG	yWh	40–45	90–100
14	Fesho	50	1.2	V-loose	D-purple	Br	38–45	75–85
15	Gea-Lamie	30	0.9	V-loose	G-green	Br	25–30	60–70
16	Gofarie	78	1.8	S-comp	PYG	yWh	45–50	90–100
17	Gommadie	75	2.0	S-comp	PYG	yWh	45–50	90–100
18	Gorradie	90	2.2	V-comp	PYG	yWh	50–55	95–120
19	Hamrawe Murri	75	1.8	V-comp	D-purple	yWh	50–55	90–100
20	Hatalla	90	2.1	V-loose	G-purple	yWh	50–55	90–115
21	Janno	75	2.0	F-loose	G-red	yWh	45–50	85–105
22	Karadebi	55	1.1	V-loose	Brown	Br	40–45	85–90
23	Kaye Agachem	77	1.8	V-comp	PYG	lBr	65–50	90–110
24	Kaye Murri	80	2.0	V-comp	D-red	yWh	45–50	90–105

(continued)

Table 10.2 (continued)

No.	Ecotype (cultivar) name	Plant height (cm)	Culm diameter (cm)	Panicle form[a]	Lemma color[b]	Seed color[c]	Days to	
							Heading	Mature
25	Manya	75	2.0	F-loose	PYG	yWh	40–45	90–110
26	Murri	95	2.3	V-comp	G-green	yWh	50–55	105–120
27	Purpurea	85	1.9	F-loose	D-red	Br	45–50	90–100
28	Rosea	75	2.0	F-loose	P-green	yWh	45–50	90–100
29	Rubicunda	85	2.4	F-loose	D-purple	yWh	45–50	90–115
30	Shawa-Gemerra	35	0.9	F-loose	PYG	Br	30–35	60–75
31	Trotteriana	70	1.6	V-comp	PYG	Br	50–55	90–95
32	Tullu Nasy	42	1.1	V-loose	PYG	poW	35–40	60–70
33	Variegata	70	1.8	F-loose	PYG	lBr	45–50	90–100
34	Viridis	75	2.2	F-loose	G-green	poW	45–50	85–95
35	Zuccagniana	65	1.3	V-comp	G-green	Br	45–50	90–100

Source: Adapted from Ebba (1975)

[a]Panicle form: *F-loose* fairly loose, *S-comp* semi-compact, *V-comp* very compact, *V-loose* very loose

[b]Lemma color: *D-purple* dark or deep purple, *D-red* dark or deep red, *G-Green* grayish, orange or olive green, *G-purple* grayish, orange or olive purple, *G-red* grayish red, *PYG* pale yellow green

[c]Seed color: *Br* brown, *mBr* medium brown, *lBr* light brown, *poW* purple orange white, *yWh* yellow white

Since this remarkable work, tef germplasm has been extensively studied using morphological, molecular and physiological parameters. As shown in Fig. 10.2, significant variabilities were reported for morphological and yield-related traits (Assefa et al. 2001b, 2003). Similarly, variabilities among diverse tef genotypes were studied using genetic markers such as microsatellites (simple sequence repeats (SSRs), genotyping by sequencing (GBS) and several other techniques (Chanyalew et al. 2013; Girma et al. 2018; Plaza-Wüthrich et al. 2013). Based on these studies, the relationships among tef germplasms were investigated using phylogenic trees constructed using morphological traits (Plaza-Wüthrich et al. 2013) and molecular markers, especially SSR markers (Assefa et al. 2015) (Fig. 10.3). Although phylogenetic trees constructed based on morphological traits provide useful information, due to inconsistencies in the values of some traits under field conditions, they are less accepted by researchers. On the contrary, those based on molecular markers provide consistent results as they are based on DNA sequences which are little altered by changes in environmental factors.

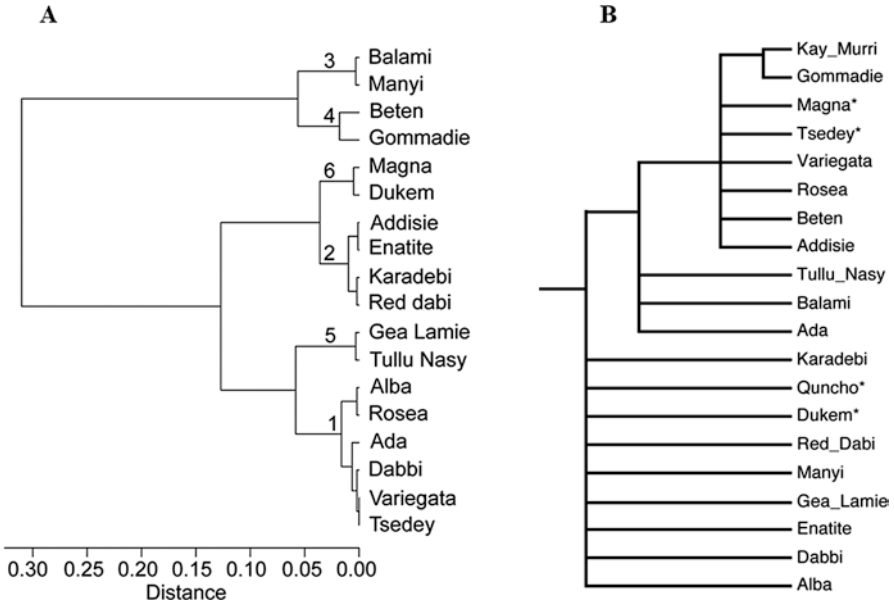

Fig. 10.3 Phylogenetic trees showing the relationship among natural accessions and improved varieties of tef. (**a**) Using morphological and yield-related traits. (Source: Plaza-Wüthrich et al. 2013). (**b**) Using SSR marker. (Source: Assefa et al. 2015)

10.4.3 Genetics and Cytogenetics

Studies involving key qualitative and quantitative traits in tef are briefly summarized as follows:

(a) Genetics of qualitative traits: Detailed studies were made to investigate the inheritance of three traits, namely lemma color, seed color and panicle form (Berhe et al. 1989a, b, c). These studies showed the involvement of multiple genes in the inheritance of the traits, and disomic inheritance patterns with no maternal effects.
(b) Genetics of quantitative traits: Several studies based on tef crosses showed that additive and epistatic gene effects controlled the inheritance of most yield and yield-related quantitative agronomic traits including grain yield (Tefera and Peat 1997a, b).
(c) Cytogenetics: Cytological studies showed that tef is an allotetraploid with 40 chromosomes (2n = 4× = 40) (Jones et al. 1978; Tavassoli 1986) although the true diploid ancestors remain unknown. Despite all tef genotypes having the same level of polyploidy, two independent flow cytometric studies using ten improved varieties and four accessions showed a genome size range of 648–926 Mbp (Ayele et al. 1996a; Hundera et al. 2000).

10.4.4 Germplasm Conservation

Tef germplasm consisting of over 5000 accessions collected from diverse tef-growing regions are preserved at the Ethiopian Biodiversity Institute (EBI). The seeds of these accessions are periodically grown and rejuvenated in the fields of research institutes in Ethiopia before their viability is drastically reduced. Research centers in the country, particularly Debre Zeit Agricultural Research Center, regularly grow improved varieties of tef at its main- or sub-station sites. Research centers belonging to respective regional research institutes also multiply seeds of improved varieties to provide to farmers and extensions agents requesting the seeds. In addition to launching periodic new collecting missions, EBI has in recent years been fostering in situ conservation of tef genetic resources in farmers' fields.

10.5 Molecular Breeding

Molecular markers provide an invaluable tool for studying genetic diversity and relationships, classification of germplasm, construction of genetic linkage maps, and in marker-assisted selection for breeding. A number of tef marker systems including restriction fragment length polymorphism (RFLP) (Zhang et al. 2001), amplified fragment length polymorphism (AFLP) (Ayele and Nguyen 2000; Ayele

et al. 1999; Bai et al. 1999a, b) and random amplified polymorphic DNA (RAPD) (Bai et al. 2000) have been developed and used for various purposes.

Mapping for quantitative trait loci (QTL) controlling key agronomic and morphological traits was made from 159 recombinant inbred lines derived from interspecific cross between *Eragrostis tef* and *E. pilosa*. The genetic map and list of traits investigated are indicated in Zeid et al. (2011). Although attempts were made in the past to develop a genetic map for tef (Yu et al. 2007), the map in Fig. 10.4 is the most comprehensive and up to date in terms of using more markers and including valuable traits. However, the resolution of this genetic map also needs to be substantially improved using additional markers already developed from recent studies. The availability of the tef genome sequence (Cannarozzi et al. 2014) enhances the discovery of new genetic markers, especially simple sequence repeat (SSR) markers. Genomic and proteomic tools have recently been employed to identify diversity and key traits in tef (Girma et al. 2018; Kamies et al. 2017).

By using expressed sequence tag (EST) from cDNA libraries, tef sequence specific markers have been developed such as expressed sequence tag derived simple sequence repeat (EST-SSR), intron fragment length polymorphism (IFLP), and single nucleotide polymorphism/insertion and deletion (SNP/INDEL) (Yu et al. 2006). Since these sequences were derived from the coding regions of genes, EST-derived markers are highly transferable to closely-related species. To that end, testing of 812 EST-derived markers from other grass species on tef revealed successful amplification of approximately 30% of the markers, and prominently EST-SSRs developed from sorghum and pearl millet (both belong to subfamily Panicoideae which is taxonomically close to the subfamily of tef, Chloridoideae) showed a transferability rate higher than 80% on tef (Assefa et al. 2017; Zeid et al. 2010).

The development of tef genomic SSR markers (gSSRs) alleviated the problem of low rate of polymorphism of EST-SSRs (Zeid et al. 2011). The genomic libraries were enriched for (AG) and (AC) dinucleotide repeats, and in tef the (AG) repeat occurs at a much higher frequency as compared to other grass species such as barley, rice and wheat. A total of 561 gSSRs were developed and 48% of the markers showed polymorphism on *Eragrostis tef* (Kaye Murri) and *E. pilosa* (Zeid et al. 2011). This indicates that the rate of polymorphism of gSSRS is twice as high as the EST-derived markers in tef (Yu et al. 2006). Presently, there are more than 1500 locus-specific tef markers available for use in genetic studies (Assefa et al. 2017).

10.6 Biotechnology

Biotechnology is a broad topic, although there is a common understanding that if refers to genetically-modified organisms (GMOs) and tissue culture techniques. Except for few preliminary investigations (Gebre et al. 2013; Mekbib et al. 2001; Mengiste 1991; Plaza-Wüthrich and Tadele 2013) detailed genetic engineering studies which resulted in plants with phenotypes of interest have not yet been found for tef.

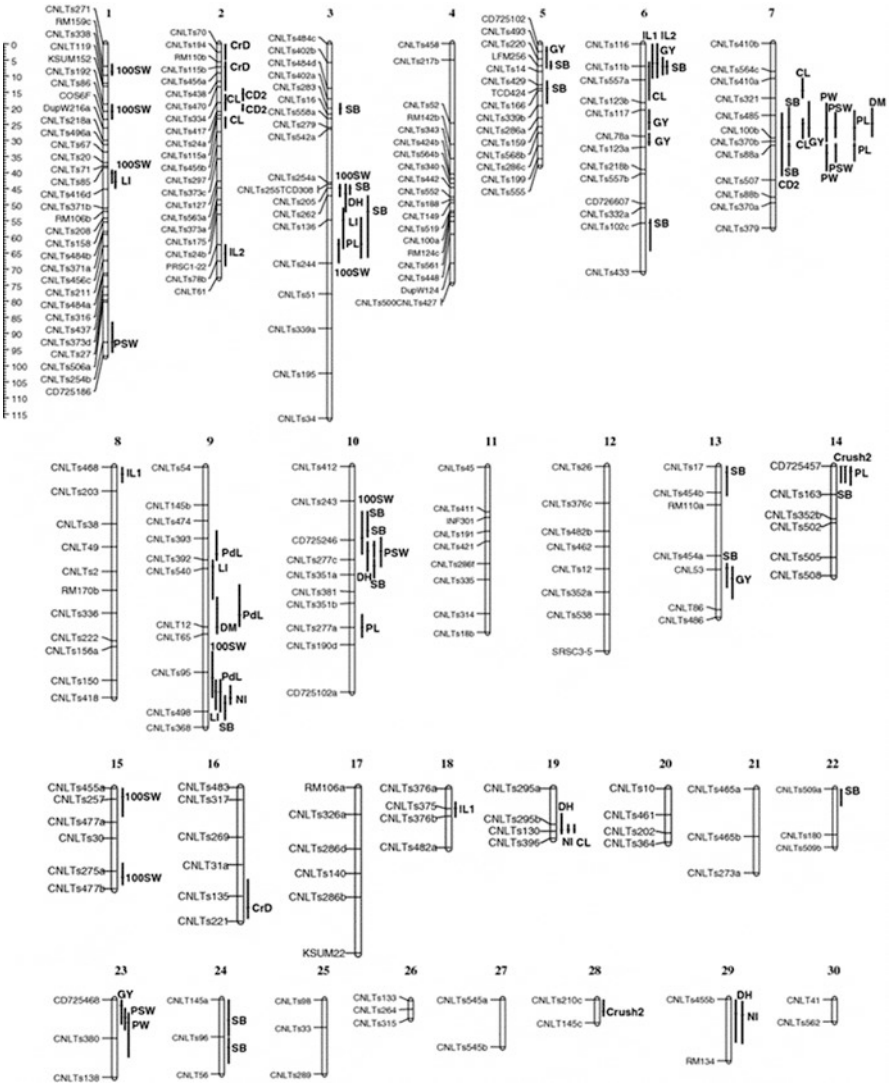

Fig. 10.4 Molecular linkage map of tef showing QTLs for important traits obtained from recombinant inbred lines *Eragrostis tef* × *E. pilosa*. Marker names are positioned equivalent to their cM distances on the left of the bars. Intervals for each QTL are indicated by the length of the vertical closed bars to the right of the linkage groups, and the name of the trait underlying each QTL is given to the right of the linkage map. Abbreviations. *100SW* 100 seed weight, *CD1* culm diameter at the 1st internode, *CD2* culm diameter at the 2nd internode, *CL* culm length, *Crush1* crushing strength at 1st internode, *Crush2* crushing strength at 2nd internode, *DH* days to heading, *DM* days to maturity, *GY* grain yield, *IL1* length of 1st internode, *IL2* length of 2nd internode, *LI* lodging index, *NI* number of internodes, *PL* panicle length, *PdL* peduncle length, *PSW* panicle seed weight, *PW* panicle weight, *SB* shoot biomass per plant. (Source: Zeid et al. 2011)

In vitro regeneration, or plant tissue culture, is an asexual method of propagation to produce clones in large quantities from an explant. This ability of the plant to develop from tissue into a whole plant after undergoing several regeneration steps is called totipotency. An efficient in vitro regeneration system is necessary for the genetic improvement of a crop which includes mutation breeding, somatic hybridization and genetic transformation. A number of in vitro regeneration studies were made on tef using a variety of explant types, genotypes and techniques. Explants from leaf parts, young seedling roots, mature seeds and immature embryos were investigated to determine the best explant for efficient regeneration (Ayele et al. 1996b; Bekele et al. 1995; Gugsa and Kumlehn 2011; Gugsa and Loerz 2013; Gugsa et al. 2006, 2009; Kebebew et al. 1998; Mekbib et al. 1997; Plaza-Wüthrich and Tadele 2012, 2013). Among these explants, immature embryos were found to be more efficient than the others (Gugsa and Kumlehn 2011; Plaza-Wüthrich et al. 2015). The procedure and the time required for each step of regeneration, starting from isolating immature embryos to obtaining fully-developed plants grown in soil are shown in Fig. 10.5. Immature embryos pass through either somatic embryos or callus before plantlets are formed. Fully-developed tef plants grown in soil can be achieved in 4 months after isolating immature embryos from tef flowers and placement on appropriate growth media.

The efficiency of regeneration is mainly dependent on the type of the genotype. Using the immature embryo technique, over 80% of explants from the natural tef accession Manya formed somatic embryogenesis while this was only 10% from the improved variety Tsedey (Plaza-Wüthrich and Tadele 2013). The proportions of explants developing into plantlets were also significantly low for Tsedey compared to Manya. Hence, it is important to first study the regeneration efficiency of diverse tef ecotypes or varieties before embarking on a large-scale study using a single or limited numbers of germplasm accessions.

Plant transformation refers to the introduction of genetic material into plant cells, tissue or organs in order to alter the trait(s) or phenotype of the plant. It is commonly

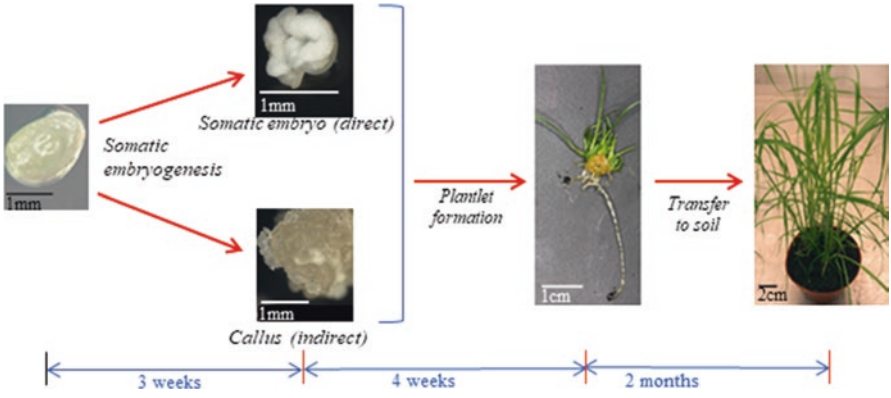

Fig. 10.5 Procedure and time taken for in vitro regenerated tef from immature embryo. (Source: Sonia Plaza-Wüthrich)

done using the *Agrobacterium* and the particle or microprojectile bombardment method. Similar to other monocot plants, tef tissue for transformation is recalcitrant to *Agrobacterium*-mediated transformation. However, an earlier study showed the attachment of *Agrobacterium* to the tef embryo, seed, seedling, leaf and callus explants, although the intensity of attachment was significantly different among the explants (Mekbib et al. 2001). Among three *Agrobacterium* strains investigated by another study, LBA4404 and EHA105 were more efficient or virulent in transient tef transformation compared to GV3101 (Plaza-Wüthrich and Tadele 2013). Stable transformation in tef was recently reported using gibberellic acid (GA) inactivating a gene under the control of triple 35S promoter (Gebre et al. 2013). According to the authors, despite inconsistencies in some results, semi-dwarf tef plants with a reduced level of endogenous GA were obtained.

Particle bombardment (also known as biolistic or gene gun) refers to delivery of the gene of interest into plant tissue using high-velocity microprojectiles that have the ability to penetrate the cell wall so that genetic material can be transferred into the cell. Only a few studies have investigated the potential of using particle bombardment in tef transformation. The efficacy of the methods were studied using a reporter gene under the control of different promoters. Transient expression of the reporter β-glucuronidase (GUS) gene using 35S promoter was noted in cell suspension cultures, callus tissue and zygotic embryos of tef (Mengiste 1991). On the other hand, an equal level of transient expression of GUS for three promoters (ubiquitin, actin, double 35S) was observed in tef callus derived from immature embryos (Plaza-Wüthrich and Tadele 2013). In general, despite some previous studies, an efficient and simple protocol for routine transformation of tef has not yet been established.

10.7 Mutation Breeding

Mutagenesis refers to the stable and heritable alteration of the genetic material of an organism. Although mutagenesis normally refers to the creation of a mutation, three categories are identified, especially in considering the utilization of mutations in crop improvement. These are mutation induction, mutation detection and mutation breeding (IAEA 2018). While mutations are induced or created using physical or chemical mutagens, the sites of mutations in the plant genome are detected using a number of molecular techniques (Tadele 2016b). The third and the most important part of mutagenesis is to incorporate the mutation into a breeding program in order to obtain a crop with enhanced trait(s) of interest.

Over the last 70 years, mutation breeding has contributed significantly to the improvement of many economically-important crops. Crops descended from using this technique were superior to the original cultivars in productivity and/or tolerance to biotic and abiotic stresses. The list of officially released and/or commercially available crop varieties originated from induced mutation are available in the Mutant Variety Database (MVD) of the Joint IAEA/FAO Program (IAEA 2018). According

to this database, since the first variety was released in 1966 in China, 3275 crop varieties derived from mutation breeding have been officially released in a large number of countries, mainly in Asia and Europe. In Africa, only 69 crop varieties were released through mutation breeding, and of these 25 are rice varieties in Cote D'Ivoire, and 15 are rice and sorghum varieties in Mali. On the other hand, Asian countries were advanced in mutation breeding by releasing 61% of the total released varieties. The three leading countries in releasing high numbers of varieties are China (810), Japan (479) and India (335). This shows that Africa benefited little from mutation breeding in improving its indigenous crops.

In the early phase of tef breeding, until the discovery of the hybridization technique in the mid 1970s (Berhe 1975), genetic improvement relied solely upon pure line or mass selection. Because of this, induced mutation techniques were introduced into the tef breeding program in 1972 through the cooperation of the International Atomic Energy Agency (IAEA) and the Food and Agriculture Organization (FAO) of the United Nations. From this work, gamma irradiation dose of 250 Krad, X-ray dose of 100–130 Krad and ethyl-methane sulfonate (EMS) concentrations of 2.5–4.0%, were recommended for seed treatment to induce mutations in tef (Ketema 1993). However, desirable mutants were not identified from either the earlier works or the consequent latter efforts made with the application of conventional induced mutation techniques.

Targeting induced local lesions in genomes (TILLING): High-throughput techniques, such as TILLING and Eco-TILLING, are attractive methods for tef improvement, since the products from these techniques do not require biosafety regulations as they are free of transgenes. TILLING is a reverse genetic technique which uses traditional mutagenesis followed by high-throughput mutation detection. While TILLING is applied to the induced mutagenized population, EcoTILLING is used in the natural population. The TILLING technique has been implemented on EMS (ethyl methanesulfonate) mutagenized tef population at the University of Bern, Switzerland, mainly to develop semi-dwarf and lodging-resistant cultivars (Esfeld et al. 2013a, b; Tadele et al. 2010). Since tef is a tetraploid, mutation in a single genome might not result in the expected phenotype, hence double crossings were made between candidate lines harboring point mutation in the two copies of the tef genome. The crossing and field testing of the breeding materials are done at the experimental site of the Debre Zeit Agricultural Research Center in Ethiopia. Unlike the above technique which uses a LiCOR machine to detect point mutations, next-generation sequencing was also applied to validate six mutations in EMS mutagenized tef population (Zhu et al. 2012). TILLING and mutation breeding enabled tef researchers to discover mutant lines with desirable traits such as semi-dwarf, lodging resistance, drought tolerance and acid soil tolerance, which were later incorporated into the national breeding program to enhance productivity (Cannarozzi et al. 2018; Desta et al. 2017; Jifar et al. 2017; Jöst et al. 2015; Zhu et al. 2012).

10.8 Hybridization and Breeding

10.8.1 Floral Biology

The paniculate tef inflorescence varies in form ranging from very compact whip-like or rat-tail-like type, with the branches appearing fused to the rachis to very loose open and laterally-spreading types (Assefa et al. 2017). Broadly, four major panicle forms are distinguished: very compact, semi-compact, fairly loose and very loose. The panicle branches bear numerous spikelets varying from 30–1070 per panicle (Assefa et al. 2001a). The spikelets are laterally compressed with a flexuous rachilla (with 3–18 nodes and about 1 mm long internodes) borne on a pedicel up to 2 mm long (Ebba 1975). The spikelets are generally linear, oblong to lanceolate in shape and each 3.0–15.0 mm long and 1.0–3.0 mm wide at the broadest part (Assefa et al. 2017). Each spikelet has 2 unequal-sized glumes at the base and a number of florets above. The color of the young glumes can generally be grayish-olive green, dark red, purple, yellow-green or variegated flecked with dark purple or dark red on a grayish yellow-green or grayish olive-green background. The tef florets (3–17 per spikelet) are characterized by asynchronous development which is basipetal on a panicle basis and acropetal on a spikelet basis. Each floret comprises a 3-nerved lemma, a 2-nerved bow-shaped palea, 3 stamens arising from near the ovary base and having very fine slender filaments apically bearing 2-celled length-wise opening anthers, and a pistil or an ovary (Assefa et al. 2017; Ebba 1975) Fig. 10.6. The ovary has 2 or in a few exceptional cases 3 styles, each ending in a plumose (feathery) yellowish white stigma. In addition to genotypic differences, the number of florets or kernels per spikelet and the size of the spikelet vary depending on the particular position along the panicle, the highest and the largest at the top and diminishing toward the base of the panicle.

Fig. 10.6 The morphology of tef flower indicating 3 stamens and a pair of hairy stigmas. (Source: Regula Blösch)

10.8.2 Breeding Behavior

As described above, the tef floret is a hermaphrodite with 3 stamens and 2 stigmas. As a result, tef is an autogamous species, and as such it was thought to be entirely cleistogamous (closed flowers) with no options for outcrossing until the discovery by (Berhe 1975) of its chasmogamous nature that revealed the opening of the florets early in the morning (about 0645–0745 h). The rate of natural outcrossing in tef is 0.2% in the field and 0.05–1.37% in the greenhouse (Kedir et al. 1992). Hence, due to this very low level of outcrossing, tef is considered as a strictly self-pollinated crop. Based on the breakthrough discovery of the chasmogamous nature of tef florets, (Berhe 1975) developed the artificial surgical hybridization method for tef which is still in use in the hybridization program. Accordingly, the conventional binocular-aided tef crossing involves emasculation of the maternal parent (by removal of the 3 stamens) the day before at about 1600–1900 h, storage of the paternal and maternal parents separately under dark and cold conditions in a refrigerator (4 °C) overnight, and collection of pollen from the paternal parent and subsequent brushing of the pollens over the stigma of the previously emasculated florets of the maternal parent. Other methods for inducing male sterility using male-selective gametocidal chemical treatments such as ethephon (ethrel) at flag leaf stage, although phytotoxic at a high concentration has shown some promise (Berhe and Miller 1978; Ketema 1983, 1993). In spite of attempts made to find alternative methods of emasculation, however, the most practicable method for tef hybridization remains the surgical binocular-aided hand emasculation and pollination technique.

10.8.3 Hybridization

In tef, hybridization involves mainly intraspecific crosses and recently some interspecific crossings, especially with *Eragrostis pilosa*. A total of about 620 crosses have been made at the Debre Zeit Agricultural Research Center. Subsequent segregating populations are handled using the combination of modified bulk population (F_2–F_5) and modified pedigree (starting from F_5) methods of breeding. However, some varieties have been developed as recombinant inbred lines (RILs) through the F_2-derived single seed descent (SSD) method. The tef breeding scheme from hybridization to variety development and dissemination is shown in Fig. 10.7.

In the SSD method, the starting materials at the F_2 generation are 500 seeds obtained from an individual F_1 plant. Promotion of a single seed to the next generation is made until the F_6 generation. This is followed by an observation nursery (ON) where selected plants at F_6 are grown, each in a single row. Selected lines from ON are promoted to PVT (pre-variety trial) where a limited number of lines are tested each on 1 m^2 plot at a minimum of 5 locations using 2 replications. At the NVT (national variety trial), 10–20 lines selected from PVT are grown, each on

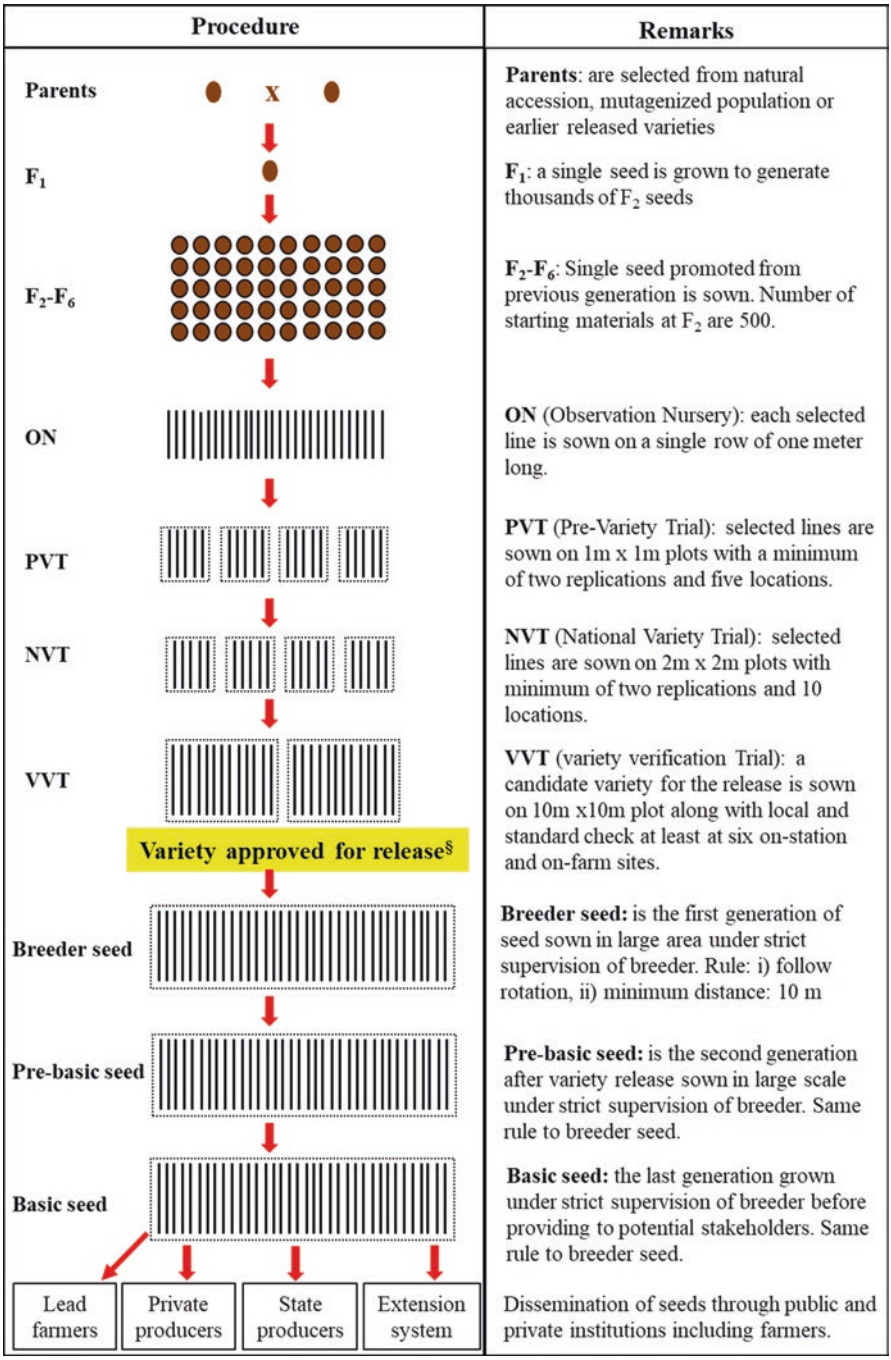

Fig. 10.7 Tef breeding scheme adopted by the National Tef Breeding Program through hybridization, early generation seed multiplication and dissemination of seeds. §Based on the field performance of the candidate variety at VVT and data from NVT and VVT, the National Variety Release Committee approves the new variety for distribution

4 m^2 plots located in at least at 10 representative tef-growing areas. Based on field performance of the candidate variety at VVT (variety verification) and data from NVT and PVT, the National Variety Release Committee approves the new variety for distribution.

10.9 Variety Development and Dissemination

10.9.1 Improved Tef Varieties

As shown in Fig. 10.7, new tef varieties are approved for release after evaluating the performance of the candidate variety at the PVT, NVT and direct field observation at VVT (variety verification). At the VVT, the candidate variety is grown along with the local and standard control varieties, each on 100 m^2 plot in at least five representative sites.

To date, 42 improved tef varieties have been released in Ethiopia through the National Agricultural Research System (MoANR 2017) (Table 10.3). Out of these, 18 were obtained through hybridization, while the remaining 24 were developed through direct selection from farmers' varieties. Except for the very early-maturing variety Simada, which resulted from the inter-specific cross of the tef line DZ-01-2785 and *Eragrostis pilosa* (accession 30-5), all the other varieties developed through hybridization are from intraspecific crosses. By center, 27 of the varieties were released by the federal research centers of EIAR, 24 by Debre Zeit, 2 by Holetta and 1 by Melkassa while those released by the regional centers were 6 by Sirinka, 5 by Adet and 2 each by Areka and Bako.

Of the total released tef varieties in the country, 30 were developed for optimum rainfall areas, 10 for terminal drought-prone areas and 2 for cool highland areas (Table 10.3). Among improved tef varieties so far, Quncho, Boset, Tsedey and Magna have received high acceptance by farmers. Quncho, with its very white seed color and high grain yield, is the most popular in almost all agro-ecological regions where tef is cultivated (Assefa et al. 2011a). Boset and Tsedey are early-maturing varieties that perform best in the vast drought-prone areas in the country, especially those which suffer from the drought during normal crop maturity. On the other hand, Magna is not a high-yielding variety but due to its extremely white seed color, the grain fetches the highest price in the market.

The genetic gain from tef breeding in Ethiopia was 0.8% per year under lodging-controlled condition by growing the varieties released up to 1995 through wire-mesh support (Teklu and Tefera 2005), while it was 0.58% per year under lodging-uncontrolled natural field conditions for the varieties released up to 2012 (Dargo and Mekbib 2017).

Increased biomass yield, plant height, panicle length, number of spikelets per panicle, grain yield per panicle and rates of phytomass production and grain filling were characteristic of improved tef varieties, while the varieties released through

Table 10.3 Improved tef varieties released in Ethiopia for different environmental conditions

Name		Variety release		Breeding method	Days to mature	Seed color	On-farm grain yield (mt ha^{-1})
Common name	Variety name	Year	Center				
Varieties for optimum rainfall areas							
Asgori	DZ-01-99	1970	Debre Zeit	Selection	80–130	Brown	1.7–2.2
Enatite	DZ-01-354	1970	Debre Zeit	Selection	85–130	Pale white	1.7–2.2
Magna	DZ-01-196	1978	Debre Zeit	Selection	80–113	Very white	1.4–1.6
Wellenkomi	DZ-01-787	1978	Debre Zeit	Selection	90–130	Pale white	1.7–2.2
Menagesha	DZ-Cr-44	1982	Debre Zeit	Hybridization	125–140	White	1.7–2.2
Melko	DZ-Cr-82	1982	Debre Zeit	Hybridization	112–119	White	1.8–2.2
Gibe	DZ-Cr-255	1993	Debre Zeit	Hybridization	114–126	White	1.6–2.2
Dukem	DZ-01-974	1995	Debre Zeit	Selection	76–138	White	2.0–2.5
Ziquala	DZ-Cr-358	1995	Debre Zeit	Hybridization	75–137	White	1.8–2.4
Holetta Key	DZ-01-2053	1999	Holetta	Selection	124–140	Brown	2.5
Ambo Toke	DZ-01-1278	2000	Holetta	Selection	125–140	White	2.7
Koye	DZ-01-1285	2002	Debre Zeit	Selection	104–118	White	1.8–2.5
Gola	DZ-01-2054	2003	Sirinka	Selection	68–100	White	1.6
Ajora	PGRC/E 205396	2004	Areka	Selection	85–110	White	1.14
Genete	DZ-01-146	2005	Sirinka	Selection	78–85	Pale white	1.55
Zobel	DZ-01-1821	2005	Sirinka	Selection	78–85	White	1.51
Yilmana	DZ-01-1868	2005	Adet	Selection	108	White	1.63
Dima	DZ-01-2423	2005	Adet	Selection	105	Brown	1.68
Quncho	DZ-Cr-387 RIL355	2006	Debre Zeit	Hybridization	80–113	Very white	2.0–2.2
Guduru	DZ-01-1880	2006	Bako	Selection	132	White	1.4–2.0
Kena	23-Tafi Adi-72	2008	Bako	Selection	110–134	Very white	1.3–2.3
Etsub	DZ-01-3186	2008	Adet	Selection	92–127	White	1.6–2.2
Kora	DZ-Cr-438 RIL133 B	2014	Debre Zeit	Hybridization	110–117	Very white	2.0–2.2
Dagim	DZ-Cr-438 RIL91A	2016	Debre Zeit	Hybridization	112–115	Very white	2.3–2-7

(continued)

Table 10.3 (continued)

Name		Variety release		Breeding method	Days to mature	Seed color	On-farm grain yield (mt ha^{-1})
Common name	Variety name	Year	Center				
Abola	DZ-Cr-438 RIL7	2016	Adet	Hybridization	98–112	Very white	1.5–1.7
Negus	DZ-Cr-429 RIL125	2017	Debre Zeit	Hybridization	102–118	Very white	2.3–2.6
Felagot	DZ-Cr-442 RIL77C	2017	Debre Zeit	Hybridization	94–102	Brown	2.0–2.6
Tesfa	DZ-Cr-457 RIL181	2017	Debre Zeit	Hybridization	99–120	White	2.0–2.7
Heber-1	DZ-Cr-419	2017	Adet	Hybridization	100–122	Very white	1.9–2.4
Areka 2		2017	Areka	Selection	84–110	White	1.6–2.0
Varieties for low rainfall (terminal drought-prone) areas							
Tsedey	DZ-Cr-37	1984	Debre Zeit	Hybridization	82–90	White	1.4–1.9
Gerado	DZ-01-1281	2002	Debre Zeit	Selection	73–95	White	1.0–1.7
Key Tena	DZ-01-1681	2002	Debre Zeit	Selection	84–93	Brown	1.6–1.9
Amarach	HO-Cr-136	2006	Debe Zeit	Hybridization	63-87	White	1.2
Mechare	Acc. 205953	2007	Sirinka	Selection	79	Pale white	1.79
Gemechis	DZ-Cr-387 RIL127	2007	Melkassa	Hybridization	62–83	White	1.4
Simada	DZ-Cr-385 RIL295	2009	Debre Zeit	Hybridization	88	White	1.4
Lakech	DZ-Cr-387 RIL273	2009	Sirinka	Hybridization	90	Very white	1.3–1.8
Boset	DZ-CRr-409 RIL 50D	2012	Debre Zeit	Hybridization	75–86	Very white	1.4–1.8
Were-Kiyu	Acc. 214746A	2014	Sirinka	Selection	94	White	–
Varieties for highland (waterlogged) areas							
Gimbichu	DZ-01-899	2005	Debre Zeit	Selection	118–137	White	1.6–2.2
Dega Tef	DZ-01-2675	2005	Debre Zeit	Selection	112–123	White	1.6–2.0

Source: Adapted from MoANR (2017)

hybridization generally exhibited 9% higher grain yield than those developed through direct selection from germplasm (Teklu and Tefera 2005).

10.9.2 Dissemination and Adoption of Improved Tef Varieties

The steady increase in the tef productivity in the last two decades (Fig. 10.1c), shows the advantages of disseminating improved varieties. The significant positive effect on productivity was observed after the year 2006 at which the yield of tef surpassed 1 mt ha^{-1}. That period coincided with the time when the popular Quncho variety was released and began to be disseminated to farmers (Assefa et al. 2011a). Widespread dissemination of improved varieties mainly depends on the commitment of the research and extension personnel in providing, not only improved varieties with high productivity, but also other essential inputs including fertilizer and herbicide. The use of lead farmers in disseminating improved tef varieties to other farmers has showed promising results (Bekele et al. 2016). A study on the adoption of improved tef varieties showed that while Quncho was adopted by 76% farmers, Magna was adopted by only 40% farmers in the Central Highlands of Ethiopia (Assefa et al. 2017). Quncho is grown on 66%, while Magna is on only 26% of the total tef area. This reflects the rapid adoption of the Quncho variety by farmers especially, by those in the Central Highlands of Ethiopia where tef is the major crop.

10.10 Conclusions and Prospects

In Ethiopia, tef improvement is carried out at federal and regional agricultural research centers, and the breeding program is chiefly at the Debre Zeit Agricultural Research Center, Ethiopian Institute of Agricultural Research. Breeding materials at different generations have been tested at over 20 sites in the country that represent diverse agro-ecological conditions. Since the scientific study of tef began about 5 decades ago, 42 improved varieties have been released. Outside Ethiopia, several academic institutions have been involved in basic and molecular studies. At present, researchers at the University of Bern, Switzerland are implementing genetic and genomic tools to identify candidate tef lines with traits of interest (Tadele 2013). Particular focus has been given to lodging resistance and drought tolerance, the two major constraints affecting tef productivity. Candidate lines obtained from the group have been introgressed into elite lines at the Debre Zeit Center where the first improved tef variety called Tesfa was recently released after several years of breeding (Cannarozzi et al. 2018).

Although tef is relatively more resilient to adverse climatic and soil conditions, the crop suffers from the prevailing extreme environmental conditions and is predicted to be severely affected from drought and other environmental conditions in the near future. In addition to boosting the productivity for this orphan crop, the

National Tef Breeding Program also focuses on developing tef varieties resilient to environmental constraints. Priority has been given to develop drought tolerant varieties. Several released varieties perform better than others in drought-prone areas (Table 10.3) mainly due to their early-maturity nature which allows them to escape from drought occurring during flowering time. A recent investigation based on climatic modelling and socioeconomic studies predicted a yield reduction of up to 0.46 mt ha^{-1} in tef by the year 2050. This magnitude of loss is equivalent to 1.19 million mt of grain and 5.4 million mt of straw for the entire country. In monetary terms, such losses are equivalent to USD 730 million for grain and USD 146 million for straw (Yumbya et al. 2014), indicating significant negative effects on even this hardy crop which normally tolerates environmental stresses better than other cereals. This calls for developing and adopting techniques which enhance the resilience of tef to expected the climate change.

Acknowledgments The tef research in Ethiopia and Switzerland is supported by Syngenta Foundation for Sustainable Agriculture, University of Bern and the Ethiopian government. We thank Sonia Plaza-Wüthrich for providing Fig. 10.5 and Regula Blösch for Fig. 10.6.

Appendices

Appendix I: Research Institutes Relevant to Tef

Institution	Specialization and research activities	Contact information and website
Ethiopian Institute of Agricultural Research (EIAR)	In addition to executing a number of research activities in the area of breeding, agronomy, crop protection, soil sciences, food sciences and economics, the Debre Zeit Agricultural Research Center of EIAR has a mandate to nationally coordinate the tef research in Ethiopia. Other EIAR centers involved in tef research include Melkassa Center on mechanization, and National Agricultural Biotechnology Research Center at Holetta	Dr. Kebebew Assefa, National Tef Research Coordinator, Debre Zeit Agricultural Research Center, P.O. Box 32, Debre Zeit, Ethiopia. Email: kebebew.assefa@yahoo.com EIAR website: http://www.eiar.gov.et/
Oromia Agricultural Research Institute (OARI)	Research on breeding, agronomy and socio economics of tef mainly at Bako Agricultural Research Center	Mr. Girma Gemeda, OARI, Bako Agricultural Research Center
Amhara Regional Agricultural Research Institute (ARARI)	Research on breeding, agronomy and socio economics of tef mainly at Adet Agricultural Research Center	Mr. Atinkut Fentahun, ARARI, Adet Agricultural Research Center
Tigray Agricultural Research Institute (TARI)	Research on breeding, agronomy and socioeconomics of tef mainly at Aksum Agricultural Research Center	Mr. Kidu Gebremeskel, TARI, Aksum Agricultural Research Center
Southern Agricultural Research Institute (SARI)	Research on breeding, agronomy and socio economics of tef mainly at Worabe Agricultural Research Center	Mr. Molalign Assefa, SARI, Worabe Agricultural Research Center
Crop Breeding & Genomics Group, Institute of Plant Sciences, University of Bern, Switzerland	Molecular and genomics studies on tef with particular focus on tackling major constraints affecting the productivity of tef	Dr. Zerihun Tadele, Tef Project Leader, University of Bern, Institute of Plant Sciences, Altenbergrain 21, 3013 Bern, Switzerland. Email: zerihun.tadele@ips.unibe.ch website: http://www.ips.unibe.ch/research/tef/index_eng.html or http://www.tef-research.org/

Appendix II: Tef Genetic Resources

Cultivar	Important traits	Cultivation location
Over 5000 tef accessions collected from diverse tef growing areas and deposited at the Ethiopian Biodiversity Institute	Huge diversity in agronomical, morphological and genomic traits	Different parts in Ethiopia
300 core tef germplasm available at Debre Zeit Agricultural Research Center, Ethiopia	For grain yield and yield related traits	Diverse tef growing areas
30 improved varieties for optimum growing rainfall areas: Abola (DZ-Cr-438 RIL7), Ajora (PGRC/E 205396), Ambo Toke (DZ-01-1278), Areka 2, Asgori (DZ-01-99), Dagim (DZ-Cr-438 RIL91A), Dima (DZ-01-2423), Dukem (DZ-01-974), Enatite (DZ-01-354), Etsub (DZ-01-3186), Felagot (DZ-Cr-442 RIL77C), Genete (DZ-01-146), Gibe (DZ-Cr-255), Gola (DZ-01-2054), Guduru (DZ-01-1880), Heber-1 (DZ-Cr-419), Holetta Key (DZ-01-2053), Kena (23-Tafi Adi-72), Kora (DZ-Cr-438 RIL133 B), Koye (DZ-01-1285), Magna (DZ-01-196), Melko (DZ-Cr-82), Menagesha (DZ-Cr-44), Negus (DZ-Cr-429 RIL125), Quncho (DZ-Cr-387 RIL355), Tesfa (DZ-Cr-457 RIL181), Wellenkomi (DZ-01-787), Yilmana (DZ-01-1868), Ziquala (DZ-Cr-358) and Zobel (DZ-01-1821)	Agronomic traits particularly yield and yield-related traits	Diverse tef growing areas
10 improved varieties for drought-prone areas: Amarach (HO-Cr-136), Boset (DZ-CRr-409 RIL 50D), Gemechis (DZ-Cr-387 RIL127), Gerado (DZ-01-1281), Key Tena (DZ-01-1681), Lakech (DZ-Cr-387 RIL273), Mechare (Acc. 205953), Simada (DZ-Cr-385 RIL295), Tsedey (DZ-Cr-37) and Were-Kiyu (Acc. 214746A)	Agronomic traits particularly yield, yield-related traits as well as drought tolerance.	Moisture scare areas
Two improved varieties for waterlogged areas: Dega Tef (DZ-01-2675) and Gimbichu (DZ-01-899)	Agronomic traits particularly yield, yield-related traits as well as waterlogging tolerance.	Waterlogged areas
Over 10,000 mutagenized tef populations available at the University of Bern in Switzerland	Diverse traits	
Kegne: semi-dwarf and lodging resistant tef line available at the University of Bern	Lodging tolerance	Universal
Kinde: semi-dwarf and lodging resistant tef line available at the University of Bern	Lodging tolerance	Universal
Dtt2 (Drought tolerant tef 2): available at the University of Bern	Drought tolerance	Drought-prone areas
Dtt13 (Drought tolerant tef): available at the University of Bern	Drought tolerance	Drought-prone areas

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Chapter 11
Triticale (x *Triticosecale* Wittmack) Breeding

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Abstract Triticale (x *Triticosecale* Wittmack) is a man-made, self-pollinated cereal crop specie developed by crossing wheat (*Triticum* spp.) and rye (*Secale cereale*). The initial goal of creating triticale was to develop a new cereal crop that would combine the superior agro-morphological and end-use quality characteristics of wheat, and the adaptability, vigor and resistance/tolerance to abiotic and biotic stresses of rye. Triticale is well adapted to a wider range of environments where wheat is grown; moreover, under stress conditions, triticale performs better. Triticale has been grown worldwide mainly for grain and forage production, and recently for bioenergy production. Although the grain quality of triticale is unsatisfactory compared to other small grain crops such as wheat, it still possesses a good level of resistance to multiple diseases and pests and many useful genes have been successfully transferred to wheat from triticale. The majority of triticale breeding programs focus on the improvement of economically-important traits such as grain and biomass yield, diseases and pest resistance, quality and agronomic traits. Several studies have demonstrated that genetic diversity within triticale germplasm is low, which

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J. M. Al-Khayri et al. (eds.), *Advances in Plant Breeding Strategies: Cereals*,
https://doi.org/10.1007/978-3-030-23108-8_11

is not unexpected. Traditional breeding methods are most commonly used in triticale improvement. Currently, modern breeding approaches, such as marker-assisted selection (MAS), genomic selection, double-haploid (DH) and genetic transformations are being explored to improve triticale. Use of molecular breeding technology and molecular markers are limited in triticale but many molecular markers of wheat and rye are conserved in the triticale genome and therefore wheat and rye genomics can be used in triticale improvement. The objective of this chapter is to summarize the current status of triticale production worldwide and provide details on different breeding approaches being used to improve triticale cultivars.

Keywords Disease resistance · Genome · Grain yield · Man-made · Marker-assisted selection · Triticale

11.1 Introduction

Triticale (x *Triticosecale* Wittmack) is a man-made, self-pollinated cereal crop. The Scottish scientist Stephen Wilson produced the first triticale by pollinating wheat (*Triticum*) with rye (*Secale*) pollen (Wilson 1873). The name *triticale* is derived from the partial combination of scientific names of wheat (*Triticum*) and rye (*Secale*). Initially, triticale was created to produce a new cereal crop that would combine the superior agro-morphological and end-use quality characteristics of wheat and the adaptability, vigor, and resistance/tolerance to abiotic and biotic stresses of rye. The first triticale plants produced had sterile pollen; therefore, the process of triticale breeding became more practical when embryo rescue and colchicine-induced chromosome doubling approaches were developed (Blakeslee and Avery 1937; Laibach 1925).

Triticale hybrids are all allopolyploid/amphipolyploid, meaning their chromosomes are derived from different taxa. Various types of triticale with different ploidy levels and chromosomal constitutions have been created and evaluated over time. Simmonds (1976) summarized the evolution and origins of many different types of triticale (Fig. 11.1). It can be tetraploid, hexaploid or octaploid. Among triticales with different ploidy levels, most cultivars are hexaploid. Although octaploid triticales show better end-use quality characteristics compared to hexaploid triticales, they suffer from genetic instability and floret sterility (Mergoum et al. 2009). It should be mentioned that teteraploid triticales suffer from floret sterility as well.

Triticales synthesized from the initial cross between wheat and rye, unmodified by further hybridization, are called primary triticales. Although the production and breeding procedures of primary triticales are difficult and slow, international and national centers and programs have been active in primary triticale productions, such as the International Maize and Wheat Improvement Center (CIMMYT) and in Eastern and Western Europe, Australia, China and Canada. Triticale breeders face many challenges to develop and improve the

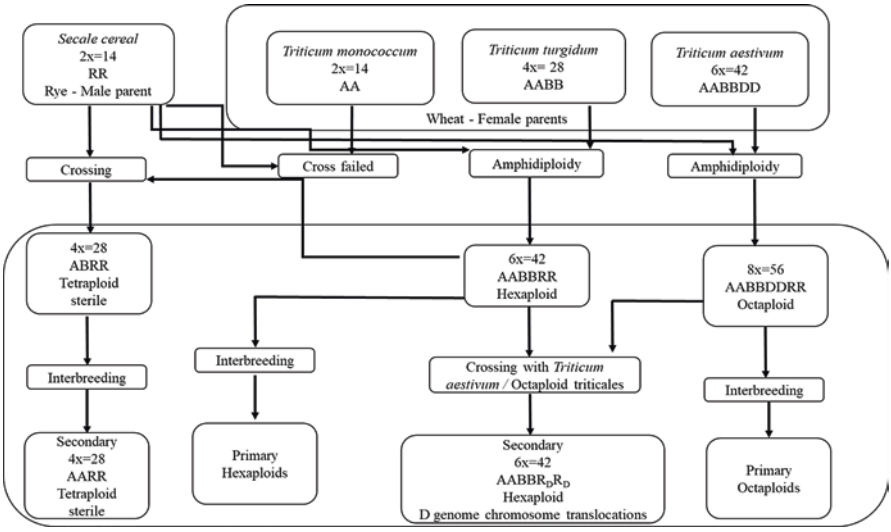

Fig. 11.1 Summary chart of the origins of different types of triticale. (Source: Simmonds 1976)

primary triticales. These challenges mainly include partial floret sterility, shriveled kernel, poor agro-morphological performance, low yield, poor end-use quality characteristics and poor adaptation. Due to these challenges, triticale breeders started to intercross different primary triticales as well as backcrossing primary triticales with wheat to improve its characteristics. The products of these approaches are triticales with new combinations of wheat and rye chromosomes and are considered as secondary triticales (Mergoum et al. 2009; Randhawa et al. 2015).

Based on rye chromosomes, triticale can be classified into two types: complete and substitute. The chromosomal constitution of a complete triticale remains unchanged from the rye parent. Generally, a complete triticale is grown in marginal agricultural areas (sandy, acidic, saline or soils with heavy metal toxicity), where rye is well adapted but other crops including wheat may not perform well. Beagle, Juanillo and Drira cvs. are some of the important complete triticales developed and released by CIMMYT. On the other hand, a substitute triticale is a triticale in which the R-genome chromosome/a segment of the R-genome chromosome has been substituted/translocated with the respective D-genome chromosome/a segment of the D-genome of bread wheat. The most important breakthrough occurred when the first substitute triticale (1B/1R) was selected from a natural outcross between a triticale and an unknown Mexican semi-dwarf bread wheat in 1967 at CIMMYT. The selected line, Armadillo, made a principle contribution to triticale improvement. Additionally, this major breakthrough allowed for improvements in bread-making quality, seed shriveling and floret ste-

rility. Furthermore, the chromosomal translocation approach provided triticale breeders with a powerful tool to improve the genetics of triticale.

According to the growth habits of triticales, they can be classified into three basic types: spring, intermediate (facultative) and winter (Mergoum et al. 2009). Spring triticale is shorter in height with shorter growth cycles, and fewer spikelets per spike compared with winter types. Additionally, spring triticales are generally insensitive to photoperiod and have limited tillering capacity (Royo et al. 1995). It should be noted that both intermediate and winter triticales were derived from their spring ancestors (Mergoum et al. 2009). Royo et al. (1995) demonstrated that spring-substituted triticales differed from both winter and spring types and showed intermediate characteristics. Mergoum et al. (2004) and Salmon et al. (1996) classified spring-substituted triticales into a separate group as intermediate (facultative) triticales.

Internationally, triticale is a well-established crop and is used as grain for human food consumption and animal feed, as well as forage for livestock in the form of grazing, silage, fodder, green-feed and hay. In addition, the European 2020 strategy for economic growth and sustainable competitiveness is considering triticale as a promising candidate among cereals to be used for both fuel alcohol and biogas production. Despite the many advantages of triticale, the world production of triticale is still low. In 2016, about 4.4 million ha of triticale were grown; Poland, Germany, Belarus, France, Russia and Hungary were the major producers (Table 11.1; FAO Stat 2016). Figure 11.2 shows the world growing areas for triticale (http://eol.org/).

Several strategies have been proposed to develop genetic variability to enable breeders to improve triticale into a more competitive crop. Oettler (1998) described systematic worldwide strategies for triticale breeding programs to create new genetic resources as well as genetic variability. Based on the expected benefit of these strategies, the author classified these strategies into highly beneficial, beneficial and no benefit for the genetic improvement of triticale with short-term, middle-term and long-term goals in triticale breeding.

Major breeding objectives for improvement of triticale include increased grain and biomass yield, shorter plant height, reduced awn size, enhanced straw strength, earlier maturity, increased grain volume weight, improved nutrients, improved water use efficiency, and tolerance/resistance to various biotic and abiotic stresses such as ergot, *Fusarium* head blight (FHB), leaf spot and drought.

Table 11.1 Worldwide triticale area harvested (ha) and production quantity (mt) in 2016

Country	Area harvested (ha)	Production (mt)	Productivity (mt/ha)
Poland	1,403,519	5,102,445	3.64
Belarus	499,568	1,641,215	3.29
Germany	396,100	2,397,300	6.05
France	334,220	1,448,116	4.33
China	238,604	431,089	1.81
Russian Federation	223,078	619,202	2.78
Spain	203,101	447,274	2.20
Hungary	139,183	504,810	3.63
Lithuania	100,367	329,455	3.28
Romania	82,566	287,326	3.48
Australia	78,400	127,393	1.62
Austria	54,886	322,560	5.88
Czechia	39,595	193,198	4.88
Turkey	37,621	125,000	3.32
Sweden	30,010	157,000	5.23
Chile	24,070	146,023	6.07
Serbia	23,191	100,301	4.32
Greece	22,287	53,670	2.41
Portugal	21,085	40,168	1.91
Croatia	19,746	81,393	4.12
Tunisia	17,905	33,587	1.88
Canada	17,400	53,900	3.10
Brazil	17,063	46,253	2.71
Italy	16,541	86,488	5.23
Bulgaria	16,096	49,265	3.06
Bosnia/Herzegovina	15,690	52,174	3.33
Mexico	11,626	36,381	3.13
United Kingdom	11,058	42,936	3.88
Latvia	10,900	37,400	3.43
Denmark	10,100	56,200	5.56
Slovakia	9714	37,858	3.90
Switzerland	8721	36,178	4.15
Belgium	5992	31,531	5.26
Estonia	5680	18,935	3.33
Slovenia	5288	24,719	4.67
Luxembourg	4609	22,843	4.96
Netherlands	1044	5387	5.16
Norway	387	1281	3.31

Source: FAO Stat (2016)

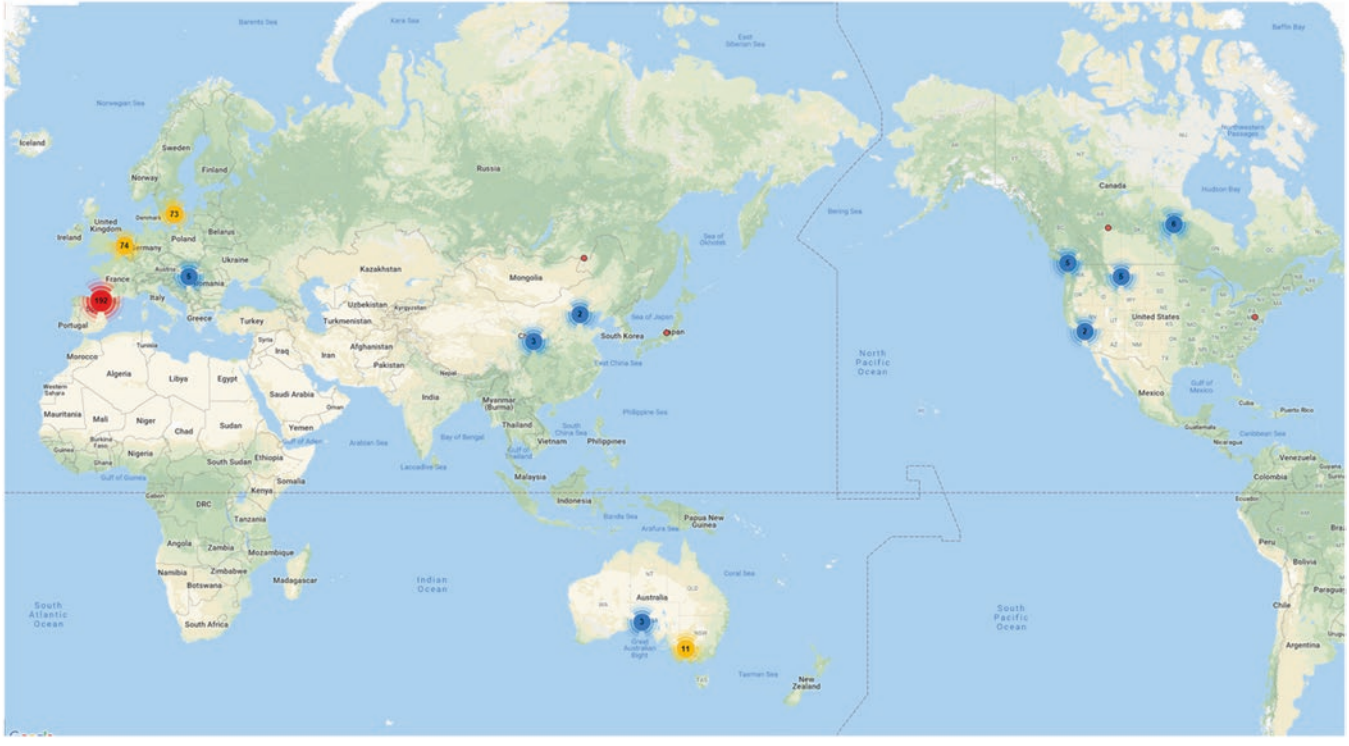

Fig. 11.2 The world's triticale growing areas (http://eol.org/). High, medium, and low growing areas are shown by red, yellow, and blue colors, respectively. (For more details see Table 11.1)

11.2 Cultivation and Traditional Breeding

11.2.1 Cultivation

The initial goal of developing triticale was to incorporate the high yielding and adaptability traits of wheat and rye, respectively. That is, to develop a single crop that can be used for the dual purpose of human food and animal feed, and could be grown on marginal lands with low inputs (Mergoum et al. 2004; Rayo et al. 1994). In general, triticale can be grown in areas suitable for wheat and rye; however, specific conditions required for triticale cultivation are largely dependent on their type, i.e., spring, facultative or winter. Spring type triticale can be grown in areas where sufficient moisture is available, either naturally or from irrigation, and winter conditions are not severe. However, winter triticale is grown in areas where a sufficient number of chilling hours are available to meet the vernalization requirement, i.e., 4–8 weeks of low temperature (above freezing, but below 9 °C). Another important factor required for successful cultivation of triticale is photoperiod. Although daylight-insensitive triticale cultivars exist and are available for cultivation, some triticale needs at least 12 h of light to initiate the reproductive stage from the vegetative stage (Mergoum et al. 2004).

11.2.1.1 Crop Adaptation

Triticale is well adapted to a wide range of environments where wheat is grown; moreover, under stress conditions, triticale performs better (Mergoum et al. 2004). The adaptation of triticale was found to be average to excellent under limited water supply conditions, but its performance is very high when produced under good soil fertility and irrigation (Blum 2014; Mergoum et al. 2004). Abiotic stresses, such as drought, cold, salinity, mineral toxicity and deficiency, waterlogging etc., are major yield-limiting factors in many cereal crop production. Under these abiotic stress conditions, triticale was found to perform better as compared to other cereal crops (Blum 2014; Mergoum et al. 2004).

Triticale cultivars carry better tolerance capacity to acidic soils and salinity (Blum 2014). Studies demonstrate that rye is more resistant to mineral toxicity, such as aluminum toxicity, as compared to wheat, barley and early triticale containing 2D/2R substitution (Blum 2014). However, hexaploid triticales developed after the mid-1970s were comparable to rye for the tolerance to mineral toxicity. Acidic soils and mineral toxicity have been a major problem for cereals production in many regions worldwide (Brazilian soils for example), but triticale has excellent tolerance to low pH and has been grown in large areas containing these types of soils (Mergoum et al. 2004).

11.2.1.2 Crop Establishment

Most triticale cultivation practices, such as seedbed preparation, seeding depth, fertilization and seeding methods, are similar to wheat (Chapman et al. 2005). Like other cereal crops, triticale should be planted in a firm seedbed and soil with optimum moisture content for better germination. The seed size of triticale is larger and has a robust embryo as compared to other cereal crops. Therefore, triticale can be planted more deeply, which in turn benefits germination by utilizing more stored soil moisture (Mergoum et al. 2004). More attention should be given during sowing and seed placement of winter triticale in areas with severe climatic conditions. Deep planting may take a longer time for seedlings to emerge, resulting in the development of a less robust crown. Zero tillage or minimum soil disturbance are beneficial for the production of both spring and winter types of triticale, mainly because it reduces soil erosion, enhances soil microbial activity, increases soil fertility, maintains soil moisture and lowers costs such as the usage of agriculture fuels (Mergoum et al. 2004; Sayre et al. 1998). Nevertheless, in areas where crop residue can harbor pathogen inoculum, crop rotation with other non-host crop species is advantageous.

11.2.1.3 Crop Management

Triticale has an extensive root system compared to other small cereal grains, which allows it to grow well in poorer soils. But, if fertilization is required, it should be done based on the fertility status of the soils, previous crops, purpose of growing triticale (grain or forage) and soil moisture level (Gibson et al. 2007; Mergoum et al. 2004). N and P are mainly required for triticale production, and winter triticale planted in the fall can utilize N left in the soil from the previous crops (Gibson et al. 2007). The vigorous growing habit of triticale, its leafiness and height allow triticale to compete well with weeds, but there are still many weeds that pose a problem for triticale production. In general, triticale has a better level of resistance to diseases and pests as compared to cereals such as wheat and rye; however, *Fusarium* head blight, ergot, leaf spot, bacterial leaf streak and rusts can cause significant economic losses (Arseniuk and Goral 2015; Randhawa et al. 2015; Sapkota 2015; Sapkota et al. 2018; Wen et al. 2018).

Since triticale is a relatively new crop, as compared to other cereal crops such as wheat and barley, there are not many herbicides or pesticides developed specifically for triticale, but most herbicides that work for wheat are recommended for triticale (Mergoum et al. 2004; Schoofs and Entz 2000; Ziveh and Mahdavi 2012). Chemical companies have started to develop and release newer herbicides and pesticides for use on triticale; generally, herbicide use in triticale is effective in weed control (Ziveh and Mahdavi 2012).

11.2.1.4 Crop Harvest and Storage

Triticale has a large seed and embryo with an elongated beak as compared to wheat, and therefore caution should be taken when harvesting it, especially when using a mechanical harvester (Mergoum et al. 2004). In general, many triticale cultivars are hard to thresh as compared to rye and wheat, but cultivars that inherited a reduced-awn and rachis from wheat are comparatively easy to thresh. Triticale grains are softer than wheat and barley, and therefore more prone to insect attack, such as by weevils (Dobie and Kilminster 1978). To ensure longevity of stored triticale, it should be warehoused in a dry and well-ventilated area. Similar to wheat, triticale should be harvested when the moisture content of the kernels is 14% or less for safe storage and longevity (Mergoum et al. 2004).

11.2.2 Agricultural Problems and Challenges

Triticale shows many promising characteristics including its good adaptation to low fertility soils and stressed environmental conditions. It also possesses a greater level of resistance to diseases and contains high amounts of essential amino acids as compared to wheat (Mergoum et al. 2009). Many countries have started to grow triticale and significant progress has been made in its improvement in recent decades. However, there exist many agricultural problems that pose obstacles in the improvement of this promising crop. One of the major problems in triticale improvement is the lack of sufficient genetic diversity (Mergoum et al. 2004; Niedziela et al. 2016). Since triticale is a relatively a new crop, natural evolution is relatively low as compared to other crops like wheat and barley (Mergoum et al. 2004), and therefore triticale breeders face challenges in developing diverse triticale germplasm. Several studies conducted using molecular data, such as microsatellite markers, demonstrated that the majority of triticale germplasm are genetically similar (De Costa et al. 2007; Kuleung et al. 2006; Niedziela et al. 2016; Tams et al. 2004; Trebichalsky et al. 2013). Tams et al. (2004) collected European triticale accessions from 13 breeding companies, and found that the germplasm was highly related with only 15.3% genetic variation among the studied accessions. Likewise, Kuleung et al. (2006) used 80 accessions of triticale collected worldwide; results demonstrated that it was not genetically divers (0.45 similarity). Overall, these studies demonstrated that genetic diversity among triticale germplasm is low which is not unexpected given the short life of this crop since its creation. Genomics and marker-assisted selection (MAS) is routine for the improvement of many cereal crops such as wheat and barley; however, early studies showed that triticale genomics has not been widely studied (Mergoum et al. 2004). Identification of markers linked to economic

traits, and the use of those markers in breeding programs will undoubtedly enhance triticale improvement, and therefore future work should focus on exploiting the genetic and genomics of triticale germplasm to benefits its improvement.

Triticale germplasm possesses a high level of resistance to diseases and pests as compared to wheat; however, with the emergence of new races in pathogen and insect populations, current triticale germplasm is vulnerable to many diseases and pests (Arseniuk 1996; Mergoum et al. 2004, 2009). Stem rust disease, caused by *Puccinia graminis* f. sp. *tritici*, was reported to be the first triticale disease in Australia that occurred in epidemic proportions (Arseniuk 1996). Other rusts (*Puccinia* sp.), *Septoria* complex, smuts (*Ustilago* sp. and *Urocystis* sp.), powdery mildew (*Blumeria graminis*), scab (*Fusarium* sp.), root rot (*Bipolaris sorokiniana),* ergot (*Cleviceps purpurea*)*,* cereal cyst nematode (*Heteroderma avenae*)*,* Hessian fly (*Mayetiola destructor*) and bacterial diseases caused mainly by the *Xanthomonas* and *Pseudomonas* sp. are among the major biotic constraints of triticale production (Arseniuk 1996; Mergoum et al. 2004, 2009; Randhawa et al. 2015; Sapkota 2015; Sapkota et al. 2018). Additionally, abiotic stresses, such as acidic and alkaline soils, micronutrient deficiency and toxicity, and moisture stress, are also considered major problems in triticale production (Mergoum et al. 2009). In addition to narrow genetic diversity and biotic/abiotic problems in triticale, the low adoption of triticale by farmers in many countries, restricted use of triticale, and as yet not fully accepted in human diet/foods such as bread, are some additional challenges that need to be addressed by the scientific community to allow triticale crop to express its potential and contribute fully to agriculture production.

11.2.3 Traditional Breeding Methodologies and Limitations

Triticale is a self-pollinating crop, like wheat and barley, and depending on its genetic and some environmental conditions, some degree of natural cross-pollination may occur. Therefore, the most commonly used traditional breeding methods for self-pollinating crops, such as backcrossing, pedigree, bulk and single-seed descent selection methods can be used in triticale improvement/breeding (Lelley 2006; Mergoum et al. 2009; Randhawa et al. 2015). Additionally, recurrent selection and hybrid triticales, which are mostly used for cross-pollinated crops, are also used in triticale breeding (Mergoum et al. 2009). Although breeding methods adopted for self-pollinated crops such as wheat and barley can be applied in triticale breeding, there are a few features that distinguish triticale breeding from the two other grains. First, when a cross is made between triticale accessions, it is highly possible that the delicate genetic make-up of triticale, containing rye and wheat genome material, is affected to produce phenotypic variation in the offspring (Lelley 2006). Therefore, breeders working on triticale improvement via conventional breeding should focus, during early generations, on restoring the balance between the wheat and rye genomes. Moreover, the chance of cross-pollination in triticale is relatively high (0–60%) as compared to

Fig. 11.3 Triticale elite lines developed using classical breeding methodologies grown in California, USA. (Photo by Wesam AbuHammad)

wheat and barley (Lelley 2006). Therefore, this may be challenging in developing pure lines. Most of the triticale germplasm released so far was developed using traditional breeding methods (Fig. 11.3). The historic cultivar Lasko, for example, was developed by Danko and released in 1982 using pedigree selection (Lelley 2006). Similarly, all cultivars developed early CIMMYT, widely grown around the world, derive from classical breeding methodologies (Mergoum et al. 2009). Traditional breeding methods were employed for triticale improvement over the last several years, and a significant amount of improvement has been made; however, traditional methods are labor intensive, take more time, genetic variations are limited and, more importantly, genetic gain is low (Zimny and Lorz 1996).

In addition to traditional methods, modern breeding approaches, such as double haploid (DH), shuttle breeding, MAS and genetic transformations are also being used in triticale breeding (Mergoum et al. 2009; Randhawa et al. 2015; Zimny and Lorz 1996). Since genomic data for triticale are still not fully available and modern breeding methods are hard to use in triticale breeding, a combination of traditional and modern breeding methods is likely the best strategy to achieve the gains in triticale (Mergoum et al. 2009).

11.3 Germplasm Collection and Conservation

Germplasm collection and conservation are important not only to preserve genetic resources, but also to enable breeders to exploit the genetic and phenotypic variation of triticale and develop superior germplasm. The concept of safeguarding existing genetic resources, including wild relatives and landraces, was initially recognized by Vavilov (Vavilov 1935) during the early twentieth century and now many

institutions around the world are working on germplasm collection, conservation and distribution. Currently, about 1.27 million accessions of Triticeae, which includes bread wheat, durum wheat, barley, rye and triticale, are conserved in 295 gene banks worldwide. Currently, the CIMMYT gene bank is the largest for wheat and triticale germplasm, where more than 70 and 17 thousand accessions of the two crops are conserved, respectively (Knüpffer 2009).

11.3.1 Genetic Resources Diversity

As a man-made and relatively new crop, triticale germplasm was used for breeding beginning in the1960s (CIMMYT 2007; Knüpffer 2009; Mergoum et al. 2004). Currently there are four different types of triticale being used in breeding programs: primary triticale (from the cross of wheat and rye), secondary triticale (from the cross of different cultivars of triticale), complete triticale (containing all 7 pairs of rye chromosomes) and substituted triticale (one or more rye chromosomes are replaced by wheat chromosomes) (Knüpffer 2009). CIMMYT, considered the primary source of genetic resources of triticale, has been working for several years predominantly to develop primary triticale (Knüpffer 2009). Therefore, scores of triticale scientists annually visit the CIMMYT triticale research and germplasm conservation programs to select germplasm for their own breeding programs (Fig. 11.4). Although triticale is considered self-pollinating for cultivar development, it is partially

Fig. 11.4 Group of world scientists visiting along with Dr. M. Mergoum (former CIMMYT triticale breeder) the CIMMYT triticale breeding and germplasm plots at Ciudad Obregon, Mexico. (Photo by M. Mergoum)

cross-pollinated, mainly due to the presence of the rye genome. This partial cross-pollinating nature of triticale can be utilized positively to develop hybrid triticale. Preliminary hybrid production showed promising results with 10–20% mid-parent heterosis for hybrid triticale. However, the lack of genetic diversity within the current triticale gene pool is a constraint for developing putative heterotic group and hybrid triticale (Niedziela et al. 2016; Tams et al. 2004). Early evaluation of triticale germplasm from American and European countries demonstrated that there exist only two major group, winter and spring types, and no diversity was found according to geographical origin (Royo et al. 1995). However, in recent years, developing facultative triticale was undertaken by many breeding programs, particularly at CIMMYT, to develop triticale for forage or dual purposes. Another study, where more than 3000 triticale genotypes collected from North American were evaluated, revealed that genetic diversity is relatively low, and differences were observed only between complete and substituted type triticale (Furman et al. 1997). Recent studies conducted using molecular markers also demonstrated narrow genetic diversity among triticale germplasm (Niedziela et al. 2016). However, genetic diversity studies among triticale germplasm using recent marker type such as single nucleotide polymorphisms (SNP) is still lacking.

11.3.2 Conservation of Triticale Germplasm

In situ and ex situ conservation are the two main approaches to preserve the genetic resources of many crops. In situ conservation involves safeguarding genetic resources in their natural habitats, whereas the ex situ approach involves maintaining genetic resources outside their natural habitats such as in gene banks (Rajpurohit and Jhang 2015). For triticale, more than 35,000 accessions are being conserved in ex-situ worldwide, with CIMMYT in Mexico the major conservation center; followed by the Vavilov Institute in St. Petersburg, Russia; Polish Gene Bank, Radzików, Poland; USDA-ARS, USA and Agri-food, Canada, each conserve more than 2000 accessions in their centers (Knüpffer 2009) (Table 11.2). In addition to these major conservation centers, many countries have small collections of triticale accessions as indicated in Table 11.2. Although many world institutions and breeders are working on triticale germplasm collection and conservation, a formal working group does not exist (Knüpffer 2009); obviously, there needs to be global networking among institutions, breeders and crop scientists to improve this promising crop.

Table 11.2 Global triticale germplasm collection and their number of accessions

Country	Institution	No. of accessions
Mexico	International Maize and Wheat Improvement Center (CIMMYT)	17,871
Russia	Vavilov Institute of Plant Industry, St. Petersburg	3744
Poland	Plant Breeding and Acclimatization Institute, Radzików	2118
USA	USDA-ARS, National Small Grains Collection, Aberdeen, Idaho	2023
Canada	Soil and Crops Research and Development Centre, Agriculture and Agri-Food Canada Sainte-Foy, Quebec	2000
Poland	Institute of Genetics and Plant Breeding, University of Agriculture, Lublin	1748
Ukraine	National Centre for Plant Genetic Resources of Ukraine, Kharkiv	1748
Germany	Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben	1571
USA	Department of Agronomy, University of Missouri, Columbia	1400
Bulgaria	Institute for Plant Genetic Resources, Sadovo	926
Slovak Republic	Research Institute of Plant Production Piestany	800
Switzerland	Agroscope Changins-Wadenswil (ACW)	750
Romania	Research Institute for Cereals and Technical Plants, Fundulea	629
Portugal	Estação Nacional Melhoramento Plantas, Elvas	600
Spain	Centro de Recursos Fitogenéticos, Alcalá de Henares	511
Austria	Austrian Agency for Health and Food Safety/Plant Production, Vienna	510
Spain	Universitat de Lleida e Institut de Recerca i Tecnologia Agroalimentàries, Centre Ud LIRTA, Lleida	501
France	Plant Breeding and Genetics Department, INRA, Clermont-Ferrand	240

Source: CIMMYT (2007) and Knüpffer (2009)

11.4 Molecular Breeding

Molecular breeding is the application of molecular tools and techniques, such as QTL mapping, MAS and genomic selection, to improve crop species. Formerly, morphological markers and protein isozymes were the most commonly used tools in plant breeding; however, with the advent of next-generation sequencing (NGS) technologies, application of DNA-based molecular markers for genome wide selection and analysis of crop species are now commonly used (Xu et al. 2012). Although genetic mapping and the use of molecular markers is a routine procedure in wheat, molecular breeding in triticale is still limited. Recently, a few research studies have been conducted on genetic map construction and QTL mapping in triticale (Tyrka et al. 2011, 2015; Wen et al. 2018).

11.4.1 Development of Genetic Maps

Genetic maps facilitate the identification of linked molecular markers with important traits, as well as revealing the genetic and genomic characteristics of the related species. Genetic mapping in triticale is expected to facilitate the understanding of the genetic behavior of genes combined from the rye and wheat genomes. Another benefit from mapping the triticale chromosomes is to underpin functions of specific traits and QTL previously identified in the rye or wheat genomes. As compared to wheat and rye, fewer genetic maps have been constructed of triticale (Gonzalez et al. 2005) which could be due to the instability of the triticale genome. The later could be due to the difference in size of the wheat and rye genomes causing replication problems and telomeric aberrations (Bento et al. 2010; Lukaszewski et al. 2004).

Gonzalez et al. (2005) constructed the first triticale genetic map which includes 356 markers with an average map density of 6.9 cM. The markers were not evenly distributed within the genomes with more than 50.7% of markers were located on the R genome. Furthermore, some of the chromosomes (1A, 4A, 3B) had only a few markers. Although this work initiated the triticale genetic maps, saturated genetic maps for triticale are necessary. Tyrka et al. (2011) reported another genetic map in triticale consisting of 21 linkage groups assigned to A, B and R genomes with using SSR (155), DArT (1385) and AFLP (28) markers covering a total of 2397 cM genetic distance. Subsequently, Tyrka et al. (2015) developed a double haploid population from a cross between Hewo and Magnat triticale cultivars, and used the resulting population to construct a genetic map of 1615 markers consisting of SSR, DArT and DArTseq markers, mapped into 20 linkage groups assigned to the A, B and R genomes. This map was compared against the consensus maps of wheat, rye and triticale and the results showed that chromosome 7R was missing; chromosomes 4B, 5A and 6A had some deletions; and also some inversions were noticed on chromosome 7B. Recently, a high density map consisting of single nucleotide polymorphic (SNP) markers was constructed using a mapping population derived from the cross of TMP16315 and AC Ultima (Dhariwal et al. 2018). This map consisted of 5274 SNPs that were mapped to 21 chromosomes of triticale with a map density of 0.48 cM/SNP. Interestingly, 1387 of the 5274 SNPs in this study were considered new markers not present on either the wheat consensus map or the previous triticale maps reported by Wang et al. (2014).

11.4.2 Quantitative Trait Loci (QTL) Mapping

Triticale genetic maps generated were used to study marker-trait association for some important economic traits. Several studies reported QTLs associated with abiotic stresses (aluminum toxicity, drought, waterlogged soils), biotic stresses

(*Fusarium* head blight, bacterial leaf streak) and agronomical traits (yield, plant height) (Alheit et al. 2014; Ayalew et al. 2018; Niedziela et al. 2012; Sapkota et al. 2018; Wen et al. 2018). Niedziela et al. (2012) utilized the association mapping (AM) approach to identify potential markers linked to aluminum tolerance in triticale and reported 37 QTLs on the 3R, 4R, 6R and 7R chromosomes significantly associated with this trait. Although the phenotypic variations explained by the QTLs were not significantly high, this work initiated the use of AM in triticale. In another study, Niedziela et al. (2014) used a different mapping approach, biparental mapping, and mapped a single major QTL on the 7R chromosome associated with aluminum tolerance in triticale. Two other F_2 biparental mapping populations were also used to identify QTL which explained 25% and 36% of the phenotypic variation in each population.

Biomass yield is a major trait in triticale which can be used in forage production and potentially for bioenergy and biofuel production (Alheit et al. 2014). Therefore, development of advanced phenotyping platforms for such a trait is important (Busemeyer et al. 2013). Although few studies focused on biomass yield in triticale, few were successful in identifying major QTLs for this trait. Busemeyer et al. (2013) employed a genome-wide association mapping approach on a set of 647 double haploid lines and reported 2 major QTLs on chromosomes 5A and 5R associated with biomass yield. Using the same mapping population and marker data, Alheit et al. (2014) employed multiple-line cross QTL (MC-QTL) mapping approach and reported 12 and 9 QTLs for plant height and biomass yield, respectively. Interestingly, a major QTL identified on the 5R chromosome was found to be associated with both traits, biomass yield and plant height, and likely to be a dwarfing gene *Ddw1*. Plant height is a complex trait and highly associated with biomass yield in triticale. Furthermore, plant height was also found to be associated with other agronomical traits in cereal crops such as lodging, grain yield and quality (Wurschum et al. 2014). Therefore, controlling triticale plant height with no influence on other traits is a major target for many breeding programs (Griffiths et al. 2012).

Fusarium head blight (FHB) is a major biotic problem in cereal crops including maize and other small grains (Miedaner et al. 2016). Unlike wheat, only few triticale studies have described QTL identification for FHB resistance (Dhariwal et al. 2018; Kalih et al. 2014, 2015; Miedaner et al. 2016). Breeding triticale for FHB resistance is challenging because FHB resistance is controlled by multiple alleles with minor effects and most resistant genes for FHB are so far identified on wheat chromosomes. In addition to FHB, bacterial infection is also among the top challenges in breeding triticale. Bacterial leaf streak (BLS) or black chaff is an important disease causing severe damage in triticale (Duveiller et al. 1997; Sapkota 2015). The source of BLS resistance is limited in wheat and only partial resistance was reported; however, a high level of BLS resistance has been reported in triticale (Duveiller et al. 1997; Johnson et al. 1987; Sapkota 2015; Sapkota et al. 2018; Wen et al. 2018). This suggests that resistance genes to BLS are provided by the rye genome in triticale and controlled by a single dominant gene in some breeding lines such as Siskiyou, M2A-Bgc and OK77842 (Johnson et al. 1987; Sapkota et al. 2018). Wen et al. (2018) developed two mapping populations derived from the cross among Siskiyou (resistant), UC-38 (susceptible) and Villax St. Jose (susceptible) and mapped a sin-

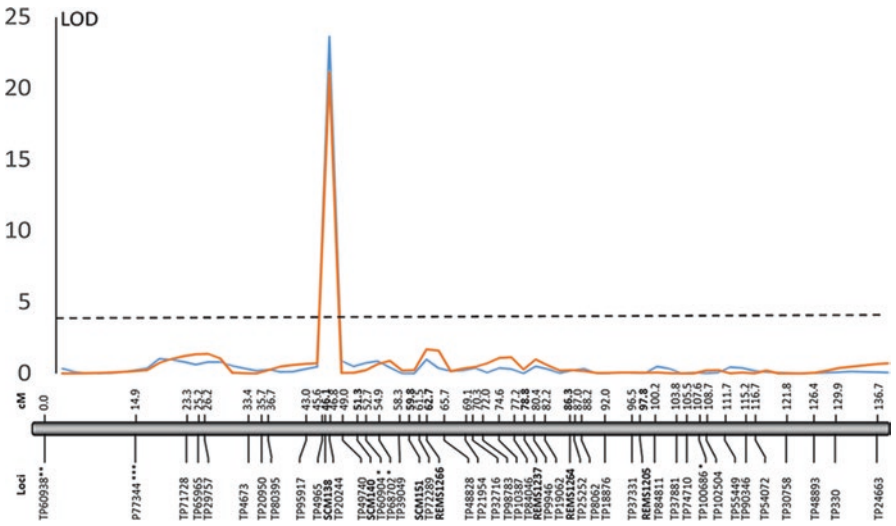

Fig. 11.5 A major QTL mapped on 5R chromosome of triticale for resistance to bacterial leaf streak disease. (Source: Wen et al. 2018)

gle major QTL on chromosome 5R for resistance to BLS in Siskiyou (Fig. 11.5). The resistance locus on the 5R chromosome was designated as *Xct1* and linked to the *XSCM138* (SSR) and *TP4965* (SNP) markers (Wen et al. 2018).

11.4.3 Marker-Assisted Selection

Marker-assisted selection (MAS) is a form of indirect selection utilizing molecular markers to select for desired traits/phenotypes. The use of molecular markers not only reduces the breeding cycle/time but is also more effective, mainly because it is not affected by the variation in environmental factors (Mergoum et al. 2009). Identification of molecular markers linked to economically-important traits and use of those markers in breeding programs is limited in triticale, as compared to crops such as wheat and rye. However, plethora of wheat and rye markers are available online which can easily be used in the triticale background (Mergoum et al. 2009). Since a large proportion of the wheat and rye genomes is conserved in triticale, this could facilitate triticale genomics for marker development and MAS (Ayalew et al. 2018; Mergoum et al. 2009). Kuleung et al. (2004) reported that 39% of rye and 57% of wheat simple sequence repeat (SSR) markers were directly transferable to triticale, indicating that triticale genomics could benefit from rye and wheat genomics. However, evolutionary processes such as allopolyploidization has created variations in wheat and rye genomes in triticale and 10–30% of wheat and up to 50% of rye genome was found to be modified in the triticale genome (Ayalew et al. 2018; Ma and Gustafson 2008; Ma et al. 2004).

Only few studies have been conducted on triticale to study the marker-trait association and QTLs were reported for some traits such as biomass and grain yield, plant height and disease resistance (Alheit et al. 2014; Liu et al. 2016; Sapkota 2015; Wen et al. 2018). However, the application of identified QTLs in the MAS is still questionable since most of the QTL studies did not validate these QTL linked markers for MAS (Ayalew et al. 2018). Although discovery of SNP markers using next generation sequencing technology (such as GBS) and construction of high density linkage maps has begun in triticale, more studies are required to identify and validate major QTLs/genes linked to economically-important traits and utilize them in the breeding program via MAS.

11.4.4 Genomic Selection

Genomic selection (GS) is a relatively new approach in molecular breeding that utilizes the marker information to predict the phenotype. GS requires the utilization of a large number of marker datasets to predict accurately the phenotype; however, with the availability of NGS technology, the cost and time required for marker development has significantly been reduced (Bhat et al. 2016). Utilization of GS to predict complex traits is very common in many crops including corn (Riedelsheimer et al. 2012; Technow et al. 2013), wheat (Heffner et al. 2011; Zhao et al. 2015) and barley (Lorenz et al. 2012; Schmidt et al. 2016). However, genomic selection is very limited in triticale and only a few research reports have been published. Wurschum et al. (2017) used a set of 647 double haploid (DH) triticale lines generated from four families and phenotyped them for grain yield, 1000-kernel weight, biomass yield, plant height, frost tolerance and *Fusarium* head blight disease (scab) resistance. Subsequentially, the DH were genotyped with 1722 DArT markers and genomic prediction results showed that the prediction accuracies were moderate to high. Although the use of GS is limited in triticale, its application to increase the genetic gain via selection, and in hybrid triticale breeding, is very promising (Marulanda et al. 2016; Wurschum et al. 2017).

11.5 Genetic Engineering

Genetic engineering is a method to alter gene or regions of the genome of an organism with the introduction of foreign genetic material and thereafter the regeneration of a healthy modified organism. Genetic modification can be used to study the change in performance and expression of the inserted gene or endogenous genes and engineer plants. There are several methods developed and used for genetic modification of plant species; however, the *Agrobacterium*-mediated and biolistic gun methods are mainly used for triticale transformation (Mergoum et al.

2009; Nadolska-Orczyk et al. 2005). Both methods involve the injection of transgene to the calli tissue of recipient followed by the selection and regeneration of plant materials carrying the transgene (Bhalla 2006). In addition to efficient gene transfer methods, the in vitro tissue and cell culture platform are equally important in triticale transformation (Maheshwari and Eudes 2015).

Integration of foreign genetic material into the triticale genome was first reported in 1995 where the β-glucuronidase gene and selectable marker gene *bar* was introduced into triticale lines using the biolistic method of transformation (Zimny et al. 1995). In another study, transgenic triticale lines were produced from the cv. Wanad (Nadolska-Orczyk et al. 2005) where the efficiency of transformation ranged from 0% to 16%. The highest number of transgenic plants was produced from the combination of the strain LBA4404 and the binary vector (pTOK233). Segregation ratios of transgenic *gus* to non-*gus* expressing lines was 30% following the Mendelian ratio, while 70% had dramatically distorted segregation lower than a 3:1 ratio. A recent transformation approach in triticale was found effective to overcome most problems of *Agrobacterium*-mediated transformation. The idea was to deliver an in vitro nano-complex independently of *Agrobacterium* (Ziemienowicz et al. 2012). The nano-complex consisted of *T*-DNA, *VerD2*, and *E. coli* recombination protein A (RecA) instead of VirE2 of *Agrobacterium* and a Tat_2 as a cell-penetrating peptide (CPP). Triticale microspores are the plant hosting cells that showed a single transgene copy of integration and with no degradation of any delivered DNA to the triticale genome. This new method of transformation using the CPP to deliver the T-DNA showed similar results in monocots to that of *Agrobacterium* in dicots. In regard to intactness of transgene, copy number and expression, the nano-complex strategy showed better integration into single locus of triticale genome. A similar idea was applied on maize calli (Hansen and Chilton 1996; Hansen et al. 1997) using the *T*-DNA, *virD1* and *virD2*, with or without *virE2* to test the efficiency of combining biolistic gun and *Agrobacterium* (agrolistic) methodologies.

Rye has proved to be a good source of resistance to multiple biotic and abiotic stresses for wheat, and useful genes from the rye genome have been successfully transferred to the wheat genome using triticale as a bridge plant (Sapkota et al. 2018; Saulescu et al. 2011). Genes for resistance to wheat bunt, barley yellow dwarf virus, leaf rust, stem rust and powdery mildew have been successfully transferred to wheat from rye with the utilization of triticale as a bridge plant (Driscoll and Jensen 1964; Saulescu et al. 2011; Stewart et al. 1968; Zeller 1973). Wheat-rye 1RS/1BL translocation lines with good adaptation to many abiotic and abiotic stresses including powdery mildew and leaf rust resistance genes have been widely employed in wheat breeding programs such as at CIMMYT (Rabinovich 1998). Recently, a report on direct transfer of leaf rust and powdery mildew resistance genes from triticale to wheat has been published (Li et al. 2018) where the wheat line Xuezao was used as the male parent and the triticale line Sorento as the female parent. The recurrent parent, Xuezao, was susceptible to leaf rust and powdery mildew whereas the triticale line Sorento was resistant to both diseases.

Genomic in situ hybridization (GISH) and fluorescence In situ hybridization (FISH) confirmed the substitution and translocations lines. Among many important traits in triticale breeding, bread-making quality is one that was exposed to direct genetic engineering. Cytogenetic engineering of rye chromosome 1R in the triticale genome was targeted to improve this trait. In this study, two low protein genes (*Sec-1*, *Sec-3*) were removed from triticale and two storage protein loci (*Gli-1*, *Glu-1*) were inserted (Lukaszewski 2006). The genetic modification method used in this study was different from the *Agrobacterium*-mediated and gene-gun method, and was performed by using a two-step cytological engineering approach (TSCEA).

Since the release of first commercial triticale cultivar in the 1970s, significant progress has been made in triticale improvement and some of the current cultivars are equally important as wheat cultivars in various end use traits (Maheshwari and Eudes 2015). In spite of low genetic diversity, many breeding programs are working on triticale improvement using traditional and biotechnological approaches. Some of the properties of triticale, such as excellent grain and biomass yield, better resistance to disease and insects, and greater tolerance to adverse environmental conditions give triticale an advantage over wheat and rye (Maheshwari and Eudes 2015; Mergoum et al. 2009). Furthermore, incorporation of novel traits using genetic engineering techniques will improve the food and feed values of triticale.

11.6 Doubled-Haploid Breeding

Doubled-haploid (DH) techniques in plant breeding enable the generation of completely homozygous plants in a single generation (Fig. 11.6). Therefore, DH accelerates the breeding cycle by saving time compared to conventional breeding (Mergoum et al. 2009; Murovec and Bohanec 2012; Wedzony et al. 2015). The DH technique has been used in the improvement of crop species including wheat, corn and rice (Murovec and Bohanec 2012). However, its utilization is limited in triticale breeding. There are several techniques that can be applied in triticale to create DH. Androgenesis (Fig. 11.6), which uses microspores to obtain haploid plants, is frequently used in triticale to generate DH. However, gynogenesis (use of egg cells) is rarely used in cereals and grasses. Although the DH production protocol in triticale was initially based on wheat, several triticale-specific modifications have recently been made (Wedzony et al. 2015). The DH production is very complex and requires successful completion of several steps, mainly: (1) selection of donor parents and growth conditions, (2) anther culture, (3) regeneration and (4) chromosome doubling (Maluszynski et al. 2003). In addition to androgenesis, crossing of triticale lines with genetically-distant parents, such as corn, was successful to produce haploid triticale (Wedzony 2003; Wedzony et al. 1998). Undoubtedly, the use of DH techniques is promising in triticale breeding but there are several problems associated with it such as albinism and genetic variation (Wedzony et al. 2015).

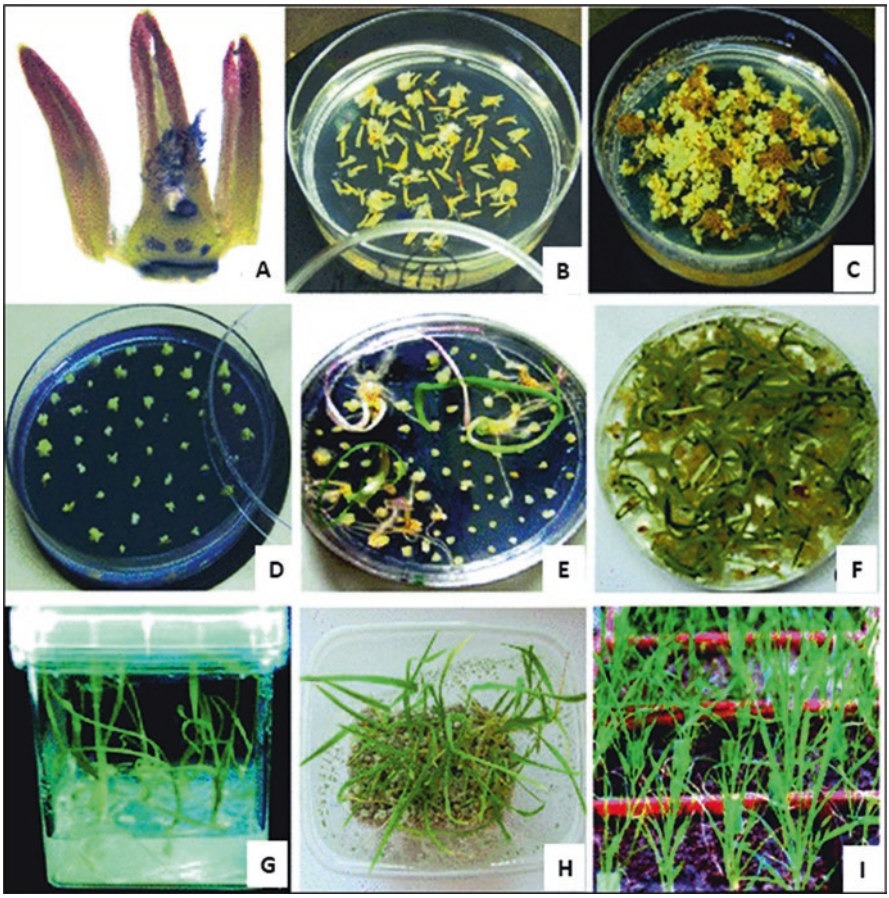

Fig. 11.6 Steps involved in anther culture during DH production in triticale. (**a**) Anthers on the induction medium, (**b**) Anthers 6 weeks after start of the induction culture, (**c**) Androgenic structures on regeneration medium, (**d**) and (**e**) Green (normal) and albino (no chlorophyll) plants after 1 week and after 4 weeks of regeneration phase, respectively, (**f**) Green normal plants transferred on rooting medium, (**g**) Acclimatization of plant at out-vitro conditions, (**h**) and (**i**) Triticale plants on soil. (Source: Wedzony et al. 2015)

Plant tissue culture is an important aspect of plant breeding and has a profound impact on agriculture (Fig. 11.6). One of the major applications of tissue culture in plant breeding is to create genetic variability within the germplasm which can then be used to develop novel superior genotypes (Hussain et al. 2012). Application of tissue culture in triticale improvement is the least studied as compared to other crops. Machczynska et al. (2015) used tissue culture in triticale to induce genetic variation using a metAFLP (methylation sensitive amplified fragment length polymorphism analysis) technique. This study demonstrated that tissue culture has the potential to cause genetic and epigenetic variation, and the metAFLP technique can be used to quantify the tissue culture induced variation in triticale.

11.7 Mutation Breeding

Genetic diversity plays a key role in any breeding program. In other words, without genetic variation, plant breeding research cannot be performed. Most strategies for breeding programs to improve the genetics of a trait of interest only account for the genetic variation of the trait itself. Most studies on the evaluation of genetic variation show a low level of genetic variability in triticale compared to its ancestors, particularly, wheat germplasm (De Costa et al. 2007; Kuleung et al. 2006; Tams et al. 2004; Trebichalský et al. 2013).

Crossing and mutation are two approaches to induce genetic variation in classical breeding for genetic improvement of desirable traits. Mutations are the ultimate source of genetic variation, and mutation breeding is the utilization of induced mutations for the genetic improvement of crops. Mutation breeding can be used as a powerful tool to induce genetic variation in both primary and secondary triticale germplasm pools. Oettler (1998) described mutation breeding using chemical or physical mutagens to induce genetic variability in triticale. The author also mentioned that mutation breeding programs can have middle-term and long-term benefits for the genetic improvement of triticale.

Mutagenesis is the process whereby sudden heritable changes occur in the genetic information of an organism through mutation-inducing agents (Roychowdhury and Tah 2013). Induced mutagenesis occurs by chemical mutagens (such as ethyl-methane sulfonate, EMS) or physical mutagens (such as gamma rays and X-rays) (Forster and Shu 2012). Until the late 1990s, mutations were only detectable based on their phenotypic performance. This required a huge population to detect homozygous mutants for every target gene. With new developments in technology, genotype-based mutation screenings have been applied in most major crops and abundant mutants have been detected (Tadele 2016). A common method of induced mutation by genotyping is targeting induced local lesions in genomes (TILLING) that uses a large offspring population derived from chemical or irradiation mutagenesis. But, instead of phenotypic screening, DNA must be isolated from the large offspring population and only the gene of interest is analyzed (Muehlbauer et al. 2006). Oladosu et al. (2016) summarized the method of mutation breeding in Fig. 11.7.

Recent new approaches of site-directed mutagenesis have been introduced to mutation breeding and are commonly known as *genome editing*. Among these approaches, clustered regularly interspaced short palindromic repeats (CRISPR) is the most efficient. CRISPR relies on adaptive immune responses of bacteria and archaebacteria to detect invading DNA by a small RNA encoded by a spacer (Sorek et al. 2013). This novel genome editing technique opens new perspectives for the practical application of mutants in crop breeding. Scheben and Edwards (2018) compared the traditional breeding pipeline using recombination or induced mutagenesis to a genome editing-based breeding pipeline using CRISPR/Cas-induced allelic series (Fig. 11.8). They also mentioned that genome editing had great potential to make plant breeding pipelines more predictable by applying a wide continuum of

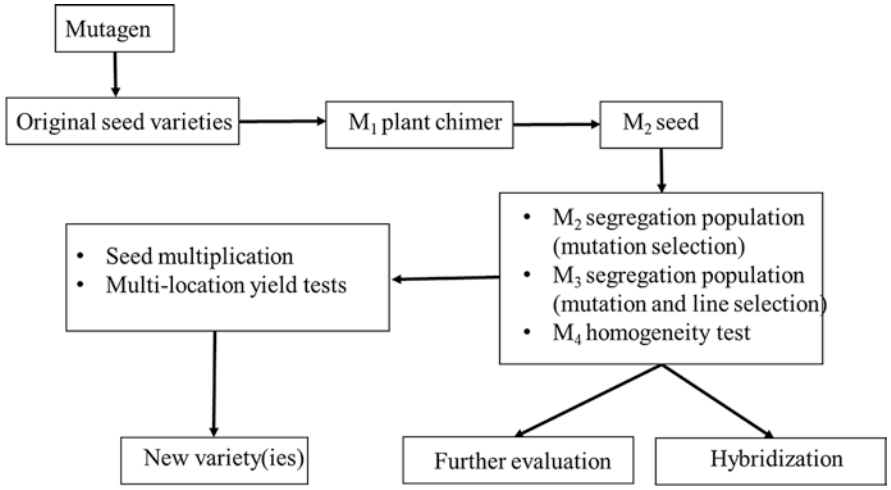

Fig. 11.7 Method of mutation breeding. (Source: Oladosu et al. 2016)

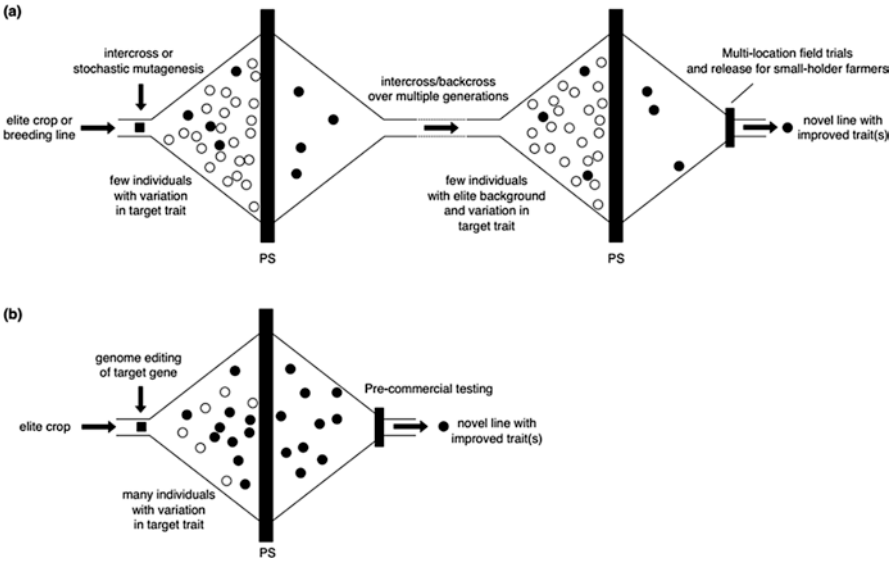

Fig. 11.8 (**a**) Comparison of the traditional breeding pipeline using recombination or induced mutagenesis, (**b**) genome-editing-based breeding pipeline. (Source: Scheben and Edwards 2018)

CRISPR/Cas-induced variants of a gene or regulatory element, rather than a smaller selection of natural or chemically/physically induced variants and this approach can increase the probability of achieving a line with a desirable phenotype.

Mutation breeding has been used widely in plant breeding programs of peas (*Pisum sativum*), rice (*Oryza sativa*), corn (*Zea mays*), soybean (*Glycine max*), wheat and many other crops (Lönnig 2005). According to the FAO/IAEA Mutant Variety Database, 2018, more than 3200 cultivars either obtained as direct mutants or derived from their crosses have been released worldwide (https://mvd.iaea.org). There appears to be limited information about mutation breeding in triticale. Pandini et al. (1997) showed plant height reduction in triticale using mutagenesis with gamma radiation and reciprocal crosses. Mutation breeding in combination with other techniques of genetic engineering as well as bioinformatics can be powerful tools to the genetic improvement of triticale.

11.8 Triticale Hybridization

One way to increase the genetic diversity of crop species is by incorporating useful genes from other species or genera. The formation of a new cultivar represents the combination of chromosomes of two different genera to combine the desirable traits into one plant. This is what is known for as *intergeneric hybridization*. Triticale from the hybridization of wheat, female parent, and rye, male parent (pollen donor), is a good example (Ammar et al. 2004). Over the years different ploidy levels have been created. The hexaploid type (2n = 42) has the A and B genomes of durum wheat and the R genome of rye. Common wheat can also be used in this type of hybridization, producing an octaploid (2n = 56) triticale with the A, B and D genomes from wheat and the R genome of rye**.** However, this type of triticale produces poor quality seed and is considered unstable (Mergoum et al. 2009). For this reason, many breeding programs have focused on hexaploid triticale for commercial use as forage and food. The tetraploid triticales, which combine the R genome of rye with the A and/or B genomes from wheat ancestors, showed no promise and were discontinued due to lack of desirable gene combinations.

11.8.1 Source of Germplasm and Cultivar Development

The first step in developing a new cultivar is the choice of desirable parents that carry the genes of interest. Usually, these adapted parents are selected from the same breeding program. If the character of interest is not available within this gene pool the breeder may explore an alternative source of germplasm that may not be as adapted, or come from the wild or even related species in an attempt to find the desired traits (Fehr 1987).

11.8.2 Breeding Goals and Strategies

The goal of a triticale breeding program is to breed improved cultivars for high forage and/or grain yield and high nutritional value. These traits are complex and controlled by multiple genes which makes them harder to breed for due to their low heritability. Triticale can also be used as human food by increasing the grain milling/baking quality traits to produce bread. A few breeding programs worldwide are working on releasing a triticale cultivar for bread making. The triticale genome, having the wheat and rye genomes, may produce irregularities during meiosis that may cause instability and incompatibility during breeding. In order to obtain a successful gene transfer from other species, the F_1 seeds from the initial cross and later generations must be viable. The early generation lines of triticale are not recommended for crossing until a certain genetic balance between wheat and rye is restored (Lelley 2006). The breeder also has to eliminate the undesirable characteristics that were transferred from the donor parents.

Several methods can be utilized in a triticale breeding program. The most common is pedigree selection. This requires a great deal of record keeping to provide catalogue information of the lines used in crosses. It is considered an effective way of selection because it is practiced and based on the phenotype and recently, the genotype of lines. Using the data provided by this selection method, the breeder advances only the superior lines carrying the genes of interest. However, the record keeping is burdensome and expensive.

Another breeding method utilized by many breeding programs is the modified bulk approach. This may be the best selection practice for triticale breeding. As previously mentioned, the goal is to develop a line that can be used in the forage market and using this method allows the breeder to select the qualitative traits such as plant height, maturity and disease resistance in the early generations. The selected plants are then harvested in bulk to form the next generation. In advanced segregating generation (F_5), selected heads from the bulk populations are planted as head rows. In subsequent generations, the rows are selected based on height, lodging, maturity and plant type. One may also take the forage weight of each selected row if the goal is to develop a forage type triticale. Harvesting of this forage row should be done at the soft or hard dough stage to insure high nutritional value. Later, the selected lines are evaluated in preliminary and advanced agronomic trials over many years and locations, compared to control cultivars and head rowed to produce breeder seed.

Male sterility facilitated recurrent selection (MSFRS) also can be used in a triticale breeding. The genetic control of this method is by single recessive gene so that the F_1 individuals is fertile. In the F_2 generation, the fertile and sterile individuals will segregate in 3:1 ratio. The breeder usually selects the sterile plants within the F_2 population that carry the trait of interest. The selected pants are tagged for easy identification at crossing. The pollen is then collected from fertile plants that could be advanced lines or adapted cultivars with unique characteristics and the sterile plants are crossed. At maturity, the crosses are collected and bulked to form the next cycle of recurrent selection or the base population. The main goal of the male sterility facilitated recurrent selection is to expedite population improvement by recur-

rent selection. In the F_2 generation, the fertile plants can be selected and advanced for further generations using the pedigree method. The recurrent part of the system occurs when the top lines selected from the population are crossed back into the population. One of the theories about MSFRS breeding is that genes for a particular trait can be placed in a large number of different backgrounds by crossing one cultivar into a broad-based population. The goal is to find an appropriate background for the genes where they will perform even better. The male sterility system is used primarily as a tool in plant breeding to eliminate emasculation in hybridization. It is worth mentioning that the stability of the male sterility system in a wide range of environments is very important to be useful for application in plant breeding.

11.9 Nutritional Value and Quality

Cereal grains are a major source of nutrients for humans and livestock. Providing enough food for the world's burgeoning human population is the first priority in the coming decades. Although expanding the global cultivation area and planting the most popular cereals may increase the annual yield, it may still not meet the increasing demands of an exponential increase of human population and may also have a negative effect on the environment and natural resources. Triticale has demonstrated high yield potential and could be a very reliable alternative for raising cereal production worldwide. Despite the high productivity of triticale, its global marketing is slowly growing, but planting area has been approximately the same for more than three decades. Triticale was used mainly for animal feed from the outset, while it can also be a good source of protein and energy for humans. The utilization of wheat in food products comes from the unique properties of grain storage proteins. Gluten is the primary storage protein of wheat grains and has primary influence on dough strength, elasticity, viscosity and cohesiveness. The high molecular weight (HMW) glutenins have a substantial influence on bread-making quality, despite being <10% of total seed protein. Scientists have frequently acknowledged the potential of hybrid triticale as a high-yielding cereal grain for human consumption; although the lack of extensive breeding and selection for storage protein quality in triticale has been a major obstacle to its use in commercial baking.

The hexaploid triticale genome (AABBRR) encodes three sets of HMW storage protein subunits, the 75 K γ-secalins of rye and other storage proteins with lower molecular weight (Igrejas et al. 1999). *Secaloglutenin* is another name for the polymeric storage proteins of triticale, consisting of HMW glutenin, HMW secalin, LMW glutenin and 75 k γ-secalin. Thus, secalogluten refers to the hydrated network of secalogluten in protein including any incorporated monomeric units (Dennett et al. 2013). The gluten and secalin network of triticale is generally weak and incohesive, with a strength between that of its progenitor species durum wheat and rye.

Triticale has lower SDS-sedimentation volume and mixing properties than hard wheat. However, variation has been reported, meaning there are some improved

modern genotypes with comparable properties to bread wheat (McGoverin et al. 2011). Triticale milling removes a significantly greater proportion of protein as compared to that of wheat because most of the removed proteins may be in the outer aleurone layer and pericarp (Serna-Saldivar et al. 2004). This suggests triticale has a thicker seed coat compared to wheat (Dennett and Trethowan 2013). The crude protein content of triticale grain is variable, 90–200 g kg^{-1} of dry matter (Boros 1999). Because of a high level of lysine, the biological value of triticale protein has been shown to be greater than wheat protein (Heger and Eggum 1991). Lysine is a limiting amino acid in cereal grains (Villegas et al. 1970), but some triticale genotypes were found to have lysine levels much higher than in either wheat or rye (Stallknecht et al. 1996). This makes triticale a rich source of lysine for human nutrition among the small grains.

Since the D-genome of bread wheat is a very important location for quality genes, such as those for the most effective gluten formation, introgressions and partial chromosome substitutions of the D chromosome is one way to enrich the triticale genomes (Budak et al. 2004; Lukaszewski 2003). The D genome has a good compensating ability in triticale (Wos et al. 2002); however, whole chromosome substitutions have reduced vigor and fertility (Xu and Joppa 2000).

The importance of the D genome is due to *Glu-D1d* allele encoding for HMW glutenin. A successful partial translocation with *Glu-D1d* was demonstrated by Lukaszewski (2006). Other studies confirm that lines carrying this chromosomal translocation produce gluten of similar quality to wheat (Wos et al. 2006); however, these translocations eliminated the locus for high root biomass and decreased the final yield (Rabiza-Swider et al. 2010). Friabilin is an approximately 15 kDa protein fraction associated with the surface of starch granules in the grain endosperm (Morris 2002). Grain hardiness is another important trait in determining the grain quality of triticale and its progenitors. Friabilin is responsible for a significant proportion of grain hardness and thus greatly influences its quality. The members of this protein family include puroindolines, grain softness protein, α-amylase/trypsin inhibitors and non-specific lipid transfer proteins. The single greatest influence on hardness in hexaploid wheat is the puroindoline (pin) locus (Martin et al. 2008). This locus is comprised of two tightly-linked genes located on chromosome 5DS, with a report of an A-genome variant (McIntosh et al. 2003). The functional puroindoline forms are designated Pina-D1a and Pinb-D1a, with other allelic forms designated Pina-D1b to Pina-D1g. Mutations at one or both of the hardness loci (Pina or Pinb) increase grain hardness (Morris 2002).

Puroindolines are highly basic proteins which are cysteine rich with a tryptophan-rich domain, and their presence results in a *soft* grain texture. Most wild type wheats produce puroindolines and grain softness protein. The pin genes have been deleted from the A and B genomes, however the grain softness protein (GSP-1) has been conserved (Bhave and Morris 2008). Since puroindolines are completely absent in durum wheat (AABB), they exhibit a very hard grain texture. The GSP-1 is also encoded on 5AS, 5BS and the distal end of 5DS (Morris 2002). They share approximately 40% identity with pin genes and all of them encode proteins with similar tertiary structure (Bhave and Morris 2008). Rye has a very soft endosperm texture,

although some variability exists (Williams 1986). In rye, the secaloindolines (Sina, Sinb) are orthologous to the puroindolines on the D genome of wheat. Grain softness proteins, GSP-R1 are also 90–95% identical to GSP in wheat (Simeone and Lafiandra 2005). Friabilins of triticale are expressed on both the durum wheat and rye genomes; however, due to the deletion of the pin loci on the A and B genomes, triticale generally exhibits moderately soft grain texture (Morris 2002). However, according to many studies (Li et al. 2006; Williams 1986), the full spectrum of grain hardness has been observed in triticale. Recent studies showed that a wide range of variation for hardness have been phenotyped in both hexaploid wheat and hexaploid triticale (Li et al. 2006). Unlike durum wheat, in triticale there is no expression for puroindolines, thus the main determinant of grain hardness are most likely the secaloindolines located on the rye genome (Morris 2002).

The largest component of cereal grains is the carbohydrate-rich endosperm. The main form of carbohydrate is starch, which is arranged in either large, lenticular A granules or smaller, spherical B granules bound by proteins and lipids. A and B granules have different physical and chemical properties such as gelatinization, pasting temperature, peak viscosity and retrogradation (Shelton and Lee 2000). There are two types of starch; amylose, which is predominantly comprised of long chains of α-(1-4) linked α-D-glucopyranosyl units; and amylopectin, a highly branched polymer with both α-(1-4) and α-(1-6) linkages. The ratio of amylose to amylopectin is controlled by a group of major and minor genes, of which the most investigated is granule-bound starch synthetase (Pham Van et al. 2006). Rye also produces starch in A and B shaped granules. The range in proportion of B granules is apparently lower in rye compared to wheat, ranging from 20% to 40% (Stoddard 1999). Like most cereals, the synthesis of starch in rye is controlled by at least one major gene and several minor or modifier genes. Since rye is an allogamous species there is considerable heterogeneity and thus few extremes of amylose content among cultivars (Mohammadkhani et al. 1999).

Non-starch polysaccharides (NSP) are other components of cereal grains involved in the structure and function of seeds, particularly in cell walls. They are polymers linked by glucodisic bonds, and the monomers such as glucose β (1-4), arabinoxylan (arabinose and xylose also known as pentosan), β-glucan, fructan, cellulose and lignin (Kumar et al. 2012). The amount of pentosan in rye is approximately twice of wheat (approximately 5% compared to 2–3%), and about 40% of this component is soluble (Ross et al. 2003). Arabinoxylan is a major part of the water-soluble NSP content in rye which are, practically, the cell wall polysaccharides. NSPs play an important role in the dough properties of rye compared to wheat due to their higher concentration and the lower strength of rye secalins (Ross et al. 2004). The genetic control of starch synthesis in triticale is a combination of expression of starch synthesis genes from both its progenitor species. The B-shaped granules in triticale lines were revealed to range from 15% to 33%; which is a lower average and smaller range, compared to the its progenitors (Stoddard 1999). Isoforms of granule-bound starch synthase (GBSS) from both wheat and rye genomes have been observed in triticale (Dennett et al. 2009; Schofield et al. 2008; Sharma et al. 2002). The range in amylose content of triticale is more than double the range observed in wheat (Dennett et al. 2009). As in wheat, GBSS genes are located on 4A and 7A in triticale,

while both the presence and absence of GBSS-4A has been observed in triticale (Dennett et al. 2009). Absence of GBSS allele in triticale is unexpected as it has largely originated from durum wheat (Soh et al. 2006). The only explanation of this phenomenon is an instability of the triticale genome or suppression of durum-derived GBSS genes in triticale by the rye genome (Schofield et al. 2008).

The amount of the non-starch polysaccharide in triticale is generally between wheat and rye (Oliete et al. 2010). Triticale has intermediate arabinoxylan content, higher fructan content than wheat and rye, while it has similar or lower β-glucan content than wheat (Rakha et al. 2011). Amylase is the primary enzyme which breaks down the starch in the cereal grains. A-amylase cleaves the α (1-4) glycosidic linkages in starch at any point along their length, while β-amylase cleaves only the non-reducing end of the chain. The α-Amylase activity in triticale is higher than both its progenitor species, approximately up to 10 times that of wheat (Mares and Oettler 1991). The gene involved in α-Amylase activity is located on chromosome 6R in triticale and removal of this chromosome results in a large increase in the falling number (Wos et al. 2002). Most of the modern triticale cultivars have low falling numbers and high α-amylase activities compared to wheat, but in some cases triticale genotypes are comparable to wheat and rye (Makarska et al. 2008). The ratio of amylose to amylopectin has a well-defined influence on quality. Low or zero amylose flour is described as *waxy*. Low amylose content causes greater water absorption capacity, higher peak viscosity of paste, lower peak viscosity temperature, lower final viscosity and greater resistance to retrogradation (Blazek and Copeland 2008; Pham Van et al. 2006). The A and B type of the starch granules have major effect on end use quality in cereal grains. Smaller B granules have a higher surface which increases water absorption, improving dough properties and loaf volume (Soh et al. 2006). Triticale has a lower proportion of B granules compared to both wheat and rye (Stoddard 1999). The milling process causes some starch granules damage. Severity of damages is directly proportional to grain texture, with harder wheats exhibiting more damaged starch. Damaged starch has a high hydration capacity, which negatively correlates with loaf volume (Oliete et al. 2010). The non-starch polysaccharides (also known as non-starch carbohydrates) play a significant role in grain texture, water absorption and baking quality in wheat (Bettge and Morris 2000) and rye (Peña 2004). The total content of non-starch polysaccharides in triticale averages between wheat and rye, which is not as important as the structure of the non-starch polysaccharides. This structure affects the viscous properties of the fiber viscosity, water binding capacity, intermolecular aggregation and oxidative gelatinization (Rakha et al. 2011). Pentosans (known as arabinoxylans or arabinogalactans) are the most important part of this structure, which forms a gel under oxidative conditions and influences many dough characters including loaf volume, crumb character and bread staling (Goesaert et al. 2005).

Soluble non-starch polysaccharides, well-known dietary fibers, have become a major subject in modern nutritional science (McGoverin et al. 2011). Dietary fibers are highly viscous and can be fermented to the shorter fatty acid chains. Purportedly, they may contribute to the prevention of coronary heart disease, cancer, high cholesterol and bowel disease (Kumar et al. 2012). Interestingly the amount of this valuable material is much higher in triticale as compared to wheat and rye (Rakha et al. 2011).

The protein content of early triticale cultivars was reported as intermediate between durum and rye, and in some cases higher than wheat (Lorenz et al. 1975). This may be due to the negative correlation between protein content and yield, or the high occurrence of non-filled or shriveled grain in early triticale (Costa and Kronstad 1994). Modern spring triticale cultivars, however, have slightly greater ability to assimilate nitrogen into the grain as compared to wheat (Erekul and Kõhn 2006). The insoluble polymeric fraction is the most important part of the grain protein in wheat and has the greatest influence on dough strength (Naeem et al. 2002). Triticale studies show that high grain protein appears to be associated with a hard endosperm, but does not necessarily result in large loaf volume (Amaya et al. 1986). Flour water absorption is low in triticale due to lower gluten content (Coşkuner and Karababa 2005). Some evidence shows that protein type exerts greater influence on loaf volume than whole grain protein quantity in triticale. For example, when the proportion of triticale gluten increases to the level found in wheat, the baking quality increases to a satisfactory level (Peña et al. 1998). Hence, improving the proportion of polymeric protein in triticale flour should also be considered as a breeding priority, alongside improving the glutenins composition. The amino acid components of the protein in triticale are an interesting subject for research. Several studies have indicated that triticale is higher in lysine compared with any other cereal grains (Heger and Eggum 1991). Due to high yield and rich lysine content, triticale produces a greater amount of desired (digestible) protein per hectare of crop compared to wheat (Heger and Eggum 1991). Protein hydrolysis and proteolysis during fermentation, crosslink them and make non-extractable polymers by disulfide bonds during kneading and baking (Horszwald et al. 2009). Since different kinds of triticale processing have different effects on the end use quality, processing methods need to be extensively investigated.

There has been very little research into the monomeric prolamins (α-, β-, ω- and γ- gliadins, ω-secalins, 40 K γ-secalins) or the proportion of polymeric to monomeric prolamins in triticale. However, solubility of the different protein fractions is roughly intermediate between its parental species in water, alcohol, ascetic acid and salt solution (Chen and Bushuk 1970). Mineral content is the total inorganic residue of flour or grain, with around 97% found in the bran. High ash content reflects minerals in flour and is associated with poor milling yield or bran contamination of flour, which leads to poor baking properties and darker color (Rasper and Walker 2000). Another form of ash content, crude ash, is highly related to agronomic conditions, such as the level of nitrogen fertilizer applied (Gulmezoglu and Kinaci 2005), which correlates positively to grain protein content (Feil and Fossati 1995).

Triticale reportedly has a higher lipid content than wheat and rye; however, lower lipid content in triticale was observed (Ao and Jane 2007). Lipids are the fat portion of the grain, consisted of non-polar glycolipids and phospholipids, comprising 2–5% in the germ of the small grain cereals. Lipids form complexes with the helical structure of amylose, hence grain amylose content is often positively correlated to lipid content (Goesaert et al. 2005). Despite making up a small proportion of grain weight, lipids can significantly influence dough prop-

erties. At lower concentrations, lipids restrict swelling of starch granules, reduce protein extractability and decrease loaf volume. At higher concentrations, however, they can increase loaf volume through stabilization of gas cells (Goesaert et al. 2005).

Although the progenitor species of triticale exhibit yellow and brown flour color (Tsvetkov et al. 2004), triticale flour is generally grayish, with a wide range of variability. White flour color is highly desirable in most major flour markets. Flour color and brightness are significantly correlated with flour protein (Kuchel et al. 2006), bran contamination and the actual color of the ground endosperm (Mares and Campbell 2001). Brightness is quantified as a L∗ value from 0 (black) to 100 (white), and creaminess/yellowness is quantified by a b∗ value ranged from +60 (yellow) to −60 (blue) (Wheat Marketing Center 2008). It is easy to conclude that, the grayish color of flour in triticale is the most important obstacle for acceptable bread baking (Salmon et al. 2004).

11.10 Conclusions and Prospects

Triticale is a man-made crop developed from the hybridization of wheat and rye with the hope of combining the high quality of wheat grain with the overall good adaptation of rye, including to marginal land with low inputs. Triticale is also a promising crop that can be grown for the dual purposes of human food and animal feed/forage. Additionally, it can be used as a genetic *bridge* and/or model to transfer useful genes, mainly disease-resistance genes, from the rye to the wheat genome. With human population increasing exponentially, more production of food crops, mainly cereals, is essential to ensure food security. Triticale has demonstrated high yield potential and represents a very reliable alternative to increase cereal production, particularly under poor and stressed environments. Triticale production and management is fortunately similar in most aspects to its progenitors, wheat and rye. This a great advantage for its adoption worldwide. However, despite the high productivity of triticale, its global marketing is slowly expanding, but planting area is approximately the same for more than three decades. This demonstrates that there are several challenges that triticale breeding and genetics research programs need to address in order to increase its grain yield/quality and forage/feed productions. Genetic diversity within triticale germplasm is relatively narrow as compared to other cereal crops such as wheat and rice. Therefore, the breeding should focus on creating genetic diversity within the available gene pool and utilization of molecular breeding approaches to improve this promising crop. Although molecular breeding approaches, such as MAS and recently GS, are crucial in the improvement of most crop species, their use in triticale is still in initial stages. Similarly, use of recently developed technologies such as CRISPER-CAS, currently used for gene editing in many crops, need to be adopted for triticale improvement. Genetic engineering and mutation breeding approaches have also been found to be effective in improving crop species, and their utilization in triticale improvement may help to achieve the

goals faster. Triticale has been used mainly for animal feed from the onset. However, triticale is a very versatile crop and demonstrated tremendous potential as a source of energy and of protein for humans as well as for animals.

Appendices

Appendix I: Research Institutes Relevant to Triticale

Institution	Specialization and research activities	Contact information and website
International Maize and Wheat Improvement Center (CIMMYT)	Germplasm collection and variety development	https://www.cimmyt.org/
The Plant Breeding and Acclimatization Institute (IHAR) – National Research Institute	Breeding	https://ihar.edu.pl/index_en.php
Danko	Breeding	Danko Hodowla Roślin Choryń 27, 64-000 Kościan Tel: +48 65 513 48 13 Fax: +48 65 513 48 06 Email: danko@danko.pl
Arizona Plant Breeders Inc.	Durum wheat, hard red spring wheat, triticale and barley breeding	Wesam AbuHammad Tel: 001 520 836-8228 www.arizonagrain.com
Australian Grain Technologies	Triticale breeding	20 Leitch Road, PO Box 341, Roseworthy, SA 5371 https://www.agtbreeding.com.au/contact
Canadian Ministry of Agriculture	Alberta- Agriculture and Forestry triticale breeding	https://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/fcd10571
Australian Government	Grain Research and Development Corporation (GRDC)	https://grdc.com.au/resources-and-publications/groundcover/ground-cover-issue-71-november-december-2007/four-new-triticale-varieties-launched
WARATAH	Seed corporation company	Smeaton, Victoria, Australia www.waratahseeds.com.au
INRA-Maroc	Agricultural Research	INRA-Maroc- Rue de la Victoire, Rabat, Morocco

Appendix II: Triticale Genetic Resources

Cultivar	Important traits	Cultivation location
Siskiyou	Resistant to bacterial leaf streak (BLS) disease	Northern California
Pi 428,736	Resistant to BLS	Manitoba, Canada
Pi 428,854	Resistant to BLS	Russia
Pi 428,913	Resistant to BLS and Common bunt	Manitoba, Canada
Pi 542,545	Resistant to BLS disease	Oregon, USA
Pi 587,229	Resistant to BLS disease	Tolbukhin Bulgaria
Sorento	Resistant to leaf rust and powdery mildew	Beijing, China
Apb113	High ADF, CP, NDF and TDN	Greece
Apb249	High ADF, CP, NDF and TDN	Central California, USA
Apb133	High ADF, CP, NDF and TDN	Utah, USA
Apb269	High ADF, CP, NDF and TDN	Utah, USA
Rosner	Yield	No representative
Welsh	Yield	University of Manitoba, Canada
Carman	Yield	SeCan Association, Canada
Oac wintri	Yield	SeCan Association
Oac triwell	Yield	SeCan Association
Oac decade	Yield	SeCan Association
Beaguelita	Yield	Agriculture and Agri-Food Canada, Oipc
Wapiti	Yield	Alberta Agriculture & Rural Development
Astute	Midseason maturing triticale, with a similar maturity to Hawkeye. Astute is widely adapted to all triticale growing regions, showing outstanding yields in higher yield potential areas of NSW	Australia
Bison	Reduced awned triticale derived from Rufustends to perform best relative to other triticale varieties in the lower to moderate yield potential environments of central NSW and SA	Australia
Fusion	Broadly adapted, mid maturing triticale selected from a unique cross between two triticale lines and Stylet (an unreleased bread wheat) suitable to all triticale growing regions of Australia, offering growers stable yield performance with a strong disease resistance package	Australia
Favorit	Yielding under pseudogley soil	Serbia
Tango	Yielding under pseudogley soil	Serbia
Odisej	Yielding under pseudogley soil	Serbia
Kg-20	Yielding under pseudogley soil	Serbia

(continued)

Cultivar	Important traits	Cultivation location
Oac trillium	Yield and other traits	SeCan Association
Frank	Yield and other traits	SeCan Association
Bura	Yield and other traits	SeCan Association
Pika	Yield and other traits	Alberta agriculture & rural development
Banjo	Yield and other traits	Farm Pure Seeds Inc., Canada
Ac polka	Yield and other traits	SeCan Association
Ac copia	Yield and other traits	Farm Pure Seeds Inc.
Ac alta	Yield and other traits	Progressive Seeds Ltd., Australia
Ac certa	Yield and other traits	Progressive Seeds Ltd.
Pronghorn	Yield and other traits	Alberta Agriculture & Rural Development
Ac william	Yield and other traits	SeCan Association
Sandro	Yield and other traits	La Coop Fédérée, Canada
Bobcat	Yield and other traits	Alberta Agriculture & Rural Development
Ac ultima	Yield and other traits	Agriculture And Agri-Food Canada, Oipc
Tyndal	Yield and other traits	Alberta Agriculture & Rural Development
Bunker	Yield and other traits	Alberta Agriculture & Rural Development
Luoma	Yield and other traits	Alberta Agriculture & Rural Development
Metzger	Yield and other traits	Alberta Agriculture & Rural Development
Bumper	Yield and other traits	Agriculture And Agri-Food Canada, Oipc
Taza	Yield and other traits	Alberta Agriculture & Rural Development
Brevis	Yield and other traits	Agriculture And Agri-Food Canada, Oipc

(continued)

Cultivar	Important traits	Cultivation location
Sunray	Yield and other traits	Agriculture And Agri-Food Canada, Oipc
Elevator	Yield and other traits	Seed-Link Inc., Canada
Thauvex	Yield and other traits	La Coop Fédérée
Sonika	Yield and other traits	La Coop Fédérée
Aac delight	Yield and other traits	Agriculture And Agri-Food Canada, Oipc
Hotshot	Yield and other traits	Seed-Link Inc.
Circuit	Yield and other traits	Seed-Link Inc.
TRINIDAD	Heading date, plant height, powdery mildew resistance and yield	German National Variety List
SANTOP	Heading date, plant height, powdery mildew resistance and yield	German National Variety List
TMP16315	Moderate FHB and ergot resistance, short height, low LDG and high TWT and YLD.	
AC Ultima	Superior in GPC	
Pronghorn	T124 Spring type with FHB resistance	Canada
Brevis	T200-Spring type with FHB resistance	Canada
LASKO	High protein content	Estonian
DAGRO	High protein content	Estonian
TEWO	High protein content	Estonian
Tatlicak 97	Drought tolerance	Turkey,
Karma 2000	Lowest drought susceptibility	Turkey
MIKHAM 2002		Turkey
CAMELOT	Spring triticale, early maturing, awnless, and short statured, leaf carriage is drooping. Necks are wavy and hairless. Heads are mid-dense, kernels are slightly wrinkled, red, elliptical, with a brush that is large and long, resistant to stripe rust, leaf rust, powdery mildew, and BYD and moderately resistant to *Septoria tritici* leaf blotch	Resource Seeds, Inc. California
MERLIN	Spring triticale, late maturing, leaves have a waxy bloom, flag leaf is twisted, kernels are amber, elliptical, and wrinkled, resistant to powdery mildew, moderately resistant to leaf rust, stripe rust, and BYD and susceptible to *Septoria tritici* leaf blotch	Resource Seeds, Inc.
PACHECO	Spring triticale for whole plant forage, day length insensitive, short plant height, very good straw strength, spikes are awned, fusiform, mid-wide, and mid-long with waxy bloom, seeds are light red, large with smooth texture, resistant to stripe rust, leaf rust, powdery mildew, and BYD and moderately resistant to *Septoria tritici* leaf blotch	Westbred LLC, Bozman, Montana

(continued)

Cultivar	Important traits	Cultivation location
TRICAL BRAND 102	Winter triticale, awnletted, late maturing, resistant to stripe rust, leaf rust and *Septoria tritici* leaf blotch	Resource Seeds, Inc.
TRICAL BRAND 105	Spring triticale, awned, medium maturing, good lodging resistance, resistant to stripe rust, leaf rust and *Septoria tritici* leaf blotch, and moderately susceptible to BYD, moderately susceptible to stripe rust	Resource Seeds, Inc.
TRICAL BRAND 118	Spring triticale, medium maturity, good lodging resistance, leaves have a waxy bloom, flag leaf is twisted, kernels are slightly wrinkled, amber, elliptical, with a brush that is midsize and mid-long, resistant to leaf rust, powdery mildew, and BYD and moderately resistant *Septoria tritici* leaf blotch, moderately susceptible to stripe rust	Resource Seeds, Inc.
TRICAL BRAND 2700	spring triticale, fully awned, with medium late maturity, flag leaf is recurved, spikes are erect, oblong, mid-wide and mid-dense, kernels are reddish tan, elliptical, and average 9 mm long and 4 mm wide, resistant to leaf rust, stripe rust, *Septoria tritici* leaf blotch, and powdery mildew, and moderately susceptible to BYD	Resource Seeds, Inc.
ALZO	Winter triticale, tall with good straw strength	Resource Seeds, Inc
BEAGLE	Spring triticale, medium maturity and is tall with fair straw strength, resistant to *Septoria tritici* leaf blotch, stripe rust, leaf rust and moderately susceptible to BYD	CIMMYT in cooperation with the Mexican Ministry of Agriculture (INIA)
BEAGUELITA 'S'	Spring triticale, medium maturity and is medium tall with good straw strength, resistant to *Septoria tritici* leaf blotch, stripe rust, and leaf rust, and moderately susceptible to BYD	CIMMYT in cooperation with INIA
CABORCA 79	Spring triticale, medium early maturity and is medium tall with fair straw strength, resistant to *Septoria tritici* leaf blotch, stripe rust, and leaf rust, and susceptible to BYD	CIMMYT in cooperation with INIA
CANANEA 79	Spring triticale, medium early maturity and is medium height with fair straw strength, resistant to *Septoria tritici* leaf blotch, stripe rust, and leaf rust, and moderately susceptible to BYD	CIMMYT) in cooperation with INIA
CELIA	Winter triticale, prostrate early growth, medium early maturity, stiff straw and good lodging resistance, spikes are awned, resistant to stripe rust, leaf rust, and *Septoria tritici* leaf blotch, moderately resistant to *Pseudocercosporella* foot rot and snow mold, and moderately susceptible to take-all disease	Oregon AES
DÉCOR	Winter triticale, mid-tall with good straw strength, resistant to leaf rust and moderately susceptible to BYD	Resource Seeds, Inc.
ELAN	Winter triticale, mid-tall with good straw strength, resistant to BYD	Resource Seeds, Inc.

(continued)

Cultivar	Important traits	Cultivation location
FLORA	Winter triticale	Oregon Agricultural Experiment Station.
FLORICO	Spring triticale, medium maturity and is tall with poor straw strength, resistant to *Septoria tritici* leaf blotch, stripe rust, and leaf rust, and moderately resistant to BYD	Nutriseed, Inc., Arizona
FORERUNNER	Spring triticale, late maturing and medium height	Resource Seeds, Inc.
GRACE	Spring triticale, medium late maturing, tall with fair straw strength, Spikes are lax and fusiform with long awns, kernels are elliptical and wrinkled, resistant to leaf rust and stem rust and tolerant of BYD	ARCO Seed Co, Pakistan
Juan	Spring triticale, tall, fair straw strength and shatter resistance, produces high forage and grain yields, long, lax spikes, Kernels are red, long, well-filled, resistant to leaf rust, powdery mildew and *Septoria tritici* leaf blotch, and moderately susceptible to BYD, susceptible to stripe rust	California AES
LANCE	Spring triticale, awnless, medium late maturing	Resource Seeds, Inc.
MAH 3600	Winter triticale, tall with good straw strength	Developed in Poland and received for evaluation from Resource Seeds, Inc.
MAH 3800	Winter triticale, mid-tall with good straw strength, resistant to leaf rust and BYD	Developed in Poland and received for evaluation from Resource Seeds, Inc.
PEACE	Spring triticale, tall with good straw strength, moderately susceptible to BYD	ARCO Seed Co.
SISKIYOU	Spring triticale, spikes are long and somewhat lax. Awns are Short and purple to black in color, kernels are somewhat wrinkled, shorter than most other triticales of the period, with a protruding germ end typical of durum wheat. Grain color is light red, generally with a high percentage of yellowberry kernels, resistant to *Septoria tritici* leaf blotch, moderately susceptible to stripe rust and leaf rust, and susceptible to BYD	CIMMYT and the California AES
SPRINGFEST	Spring triticale, late maturing and is tall with good straw strength, resistant to *Septoria tritici* leaf blotch, stripe rust, and leaf rust, and moderately resistant to BYD	Nutriseed, Inc.
TRICAL BRAND 96	Spring triticale, medium early maturing and has excellent straw strength, resistant to leaf rust and BYD and susceptible to *Septoria tritici* blotch and stripe rust	Resource Seeds, Inc.

(continued)

Cultivar	Important traits	Cultivation location
TRICAL BRAND 98	Spring triticale, early maturing, good lodging resistance, heads are mid-dense and fusiform, with yellow awns, kernels are slightly wrinkled, amber, elliptical, with a brush that is midsize and mid-long, resistant to leaf rust, powdery mildew, and BYD, moderately susceptible to stripe rust, and susceptible to *Septoria tritici* leaf blotch	Resource Seeds, Inc.
TRICAL BRAND 103BB	Winter triticale, late maturing with fair straw strength, resistant to stripe rust, leaf rust, and *Septoria tritici* leaf blotch	Resource Seeds, Inc.
TRICAL BRAND 110	Spring triticale, midseason maturity, good straw strength, resistant to stripe rust, leaf rust, powdery mildew and BYD, and moderately susceptible to *Septoria tritici* leaf blotch	Resource Seeds, Inc.
TRICAL BRAND 111	Spring triticale, awned, medium maturing, and has good lodging resistance, resistant to stripe rust, leaf rust, *Septoria tritici* leaf blotch, powdery mildew and BYD	Resource Seeds, Inc.
TRICAL BRAND 116	Spring triticale, medium early maturity and good lodging resistance, flag leaf is twisted, heads are mid-dense and fusiform, with tan awns, kernels are slightly wrinkled, amber, elliptical, with a brush that is large and mid-long, resistant to leaf rust and BYD, and moderately susceptible to stripe rust and *Septoria tritici* leaf blotch	Resource Seeds, Inc.
TRIMARK 336	Winter triticale, tall with good straw strength, resistant to BYD and leaf rust	Resource Seeds, Inc.
WYTCH	Spring triticale, medium maturity and is tall with poor straw	ARCO Seed Co.
	Strength, moderately susceptible to BYD	
YUMA	Spring triticale, medium maturing and is tall with fair straw strength, resistant to stripe rust and moderately susceptible to BYD	Nutriseed, Inc. (AZ).
Alamos	Yield, diseases resistant	Mexico/CIMMYT
Armadillo	Yield, diseases resistant	Mexico/CIMMYT
Yoreme	Yield, diseases resistant	Mexico/CIMMYT
Cananea	Yield, diseases resistant	Mexico/CIMMYT
Fahad-5	Yield, diseases resistant	Mexico/CIMMYT
Pollmer	Yield, diseases resistant	Mexico/CIMMYT
Rhino	Yield, diseases resistant	Mexico/CIMMYT
Eronga-83	Yield, diseases resistant	Mexico/CIMMYT
Juanillo	Yield, diseases resistant	Mexico/CIMMYT/ Morocco
Beaguelita	Yield, diseases resistant	Mexico/CIMMYT/ Morocco
Beagle	Yield, diseases resistant	Mexico/CIMMYT/ Morocco

(continued)

Cultivar	Important traits	Cultivation location
Drira out cross	Yield, diseases resistant	Mexico/CIMMYT/ Morocco
Borhane	Yield, diseases resistant	Morocco
Mpoumtaz	Yield, diseases resistant	Morocco
Milenio TCL3	Yield, diseases resistant	Mexico/CIMMYT
Siglo-TCL21	Yield, diseases resistant	Mexico/CIMMYT
Sup[remo TCL2000	Yield, diseases resistant	Mexico/CIMMYT
Quebrantahuesos-TCL99	Yield, diseases resistant	Mexico/CIMMYT
Maravilla-TCL99	Yield, diseases resistant	Mexico/CIMMYT
Cerrillo-TCL99	Yield, diseases resistant	Mexico/CIMMYT
AN31	Yield, diseases resistant	Mexico/CIMMYT
An34	Yield, diseases resistant	Mexico/CIMMYT
TCLW-Anapelon	Yield, diseases resistant	Mexico/CIMMYT
TCLF-AN38	Yield, diseases resistant	Mexico/CIMMYT
TCLF-AN105	Yield, diseases resistant	Mexico/CIMMYT
Focus	Winter triticale	Turkey
Mikham-2002	Winter triticale	Turkey
Tacettinbey	Spring triticale	Turkey
Tatlıcak-97	Winter triticale	Turkey
Ege Yıldızı	Spring triticale	Turkey
Melez-2001	Winter triticale	Turkey
KARMA-2000	Spring triticale	The Anatolia Agricultural Research Institute (AARI)
PRESTO	Spring triticale	AARI
TACETTİNBEY	Spring triticale	Cukurova University (CU)

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Chapter 12
CRISPR/Cas9 Genome Editing in Bread Wheat (*Triticum aestivum* L.) Genetic Improvement

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Abstract Bread wheat (*Triticum aestivum* L.) is the most important staple crop worldwide. Wheat has a large and allohexaploid genome with more than 107 thousand gene models that expand over 21 chromosomes with 3 replicates. The high complexity of the wheat genome has restricted the success of conventional breeding programs. Wheat genome modification by biotechnological methods has been hindered due to the current methods limitations and safety issues over genetically-modified crops. CRISPR/Cas9 is an emerging biotechnological tool that holds promises for multiplexed, sequence-specific, efficient and rapid manipulation of large genomes such as that of wheat. The CRISPR/Cas9 system introduces sequence-specific double-strand breaks (DSBs) in DNA by synthetic nucleases. The targeted genomic loci are then fixed by DNA repair mechanisms such as non-homologous end-joining (NHEJ) or homology-directed repair (HDR). The system and its improved sub-techniques have achieved significant successes in addressing biosafety and legal concerns over genetically-modified plant production. In this chapter, the history, potentials and the latest results of CRISPR/Cas9-based genetic manipulations in bread wheat is reviewed.

Keywords Common wheat · Polyploidy genomes · Multiplexed · Sequence-specific genetic editing

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J. M. Al-Khayri et al. (eds.), *Advances in Plant Breeding Strategies: Cereals*,
https://doi.org/10.1007/978-3-030-23108-8_12

12.1 Introduction

The rapid pace of global human population growth has raised serious concerns over food supply in the near future. Therefore, producing more food using the already-depleting natural resources including, water, arable lands and energy is very important. However, the adverse effects of global warming due to climate change, advancement of desertification and deforestation phenomena, and the water crisis are just a few problems that have hindered the efforts to increase agricultural efficiency and food production worldwide. It is estimated that the global food production requires an increase about 50% by 2030 and 70–100% by 2050 to satisfy the world population demand for food and feed (Godfray et al. 2010). Regarding the rapid decline in natural resources, and anthropogenic and nature-made crises, it is necessary to develop strategies to introduce tolerant and adaptable crop species to environmental stresses, with higher yield and quality (Haque et al. 2018).

Bread wheat (*Triticum aestivum* L.) is the second most important crop plant, after rice, and a staple food since the beginning of agriculture history. The plant is globally cultivated under various climatic conditions and provides about 20% of human daily calories. However, the annual increase in wheat production is less than 1% that cannot support the soaring demands for wheat-based foods (Bhowmik et al. 2018). The complex and gigantic genome structure (~17 Gb) (Krasileva et al. 2017), high ploidy level, high repetitive DNA content (80–90%) and monocot nature have made wheat a recalcitrant species for conventional forward and reverse genetic studies, and genome modification (Dvořák 2009; Uauy et al. 2017). Although it makes the plant's genome an interesting model in genetic and modern biotechnology studies (Kim et al. 2018), the conventional and non-specific genetic methods have not yet offered any significant contribution to wheat yield. Therefore, the adoption of innovative, precise and reliable genetic approaches for analysis and modification of the wheat genome is required to improve the crop production in terms of quality and yield (Bhowmik et al. 2018; Liang et al. 2018).

Conventional improvement methods are heavily dependent on non-plant gene-delivery tools such as *Agrobacterium* that can be integrated into the target plant genome (i.e., foreign DNA), passed down to plant progeny and could be constituently expressed, which is a bio-safety issue. Recently, methods have been developed allowing precise, tissue-specific, high-frequency mutation or multiplexed, and efficient genome modification with transiently-expressed mediators that have significantly contributed to fast breeding of plants with polyploidy and large genomes. In this chapter, the potential of newly-developed editing tools for modification of wheat complex and polyploid genome are briefly reviewed.

12.2 Manipulation of Large and Polyploid Genomes

Polyploidy is an important evolutionary phenomenon and refers to the presence of more than two paired (homologous) sets of chromosomes in a genome. This is the characteristics of many important crops and industrial plant species, such as wheat,

oilseeds, potato, cotton and peanut with three sets (triploids), four sets (tetraploids), or six sets (hexaploids) of chromosomes. The high level of complexity and ploidy in many crop plants like hexaploid wheat, presents serious challenges in genetic studies because there are three copies of each gene. Any mutation or modification in one gene will be neutralized by the other two functional genes. Therefore, all copies of a target gene need to be modified. Conventional methods or genome editing with an engineered nuclease system, such as T-DNA insertions, ethyl methanesulfonate (EMS) mutagenesis and gamma (γ) radiation have been used but they have not been successful to address this issue due to their random targeting and inability of precise and multi-locus or multiplexed modification of polyploid genomes (Belhaj et al. 2015). In fact, these systems use restriction endonucleases and ligase enzymes that could target, cut and rejoin small DNA sequences, such as viral and bacterial genomes. In addition, they are incapable of precise and multi-editing of genetic loci of higher plants. The latest solution for this challenge is multiplexed editing. The multiplexed genome editing is the process of simultaneously targeting and modifying more than one genomic locus. It has revolutionized the current genome engineering techniques, such as multi-gene knockouts, chromosomal deletion and translocation, gene or promoter knock-in, gene activation and repression, or epigenome modifications (Minkenberg et al. 2017).

The discovery of new genome editing tools, such as transcription activator-like effector nucleases (TALENs), zinc finger nucleases (ZFNs) and clustered regularly interspaced short palindromic repeats/associated Cas9 protein (CRISPR/Cas9) has greatly contributed to overcome the problem of large genomes and multiplex editing. These systems use artificial enzymes or short oligonucleotides sequences that are designed to selectively target specific gnome loci or sequences on genomes and cleave the DNA in those regions. Table 12.1 summarizes the technical characteristics of four recent genome editing tools based on the sequence-specific nucleases approach. These systems offer advantages or faced drawbacks with respect to cloning process, number of simultaneously-expressed gRNAs or genomic loci targeted and editing efficiency (Kamburova et al. 2017). The TALENs method is reported to be the most precise system for genome editing in terms of both targeting the correct genomic loci and causing less cytotoxicity due to off-targeting effects (Weinthal and Gürel 2016). The ZFNs system has been reported to be less efficient than CRISPR and TALENs.

The utilization of sequence-specific, multiplexed genome editing tools, including ZFNs and TALENs have been successfully implemented in both animals and plants (Gaj et al. 2013; Joung and Sander 2013). However, their implementation and gene editing design require sophisticated protein engineering, which is an expensive and delicate task (Bortesi and Fischer 2015; Gaj et al. 2013). Briefly, in these methods, a sequence-specific DNA binding domain is designed and fused with *Fok*1 DNA nuclease (Carroll et al. 2006; Christian et al. 2010). The nuclease then modifies the genome by creating double-strand breaks (DSBs). The breaks are then repaired by either homologous recombination (HR) or non-homologous end joining (NHEJ). The HR is a sequence replacement of correction pathway with lower error-rate, while NHEJ is subjected to some errors (Symington and Gautier 2011). The mode of action in both TALENs and ZFNs is through the tandem repeats in their DNA binding sites. These tandems could be engineered to find specific DNA sequences

Table 12.1 Comparison of sequence-specific genome editing tools

Parameter	ZFNs[a]	TALENs	ODM	CRISPR/Cas9
Components	Zn finger domains Non-specific *Fok*I nuclease domain	TALE DNA-binding domains non-specific *Fok*I nuclease domain	Exogenous polynucleotide(chimeraplast)	crRNA, Cas9 proteins
Structural proteins	Dimeric protein	Dimeric protein	Non-protein nature	Monomeric protein
Catalytic domain	Restriction endonuclease *Fok*I	Restriction endonuclease *Fok*I	No catalytic domain	RUVC and HNH
Length of target sequence (bp)	24–36	24–59	68–88	20–22
Protein engineering	Required	Required	Not required	sgRNA could be applied
Cloning	Necessary	Necessary	Not necessary	Not necessary
sgRNA production	Not applicable	Not applicable	Not required	Easy to produce
Mode of action	Double-strand breaks in target DNA	Double-strand breaks in target DNA	Information strand directs conversion(s) within target region	Double-strand breaks or single-strand nicks in target DNA
Target recognition efficiency	High	High	High	High
Mutation rate	High	Middle	Middle	Low
Protein engineering	Required	Required	Not applied	Not applied
Target sites range	Limited	Limited	Limited	Unlimited (Only limited by PAM[a] sequences)
Delivery of the vector construct into cells	Long and highly repetitive encoding vector	Long and highly repetitive encoding vector	Double stranded Chimeraplast containing DAN-RNA sequences	Short sgRNA vector
Multiplexing	Possible: Difficult	Possible: Difficult	Technically difficult	Possible: Easy

[a]*ZFNs* Zinc finger nucleases, *TALENs* Transcription activator-like effector nucleases, *ODM* Oligonucleotide-directed mutagenesis, *CRISPR/Cas9* Clustered regularly interspaced short palindromic repeats/associated Cas9 protein, *PAM* Protospacer adjacent motif
Source: Adopted from Kamburova et al. (2017) with modifications

on the host genome. The designed chimeric nucleases are then guided to any desired genomic loci to generate DSBs. This step requires designing and construction of specific TALEN or ZFN chimeric proteins for each target site, which is a main obstacle.

12.3 CRISPR/Cas9: A Multiplexed, Sequence-Specific Tool

Clustered regularly interspaced short palindromic repeats/associated Cas9 protein (CRISPR/Cas9) is an emerging sequence-specific editing technique that has gain considerable reputation for its advantages over both conventional and other recently-developed gene editing methods. Since its advent, CRISPR-Cas9 has been successfully utilized for human, animal and plant genome editing such as human embryo and stem cells (Freiermuth et al. 2018; Schenkwein and Ylä-Herttuala 2018), mice (Platt et al. 2014), *Arabidopsis thaliana* (Feng et al. 2014; Zhang et al. 2016b), tobacco (Gao et al. 2015; Jiang et al. 2013), tomato (Čermák et al. 2015), barley (Lawrenson et al. 2015), *Camelina sativa* (Morineau et al. 2017), wheat (Gil-Humanes et al. 2017; Liang et al. 2017; Upadhyay et al. 2013; Wang et al. 2014b; Zhang et al. 2016a), rice (Endo et al. 2015; Jiang et al. 2013), sorghum (Jiang et al. 2013) and maize (Svitashev et al. 2016). CRISPR/Cas9 is one of the multiplexed genome editing tools that hold promises for successful manipulation of important agronomic traits under multiple genes control (Bortesi and Fischer 2015).

The CRISPR/Cas9 system like TALENs or ZFNs stimulates genome editing by creating sequence-specific DNA DSBs, and NHEJ or HDR repair at targeted loci. This is basically a bacterial defense mechanism against bacteriophages in many prokaryotic cells, and destroys foreign DNA or RNA sequences to protect the cell against viral or mobile genetic elements (Barrangou et al. 2007). The genetic region on the DNA that is responsible for this mechanism is called CRISPR (Jansen et al. 2002). It is found on chromosomal and plasmid DNA (Rath et al. 2015). The CRISPR genetic locus was first reported in *Escherichia coli*, and has been identified in more than 84% and 45% of the known archaea and bacteria species, respectively (Al-Attar et al. 2011).

The mechanism of CRISPR/Cas9 action is thoroughly reviewed by Thurtle-Schmidt and Lo (2018). Briefly, the system includes a cascade of few proteins with different and complementing roles. Most of the cascade proteins in *Streptococcus pyogenes* are designated as an integrated self-operating protein complex known as Cas9. The Cas9 is responsible for enabling sgRNA binding, finding the complementary sequence and cleaving the target sequence on the DNA strands. In CRISPR, protospacer adjacent motif (PAM) recognition domain is an important component, which is responsible for distinguishing the bacterial encoding RNA from the bacteriophage target sequence. The PAM sequence needs to be present at the NGG downstream of the targeted sequence. The Cas9 first binds the PAM sequence, and then opens the DNA allowing RNA/DNA hybridization or R-loop formation, and cleaves both DNA/RNA and ssDNA strands (Fig. 12.1).

The application of nucleotide sequences known as guide RNA (gRNA) is one of the advantages of this system over the ZFN and TALEN editing systems, which use protein sequences for precise targeting. In fact, the system uses a single short guide RNA (sgRNA) to send the Cas9 endonuclease to its complementary target DNA. This makes the method inexpensive and easy to design and implement, because only a nucleotide sequence is required for a target domain (Mao et al. 2013; Upadhyay et al. 2013).

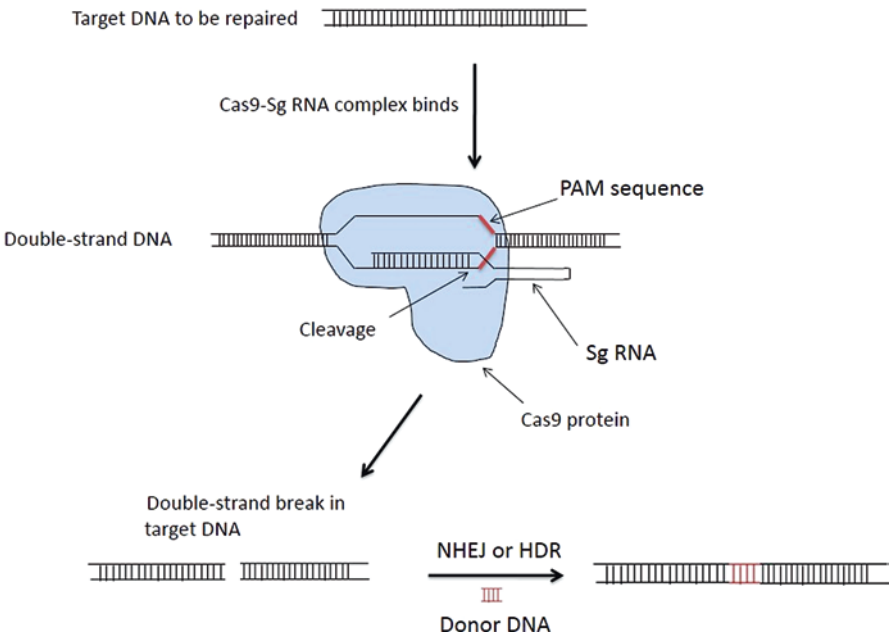

Fig. 12.1 A schematic presentation of CRISPR/Cas9 genome editing technique
Cas9 (CRISPR associated protein 9): an RNA-guided DNA endonuclease enzyme associated with the CRISPR;
PAM (Protospacer Adjacent Motif): a 2–6 base pair immediately following the DNA sequence targeted by the Cas9;
Sg RNA: Single guide RNA used to direct the Cas9 protein to bind and cleave a particular DNA sequence for genome editing;
NHEJ: Non-Homologous End Joining pathway that generates insertions and deletions during double-stranded break repair;
HDR: Homology-Directed Repair pathway that seals the double-stranded break in an error-free manner

CRISPR/Cas9 is an emerging technique in genetic engineering with exceptional advantages over similar techniques such as ZFNs and TALENs (Table 12.1). However, the system suffers from some limitations, including PAM sequences identification, low homologous recombination ratio, off-target mutagenesis and recalcitrant sgRNA/target binding (Richardson et al. 2016; Shan et al. 2014). It is also suggested that the Cas9 size was still an issue subject to more enhancement. Despite the limitations, CRISPR/Cas9 currently offers the most efficient and hassle-free system for multiplexed genome editing. The non-specific editing or low specificity that is a very important qualitative characteristic of targeted-modification is rarely reported in this system (Fu et al. 2013). Several approaches, such as dCas9-fok (Weinthal and Gürel 2016) and CRISPR-SKIP (Gapinske et al. 2018) have been suggested to improve the system efficiency. For example, the improvement in the CRISPR-SKIP method involves the application of single-base editors that regulates genetic loci without breaking genomic DNA at unwanted locations. The plasmid-mediated delivery of genome editing parts such as CRISPR/Cas9 ribonucleopro-

teins (RNPs) into target cells is a critical issue. The conventional methods might cause random integration of plasmid sequences into the host cell genome. This has raised serious concerns among legal and social communities over the food and bio systems' safety of CRISPR-edited plants. Malnoy et al. (2016) devised a method that could deliver purified RNPs into apple protoplast. Therefore, the chances of off-target mutations or introduction of alien genome sequences into target cells would significantly decrease. The result of such improvements could lead to producing safer and high quality genetically modified plants.

12.4 CRISPR/Cas9 Genome Editing in Wheat

There are six described wheat species with different levels of polyploidy (Table 12.2) (Dvorak 2001). About 95% of wheat cultivation consists of common wheat, and 5% durum wheat. The common wheat (*Triticum aestivum* L.) genome is complex due to its allohexaploid structure or inclusion of three different genomes (2n = 6× = 42, AABBDD) (Ling et al. 2013). The species is considered a young polyploid species that originated around 10,000 years ago by hybridization of a tetraploid species with *Aegilops tauschii* (diploid, DD), followed by domestication (Dubcovsky and Dvorak 2007). The main sources of wheat allelic (gene) variation include the natural diversity of wild ancestors (Jordan et al. 2015; Wang et al. 2014a) or *in vitro* induced mutants (Krasileva et al. 2017). The variation in genes that control agronomic and qualitative traits and local recombination at their genomic loci could also occur in the wheat genome (Wang et al. 2018).

The application of the CRISPR/Cas9 system for wheat genome modification is expected to bring about interesting results. However, it is still in its infancy and studies about the successful manipulation of wheat genome by the CRISPR method are just being published (Table 12.3). One reason could be that the main components of the CRISPR system, such as the version of Cas9 are still being improved to meet the specific characteristics of wheat genome. Moreover, despite several advantages of the CRISPR system for wheat genome editing, the monocot nature of the species is still a challenge for vector delivery into cells and the regeneration of transgene cells.

The gene loci *TaMLO* in wheat is responsible for encoding proteins that repress the defense responses against powdery mildew diseases in plants (Araki and Ishii 2015). The *MLO* loci are highly conserved in plant genomes (Kusch et al. 2016). This gene has about three homologus loci across the wheat genome. Shan et al. (2013) were the first who reported the application of CRISPR/Cas9 method to knock-out all the three alleles of *TaMLO* in wheat genome to induce heritable mildew resistance in genetically-modified wheat. They compared the CRISPR and TALENS systems and reported that despite the lower mutagenesis frequency in CRISPR (26%) compared to that of TALENS (36.5%), the CRISPR system was easier to design and implement. However, they did not indicate the inheritance of the mutant alleles in transgenic lines. In another study, Wang et al. (2014b) knocked-out the three homoeoalleles of *MLO* gene, and produced stable transgenic lines

Table 12.2 Wheat (*Triticum* spp.) species and subspecies with indication to ploidy level and domestication status

Species	Ploidy	Domestication status	Subspecies
T. urartu	Diploid (2x)	Wild	–
T. monococcum	Diploid (2x)	Wild	Wild einkorn wheat (*aegilopoides*)
T. monococcum	Diploid (2x)	Cultivated	Wild einkorn wheat (*monococcum*)
T. turgidum	Tetraploid (4x)	Wild	Wild emmer wheat (*dicoccoides*)
T. turgidum	Tetraploid (4x)	Cultivated	Cultivated emmer wheat (*dicoccom*)
T. turgidum	Tetraploid (4x)	Cultivated	Durum (durum), *turgidum*, *turancium*, *plonicum*, *carthicum* (Persian wheat), *isphahanicum*
T. timopheevii	Hexaploid (6x)	Wild	*armeciaeum*
T. timopheevii	Hexaploid (6x)	Cultivated	*timopheevii*
T. aestivum	Hexaploid (6x)	Cultivated	*macha*, *tibetanum*
T. aestivum	Hexaploid (6x)	Cultivated	*spelta*, *vavilovii*, *yunanese*
T. aestivum	Hexaploid (6x)	Cultivated	*aestivum* (bread wheat), *compactum*, *sphaerococcum*, *petropavlovsky*
T. zhukovskyi	Hexaploid (6x)	Cultivated	–

resistant to the powdery mildew fungus. They also investigated the efficiency of TALENs and CRISPR/Cas9, and again asserted CRISPR's acceptable efficiency and ease of use. Gil-Humanes et al. (2017) investigated the replication and protein expression of the wheat dwarf virus (WDV) system in wheat cells, and compared different replicon architectures to optimize WDV as a vector for delivering CRISPR/Cas9 reagents and donor templates. They achieved a 110-fold increase in the expression of a reporter gene relative to non-replicating controls under the CRISPR/Cas9 system for targeting the *TaMLO* in bread wheat with gene targeting efficiency of ~1%. The *TaMLO* multiplexed mutation in the wheat genome was also investigated by other researchers (Gil-Humanes et al. 2017; Wang et al. 2016).

Despite achieving higher resistance against powdery mildew in *MLO* mutant plants, a few negative pleiotropic phenotypes such as spontaneous cell death, early senescence and leaf chlorosis were reported. The enhanced disease resistance 1 (EDR1) is another gene that negatively affects the plant defense response against powdery mildew disease. The EDR1 is well characterized in *Arabidopsis*, but little is known about wheat EDR1. Zhang et al. (2017) showed that the down-regulation of *TaEDR1* in wheat by VIGS or RNAi methods increased resistance to powdery mildew isolates. Consequently, they used CRISPR/Cas9 to knock-out the three

Table 12.3 A summary of research progress in CRISPR/Cas9 genome editing of bread wheat (*Triticum aestivum*)

Target genes	Trait	Cas9 promoter	sgRNA Promoter	Version of Cas9	Reference
Inositol oxygenase (*TaInox*), phytoene desaturase (*TaPds*)	Chlorophyll synthesis	*CaMV* 35S	*CaMV* 35S	Human codon-optimized Cas9	Upadhyay et al. (2013)
Protoplast *TaMLO*	Resistance to powdery mildew	2 × 35S	*TaU6*	Rice codon-optimized Cas9	Shan et al. (2013)
TaLOX2	Grain development	2 × 35S	*TaU6*	Rice codon-optimized Cas9	Shan et al. (2014)
TaMLOA1, TaMLOB1, TaMLOD1	Resistance to powdery mildew	Ubi	*TaU6*	Plant codon-optimized Cas9	Wang et al. (2014b)
TaGASR7, TaGW2, TaDEP1 (bread wheat), *TdGASR7* (durum wheat)	Grain and kernel length and weight, storability, plant height	pJIT163-Ubi	*TaU6*	nr[a]	Zhang et al. (2016a)
Q, *TaGW2*, *TaLpx-1, TaMLO*	Kernel width and weight; resistance to powdery mildew, domestication trait	Ubi	*TaU6*	Wheat codon optimized Cas9	Wang et al. (2016)
TaMLO	Resistance to powdery mildew	Zm-Ubi	*TaU6*	Wheat codon-optimized Cas9	Gil-Humanes et al. (2017)
TaGW2, TaGASR7	Grain and kernel length and weight	nr	nr	nr	Liang et al. (2017)
α-gliadin, gamma-gliadins	Removing gliadins	nr	nr	nr	Smulders et al. (2017)
TaLOX2	Grain development	Ubi	*TaU6*	nr	Zong et al. (2017)
TaEDR1	Powdery mildew resistance	pJIT163-Ubi	*TaU6*	nr	Zhang et al. (2017)
TaLOX2, TaUbil1	Grain development	pJIT163-Ubi	*TaU6*	nr	Bhowmik et al. (2018)
TaDREB2, *TaERF3*	Drought signaling	pJIT163-Ubi	*TaU6*	nr	Kim et al. (2018)
TaCER9, TaLOX2, TaGW2	Grain development	pJIT163-Ubi	*TaU6*	nr	Liang et al. (2018)
TaGW2, TaLpx-1, TaMLO	Kernel width and weight; resistance to powdery mildew	nr	*TaU6*	nr	Wang et al. (2018)

(continued)

Table 12.3 (continued)

Target genes	Trait	Cas9 promoter	sgRNA Promoter	Version of Cas9	Reference
α-gliadin genes	Low-gluten wheat	Ubi	*TaU6*	nr	Sánchez-León et al. (2018)
TaMs45	Male fertility	Ubi	*TaU6*	nr	Singh et al. (2018)

[a]nr: not reported

homologs of *TaEDR1*. The *TaEDR1* mutant plants showed higher resistance to powdery mildew while none of the mildew-induced negative effects occurred.

Grain weight is an important agronomic trait in cereals that is directly correlated with crop yield. Zhang et al. (2016a) developed two transient expression methods based on CRISPR/Cas9 DNA or IVTs to introduce heritable mutations in all the three homologs (six alleles) of *TaGASR7*, *TaGW2* and *TaLOX2* genes in hexaploid bread wheat, and *TdGASR7* in tetraploid durum wheat mutants. All three genes negatively affect the grain and kernel length and weight. The grain length and weight in wheat is suggested to be under the control of *TaGASR7* gene (Dong et al. 2014; Ling et al. 2013). The *TaGW2* gene is a negative regulator of grain kernel width and weight in bread wheat (Yang et al. 2012), and *TaLOX2* gene expression is correlated with grain development. It also might regulate the grain storability (Feng et al. 2010). The results of Feng et al. (2010) showed that the *TaGASR7* mutant plants had higher thousand-kernel weight (TKW). They also investigated the effect of *TaDEP1* mutation that led to a very dwarf phenotype compared to wild types with a mean plant height of 36.7 cm and 56.0 cm, respectively. Shan et al. (2014) and Zong et al. (2017) focused on efficient methods for multiplexed mutation of *TaLOX2* alleles in wheat by CRISPR/Cas9 system. The microscopy method and CRISPR/Cas9 technique was also reported in *TaLOX2* gene manipulation. An innovative study was conducted by Bhowmik et al. (2018) who applied the CRISPR/Cas9 system with microspore technology to induce haploid mutagenesis in wheat *TaLOX2* gene. They used haploid single-celled microspores to shortcut the genetic engineering in plant species with long life cycles or recalcitrant nature. It was an achievement because producing stable mutations usually takes place over multiple generations. Regarding the importance of *TaLOX2* in the wheat grain development and crop yield, the focus of a few studies have been on improving the efficiency of the CRISPR method for its multiplexed manipulation (Liang et al. 2018; Shan et al. 2014; Zong et al. 2017).

The nutritional and health characteristics of cereal grains are critically important. Cereal-based foods may cause allergic responses such as coeliac disease (CD), which affects about 1–2% of world population. Currently, a gluten-free food regime is strictly prescribed for people prone to such diseases. There are storage proteins such as gliadins and glutenin proteins in cereal grains that could cause CD. They are controlled by multi-locus gene families, such as α-gliadin and gamma-gliadins genes (Smulders et al. 2017). CRISPR/Cas9 gene editing has been implemented to produce α-gliadin, gamma-gliadins mutant wheat lines (Sánchez-León

et al. 2018; Smulders et al. 2017). The CRISPR/Cas9 system has also been utilized to improve other agronomically-important traits in hexaploid wheat, including plant signaling under drought stress (Kim et al. 2018), chlorophyll synthesis (Upadhyay et al. 2013), domestication trait (Q) (Wang et al. 2016) and male fertility (Singh et al. 2018) (Table 12.3).

CRISPR/Cas9-mediated genome editing is still in its infancy, especially for wheat breeding. There are different types of Cas9 promoters such as 35S PPDK: 35S enhancer fused with pyruvate orthophosphate dikinase basal promoter (Li et al. 2013), ICU2: incurvata 2 promoter (Hyun et al. 2015), and EF1A2: elongation factor-1 alpha 2 (Li et al. 2015) that have been used in CRISPR-based gene editing, but most studies on wheat have used similar Cas9 promoter systems (Table 12.3). Moreover, an efficient wheat-codon optimized Cas9 structure is still being developed. The success in wheat genome sequencing and publication of its long-awaited fully annotated reference genome opened new horizons in wheat breeding (Appels et al. 2018). Therefore, it is expected that sequence-specific and multiplexed genome editing tools such as CRISPR/Cas9 could more significantly and efficiently contribute to wheat breeding programs in near future.

12.5 Biosafety Considerations for CRISPR Genome Editing

Modern genome editing using engineered nucleases (GEEN) is increasingly being utilized and is expected to, first, preserve the natural genetic structure of the species, and second, to introduce completely transgene-free crop varieties with improved agroeconomic importance (Xu et al. 2015). However, as Wolt (2017) reviewed the latest development in safety, security and policy considerations for plant genome editing, this fact might not be fully implemented, and there are public concerns over the quality and biosafety of the genome-edited crops.

The novel genome manipulation systems, such as TALENs and ZFNs are more efficient and faster than the conventional breeding techniques that could facilitate breeding programs of recalcitrant crops. One issue with these techniques is the chances of introducing non-target effects by synthetic nucleases, or antibiotic-resistant marker genes in the target genome (Kamburova et al. 2017). The CRISPR-Cas9 system has offered the advantage of obtaining marker-free genetic modifications, and the elimination of transgene or small DNA insertions into the target genome (Jones 2015; Kanchiswamy et al. 2015). Unfortunately, this is not true in most cases, because CRISPR/Cas9 is still dependent on bacterial (*Agrobacterium tumefaciens*) mediated T-DNA transfer or biolistic bombardment for its DNA construct delivery into the target cell. Consequently, there are chances of the CRISPR/Cas9 bacterial constructs being integrated into the target genome that could result in introducing unwanted genetic modification and off-target mutations. This also suggests that degraded CRISPR/Cas9 sequence inside the host cell might interfere in DSBs repair process and enter the genomic sites (Liang et al. 2017). However, as mentioned earlier, new modifications and improvements could address such issues.

The development of quality control steps and improving the genome modification strategies are strongly required to minimize the accidental and unwanted off-target effects. One crucial step could be accurate site selection for DSBs introduction and considering repeated sequences and sites having a high homology with the other regions through advanced bioinformatics analysis (Lombardo et al. 2011). Moreover, the current regulations for genetically-modified plants should be revised to include efficient strategies to identify any unintended mishaps during plant genome modifications by the modern techniques such as CRISPR/Cas9.

12.6 Conclusions and Prospects

The increasing demand for wheat production due to human population growth, and environmental and climate change issues require proper plans and actions by all stakeholders, including agricultural scientific and breeding communities, economists and governmental policy makers. The advent of precision agriculture accompanied by utilizing crops with improved agronomic traits, and integrated pest management are necessary to achieve a sustainable global food supply. Wheat is one the most important staple crops in many parts of the world. The polyploidy and recalcitrant nature of the species presents serious challenges in breeding programs. Therefore, new strategies are needed to preserve the species under cultivation and wild genetic diversity to support the constant need for new allelic variation in both conventional breeding and precision biotechnology techniques. Moreover, since most of the important traits in wheat are controlled by different genetic loci, the simple random mutagenesis methods are not appropriate. It is critical to develop highly efficient techniques for introducing new cultivated varieties that are more adaptable to regional and global cultivation practices, and resistant to abiotic and biotic stresses.

The engineered nucleases in genome editing systems (GEEN) are becoming popular in genetic studies because of interesting features such as high efficiency, improved specificity, simplicity and multiplexing. The main purpose of these systems is producing knockout plants to improve specific traits such as biotic/abiotic stress tolerance, yield improvement or crop quality. They revolutionized the contemporary breeding approaches, and opened new horizons for the next generation of breeding programs.

Overall, the application of rapid and high-throughput methods in developing, testing and breeding crop plants are critically important for scientific communities to be able to feed the world's population in 2050 and beyond. The CRISPR/Cas9 is one technique that holds more promises for precision, cost and time efficient genome modifications in higher plants with high genome size and ploidy levels. There are already promising solutions to address some drawbacks of CRISPR such as off-target mutations or vector-mediated cell delivery. In the long term, more improvements in its method and delivery could be introduced that lead to the CRISPR/Cas9 nomination as an elite technique for high precision multiplexed genome editing in wheat.

Appendix I: Research Institutes Relevant to Wheat Biotechnology

Institution	Specialization and research activities	Website
International Maize and Wheat Improvement Center (CIMMYT), Mexico	The development of improved varieties of wheat and maize	www.cimmyt.org/
The Nottingham/ BBSRC Wheat Research Centre (WISP), UK	Creating genetic variation for agronomically and scientifically important traits from wild and distantly related species into wheat	http://www.wheatisp.org/
Wheat initiative institutions, Berlin, Germany	International collaboration for wheat breeding	http://www.wheatinitiative.org/about-us/countries-international-research-centres
Institute of Agrobiological Sciences, NARO, Japan	Understanding the biological phenomena of wheat transformation	ww.naro.affrc.go.jp
Institute of Molecular Plant Biology, ETH, Zurich	Improving wheat nutritional qualities as well as understanding the molecular processes that play a key role in protecting the crop against biotic and abiotic stresses	http://www.impb.ethz.ch
The International Service for the Acquisition of Agri-Biotech Applications (ISAAA)	To share the benefits of crop biotechnology to various stakeholders, particularly resource-poor farmers in developing countries, through knowledge sharing initiatives and the transfer and delivery of proprietary biotechnology applications	http://www.isaaa.org
BASF Plant Science, USA	Wheat genetic engineering	https://agriculture.basf.com
John Innes Centre, UK	To develop new wheat germplasm containing the next generation of key traits	https://www.jic.ac.uk
Rothamsted Research (RRES), UK	Improving the environmental resilience of the wheat crop through genetics and targeted traits analysis	https://www.rothamsted.ac.uk
National Institute of Agricultural Botany (NIAB)	Functional analysis of wheat genes for breeding new traits for commercial exploitation through traditional breeding techniques	http://www.niab.com
Earlham Institute (EI), UK	Genetic diversity in wheat and Sequencing the wheat genome	http://www.earlham.ac.uk
European Bioinformatics Institute (EBI)	Analysis of the bread wheat genome	https://www.ebi.ac.uk
International Wheat Genome Sequencing Consortium, IWGSC	To establish a high quality reference sequence of the wheat genome anchored to the genetic/phenotypic maps	http://www.wheatgenome.org/

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Chapter 13
Durum Wheat (*Triticum turgidum* ssp. *durum*) Breeding to Meet the Challenge of Climate Change

Pasquale De Vita and Francesca Taranto

Abstract Durum wheat (*Triticum turgidum* ssp. *durum*) is a tetraploid wheat species (2n = 28) planted annually on an estimated area of 18 million ha, representing approximately 8–10% of all the wheat cultivated area in the world, and an annual production ranging from 35–40 million mt. Regions of the Mediterranean Basin and North America (i.e. Canada) produce about 60% of world durum wheat production, mainly used for human consumption as pasta, bulgur, couscous and some breads. In general durum wheat is better adapted to high temperatures and to semiarid climates than bread wheat. In spite of its relatively high adaptability to the marginal and drought environments, the production of durum wheat is threatened by the impacts of climate change, and the need for more sustainable development. This chapter provides an update on progress in genetic improvement of durum wheat and on the tools and strategies to maintain productivity and strengthen food security despite increasing water scarcity, higher temperatures, and the emergence of new pests and diseases. These demand changes in the approaches to crop improvement and require implementing novel approaches in gene discovery and plant breeding. Conventional and modern breeding strategies are discussed to integrate new target traits into varieties. Modelling provides a rational approach to identify desirable traits or combination of traits potentially leading to the specification of wheat ideotypes optimized for target environmental and future climatic conditions. The integration of genomics and phenomics is promising to revolutionize plant breeding providing an exceptional opportunity to identify genetic variation that can be employed in durum wheat breeding programs.

Keywords Climate change · Durum wheat · Marker-assisted breeding · Speed breeding · High-throughput phenotyping · Genomic selection · KASP markers

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J. M. Al-Khayri et al. (eds.), *Advances in Plant Breeding Strategies: Cereals*,
https://doi.org/10.1007/978-3-030-23108-8_13

13.1 Introduction

Global worming will impact the crop productions at various rates in different parts of the world, particularly in those areas, such as the Mediterranean Basin, already considered as one of the most critical and vulnerable geographic zones (Handmer et al. 2012).

Durum wheat is a strategic crop across many of these areas, cultivated on marginal lands with poor soils and limited water and, although it is regarded as one of the most drought-tolerant cereal crops, can be negatively impacted by high temperatures and drought. Considering the limitations on expanding crop-growing areas, a significant increase in crop productivity will be required to achieve this target (Ainsworth and Ort 2010). In this new scenario, durum wheat breeding may help by developing new cultivars with enhanced traits better suited to adapt to climate change conditions.

This chapter provides an update on progress in genetic improvement of durum wheat and on tools and strategies to maintain productivity and strengthen food security despite increasing water scarcity, higher temperatures, and the emergence of new pests and diseases (Fig. 13.1). This demands changes in the approaches to crop improvement and require implementing novel approaches for quantitative analysis, gene discovery and breeding approaches.

Conventional and modern breeding techniques will be discussed to integrate new target traits into varieties. Breeding wheat for adaptable varieties offers a practical defense to reduce the negative effects of climate change on crop production despite increasing water scarcity, higher temperatures, and the emergence of new pests and disease. The integration of genomics and phenomics have promised to revolutionize the field of plant breeding, providing an exceptional opportunity to identify genetic variation that can be employed in durum wheat breeding programs. Some of the

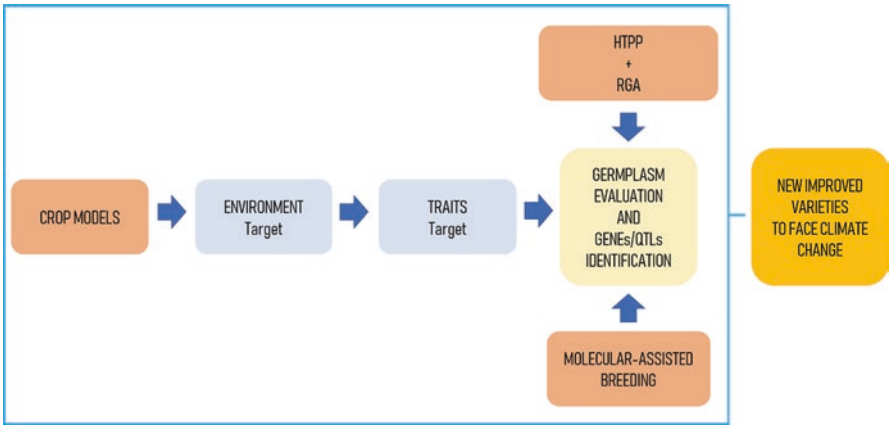

Fig. 13.1 Flowchart of strategies and tools for a durum wheat breeding program to face climate change. *HTPP* = high-throughput phenotyping platform, *RGA* = rapid generation advance

routine molecular assisted-based methods as well as recent rapid generation advance to transfer novel genes into adapted germplasm and speed up the selection process represent a good opportunity for development better adapted varieties. More recent trends in crop models, leading to the specification of wheat ideotypes optimized for target environments and future climatic conditions, are proving useful evidences for improving the efficiency and accuracy of modern breeding activities (Fig. 13.1).

13.1.1 Botanical Classification

Durum wheat (*Triticum turgidum* ssp. *durum*) is a monocotyledonous plant of the Gramineae family, Triticeae tribe and *Triticum* genus. The classification of *Triticum* and related genera within the tribe Triticeae has been strongly debated (Goncharov 2011). Currently, most of the scientific community uses a revision of Mac Key's classification made by van Slageren (1994) according to which *Triticum* comprises only 5 main species (Table 13.1), including diploid *Triticum monococcum* L. (2n = 14), tetraploid *Triticum turgidum* L. (2n = 28) and hexaploid *Triticum aestivum* L. (2n = 42) species. Tetraploid wheats are represented by 2 wild and 9 cultivated species (Matsuoka 2011). At present, only 5 of them, namely *Triticum turgidum* L. ssp. *durum* (Desf.), ssp. *turgidum* L., ssp. *dicoccon* (Schrank) Thell., ssp. *aethiopicum* Jakubz. and ssp. *turanicum* (Jakubz.) are cultivated.

13.1.2 Distribution and Importance

Worldwide durum wheat production ranges from 35–40 million mt annually, accounting for about 3–5% of the global wheat production (Fig. 13.2). Differently from bread wheat, which is basically cropped everywhere in the world with the exception of the tropical areas, durum wheat is an economically important crop in the Mediterranean area, with a total annual production ranging on average of 14–18 million mt.

In the world, durum wheat is used predominantly for the manufacture of pasta, and to a minor extends, for baked raised breads in the southern Mediterranean. Alternatively, in areas of West Asia and North Africa, durum wheat has traditionally been used in the production of single- and two-layered flat breads, couscous, freekeh and burghul or bulgur (Cubadda 1989). Currently, world demand for durum wheat is increasing, in particular for excellent-quality durum wheat, particularly in the European markets.

According to Table 13.2, the EU is the top producer in global durum wheat production, which amounted to 8.7 million mt in 2016/2017 (IGC 2016). Among the European countries, Italy is the largest producer of durum wheat with about 4–4.5 million mt on average. Turkey and France follow with averages of 4 and 2.1 million mt, respectively, in 2016/2017. Algeria follows with a production of 2.5 million mt

Table 13.1 Taxonomy of wheat according to van Slageren (1994) and grouped according to ploidy level

Species	Genome	Subspecies	Common name	Domesticated status
Diploid (2x)				
Triticum urartu Tumanian ex Gandilyan	A^u	Triticum *urartu* Tumanian ex Gandilyan	Wild urartu einkorn	Wild, hulled
Triticum monococcum L.	A^m	ssp. *aegilopoides* (Link) Thell.	Wild einkorn	Wild, hulled
	A^m	ssp. *monococcum*	Cultivated einkorn	Cultivated, hulled
	A^b	ssp. *sinskayae* A. Filat. & Kurkiev	Sinskaya einkorn	Domesticated, free-threshing
Tetraploid (4x)				
Triticum turgidum L.	BA^u	ssp. *dicoccoides* (Korn. ex Asch. & Graebn.) Thell.	Wild emmer	Wild, hulled
	BA^u	ssp. *dicoccum* (Schrank ex Schübl.) Thell.	Emmer	Domesticated, hulled
	BA^u	ssp. *paleocolchicum* Á. & D. Löve		Domesticated, hulled
	BA^u	ssp. *durum* (Desf.) Husn.	Durum wheat	Domesticated, free-threshing
	BA^u	ssp. *turgidum*	Rivet wheat	Domesticated, free-threshing
	BA^u	ssp. *polonicum* (L.) Thell.	Polish wheat	Domesticated, free-threshing
	BA^u	ssp. *turanicum* (Jakubz.) Á. & D. Löve	Khorasan wheat	Domesticated, free-threshing
	BA^u	ssp. *carthlicum* (Nevski) Á. & D. Löve	Persian wheat	Domesticated, free-threshing
Triticum timopheevii	GA^m	ssp. *armeniacum* (Jakubz.) Slageren		Wild, hulled
	GA^m	ssp. *timopheevii* (Zhuk.) Zhuk.		Domesticated, hulled
Hexaploid (6x)				
Triticum aestivum L.	BA^uD	ssp. *spelta* (L.) Thell.	Spelt wheat	Domesticated, hulled
	BA^uD	ssp. *macha* (Dekapr. & A. M. Menabde) Mackey		Domesticated, hulled
	BA^uD	ssp. *compactum* (Host) Mackey	Club wheat	Domesticated, free-threshing
	BA^uD	ssp. *sphaerococcum* (Percival) Mackey	Indian dwarf or shot wheat	Domesticated, free-threshing
	BA^uD	ssp. *aestivum*	Common or bread wheat	Domesticated, free-threshing

Source: Modified from https://en.wikipedia.org/wiki/Taxonomy_of_wheat

Fig. 13.2 Worldwide durum wheat production area (marked blue areas)

Table 13.2 Word durum wheat production (million mt)

Country	2013/2014	2014/2015	2015/2016	2016/2017
European Union (EU)	8.1	7.6	8.5	9.1
France	1.8	1.5	1.8	1.7
Greece	1.0	0.8	1.0	1.0
Italy	3.9	3.9	4.2	4.8
Spain	0.9	0.8	0.9	1.0
Kazakhstan	2.0	2.0	2.1	2.1
Canada	6.5	5.2	5.4	6.5
Mexico	2.3	2.3	2.3	2.5
USA	1.6	1.5	2.2	2.3
Argentina	0.1	0.2	0.3	0.2
Syria	1.5	0.8	1.4	1.3
Turkey	4.1	3.3	4.1	3.6
India	1.2	1.3	1.2	0.9
Algeria	2.5	1.3	2.5	1.9
Libya	0.1	0.1	0.1	0.1
Morocco	1.9	1.4	2.4	0.9
Tunisia	0.8	1.2	0.8	1.0
Australia	0.5	0.5	0.5	0.5
Others	5.7	5.9	5.7	5.9
Total durum wheat	38.8	34.5	39.3	38.3

Source: International Grains Council (IGC) (2016)

in 2015/2016; production is highly instable due to the irregular precipitation during various phases of the growing season, while imports of durum always remain at very high levels, ranging from 1.5–2 million mt.

After the EU, Canada is the largest producer of durum wheat, with an annual production of 4.5–6 million mt, depending on the climatic conditions recorded during the months May–September.

Over 67% of the durum wheat hectarage in the United States is located in North Dakota where the annual durum wheat production (2008–2010) averaged 1.6 million mt. The remaining percentage of durum wheat production occurs in Arizona, California, Minnesota, Montana and South Dakota. Desert durum™ is a registered brand name for durum wheat grown in California and Arizona with irrigation water supplement. Approximately, 500–600 thousand mt of the durum wheat is grown in California and Arizona each year, although in 2017 the production acreage was less than in 2016, due largely to lower prices available at planting time (CWC 2017). From the qualitative point of view, Desert durum™ is considered one of the best durum wheat in the world for its high protein content, gluten toughness and semolina color, but above all for the stability of the quality parameters of the supplies.

In Mexico, high-quality durum wheat is also produced mainly in the northwestern states of Sonora and Baja California Norte. The Yaqui Valley in the state of Sonora is essentially a desert but thanks to irrigation, it provides ideal conditions for growing durum wheat. These particular conditions make the cultivation of durum wheat more convenient for farmers than bread wheat, due to greater yields (Meisner et al. 1992). There are other much smaller areas where durum wheat is cultivated including the Russian Federation, Kazakhstan, Australia, Argentina, India and Ethiopia.

13.1.3 Climate in Growing Areas

Durum wheat is grown under several different ecological conditions and the breeders of durum must take these conditions into account. The fundamental denominators of these conditions are the temperature and the rainfall patterns during the growing season. In the Mediterranean Basin, the traditional site of durum wheat production (Bozzini et al. 1988), the agronomic yields and grain quality are generally constrained by dry growing-season precipitation, as well as by higher temperatures towards the end of the crop cycle (Rharrabti et al. 2003). There is, in fact, a marked climatic heterogeneity between zones and years, in which long drought periods are alternated with short periods of more or less intense rainfall (Luterbacher et al. 2006), with consequent risk of desertification (Greco et al. 2005). In many Mediterranean countries, the rainfall is concentrated in winter (Royo et al. 2014), while in others mainly in the early spring, that if associated with anticipated changes in temperature around flowering time can result in moderate stress for rainfed wheat. In southern Italy, for example, the erratic rainfall, lead to a high degree of variability of grain yield ranging from 0–6 mt ha^{-1} from year to year (Basso et al. 2012) with a

great impact on the qualitative aspects of the grain, especially on protein content and test weight. Another important Mediterranean country for the consumption and production of durum wheat is Algeria, where the main climatic problems are related to the abiotic stresses that occur during the growing season as drought recurrence: low and erratic rainfalls to which is added the problem of high temperatures in the period of high ant populations and of cold in spring. In addition, historically, in all Mediterranean environments the degree of drought stress increases throughout the period of grain filling (Araus 2002).

The cold semiarid climate, found in the Northern Great Plains of the USA (North Dakota, Montana) and southwestern Canada (Saskatchewan), characterized by warm days and regular rainfall during the growing season, as well as dry days before harvest, represents a good combination for spring durum. However, in Canada, in contrast to Mediterranean region, the spring type is sown between mid-April and the end of May, allowing a harvest between August and September. A shorter growing season is the main factors that limit the yield potential of spring wheat in Canada. Average yields are typical of short-grain cycles and are slightly higher than 2 mt/ha. In addition to potentially reducing yields, during the harvest time, climatic conditions predispose the crop to fusarium infections with the consequent development of mycotoxins on the grain.

In Australia the durum production is mainly confined to the states of New South Wales (NWS), South Australia, and Queensland and to a lesser extent Western Australia. The climatic conditions of South Australia and Western Australia are very similar to those in the Mediterranean Basin.

In general, in all major world production areas, periodic droughts and unseasonably hot or cool weather not only affect yield and grain quality directly, but also change the disease complex. Thus, while the varieties grown in a locality may have some resistance to the prevalent diseases or races (strains) of diseases, if the season is cooler or wetter than usual, other diseases or races may predominate and destroy the crop. These issues are becoming even more important in light of the ongoing climatic changes and in particular the increase in extreme events that emphasize the impact on durum wheat production.

13.1.4 Domestication, Selection and Early Genetic Improvement

Durum wheat history began with the domestication of wild emmer (*Triticum turgidum* ssp. *dicoccoides*) about 12,000 years ago by ancient farming communities in the Fertile Crescent region, through the loss of fragility of the rachis. This means that cultivated emmer does not shed its seed as readily as the wild form (Salamini et al. 2002).

This first step in durum wheat domestication was probably a gradual process as suggested by both genetic and the archaeological evidence (Tanno and Willcox 2006). The loss of fragility gave rise to the first known domesticated wheat, *Triticum*

turgidum ssp. *dicoccum*, or emmer wheat. This latter, like its wild progenitor, is a hulled wheat with strong glumes that enclose the grains and only 2 kernels per spikelet, but its spike is not fragile. The transition from emmer to modern durum wheat involved the acquisition of free-threshing and, the appearance of naked kernels was the second most important step in durum wheat domestication after non-brittle spikes (Fig. 13.3) (Peleg et al. 2011; Simonetti et al. 1999; Simons et al. 2006).

Due to its larger grains and higher productivity, durum gradually replaced emmer in most of the wheat growing areas becoming, by the second millennium BC, the most widely cultivated tetraploid wheat specie (Maier 1996), spreading throughout the Mediterranean Basin. Archaeological evidence confirms this hypothesis showing a very sporadic appearance of durum-like wheat about 7000 years ago in the Near East, but it was only about 2500 years ago that durum become a major crop in the Mediterranean Basin (Feldman and Millet 2001). Actually, although the origin of the free-threshing trait of durum remains uncertain, whether directly from *Triticum turgidum* ssp. *dicoccum* or via hybridization with other species, genetic analysis shows a bottleneck in the origin of durum that was later isolated from its Near-Eastern emmer wheat center of origin (Oliveira et al. 2012; Özkan et al. 2011).

Ethiopia is known as a secondary center of durum wheat diversity (Harlan and Zohary 1966). Landraces from this country have unique morphology (Mengistu et al. 2015) and represent a separate subspecies, *Triticum durum* ssp. *abyssinicum* (Kabbaj et al. 2017).

Thousands of years of cultivation aided by natural and human selection favored the cultivation and utilization of diverse wheat genetic resources, constituting what are now known as *landraces*. Durum wheat landraces are considered an evolutionary link between wild emmer wheat and modern wheat cultivars (Jaradat 2014).

Fig. 13.3 Domesticated emmer and durum wheat with hulled (**a**) and nuked (**b**) kernels

Modern durum wheat breeding programs started at the beginning of twentieth century in Italy, with the pioneering work of N. Strampelli who exploited the genetic variation in landraces from southern Italy, North Africa and West Asia (Scarascia Mugnozza 2005). The most successful result was the cultivar Cappelli, released in 1915 (Laidò et al. 2013). This cultivar represented a milestone in durum wheat breeding; its cultivation covered more than 60% of the durum-wheat growing area in Italy, and was extensively cultivated in all Mediterranean countries, including Turkey and Spain. Laidò et al. (2013) have highlighted the presence of the genetic background of Cappelli in the pedigree of almost all modern durum wheat varieties. This situation changed with the adoption of the new semi-dwarf varieties developed at CIMMYT in Mexico during the 1960s, crossing the tall durum wheat varieties with bread wheats carrying the Norin 10 gene (Borlaug 2007). This resulted in the release of several semi-dwarf high-yielding cultivars that are the progenitors of most of currently grown varieties (Ortiz et al. 2007).

During the evolution of tetraploid wheats, two potential bottlenecks occurred in the genetic diversity of durum wheat. The first relates to the recent origin of tetraploid wheats and the presumption that there are relatively few diploid progenitor crosses. The second one occurred in developing of local varieties well adapted starting from genetic materials based a relatively limited number of lines. This latter helps to explain the value of germplasm exchange and the use of landraces (Lopes et al. 2015). The bottleneck decreases genetic diversity in neutral and unselected genes, but selection decreases diversity beyond that caused by the bottleneck effect alone (Ross-Ibarra et al. 2007). The loss of nucleotide diversity during wheat domestication is one of the largest reported so far for crop species. In durum wheat, 84% of the nucleotide diversity originally present in *Triticum dicoccoides* has been lost (Haudry et al. 2007).

Molecular marker analysis has shown that durum wheat history was associated with a decrease in the level of genetic diversity (Sahri et al. 2014), from the wild ancestor to the most recent modern varieties (Haudry et al. 2007; Thuillet et al. 2005). Modern durum wheat genotypes do not have the wide adaptation and the diverse genetic background already present in landraces (Mohammadi et al. 2014; Royo et al. 2014). This genetic narrowing has led to a reduction in allelic plasticity, hence to a germplasm less prone to adapt to climate change and new emerging threats from diseases, weeds and pests (Duveiller et al. 2007).

13.2 Impact of Climate Change

Future impacts of climate change will vary greatly depending on the geographic region, and they will not be evenly distributed. Adverse weather conditions for European wheat production will become more frequent with climate change (Trnka et al. 2014). Numerous recent scientific works have confirmed the link between global warming and an increase in extreme events, temperatures and precipitation (Lobell et al. 2012; Rosenzweig et al. 2001).

Climate change affects crop species directly by altering their physiology and function, but also indirectly by altering interactions with insects, diseases, and weeds (Chakraborty et al. 2000; Ramesh et al. 2017). As a result, competition-mediated indirect effects may alter crop response to environmental change.

13.2.1 Direct Effects of Climate Change

Direct effects of climate change are related to changes in temperature, precipitation, humidity and atmospheric CO_2 concentrations. Each of these variables has a direct influence on the growth and development of durum wheat. Climatic and environmental variations highly and sensitively affect wheat production (Porter and Semenov 2005), but their positive or negative effects strongly depend on geographic location (Porter and Gawith 1999). A global analysis of wheat yields has shown that the increasing mean temperatures in recent decades have had a negative effect on yield. Similar effects have been observed in various European countries. In France, the increasing temperatures affect the lack of increase in winter wheat production, despite large investments in genetic improvement activities. In addition, both in France and Italy, there is also a tendency towards increasing yield variability over the years, due to the increased frequency of heat waves and water scarcity. Similar effects of heat waves and droughts have been observed globally, whereas floods and intense rainfall have not been seen to affect overall crop production. In Italy and South-central Europe, the potential crop yields of wheat, maize and barley significantly decreased over the period 1976–2005 owing to temperature and radiation change effects. On the other hand, climate change has also shown positive effects on yields. In parts of the UK and north-central Europe, the yield potential of wheat and maize has increased since 1976. Grain yields in maize have been steadily increasing in northern Europe, most likely linked to the warmer climate.

In addition to production quantity, climate change affects the quality of durum wheat, mainly due to the increase in atmospheric CO_2. For example, plants grown under elevated CO_2 have reduced nitrogen (N) content (Cotrufo et al. 1998), a critical agricultural crop nutrient. Nitrogen, in fact, in addition to affecting unit yield, is responsible for the ability of semolina to produce good quality pasta and bread (Sissons 2008). The mechanism for this is unclear, however, durum wheats grown under elevated CO_2 often have lower protein (Fares et al. 2016) and mineral contents (Beleggia et al. 2018) which will affect the technological and nutritional performance of raw material.

13.2.2 Indirect Effects of Climate Change

Indirect effects may have as large an impact on crop productivity as the direct impacts and may also amplify or counteract the direct effects on yield and grain quality of durum wheat. To fully appreciate the impacts of climate change on plants it is important to understand how indirect effects impact durum wheat diseases, insects and weeds.

13.2.2.1 Diseases

A change in environment will definitely impact the disease triangle involving host, pathogen and environment (Jeger and Pautasso 2008). Increases in temperature favor populations of pathogens because the growth rate of these organisms is temperature dependent. It has been shown that wheat becomes more susceptible to rust disease with increased temperature (Milus et al. 2009). Generally, disease-causing fungi will be invasive at moderately high temperatures. Moisture can also affect both host plants as well as pathogens. In particular, high moisture could favor foliar diseases and some soil borne pathogens. Many climate change models predict that higher atmospheric moisture with increased temperature could favor pathogen and disease development. However, a limited amount of information on the potential impacts of climate change on plant diseases is available (Garrett et al. 2006). Climate changes may affect the habitats of crops and pathogens, which must either adapt or migrate to areas with more favorable conditions and create the environmental conditions to alter the levels of pathogen inoculation and of new vectors responsible for epidemic dynamics (Garrett et al. 2011; Jeger and Pautasso 2008).

Waalwijk et al. (2003) reported the results of surveys carried out in Europe showing an increased prevalence of the cereal head blight pathogen *Fusarium graminearum*, with higher temperature optimal, over *F. culmorum* and *Microdochium nivale* as temperatures warm. Mycotoxin producing *Fusarium* species occurr in winter wheat in Belgium (Flanders) during 2002–2005. The occurrence of *F. langsethiae* on many cereal crops has recently caused great concern in Europe due to the production by this specie of trichotecene mycotoxins that are toxic to humans and animals (Edwards et al. 2009). In Italy, *F. langsethiae* has been recently isolated from wheat kernels cultivated in central and southern Italy (Infantino et al. 2015). In addition, fusarium root and fusarium crown rot recently became more prevalent and are nowadays considered to account for a substantial part of *Fusarium* infections in wheat (Dyer et al. 2009). In recent years, wheat stem rust, caused by the fungal pathogen *Puccinia graminis* f. sp. *tritici*, has re-emerged in Europe as new virulence traits have evolved in stem rust populations demonstrating the vulnerability of broadly-used wheat cultivars (Singh et al. 2015b).

The Ug99 race, a lineage of wheat stem rust, originally detected in Uganda in 1998 has spread throughout Africa and the Middle East, generating significant concern, suggesting that 90% of wheat varieties in the world are susceptible

(Singh et al. 2011). In 2016, another *broadly* virulent race was detected in an outbreak in Sicily (Bhattacharya 2017). Stripe rust, also known as yellow rust, is currently the most economically important wheat rust disease and can cause 100% yield loss in susceptible cultivars if infection occurs in early growth stages (Chen 2005). The presence and severity of this fungal disease in Mediterranean and temperate cultivars has not been important until recently. However, the presence of a new stem rust race in the UK, Germany, Denmark, France and the Scandinavian countries, has severely affected wheat production in recent years. The emergence of this race has led to the formation of the Borlaug Global Rust Initiative, an internationally-coordinated program to develop resistant varieties (BGRI, http://www.globalrust.org).

13.2.2.2 Weeds

Weeds have a much greater response to increasing CO_2 concentrations than has been observed in cultivated plants (Ziska and George 2004). This means that in the near future weeds control may become a very complex problem to be solved because climate change could make it more vigorous and reduce plant productivity (Ramesh et al. 2017). Temperature controls weed species success with the same effects as on cultivated plants (Nichols et al. 2015). Temperature increase is also expanding the habitat of weeds into new geographic locations. It is speculated that switch grass, a perennial and robust grass weed, currently widespread to the coastal plain of North and South Carolina, may occupy the entire Corn Belt with an increase in temperature of 3 °C.

Extreme weather and climate events could also alter the efficacy of chemical agents control on weeds alternating their growth and development (Varanasi et al. 2016). In addition, high temperatures along with long periods of drought could alter the thickness of the cuticle and the pubescence of the leaves and, consequently, reduce the effect of herbicides.

13.2.2.3 Insects

Overall, climate induces changes in pest activity affecting agricultural production in several ways through increasing of types and numbers of insects, number of generations and promoting the development of resistant biotypes. (Woiwod 1997). This would certainly increase the damage caused by the insect to crops and increase the cost of crop protection because of the minor effect of certain classes of pesticides at higher temperatures (Glunt et al. 2014). Lower winter mortality of insects due to warmer winter temperature could also be result in an increase of insect populations (DeLucia et al. 2012). It has also been speculated that interactions of elevated CO_2 with temperature and precipitation may act as the key components for estimating the damages produced by pathogens on crops in the next future (Donatelli et al.

2017). Increasing air temperatures also affects the postharvest insects that are responsible for serious crop losses (Moses et al. 2015).

13.3 Improving Durum Wheat under Climate Change

In the predicted new climatic scenario, genotypic adaptation is expected to be one of the most important strategies to future climate change (Semenov et al. 2014). However, to assure that modern breeding strategies and new technologies help in developing new durum wheat cultivars it is necessary to identify the most suitable traits to limit the effect of climate changes. These include drought and temperature stress tolerance, resistance to pests and diseases, tolerance to salinity and increase in nutrient efficiency. Opportunities for new cultivars with increased drought tolerance include changes in phenology or enhanced responses to elevated CO_2. Matching phenology to growing-season length through changes in cultivar day-length and temperature response (Kumudini et al. 2014) could be a useful prospect of adaptation to climate change. Changes in root system architecture could allow better access to soil water (Lynch and Wojciechowski 2015) maintaining a high transpiration rates (Messina et al. 2015). For these reasons, it could be useful to increase the knowledge of durum wheat mechanisms that regulate the expression of the main morpho-phenological and physiological traits to improve yield and grain quality performances of durum wheat under the effects of ongoing climate change.

13.3.1 Drought and Heat Tolerance

Drought and heat are the two most important abiotic stresses affecting the main durum wheat areas and frequently they occur together (Ortiz et al. 2008). Early drought during the vegetative phase reduces the plants emergence and the crop establishment influencing also the tillering ability of durum wheat. Water deficit around the period of floral initiation can decrease the number of spikelet primordia compromising the number of kernels. Moreover, heat and water stress a few days before spike emergence decreases the number of spikelets per spike of fertile tillers, reducing anther dehiscence and pollen fertility rate. Furthermore, the water deficit during the post-flowering phase reduces the grain weight by anticipating the senescence phase. In addition, high temperature and drought occur frequently in the grain-filling period of wheat growth, reducing the length of the grain-filling period specifically in dry land and rainfed cropping areas (Altenbach et al. 2003; Hossain et al. 2012). For such areas, the main challenge faced by wheat breeders is to identify genotypes able to tolerate multiple stresses that occur simultaneously (Reynolds

et al. 2012). Therefore, there are various traits that could be used as proxies to select germplasm with enhanced adaptation to drought-prone environments. Early flowering could provide partial relief to water shortage during grain filling, early vigorous growth could improve the crop establishment and reduce soil evaporation; root architecture and size could optimize water and nutrient harvest (Araus et al. 2008). As noted, wheat performance under drought stress is controlled by quantitative traits, which may often be confounded by plant phenology (Fleury et al. 2010) and, for these reasons, it is not possible to improve wheat yields in environments affected by water scarcity. In spite of an interest by wheat breeders in traits with high heritability and showing low genotype-by-environment interactions, few cultivars have been developed based on the above traits, due to the difficulty of breeding programs to know the actual economic benefit of screening one trait against another and to validate results through relevant field experiments. To control water availability and to remove the impact of seasonal changes and timing of rainfall on the performance of trial crops, managed environment facilities may need to be used. Accurate measurements can be helpful to attribute phenotypic effects and to explain the underlying genetics.

13.3.2 Root System Architecture

An important selection criterion in a breeding program is root characteristics, even if it is difficult to select for root-associated traits because of the complex nature of root architecture and interactions of roots with the surrounding rhizosphere. A deep root structure is critical for crops to be able to use nitrates and water in deeper soil layers, particularly under abiotic stress conditions. (Koevoets et al. 2016).

Recent advances in high-throughput genotyping and phenotyping approaches and genetic engineering have shown promising improvements in drought tolerance of several crops. The root system architecture (RSA) refers to the spatial organization of the root system in its growing environment, which reflects its capability to extract resources (Wang et al. 2006). Eco-physiological simulation models have suggested that changes in RSA will play a central role to achieve plant growth and development capable of ensuring high yields. (de Dorlodot et al. 2007). In addition, association of plant roots with soil microbes like arbuscular mycorrhizal fungi (AMF) or plant growth promoting bacteria (PGPR) increases total plant carbohydrate budget by 10–20% and, in return, AM fungi facilitate plant uptake of phosphorus and nitrogen by increasing the absorbing root surface area and by mobilizing available nutrients (Johnson and Gehring 2007). On the basis of mycorrhizal associations, genetic variations have been demonstrated in a number of plant species (Zangaro et al. 2008), which offer the potential to select for AMF responsiveness. Recently, De Vita et al. (2018) found good variability for this character in durum wheat. Therefore, a better understanding of the genetic controls of AM root colonization is required to allow this synergistic relationship to be manipulated.

13.3.3 Water Use Efficiency

Under drought soil conditions, besides water extraction ability through the roots, the ability to use water resources is also important. This is called water use efficiency (WUE) and reflects the balance between mols of CO_2 assimilated and mols of water transpired. The common method to measure WUE is carbon isotope discrimination (Δ) (Farquhar et al. 1989). Genetic variation exists in crop plants for these traits, but more research is needed since numerous secondary traits affect WUE.

Actually, WUE can be improved in wheat through the synergistic action of different characters such as deep and dense roots, long coleoptile, vigorous juvenile growth, tiller inhibition and high transpiration efficiency (Reynolds et al. 2005).

13.3.4 Salt Tolerance

Durum wheat could be considered moderately tolerant to salinity, but less than barley that is one of the most tolerant crops (Munns et al. 2006). Extensive irrigation, drought and the increasing level of seawater are making this kind of stress very widespread even in the main durum wheat cultivated areas (Dajic 2006). The presence of salt in the soil at sowing decreases the germination rate (Foolad 1999) whereas high concentrations of salt during the growing season inhibits plant growth leading to the appearance of leaf chlorosis and yield decrease (Parida and Das 2005).

Plant growth can be inhibited by the presence of high concentrations of salt both in the soil, reducing the ability of plants to absorb water and, in plant cells damaging the metabolic and photosynthetic capacity which results in in a decline of grain yield (Läuchli and Grattan 2007).

13.3.5 Nitrogen Use Efficiency

Under climate change, higher yields will be required to improve food security, reducing the external input and increasing the uptake of most of the essential nutrients from soil (Cordell et al. 2009). Although fertilizer application can alleviate the soil nutrients deficiency, the efficacy of mineral fertilization declined with increasing of drought stress (Fahad et al. 2017). For example, N application in soils with low moisture content (i.e. drought-affected areas in the world) did not increase yield as much as application in well-irrigated areas (Hu and Schmidhalter 2005). This represents a serious problem for durum wheat, given that for this species the availability of N in the soil is also responsible for the grain quality standard. The grain protein content determines, in fact, the technological and rheological parameters of the dough and consequently the pasta quality (Sissons 2008).

Nutrient concentrations in plants are also responsive to elevated atmospheric CO_2. Nitrogen concentrations, for example, are consistently lower in leaves and grain of plants grown under elevated levels of CO_2 (Taub and Wang 2008). In durum wheat, elevated atmospheric CO_2 decreases the grain quality with the main negative effect being on gluten content (Fares et al. 2016). Reductions in grain N concentrations have also been associated with reductions in gluten content that is important for breadmaking quality (Nuttall et al. 2017; Verrillo et al. 2017). Elevated CO_2 negatively impacted wheat flour reducing the content of gliadins and glutenins of 15 and 20%, respectively (Wieser et al. 2008). Among crop plants, elevated CO_2 has been reported to lower the concentrations of S, Zn and Fe in the grain (Fangmeier et al. 1997). Determining the concentrations of 10 minerals in durum wheat grain grown under free air CO_2 enrichment conditions, Beleggia et al. (2018) showed strong and significant decreases of −20 to −40% for all minerals evaluated.

13.3.6 Cold Stress Tolerance

One of the most decisive factors limiting the cultivation of durum wheat in the coldest areas of the world is its poor resistance to extreme cold conditions. In central northern Europe, for example, thermal fluctuations can reach very high values and irreversibly impede the growth and development of the crop. Under these conditions, the wheat suffers losses of production for which the breeders are faced with the task of developing winter-hardy cereal varieties capable of surviving the most severe winters occurring in a given region with a low level of damage (Longin et al. 2013).

13.3.7 Disease Resistance

Disease resistance was one of the most important objectives in crop improvement programs in the past and will be even more important in the near future due to climate change. While breeders focus on identifying new genetic materials with durable resistance genes, pathogen populations continue to evolve producing new virulent races. Therefore, for sustainable food production, disease management strategies should be reoriented to a changing climate, trying to focus on important disease complexes (Fig. 13.4).

13.3.7.1 Rust and Blotch Diseases

Rust diseases represent the most economically significant fungal diseases in wheat and are widely distributed across durum wheat growing regions. Three distinct rust diseases (stem rust, stripe rust and leaf rust) occur on durum wheat (Fig. 13.4).

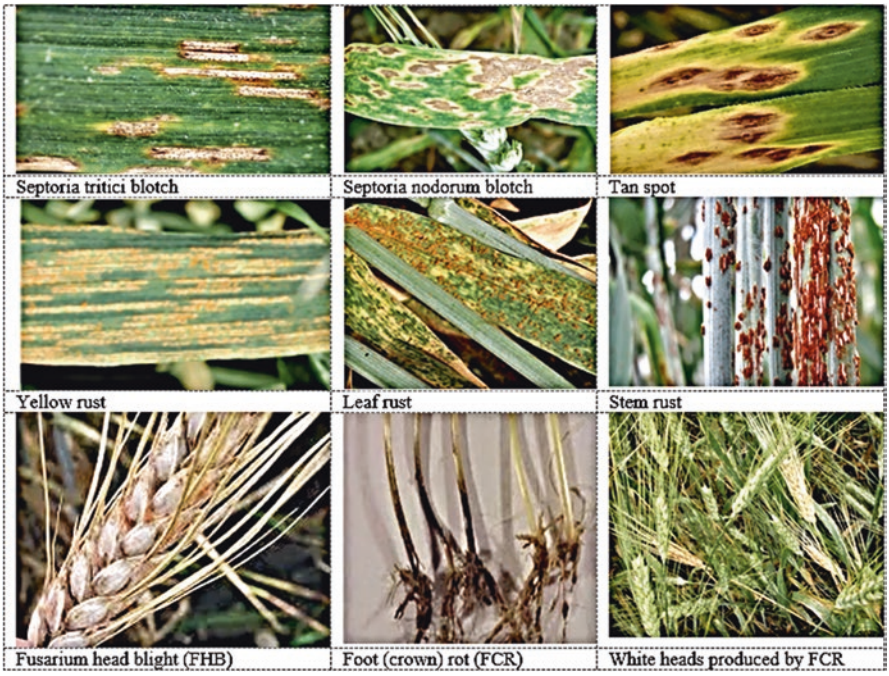

Fig. 13.4 Most important diseases in durum wheat

Puccinia graminis f. sp. *tritici* Ericks and Henn. (Pgt), the causal agent of stem rust, is a devastating fungal disease of both bread and durum wheats, and can culminate in significant yield losses worldwide (Singh et al. 2015a). Stem rust is usually found in regions where warm and moist environments prevail and symptoms of infection are generally manifested 7–15 days after infection when the oval pustules (uredinia) break through the epidermis of leaf sheaths, stems, glumes and awns of susceptible plants (Kolmer 2005). Explosive epidemics can occur during favorable environmental conditions, resulting in severe yield losses up to 70% (Huerta-Espino et al. 2011).

Stripe rust, caused by the fungus *Puccinia striiformis* Westend. f. sp. *tritici* (Pst), is an important disease of wheat, especially in cool climates (Chen et al. 2014). The presence and severity of this fungal disease in Mediterranean and temperate cultivars has not been important until recently; it is the most common and widely distributed of the three wheat rust diseases (Bolton et al. 2008). Stripe rust is usually evident earlier in the season than leaf rust because stripe rust pathogen requires colder temperatures for development. Yield losses associated with infection are attributed to a reduction of kernel weight and numbers of grain per head. *Puccinia triticina Eriks.* (Pt) is the causal agent of leaf rust, one of the most common rust disease of wheat widely distributed in wheat growing regions (Huerta-Espino et al. 2011, Bolton et al. 2008). Leaf rust is prevalent in areas with mild temperatures and moist conditions. In some areas, grain yield losses in the presence of leaf rust have surpassed 50%,

depending on the susceptibility of the varieties and the severity of the epidemics. Because of high pathogen diversity, leaf rust shows a high levels of pathogenic variation and adaptability to a wide range of climates (Huerta-Espino et al. 2011).

Another leaf disease of wheat in temperate growing regions is *Zymoseptoria tritici* (formerly known as *Mycosphaerella graminicola* or *Septoria tritici*) (Zt); the causal agent of *Septoria tritici* blotch (STB) is shown in Fig. 13.4 (Berraies et al. 2014; Eyal 1999). In Europe, STB is currently regarded as the primary threat to wheat production although the actual losses associated with it are less clear in other growing regions (Fones and Gurr 2015). *Stagonospora nodorum* blotch (SNB) is a major necrotrophic pathogen of wheat occurring all over the world (Eyal 1999), with direct damage to kernel quality causing heavy yield losses (Berraies et al. 2014). Tan spot (TS), caused by the ascomycete fungal pathogen *Pyrenophora tritici-repentis* (Ptr), is taxonomically closer *S. nodorum* and, together with *S. tritici*, have similar symptoms and frequently co-infect wheat (Abdullah et al. 2017). The complex of foliar diseases that includes tan spot has increased over the last several decades due to the great diffusion of conservative agriculture practices as minimum or zero tillage (Singh et al. 2008).

13.3.7.2 Fusarium Diseases

Another destructive disease in wheat is *Fusarium* head blight (FHB) caused by *Fusarium graminearum* (Fig. 13.4) (Goswami and Kistler 2004), which greatly reduces yield and kernel quality in epidemic years. The serious problem is that it is almost uncontrollable due to the lack of immune germplasm. Furthermore, fungal mycotoxin contaminated kernels render the grains unsuitable for food or feed. In many countries, legal limits are in place on the permitted mycotoxin levels for the various end uses (Knutsen et al. 2017). The intensification of crop systems in recent decades has probably increased the pressure of *Fusarium* diseases including head blight (FHB), crown (FCR) and root rot (FRR). Contrarily to the FHB, which has been the focus of numerous researches for the identification of *Fusarium* resistance genes and the development of new genetic materials tolerant to soil-borne fusarium diseases have been much less explored. Recently, FCR and FRR become more common in the main wheat growing areas and represent a significant part of *Fusarium* infections in wheat (Smiley et al. 2005). The major agents for FCR and FRR in cereal crops are *F. culmorum* and *F. pseudo-graminearum*, respectively, while *F. graminearum* is, so far, only sporadically related to these diseases (Scherm et al. 2013).

13.4 Conventional and Modern Breeding Approaches

Durum wheat improvement in the twentieth century, using phenotypic, pedigree and performance data, was very successful (De Vita et al. 2007). However, signs of yield stagnation in grain crops, especially in drought-stressed and semiarid regions,

were evident (Ray et al. 2012). Conventional breeding approaches have demonstrated that the manipulation of stress-related traits is particularly complex as they are controlled by a high number of genes acting additively and synergistically. The advancement of alternative breeding strategies to increase the selection gain per year is a continuous challenge for plant breeders (Longin et al. 2015) and to molecular-assisted breeding approaches. In this perspective, marker-assisted (MAS) and genomic selection (GS) provide powerful tools to improve breeding efficiency under a climate change scenario (Heffner et al. 2010).

13.4.1 Conventional Breeding Approaches

Considering the main distribution area of durum wheat in the world, the genetic improvement of stress-related traits has usually been approached by breeding directly for yield in low-potential environments subject to abiotic stress. Under these conditions, the selection efficiency decreases as the difference between the environment being selected in and the target environment increases, due to high genotype x environment (G x E) interactions (Ceccarelli 1994). Thus, genotypes developed under optimal conditions do not ensure the same performance under unfavorable environmental conditions as marginal dry areas (Ceccarelli et al. 1998). It is however true that selection for high-yield potential has often led to high production in a wide range of environments (Araus et al. 2008). There are arguments and evidence that selection in high-potential environments does lead to higher yields in poorer ones (Araus 2002), but only where those are characterized by frequent mild or moderate stresses.

The first significant success of grain breeding was the introduction of the reduced height (*Rht*) dwarfing genes (*Rht-B1*, *Rht-D1*) in commercial bread and durum wheat varieties, and the adoption of shuttle-breeding schemes in the breeding programs of CIMMYT, Mexico, to accelerate varietal improvement (Reynolds and Borlaug 2006). The development of semi-dwarf wheat varieties, spread all over the world since the 1960s, is a concrete demonstration of the effectiveness of this approach.

New genetic materials were established in the main areas of cultivation of durum wheat with climatic conditions, even extreme and very different from those of origin. In this context, the adaptation genes played a major role in plant response to environmental stresses (Bentley et al. 2011) and yield potential. In addition, to reduction of plant height, flowering time was one of the key adaptive traits. To realize optimal adaptation to local eco-geographic region, farmers and breeders have selected a complex of different alleles for adaptation controlling vernalization requirement (*Vrn* genes), day-length or photoperiod response (*Ppd* genes) and, to a lesser degree, earliness per se (*Eps*) *loci*, to modulate the flowering time and adapt varieties to a wide range of growing regions and sowing times (Distelfeld et al. 2009). This latter is controlled by at least 20 genes dispersed over the wheat genome

(Sanna et al. 2014). Hence, the adaptation of a genotype to a particular environment depends on the interaction of these three groups of genes.

The concept of photoperiod insensitivity was unknown to science when the CIMMYT shuttle-breeding program was initiated. This was an empirical approach and it only became apparent when Norman Borlaug began growing two crops of wheat a year in contrasting growing conditions in the early 1960s. In Asia, Mediterranean and North African regions, most landraces are sensitive to photoperiod; on the contrary the intensive selection for photoperiod insensitivity conducted during the twentieth century, resulted in the selection of early maturity cultivars with high yield potential (Ferrara et al. 1998). The photoperiod insensitive gene, *Ppd1*, is particularly abundant in the CIMMYT material, and along with the reduced height genes, *Rht1*, resulted in a new plant ideotype, with short stature, lodging tolerance, early heading and a yield more than doubled compared to traditional varieties. In subsequent decades, photoperiod insensitivity was selected by wheat breeders to enhance yield under certain climatic conditions and to circumvent drought and heat stresses in bread and durum wheat. Drought escape (related to shorter maturity durations) was the most widely used strategy (Shavrukov et al. 2017). However, it was difficult to develop a generalized methodology (Ceccarelli et al. 2010), and, despite some successes (Kumar et al. 2008), direct selection for yield in drought-prone environments has generally proven elusive (Blum 2011).

Ceccarelli et al. (1998) demonstrated that, under extreme drought conditions (yield potential <1 mt ha^{-1}), and by using locally-adapted germplasm, breeding cereals for survival (tolerance to severe stress) has been successful rather than for production.

Subsequently, an analytical approach based on the indirect selection of morpho-physiological traits of interest was proposed by Monneveux et al. (2012). Various traits associated to yield in arid environments were considered, especially in drought environments such as the Mediterranean Basin, in particular: broad and waxy leaves that ensure initial biomass, reduced photoinhibition and lower transpiration rate (Rebetzke and Richards 1999); a long coleoptile to allow emergence from deep sowing (Rebetzke et al. 1999); early ground cover that decrease the soil evaporation and increase the radiation-use efficiency (Condon et al. 2002) and, canopy temperature depression and higher stomatal conductance to guarantee greater extraction of water from soil (Loss and Siddique 1994; Reynolds et al. 2000).

In Australia, breeding programs based on indirect selection of morpho-physiological characters developed some current commercial bread wheat varieties such as Axe, a cultivar with good early vigor, Drysdale, a high transpiration-efficiency cultivar, and Gladius, a drought-tolerant cultivar with broad, erect, waxy leaves (Izanloo et al. 2008). According to these authors, heritability of the secondary traits should be higher than that of yield, and the genetic correlation of these traits with yield should also be high in the target environment.

At this time, we are unaware of durum wheat varieties selected on the basis of secondary traits, other than earliness, although many durum wheat breeding programs adopt the criteria of indirect selection.

13.4.2 Mutation Breeding

Mutation breeding is the utilization of induced mutation in crop improvement. It is a relatively cost-effective, quick, proven and robust breeding technique to generate new genetic variability through chemical and physical mutagenesis. In the past, induced mutations have been successfully utilized to create high-yielding varieties in more than 210 plant species from over 70 countries (FAO/IAEA 2018) and hundreds of semi-dwarf and early cereal varieties. This was possible as both these characters have been found in mutated generations with a relatively high frequency. Mutagenic treatment of wheat seeds has often been used to obtain short, stiff-straw, early lines, especially in locally, well-adapted varieties of cereals, traditionally grown in particular regions of the world. Indeed, the increase of durum wheat productivity in Italy was achieved through the introduction of new semi-dwarf genes obtained by physical mutagenesis. Lodging resistant mutant lines Cp B132, Cp B144 and Gr A145, developed after thermal and fast-neutron irradiation of varieties Cappelli and Grifoni, opened a new era in durum wheat breeding in Italy (Scarascia Mugnozza 2005). The value of a mutant line, and its potential as a new variety, depend on the level of background mutations. Considering that mutations occur randomly with respect to the effects produced, across the genome there will always be additional mutations with negative effects on the crop. The Cp B144 mutant line, in fact, was not used directly as a commercial variety but subjected to multiple crosses leading to the development of Creso, the most successful Italian variety, officially released in 1974 (De Vita et al. 2007). The introduction of this variety almost doubled the durum wheat yield in Italy, expanding the cultivation thanks to its resistance to lodging and to leaf rust, and good grain quality for pasta making. From the late 1960s, using different types of mutagens, γ-rays, X-rays, neutrons, sodium azide and ethyl methane sulfonate (EMS), 31 mutant durum wheat varieties have officially been released for commercial use (FAO/IAEA 2018), both through direct use of an induced mutant or for crossing with mutants.

The main improved attributes of mutant varieties were reduced plant height, resistance to lodging, high yield, adaptability, early maturity, tolerance to low temperature, resistance to rusts and powdery mildew, high thousand-kernel weight, high yellow semolina color and good quality. A new durum variety to better express its genetic yield potential, should have a proper combination of disease resistance, stress tolerance, plant height, tiller ability, kernel weight and grain quality attributes. Many quantitative characters of wheat are controlled by multiple genes and/or influenced by the environment. Mutagenic treatment acting randomly on the genome can easily alter positively or negatively all the genes involved in the response of the plant. Desirable mutant individuals can be isolated using good selection procedures, which in polyploid species such as durum wheat, can only begin in the M2 generation after the mutagenic treatment of seeds, even if first generation (M1) dominant mutations can be identified. However, many new mutants could be selected in M3 and M4 generations as in the M2 recessive mutant genes are not yet present in a homozygous condition (Jankowicz-Cieslak et al. 2016). Generally, three main types

of screening/selection techniques are used: mechanical, to determine the shape, size and weight of kernels using appropriate sieving machinery; phenotypic screening in selection for plant height, adaptation, flowering time and disease resistances; physiological, biochemical, chemical and physio-chemical procedures to screen for selection of certain types of mutants (Pathirana 2011).

Today, mutation techniques for crop improvement are mainly used by advanced laboratories to obtain new genetic sources for biotic and abiotic stresses or herbicide resistance and for improvement of specific quality traits (type of starch, carotenoids, dietary fiber, polyphenols or other compounds with antioxidant activity). After booming in the 1960s, mutation breeding is still used, for example, by seed companies to create crops for markets that reject genetically-engineered crops (Eriksson and Amman 2017). With respect to this last point, particular attention was paid to the development of herbicide-tolerant crops because they have several advantages over other weed management systems.

Imidazolinone (IMI) tolerant wheat was developed from seed mutagenesis projects using chemical mutagens. The M2 plants were sprayed with a specific herbicide as the selection agent and the imidazolinone-tolerant bread wheat varieties that have been marketed as Clearfield® wheat since 2001 (Pozniak and Hucl 2004). Herbicide-tolerant varieties have an enhanced ability to metabolize IMI herbicides before they reach the target site, so in combination with non-selective and of broad spectrum herbicides are able to effectively kill or suppress all types of plants. Now they are durum wheat varieties released with Clearfield® technology in several countries (Domínguez-Mendez et al. 2017).

Unfortunately, mutation breeding also has limitations and disadvantages, as compared to modern molecular breeding approaches, mainly related to the very low frequency of desirable mutations. Breeders have to screen large populations for desirable mutations. Certain types of mutations have pleiotropic effects influencing several traits simultaneously; other mutations have chromosomal aberrations and deletions. To minimize undesirable genomic segments linked to the target genes, mutant lines often have to be backcrossed to parents or adapted varieties. Backcrossing is time-consuming work and linkages between genes cannot be broken down easily (Pathirana 2011).

The recent developments in reverse genetics and gene discovery technologies have brought about renewed interest in mutation techniques and expanded their use beyond direct application in breeding and classical genetic analysis (Jankowicz-Cieslak et al. 2016). This becomes particularly important even after the sequencing of the reference genomes in wild emmer, durum and bread wheat, whose main objective in future will be to join the DNA sequence with its function. TILLING (targeting induced local lesions in genomes) a developed general reverse genetic strategy allows direct identification of mutations in a specific gene with known functions and their use as the genetic markers for selection (Slade et al. 2012). TILLING has been used for manipulation of starch composition in durum wheat for high amylose and waxy starch composition (Sestili et al. 2010), carotenoid content (Colasuonno et al. 2016) and thousand-grain weight (Simmonds et al. 2016). Because the TILLING population is a permanent resource for the genetic and

molecular analyses, the genetic materials and information produced by the application of this protocol be efficiently translated into breeding programs.

13.4.3 Modern Plant Breeding Approaches

To meet the challenge of climate change, durum wheat needs much better environmental adaptation, including innate resilience to cope with more extreme abiotic stresses as well as tolerance to combined stresses. Development of genomic strategies and tools will need to consider responses of different cereals to climate changes, some of which could be crop-specific while others may be shared among different crops. It has become essential to identify positive alleles, genes and useful haplotypes to transfer to elite cultivars, based on various germplasm, including landraces and wild relatives. Novel strategies to combine molecular markers with accelerated development of elite germplasm (including techniques like doubled haploidy, speed breeding and high-throughput phenotyping) are needed to fast-track development and delivery of improved germplasm.

13.4.3.1 Molecular Marker-Assisted Breeding

Marker-assisted selection is a pivotal component of modern breeding strategies, which utilize full genome sequencing and genome-wide molecular markers to accelerate the whole process of durum wheat genetic improvement (Taranto et al. 2018). At present, molecular markers based on single nucleotide polymorphisms (SNPs), whose diversity comes from mutation of a single nucleotide, are widely utilized. In wheat, SNP arrays are powerful tools to reveal genomic diversity. One widely used SNP-array is the 90 K array, developed from 19 bread wheat accessions of diverse geographic origin (Wang et al. 2014). This array contains a large number of SNPs distributed across the common wheat genome and it is currently used also in durum wheat. The most recent SNP array contains about 630,517 SNPs (660 K array) developed by low coverage sequencing of 192 accessions of bread wheat and other related species (Cui et al. 2017).

In 2017, the total assembly size of 10.5 gigabase resulted in the 14 chromosomes of wild emmer (*Triticum turgidum* ssp. *dicoccoides*) was published and provided a roadmap to enhance genomics-assisted breeding and improvement of modern wheat varieties (Avni et al. 2017). In 2018, the 21 chromosomes of bread wheat (17 gibabase) was released (Appels et al. 2018). In 2019, an international consortium has generated a high quality reference sequence of the modern durum wheat cultivar Svevo (Maccaferri et al. 2019) (https://www.interomics.eu/durum-wheat-genome). The completion of genome sequencing of these species represents a milestone for wheat biology and provide long-term resources for wheat functional genomics. The next challenge for durum wheat, as well as for bread wheat (Montenegro et al. 2017), will be that of pan-genomes to capture the genetic diversity of the major

global durum wheat growing regions. An alternative, efficient and cost-effective approach to dissect the genetic diversity of wheat accessions is genotyping-by-sequencing (GBS) (Poland et al. 2012). This method is based on reducing genome complexity with restriction enzymes and generating randomly, polymorphisms (SNPs) across the genome, such that it can detect population-specific variants.

The molecular markers discovered by sequencing approaches can be used in many areas of durum wheat breeding programs: i) germplasm identification and genetic diversity analysis; ii) genetic linkage map construction and gene mapping; iii) map-based cloning and iv) marker-assisted selection breeding.

13.4.3.2 Germplasm Identification and Genetic Diversity Analysis

Germplasm collections, including elite wheat cultivars, landraces, progenitors and ancestral species, represent a suitable platform on which to test molecular markers. Currently, SNP array-based technologies are used to explore the genetic diversity of durum wheat, its origin and domestication. SNP and DArTseq markers were used to assess the genetic diversity of durum wheat landraces from Turkey and Syria, revealing that the population structure reflected the exchange of genetic material due to unconscious farmer selection and lack of the commercial varieties (Baloch et al. 2017). Kabbaj et al. (2017) investigated the population structure and genetic diversity within Ethiopian germplasm, including elites and landraces, using 8173 segregating SNPs, generated by the Axiom 35 K array. Genetic analysis split the population into 10 groups, dividing modern cultivars and landraces into 6 and 4 groups, respectively, according to geographic origin. Phylogenetic analysis among landraces elucidated that Ethiopian germplasm is distinct from those of the primary region of origin of durum wheat and may represent a second center of origin of durum wheat.

In two separate studies, Soriano et al. (2016, 2018) showed a reliable relationship between genetic and phenotypic population structures in a durum wheat collection, including 172 durum wheat landraces from 21 Mediterranean countries and 20 modern cultivars, which were growth in 6 environments, and the connection of both with the geographic origin of the landraces. These results support the hypothesis that durum wheat, during the migration phase through the Mediterranean Basin, has adapted to the new environmental conditions by modifying the morpho-phenological yield-related traits. To date, numerous studies have shown a loss of genetic diversity due to domestication and the selection made for the development of new varieties in durum wheat (Laidò et al. 2014).

Genotyping a worldwide durum wheat collection with SNP markers, Ren et al. (2013) showed a reduction in genetic diversity during the early periods of the Green Revolution, while in the last decades of last twentieth century the new released cultivars showed an increase in genetic diversity. This trend was also greatly impacted by environmental conditions, breeding methods and gene flows via human activities.

Introgression of useful alleles from landraces, adapted to their local environmental conditions through natural selection and traditional breeding, into modern cultivars represents a valuable genetic resource to be used in modern assisted breeding programs for suboptimal environments, such as those prevalent in the Mediterranean Basin. Lopes et al. (2015) supported the hypothesis that landraces can provide sources of increased biomass and thousand-kernel weight which are important traits for adaptation to tolerate drought and heat.

13.4.3.3 Genetic Linkage Map Construction and Gene Mapping

In conjunction with genomic tool development, linkage map and QTL mapping are useful tools to discover genes controlling important agronomic traits. The progressive addition to linkage maps of new and abundant types of molecular markers suggests that the construction of a genetic map with desirable density is highly achievable.

In durum wheat, several linkage maps have been published and available to dissect out the genetic basis of traits of agronomic relevance for the genetic improvement of durum wheat. The first linkage consensus maps able to ensure good genome coverage were constructed with SSR and DArT markers (Marone et al. 2012). Recently, several independent tetraploid wheat mapping populations were developed using with the Illumina 9, 15 and 90 K SNP arrays (Maccaferri et al. 2015). The construction of linkage maps is often associated with low resolution, low saturation and excessive distance between markers, resulting in limited application value. In order to solve these problems, a consensus genetic map provide a higher marker density and a greater genome coverage when compared to the individual maps and increase the effectiveness of genome-wide association studies and QTL meta-analyses (Swamy and Sarla 2011). Thanks to the recently released consensus linkage maps for bread wheat (Wang et al. 2014) and durum (Maccaferri et al. 2015), development and saturation of genetic maps for detection of QTLs underlying agronomic traits has rapidly increased in genetics studies.

The development of genetic maps (genotyping) and the uniform phenotyping of segregant populations including (RILs), backcross inbred lines (BILs), advanced backcross lines (ABLs), and doubled haploid (DH) lines are the required steps for linkage and QTL mapping.

Major QTLs for traits such as flowering time (Sanna et al. 2014) and high salinity (Munns and Tester 2008) have been detected. QTLs for stomatal conductance, carbon isotope discrimination (Panio et al. 2013) and stay-green traits (Kumar et al. 2010) were identified in durum wheat as the easiest indicator for evaluation of drought tolerance. A heat-susceptibility index was mapped as heat tolerance surrogate through grain fill duration and canopy temperature depression (Paliwal et al. 2012).

Unfortunately, the identification of QTLs related to complex characters such as heat and drought is strongly affected by mapping populations used. In addition, some phenological traits may potentially mask the effect of traits that directly con-

tribute to increased performance under drought (Olivares-Villegas et al. 2007). For this reason, in association mapping studies could be very useful to use homogeneous genetic materials for flowering time and plant height in order to increase the possibility to identify QTLs that can be masked by these confounding effects (Pinto et al. 2010). To date, QTLs that control tillering and spike fertility traits have been found in wheat, generally in bread (Xie et al. 2016) and durum wheat (Giunta et al. 2018); however, in only a few cases have any attempts been made to relate these QTLs to flowering genes.

The main challenge in this area will be to identify stable QTLs in different genetic background and in typical climatic conditions of durum wheat growing areas (Yang et al. 2002). Maccaferri et al. (2008) evaluated a durum wheat population of 249 recombinant inbred lines (RIL) in 16 environments characterized by a broad range of water availability and detected 16 QTLs that affected grain yield (GY), heading date (HD) and plant height (PH).

To overcome this problem, meta-QTL analysis was used to identify consensus QTLs across different populations and environments (Zurek et al. 2015). This method has been applied in determining consensus QTLs for grain yield and related traits (Zhang et al. 2010), flowering time (Zanke et al. 2014), disease resistance (Marone et al. 2013), root architecture in durum wheat (Iannucci et al. 2017) and adaptation to drought and heat stresses (Acuña-Galindo et al. 2015).

Meta-QTL analysis conducted to identify *loci* associated to drought yield revealed that different QTLs co-localized with QTL associated with root morphology (Swamy and Sarla 2011). The importance of root system characteristics in relation to water stress in cereals has been clearly established (Watt et al. 2005). Petrarulo et al. (2015) and Iannucci et al. (2017), using a set of RILs derived from tetraploid wheats, identified the genomic regions involved in the control of root morphological traits in durum wheat.

Along with advances in sequencing technologies, and also a decrease in sequencing cost, the association mapping approach of (i) candidate-gene association mapping by studying single nucleotide polymorphisms within candidate genes and (ii) genome-wide association studies (GWAS) using molecular markers distributed across the whole genome has been adopted. GWAS became more common in identifying SNPs or indels associated with stress tolerance and is now extensively used in wheat without creating mapping populations, but instead by utilizing varieties and landraces available in germplasm collections. In durum wheat, sequence-based GWAS are still rare. In wheats most works were conducted using diversity arrays technology (DArT) and SSR markers.

Association mapping (AM) was used to dissect the genetic basis of drought-adaptive traits and grain yield in durum wheat across 15 environments of highly different water availability during the crop cycle (Maccaferri et al. 2011). The number of markers affecting GY decreased considerably under drought conditions, suggesting a limited effectiveness of AM to identify *loci* for traits of interest under these conditions. The study confirmed the role of major *loci* for phenology previously described in biparental mapping populations. The same accessions were evaluated in order to identify QTLs for root system architecture (RSA) and, seminal

root angle and root number appear the most promising traits for adaptation in different environments characterized by soils with a limited moisture content (Canè et al. 2014; Maccaferri et al. 2016).

Numerous association mapping studies have been carried out to characterize resistance to diseases in durum wheat. Laidò et al. (2015) identified new resistance *loci* to the African stem rust race TTKSK in tetraploid wheats, based on linkage and genome-wide association mapping. To validate association mapping results, a biparental population of 146 recombinant inbred lines was also considered and the stem rust resistance gene *Sr13* on the distal region of chromosome arm 6AL was confirmed. A novel source of stripe-rust resistance was identified by GWAS in an Ethiopian durum wheat population by Liu et al. (2017). In this study, landraces were less resistant than cultivars at the adult-plant stages. Aoun et al. (2016) identified sources of resistance to several leaf rust races from a worldwide durum wheat collection of 496 accessions through experiments conducted both in the field and in the greenhouse. Ghavami et al. (2011) applied a mixed model association mapping approach for fusarium head blight resistance in Tunisian-derived durum wheat populations.

The discovery of new disease resistance *loci* through AM represents a very important step to broaden the leaf-rust resistance spectrum in durum wheat germplasm.

Improving nitrogen use efficiency is a key factor to sustainably ensure global production increase under climate change. Physiological and molecular studies on N uptake and assimilation pathways reveal the high complexity of N use in plant and high-throughput screening methods remain at the developmental stage; genetic progress may be mainly driven by marker-assisted selection (Cormier et al. 2016; Hirel et al. 2007; Le Gouis 2011). Cormier et al. (2014) characterized 214 elite European varieties for 28 traits related to NUE in 8 environments under 2 different nitrogen fertilization levels, using 23,603 SNPs for GWAS. Numerous co-localizations were observed with previously published genomic regions and, particularly interesting were those between agronomic and phenological genes as *Ppd-D1*, *Rht-D1* with *loci* controlling nitrogen status as *NADH-Gogat* and *GSe*.

Exploiting this knowledge, Nigro et al. (2017) identified new allelic variants in durum wheat for both *GS2* and *GOGAT* genes, and regression analysis demonstrated that some variations are positively and significantly related to the grain protein content. Moreover, in the same chromosomal regions, several authors have located important QTLs for grain protein content (Bogard et al. 2013; Fontaine et al. 2009; Gadaleta et al. 2011).

13.4.3.4 Map-Based Cloning

Map-based cloning is the process of identifying a targeted-gene location by looking for the marker and the tightly-linked gene of interest whose physical location in the genome is known. Map-based cloning has been commonly used in bread wheat and wild emmer wheat where high-density genetic maps, large physical maps, broad

sequences in the gene libraries, and whole-genome sequence have been available for some years, allowing the applications of map-based cloning (Krattinger et al. 2009a).

Wheat's large genome size and its genomic complexity make it a challenge to clone genes from wheat without a reference sequence or an extensive physical map and high content of repetitive sequences; nevertheless, a growing number of genes have been isolated from wheat species through positional cloning. Many of them are genes conferring resistance to diseases such as stem rust *Sr 35* and *Sr 33* (Periyannan et al. 2013; Saintenac et al. 2013), leaf rust *Lr 1*, *10*, *21* and *34* (Cloutier et al. 2007; Feuillet et al. 2003; Huang et al. 2003; Krattinger et al. 2009b) and yellow rust *Yr36*, (Fu et al. 2009). Some of these are adaptation genes, i.e. vernalization genes *VRN1*, *VRN2*, *VRN3* (Yan et al. 2003, 2004, 2006) and the *GPC-B1* gene (Uauy et al. 2006) associated with increased grain protein, zinc and iron content in wheat grain. Exploiting the Chinese Spring 1IBS Arm-specific BAC library the *Stagonospora nodorum* susceptibility gene *snn1* in wheat was map-based cloned (Shi et al. 2016). A new strategy called targeted chromosome-based cloning via long-range assembly (TACCA) was successfully applied in cloning the wheat leaf rust resistance gene *Lr22a* in only 4 months (Steuernagel et al. 2016).

The change in the wheat-genomics landscape with resources which include complete reference genome and high-quality gene models (Appels et al. 2018), transcriptomics databases (Borrill et al. 2016; Pearce et al. 2015) and high-density SNP arrays (Wang et al. 2014) will make it much easier to conduct map-based cloning on specific genes to meet the challenges of climate change.

13.4.3.5 Marker-Assisted Selection (MAS) Breeding

Although many quantitative trait *loci* (QTLs) have been identified in bread and durum wheat, the impact of marker-assisted selection for improving truly quantitative traits in durum wheat breeding is limited (Habash et al. 2009) particularly for typical quantitative traits as drought response, heat stress and water-use efficiency. This could be attributed to the low proportion of variance explained by most of the identified QTLs, as well as the fact that many identified QTLs are specific to a particular phenotyping environment or genetic background.

To date, MAS has been most successful in the selection of resistance to diseases and for improving grain quality (www.maswheat.com). MAS-derived varieties and advanced lines combining resistance to biotic and abiotic stresses or improved grain quality have been reported in wheat (Randhawa et al. 2013), but it is expected that many more successful applications already exist but remain within the confidentiality restrictions of commercial breeding companies around the world. To meet the climatic challenges of the near future, it is necessary to find tools that can contribute substantively to the development of durum wheat varieties adapted to the new predicted climate scenarios.

SNP arrays represent convenient platforms to genotype large numbers of wheat accessions and are ideal for breeding applications, and now are preferred over other

molecular marker systems due to their relative abundance (Akhunov et al. 2009; Cavanagh et al. 2013). The critical step for effective deployment of MAS requires the development of easy-to-use molecular markers like single nucleotide polymorphisms (SNPs).

Recently, genome-specific KASP (Kompetitive allele specific PCR) markers (Ramirez-Gonzalez et al. 2015) were developed for the identification of functional markers in wheat for several agronomically-important traits like plant height, thousand-kernel weight, grain length, pre-harvest sprouting, drought tolerance, rust and blight diseases as well as some genes related to starch synthesis, phytoene synthase and others (Chandra et al. 2017). For wheat MAS, KASP genotyping assays have been developed for several traits of interest.

A comprehensively list of KASP markers developed and validated in bread and durum wheat for the development of precision breeding is provided in Table 13.3. SNP markers mapped on the A and B genomes are reported. In detail, the list

Table 13.3 Kompetitive allele-specific PCR (KASP) assays for major determinants of durum wheat adaptation to change in climate

Trait	Gene	SNP_ID	Source	Reference
Photoperiod	*Ppd-A1*	wMAS000029	CerealsdB	Beales et al. (2007)
	Ppd-A1	wMAS000030	CerealsdB	Wilhelm et al. (2009)
	Ppd-A1	wMAS000031	CerealsdB	Wilhelm et al. (2009)
	Ppd-B1	wMAS000027	CerealsdB	Beales et al. (2007)
Plant height	*Rht-B1*	wMAS000001	CerealsdB	Ellis et al. (2002)
	Rht-B1	Rht-B1_160IND	Rasheed et al. (2016)	Wilhelm et al. (2013)
	Rht-B1	Rht-B1_197IND	Rasheed et al. (2016)	Wilhelm et al. (2013)
Vernalization	*Vrn-A1*	wMAS000033	CerealsdB	Rasheed et al. (2016)
	Vrn-A1	wMAS000034	CerealsdB	Chen et al. (2009)
	Vrn-A1	wMAS000035		Yan et al. (2004)
	Vrn-B1	wMAS000036	CerealsdB	Milec et al. (2012)
	Vrn-B1	wMAS000037	CerealsdB	Milec et al. (2012)
Leaf Rust	*Lr47*	Lr47-1	Rasheed et al. (2016)	–
	Lr68	Lr68-2	Rasheed et al. (2016)	Rasheed et al. (2016)
Stem rust	*Sr2*	wMAS000005	CerealsdB	Mago et al. (2011)
	Sr36/Pm6	wMAS000015	CerealsdB	Distelfeld et al. (2006)
Fusarium	*Fhb1*	wMAS000008	–	Bernardo et al. (2012)
Fusarium	*Fhb1*	wMAS000009	–	Liu et al. (2008)
HMW-GS	*Glu-A1*	wMAS000012	CerealsdB	Liu et al. (2008)
	Glu-A1	wMAS000013	CerealsdB	Liu et al. (2008)
	Glu-A1	CIMwMAS0011	CerealsdB	Liu et al. (2008)
	Glu-A1	CIMwMAS0012	CerealsdB	Liu et al. (2008)
	Glu-B1	BX13-1510	Rasheed et al. (2016)	Ragupathy et al. (2008)
	Glu-B1	7OE-866	Rasheed et al. (2016)	JN982368.1[a]
Grain protein	*Gpc-B1*	wMAS000017	CerealsdB	Distelfeld et al. (2006)

CerealsdB: www.cerealsdb.uk.net/cerealgenomics/CerealsDB/Excel/MAS_data_May_2013.xls
[a]=NCBI accession number

includes KASP markers retrieved from the Cerealsdb database (www.cerealsdb.uk.net/cerealgenomics/CerealsDB/Excel/MAS_data_May_2013.xls) and developed by Rasheed et al. (2016) and SNP markers currently in use at CIMMYT (http://repository.cimmyt.org/dvn). This set of KASP markers represents a toolkit ready to use in MAS for adaptive genes, grain quality traits and diseases resistance genes. The future climatic challenge for durum wheat breeding will be to build upon developing new varieties to enhance concomitantly-positive alleles for complex traits.

13.4.3.6 Genomic Selection

MAS uses molecular markers in linkage disequilibrium (LD) with QTLs and it is efficient only if the trait under consideration is controlled by a limited number of QTLs with large contributions to phenotypic variation, but is inferior to traditional phenotypic selection in dealing with complex agronomic traits controlled by many QTLs with small effects (Bernardo 2008). One major reason is that estimates of QTL effects for minor QTLs are often biased.

Genomic selection (GS) provides an alternative method to use genomic information in breeding decisions (Meuwissen et al. 2001). Instead of using only significant marker-trait associations to build up the prediction model, genomic selection uses all markers simultaneously (Bassi et al. 2015). This approach has been evaluated in most major animal and plant species (for a review see: Desta and Ortiz 2014) and is or is becoming a routine tool in commercial and public breeding programs.

The basic steps can be summarized in: (i) phenotype and genotype a training population, (ii) build a GS model estimating regression coefficients for all markers and (iii) based on this model, calculate the genomic estimated breeding values (GEBVs) in a training population and select without phenotyping in the following generations. Gain from selection during GS is proportional to GEBV accuracy. The main advantage of GS is that candidates can be evaluated and therefore selected without their own phenotypic information (Spindel et al. 2016). Until today, the limitation of GS has been the large number of markers required and the cost of genotyping. However, with the availability of cost-effective whole-genome SNP panels for wheat species, GS is now leading the way to a paradigm shift in durum wheat breeding (Jannink et al. 2010).

To date, several GS studies have been published on bread wheat (Crossa et al. 2017) with the main objective of comparing the genomic prediction accuracy within and across environments (Crossa et al. 2016) for yield, or to compare the genomic selection and marker-assisted selection in their ability to predict traits associated with some diseases such as resistance to *Fusarium* head blight (FHB) in bread (Arruda et al. 2016) and durum wheat (Miedaner et al. 2017). To predict rust resistance, a genomic best linear unbiased prediction (GBLUP) and a Bayesian regression method was applied in 206 wheat landraces from 32 countries, using 5568 SNPs, with a prediction accuracy of 3 traits ranged from 0.27 to 0.44 (Daetwyler et al. 2014). Juliana et al. (2017) tested different prediction models for leaf spotting diseases and found that using genomic prediction models gave a 48% average

increase in accuracy. Sun et al. (2017) found that secondary traits improved genomic predictive ability for grain yield in wheat by an average of 70%. In addition, the phenotyping of secondary traits at early growth stages facilitates selection prior to harvest, thus improving testing efficiency. At CIMMYT, GS has been applied to predict grain quality traits routinely evaluated in the wheat breeding programs. Battenfield et al. (2016) developed and validated whole-genome prediction models for end-use quality phenotypes in the CIMMYT bread-wheat breeding program. Similarly, Guzman et al. (2016) demonstrated that the development of most grain quality traits are time-consuming and costly. Dough rheology and baking final product can be predicted using genomic selection with a high degree of confidence.

However, apart some examples, there are still very few studies that propose practical applications of GS in active wheat-breeding program (Song et al. 2017).

In durum wheat, limited information is available on the selection of grain quality traits. Fiedler et al. (2017) explored the potential application of GS in a durum wheat breeding program to enhance the selection of major durum wheat quality trait (test weight, semolina color, gluten strength). The results showed the potential of grain and semolina quality traits to be selected more efficiently through MAS and GS with further refinement. Haile et al. (2018) assessed the potential of single-trait and multi-trait (MT) genomic prediction models for durum wheat on yield and quality traits using a breeding panel of 170 varieties and advanced breeding lines, and a DH population of 154 lines. Rapp et al. (2018) investigating the potential of different indices to simultaneously improve grain yield and protein content in durum wheat showed that GS appeared promising, but the performances of the model adopted were extremely dependent on the relationship between training population and prediction sets.

The accuracy of GS also depends on training population (TP) size, marker density, heritability, trait architecture, magnitude of LD and the interaction of these factors. Using the same GS method, prediction accuracy of a high-heritability trait is often higher than that of a low-heritability trait (such as grain yield, drought and heat tolerance). G × E interaction is another important factor that must be taken into consideration. Even thought, at the beginning GS models were implemented for a single trait evaluated in a single environment, and most analyses published so far are based on within-environment analyses. Burgueño et al. (2012) extend the GS approach to a multi-environment framework, reporting very promising results in terms of prediction accuracy. More recently, Heslot et al. (2012) and Jarquín et al. (2014) showed that modelling G × E can give substantial gains in prediction accuracy.

13.4.3.7 Rapid Generation Advance

Durum wheat breeding programs typically require 10–15 years to transfer novel genes into adapted germplasm. In the 1950–1960s, an approach called *shuttle breeding*, introduced by Norman Borlaug at CIMMYT in Mexico, allowed breeders to grow two generations of wheat in a year in two locations differing in elevation,

latitude and climate in Mexico (Ortiz et al. 2007), reducing the time needed to develop new varieties and ensuring a wide adaptation, resistance to wheat diseases and greater annual yield gain.

Alternatively, in order to develop recombinant inbred lines by acceleration via single seed descent (SSD), the plants can grow under conditions that anticipate the time of flowering and seed maturation by the rapid generation advance (RGA) system in glasshouse or controlled environment facilities. Recently such methods have been referred to as faster generation cycling system (FGCS) or rapid cycle breeding (RCB) or simply rapid generation advancement (RGA), by different researchers (De La Fuente et al. 2013; Yan et al. 2017). For these methods, supplementary lighting is not the only basis for rapid generation advance in plants. Embryo rescue, in which immature seeds are harvested and induced to germinate on culture medium, represents another common feature in many rapid cycling methods. Zheng et al. (2013) reported up to 8 generations per year for wheat without PGRs. Unfortunately, these approaches require species-specific protocols and can also be labor intensive for large populations, especially those requiring removal of the embryos from the seed coat; moreover, skills and extensive testing are needed for any plant species to optimize such applications.

13.4.3.8 Doubled Haploid Technology

The production of doubled haploid (DH) plants has become a key tool in advanced plant breeding. The primary advantage of the DH approach is in instantaneous development of homozygous lines/varieties from crops with any degree of heterogeneity in a single generation, which is a major breakthrough to speeding up wheat cultivar development (Murovec and Bohanec 2012; Szechy et al. 2009). Researchers have applied different methods for the production of DHs in wheat which includes anther culture, ovule culture, chromosome elimination following wide hybridization, haploid inducer gene/s and chemical treatments (Niu et al. 2014). The regeneration of wheat through anther culture is used in many cereal breeding programs to obtain DHs, because of it is more cost-effective than intergeneric (Cistué et al. 2009). However, the application of anther culture in wheat breeding has several disadvantages, being highly dependent on genotypes (Tadesse et al. 2012). The haploid production response varies within a given genus. In tetraploid durum wheat, several authors have reported its recalcitrant nature to anther culture (García-Llamas et al. 2004), with low regeneration rates and a very high frequency of albino plants (Labbani et al. 2005).

For these reasons, until now, the system used to obtain durum wheat haploid plants has been the intergeneric crossing with maize (*Zea mays* L.) (Jauhar and Peterson 2001). In this method, the induction of haploid embryos is produced when wheat is pollinated with maize. This technique is a success as the maize pollen is insensitive to the cross-ability inhibitor genes (Laurie and Bennett 1986) and could be applied to a wide range of wheat as well as maize genotypes (Pratap et al. 2006; Singh et al. 2004). The wheat × maize system is not affected by the change of

ploidy; however, the exogenous supply of growth hormones or chemicals also determines the efficiency of haploid induction (Bokore et al. 2016). In order to improve this protocol, more research is currently underway on different aspects. Because DH technology can reduce the time to develop genetically-stable lines from a new cross to a single generation, it can save significant time and resources for implementing genetic studies and/or molecular breeding projects, including genetic mapping, QTL discovery and estimation of genetic effects (Xu et al. 2017). Because they are completely homozygous, DH lines can be easily replicated and maintained through self-pollination (O'Donoughue and Bennett 1994). Haploid technology has tremendous potential for accelerating breeding technologies when combined with MAS. In combination with doubled haploidy, MAS is a time-saving method of performing backcross conversions to select an elite line with a particular trait.

13.4.3.9 Speed Breeding

The recent development of a speed-breeding protocol, to achieve rapid generation cycling in fully enclosed growth rooms or glasshouses, simplifies and makes the procedure widely applicable for large-scale application in crop-breeding programs at low cost and without particular technical skills. Exposing plants to continuous light or prolonged photoperiod can significantly reduce generation time for a broad range of plants (Sysoeva et al. 2010). RGA systems were implemented in temperature-controlled glasshouses using 22 h supplementary LED lighting, temperature and photoperiod regime (22 °C/17 °C 179 (22 h/2 h) and successfully applied in spring bread wheat (*Triticum aestivum* L.), providing a useful tool for crop improvement (Hickey et al. 2012). This provides a highly flexible platform to achieve rapid generation advancement, irrespective of genetic background, where up to 4–7 generations per year can be achieved in several crop species, including durum wheat (Alahmad et al. 2018; Watson et al. 2018). A threefold increase in the shuttle-breeding techniques is currently used by breeders and researchers; the technique was first introduced after World War II as part of the Green Revolution.

The protocol for rapid trait introgression in wheat was developed combining the use of speed breeding and high-throughput phenotypic selections. The approach was initially tested by selecting for resistance to tan spot in wheat and then expanded to a number of important wheat traits, including: grain dormancy (Hickey et al. 2010), stripe rust (Hickey et al. 2012) and leaf rust (Riaz et al. 2016). In Australia, DS Faraday was the first bread wheat variety to be developed using the speed-breeding method in which genes for grain dormancy were introduced to ensure tolerance to preharvest sprouting (Watson et al. 2018). Alahmad et al. (2018), for the first time applied this methodology in durum wheat by integrating selection for key agronomic traits such as seminal root angle (RA), seminal root number (RN), tolerance to crown rot (CR), resistance to leaf rust (LR) and plant height (PH). The results strengthened the suggestion that, when selection is coupled with the speed-breeding system, useful alleles will be introgressed much faster into elite breeding populations to develop new improved varieties. The protocol has also been adapted

for high-density plant production systems for SSD programs (Ghosh et al. 2018). The combination of speed breeding and other leading-edge plant breeding technologies with strategic global partnerships, has the potential to achieve the genetic gain targets required to deliver more productive future crops (Li et al. 2018).

13.4.3.10 Gene Editing

Induced mutations in breeding, based on physical or chemical mutagens, generates random mutations across genomes, introducing a wide range of changes in target genes. In this way, a single plant can contain a large number of different mutations simultaneously (Parry et al. 2009). On the other hand, the critical point of chemical and physical mutagenesis is its stochastic nature. To overcome this limitation, new breeding techniques, also called new genetic engineering techniques (i.e gene/genome editing, cisgenesis), are a suite of genetic engineering methods that could increase and accelerate the development of new traits in plant breeding (Zhang et al. 2016). These new techniques have the ability to move from random mutation breeding to point-specific mutations, advancing towards a new generation of mutagenic agents.

Recently-developed genome editing techniques, despite often using similar molecular tools as in the transgenic methodology, provide products that are often difficult to distinguish from those of the conventional mutation breeding methods (Cardi et al. 2017). The new generation of mutagenic agents, based on the more recent clustered regularly interspaced short palindromic repeats, CRISPR/Cas9, has been developed and has very rapidly been integrated into plant breeding programs, largely replacing older versions, such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) (Zhang et al. 2016). Although many experimental research studies have been performed in numerous crop species (Cardi et al. 2017), CRISPR/Cas9 technology in durum wheat is still poorly used, due to its variable transformation efficiency. However, the positive experiences carried out in common wheat open a very promising scenario also for durum wheat. CRISPR/Cas9 technology was successful applied in wheat protoplasts, inducing mutations in resistance gene *TaMLO*, and generating plants with resistance to powdery mildew (Shan et al. 2014). The durum wheat *TdGASR7* gene associated with grain yield or disease resistance was edited by CRISPR/Cas9, (Zhang et al. 2016). CRISPR/Cas9 technology was used to specifically reduce the amount of α-gliadins in the kernels, developing transgene-free wheat lines with low-gluten and thus reduced immunoreactivity for gluten-intolerant consumers (Sánchez-León et al. 2018). In the near future, to achieve relevant results in developing genetically-engineered bread and durum wheat, it will be necessary to increase transformation efficiency and the accuracy of wheat genome editing, through the development of specific protocols.

13.5 High-Throughput Phenotyping

In contrast to the advancement of sequencing technologies, a critical point for accelerating the development of new and improved cultivars is the assessment of high-throughput phenotyping of thousands of breeding lines over time (Fu 2015). At present, the efforts of the plant science community are addressing the gap between genotype and phenotype in order to explore existing genetic resources for their interaction with the environment. Advances in plant phenotyping are even more crucial in scenarios of high environmental variability occurring under climate change (Araus et al. 2018). High-throughput phenotyping platforms (HTPP) are capable of simultaneously taking multiple measurements of plant characteristics to capture and provide reliable estimates of trait phenotypes. In recent years, there has been increasing interest in establishing HTPPs under different growth conditions. In addition, particular attention was paid to develop field-phenotyping low-cost platforms to overcome the spatio-temporal limitations imposed by the greenhouses and growth chambers (Masuka et al. 2012). Significant progress has been achieved thanks to the development of novel sensor modules, imaging devices, automated systems, drones, LED lightings and portable devices for a wide range of traits, organs and situations (Fiorani and Schurr 2013) (Fig. 13.5). Generally the traits taken into consideration with HTP tools are not those that have a direct agronomic interest such as grain yield but secondary traits, indirectly connected with the main ones. Secondary traits of adaptive value under abiotic stresses are collected to facilitate the prediction of yield or to improve the knowledge on the genetic bases that regulate the primary traits (van Eeuwijk et al. 2018). High-throughput, automatic and reliable phenotyping platforms have been developed to meet the objectives of ongoing research and are arousing interest in the scientific community to launch national, regional and international initiatives (Fig. 13.5). For example, the Australian Plant Phenomics Facility (APPF) is a world-leading infrastructure facility leading innovative research to accelerate the development of new and improved crops. The EU has launched the European Plant Phenotyping Project (EPPN) to establish a phenotyping infrastructure in the region. This network includes projects such as the International Plant Phenotyping Network (IPPN), Deutsche Pflanzen Phänotypisierungs Netzwerk (DPPN), Phenome French Plant Phenotyping Network (FPPN), the UK Plant Phenomics Network and the Italian Plant Phenotyping Consortium (Phen-Italy). Features and potentialities of these tools are widely documented (Araus et al. 2014); herein, we briefly list which implications (benefits and constraints) have emerged from their use on bread and durum wheat breeding programs, considering the HT phenotyping experiments conducted in the open field that represent well the response of the genotypes to environmental conditions. The most important challenge, from the technological point of view, is to develop non-destructive methods that can be used to screen large numbers of genotypes directly in the field with quick, precise, accurate and low-cost measurements. The potential to measure parameters of field trials such as crop establishment, plant height,

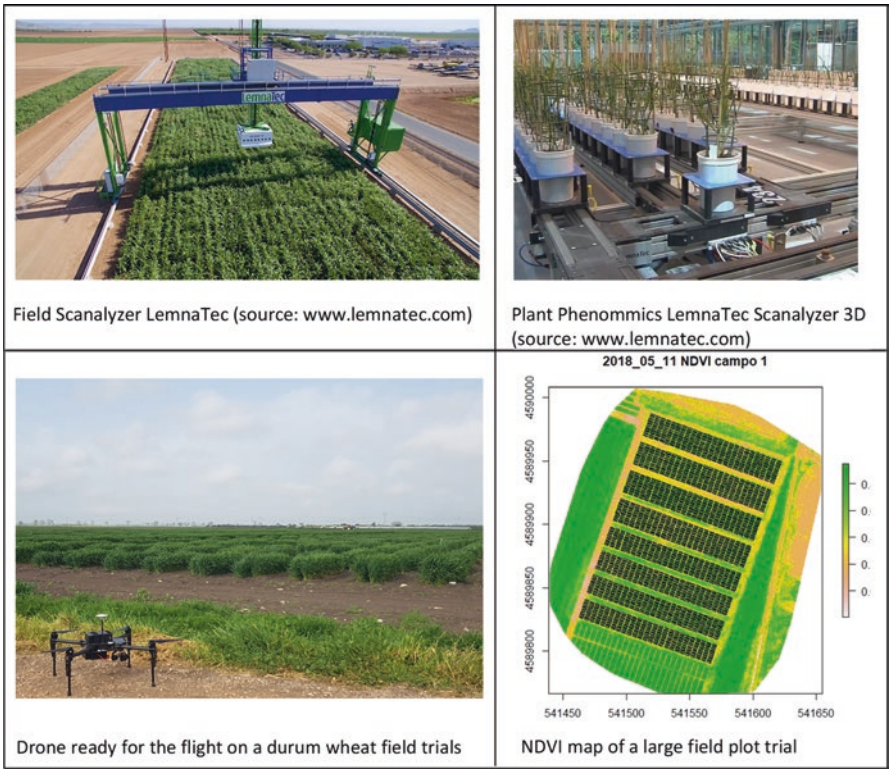

Fig. 13.5 Innovative technologies for high-throughput phenotyping

biomass, diseases, intensity of abiotic stress and nutritional status can be explored using this technology.

In recent years there have been a large number of scientific studies whose main objectives were to validate data collected on the ground with that recorded in the immediate area using various types of platforms (UAV-based remote sensing versus ground-based HTPP). There have been numerous studies conducted on wheat to compare different active and passive spectral sensors in estimating and discriminating adaptive characters such as biomass, vegetation indices (i.e. normalized difference vegetation index, NDVI) (Condorelli et al. 2018; Khan et al. 2018; Tattaris et al. 2016), yield (Haghighattalab et al. 2016) canopy traits (Bai et al. 2016), early plant vigor (Kipp et al. 2014) and plant height (Madec et al. 2017). For many of these traits such as establishment, height, biomass, stress, disease and nutritional status, the results suggested in early selection cycles, enhanced high-throughput phenotyping might be single or in multiple rows.

The detection and identification of plant diseases is a fundamental task in sustainable durum wheat production. Exploiting the technologies present on the HTP platforms, numerous studies have been carried out to screen wheat germplasm collection for the main diseases such as yellow rusts (Zhou et al. 2015), *Fusarium* head

blight (FHB) resistance (Alisaac et al. 2018); and spot blotch disease (Kumar et al. 2016), recording NDVI data as a substitute for visual observation of the percentage of disease occurrence.

Currently the main limitation to the application of these technologies, for the selection in the open field, is linked to the wide spatial variability of the chemical and physical properties of the underlying soil. It is therefore necessary to keep under control the experimental error related to environmental variability. This can be done through the development of new high-throughput tools able to accurately characterize the environment in which the experimentation is conducted.

13.6 Modelling and Durum Wheat Improvement

Crop models are used to predict growth and yield as influenced by the growing environment and agronomic practices, and can be used to identify desirable traits, or a combination of traits, potentially leading to the specification of crop ideotypes (Gouache et al. 2015; Ramirez-Villegas et al. 2015; Tao et al. 2016). Wheat crop models have been evolving since the 1960s (Chenu et al. 2017) and have been used over decades for agronomic purposes. More recently, modelling has become important for supporting plant breeding, in particular in designing ideotypes, for target environments and future climatic conditions (Semenov et al. 2014). In a review, Chenu et al. (2017) examined the main limitations of crop simulation modelling to support ideotype breeding, and described developments in cultivar traits in response to climate variations. Semenov et al. (2014), using a crop model, predicted an increase in frequency of heat stress at meiosis and anthesis, and quantified the effects of heat and drought at booting and flowering on grain numbers and potential grain size. Dettori et al. (2017) used the CERES-Wheat crop model to simulate grain yield durum wheat under climate change projections in a typical Mediterranean environment. Based on this information, the authors estimated the impacts of climate projections on different varieties and locations and provide guidelines for realistic adaptation strategies in a typical Mediterranean area Mereu et al. (2011).

One of the most significant effects of climate change and global warming trend is the advance of the phenological phases of crops (Rezaei et al. 2018).

Therefore, by modulating the sowing dates and choosing the most suitable cultivars, it is possible to adapt the agricultural crop system to the new climate scenario. For example, in China new cultivars of wheat with higher vernalization requirements and longer growing cycle were introduced to compensate the advancement in crop phenology caused by increased temperatures (Rezaei et al. 2018). Analyses showed that the shift of the ordinary sowing date could be a reliable and efficient adaptation strategy for wheat cultivation in the Mediterranean area, reducing the negative effect on yield due to changed climatic conditions (Mereu et al. 2011). According to He et al. (2012), the extent of projected climate change in Saskatchewan, one of the most important growing areas of durum wheat in Canada, indicated that growers in this region have the potential of earlier seeding or selection of the earliest

varieties. These strategies could help producers mitigate the impact of climate change on durum wheat.

The uncertainties inherent in climate and impact projections present a particular challenge for translating climate science into actionable outcomes for agriculture. In any case, there could be a significant incentive to develop a multidisciplinary approach involving breeders, climate scientists and crop modelers in order to address the challenges of climate change (Taranto et al. 2018).

13.7 Conclusions and Prospects

Climatic unpredictability remains one of the main challenges for the genetic improvement of durum wheat in the near future. The frequency and the intensity of extreme weather events are concomitant with changes in global climate, making difficult the selection of superior genotypes. It is therefore necessary to exploit the best of modern technologies and the entire methodological arsenal currently available to prevent the stagnation of wheat production. Identifying allelic variants associated with tolerance to abiotic stress and resistance to the major diseases of durum wheat is a priority. To this end, it is necessary to explore the genetic variability existing within wheat species, including landraces, traditional varieties and wild relatives. This to incorporate novel alleles from the primary gene pool into elite varieties. In this respect, advances in genomics, precise phenotyping and plant physiology, coupled with new developments in crop modelling will provide the means to dissect integrative traits that affect adaptation to stressful environments. Finally, the release of the durum wheat genome (Maccaferri et al. 2019) will provide a valuable insight into the cultivated tetraploid wheat genome, and an enormous opportunity to elucidate the genetic basis of variation for the most important agronomic traits. Moreover, this will expand our knowledge of durum wheat at the nucleotide level, contributing to improve molecular-marker discovery that should facilitate the identification of desirable alleles to use in future climate-smart plant breeding programs.

Acknowledgement This work was supported by the Italian Ministry of Economic Development (MISE) for funding the project HORIZON 2020 PON I&C 2014–2020, INNOGRANO N. F/050393/00/X32.

Appendices

Appendix I: Research Institutes Relevant to Durum Wheat

Institute	Specialization and research activities	Contact information and website
CREA Research Centre for Cereal and Industrial Crops	Genetics and breeding of durum wheat	Pasquale de Vita SS 673 km 25 + 200–71,122 Foggia, Italy pasquale.devita@crea.gov.it https://www.crea.gov.it/it
Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, University of Bologna, Italy	Genetics and genomics in durum wheat	Roberto Tuberosa Viale Fanin 44, Bologna, Italy roberto.tuberosa@unibo.it https://www.unibo.it
Plant Sciences, Crop Development Centre, University of Saskatchewan, Canada	Genetics and genomics in durum wheat	Curtis Pozniak Curtis.pozniak@usas.ca Crop Development Centre, University of Saskatchewan 2E64 – Agriculture Building 51 Campus Drive, Saskatoon, SK, Canada https://agbio.usask.ca/departments/plant-sciences.php
International Maize and Wheat Improvement Center (CIMMYT), Mexico	Head of durum wheat and triticale breeding at CIMMYT	Karim Ammar Km. 45, Carretera, México-Veracruz, El Batán, Texcoco CP 56237, Edo. de México, Mexico k.ammar@CGIAR.ORG www.cimmyt.org
International Center for Agricultural Research in the Dry Areas (ICARDA), Morocco	Genetics and breeding of durum wheat	Filippo Bassi F.Bassi@cgiar.org Dalia Building 2nd Floor, Bashir El Kassar Street, Verdun, Beirut, Lebanon 1108-2010 www.icarda.org
Swift Current Research and Development Centre Agriculture and Agri-Food Canada	Genetic, genomic and breeding of durum wheat	Ron Knox 1 Airport Road, PO Box 1030, Swift Current, Saskatchewan S9H 3X2 ron.knox@agr.gc.ca http://www.agr.gc.ca

(continued)

Institute	Specialization and research activities	Contact information and website
University of New England, Australia	Cereal chemistry of durum wheat and wheat breeding for quality	Mike Sissons Tamworth Agricultural Institute, 4 Marsden Park Road, Calala, NSW 2340, Australia. mike.sissons@dpi.nsw.gov.au https://www.dpi.nsw.gov.au
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Appendix II: Durum Wheat Genetic Resources

Cultivar	Important traits	Cultivation location
Antalis	Yield potential	Italy
Iride	Yield potential	Italy
Claudio	Yield potential	Italy/France
Svevo	Yield potential and grain quality	Italy
Miradoux	Yield potential and grain quality, diseases resistance	France/Germany
Anvergur	Yield potential and grain quality, diseases resistance	France
Tempodur	Yield potential, diseases resistance	Germany and Austria
AC Avonlea	Grain quality	Canada
AC Navigator	Grain quality	Canada
Strongfield	Grain quality	Canada

(continued)

Cultivar	Important traits	Cultivation location
Commander	Grain quality	Canada
Kamilaroi	Yield stability and grain quality	Australia
Yallaroi	Yield stability and grain quality	Australia
Wollaroi	Yield stability and grain quality	Australia
Sredetz[a]	Resistance to lodging and tolerance to low temperature	Bulgaria
Castelporziano[a]	Resistance to lodging and high yield	Italy
Grandur[a]	Short straw, resistance to lodging, high yield,	Austria
Attila[a]	Resistance to lodging, high yellow pigment and good quality	Austria
Cargidurox[a]	Semi-dwarfness and resistance to lodging	France
Antoñín[b]	Resistance to imazamox herbicide	Spain

[a]Mutant variety
[b]Clearfield® variety

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Chapter 14
Spelt (*Triticum spelta* L.) In Vitro Androgenesis Breeding for Special Food Quality Parameters

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Abstract Consuming gluten-containing foods derived from wheat, barley, rye and possibly oats can result in health problems for a significant proportion of people. However, in the case of several types of disorders related to consuming gluten-containing cereals, not only the gluten components are trigger compounds. According to medical experts the majority of people suffering from health problems because of gluten— except for celiac disease patients—instead of consuming gluten-free food have the option to choose food products containing healthier, low levels of fermentable oligosaccharides abbreviated FODMAP. In order to meet the health-related special needs of these particular consumer groups, cereal breeders aim to develop new germplasm, suitable for the food industry to produce healthier products. This chapter provides a summary of the latest developments in this booming research field, including: (i) describing the actual knowledge on cereal-related health problems, (ii) describing the current status of celiac-safe cereal breeding, (iii) enhancing the importance of developing healthier spelt-based cereal products through the advancement of an ongoing spelt breeding program and finally (iv) developing plant biotechnology improvements relative to special food quality parameters.

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J. M. Al-Khayri et al. (eds.), *Advances in Plant Breeding Strategies: Cereals*,
https://doi.org/10.1007/978-3-030-23108-8_14

Keywords Celiac disease · Irritable bowel syndrome (IBS) · In vitro androgenesis · Non-celiac gluten sensitivity · Spelt · Wheat allergy

14.1 Introduction

Islam et al. (2011), defined wheat gluten as a protein-lipid-carbohydrate complex formed through particular non-covalent and covalent correlations among flour constituents during dough making, as the constituents are hydrated and where the mixing process provides the mechanical energy input. Nevertheless, these days the use of technical terms such as *gluten-free* and *gluten* are more common (but inaccurate) among lay people. Gluten-free roughly refers to food products free from cereal prolamin proteins, i.e. wheat glutenin and gliadin, which are similar.

Consuming food with gluten content derived from wheat, barley, rye and possibly oats can result in health problems for a significant proportion of people. In this regard, considering oats as a *harmless cereal* is one of the oldest and open disputes among specialists. Some experts assert that a certain percentage of patients suffering from celiac disease are sensitive to epitopes located in the so-called avenins, the prolamin type storage proteins in oat (Comino et al. 2016; Pulido et al. 2009), while other publications explain its potential danger through contamination with other cereal.

However, in the case of several types of disorders related to consuming gluten-containing cereals, the gluten components are not the trigger compounds. We do not have enough specific knowledge about the reasons for different symptoms or diseases and the precise meaning of terms like *gluten*, *prolamins*, *gliadin* or *glutenin*, neither for average consumers nor for medical experts (Branchi et al. 2015).

Nowadays in most Western countries the general public has learned of the perceived dangers and adverse influence of cereals containing gluten, thanks to reports appearing in the lay press (Braly and Hoggan 2002; Ford 2008; Wangen 2009) recommending gluten-free diets. Many of these reports are not able to define the nature of the gluten *intolerance* an individual may have or enhance the significance of appropriate diagnosis. In this way they portray a severe public relations threat to the grain industry.

In Western countries the gluten-free food business has improved in the last 5 years. In 2013 the US retail trade in gluten-free products reached USD 10.5 billion, and up to USD 15 billion was predicted for 2016. It is forecast in Australia that in the following 5 years the gluten-free retail sales will exceed USD 100 million, where in 2014 already more than 20% of the newest baking products were launched in the market as being gluten free (Jargon 2014).

As information about mechanisms of certain disorders has been accumulating, it has been revealed that the causative components of cereal products are often the soluble proteins (albumins and globulins) or the soluble oligosaccharides instead of

gluten proteins. This fact enhances the importance of research dealing with an appropriate diet for different types of gluten-sensitive people, instead of just suggesting a gluten-free diet.

Marketing activities associated with *wheat allergy* or *gluten toxicity* is frequently concentrated on gluten-free products. However, to meet the needs of different consumer groups, the medium or long-term solution based on more and more information collected in the medical research field is not simply having gluten free and traditional products on the shelves of the stores. A gluten-free diet is incomparably more expensive but not healthier than consuming gluten-containing foods (Missbach et al. 2015). Except for individuals who suffer from celiac disease (about 1% of the Caucasian population), people with some kind of cereal-related health problems can consume foods with lower levels and/or modified gluten content and decreased amounts of certain soluble carbohydrate (FODMAPs) components (Halmos et al. 2015).

To meet the special health related needs of certain consumer groups, cereal breeding has the duty to develop new germplasm, suitable for the food industry to produce new, healthier products. Setting achievable goals, comprehending the numerous health-related disorders, their presence and trigger composites, is essential not only in the current basic research but also in profit-based cereal breeding as well. In this chapter we present a summary of the latest developments in this booming research field, describing the actual knowledge on cereal-related health problems, describing the current status of celiac-safe cereal breeding and finally—through an example— enhancing the importance of developing healthier spelt-based cereal products through the advancement of an ongoing spelt breeding program.

14.2 Cereal-Related Health Disorders

Health problems caused by gluten can be classified basically into three types: allergic, autoimmune and nonallergic - not autoimmune disorders (Fig. 14.1, Sapone et al. 2012). The first two of these, are quite well researched; although, to reveal mechanisms related to various symptoms of allergic reactions, further studies are required. The allergic reactions comprise food allergy (Mills et al. 2004), respiratory allergy (Amano et al. 1998), contact urticaria (Lahti 1986) and wheat-dependent exercise-induced anaphylaxis (Armentia et al. 1990). The innate and adaptive pathways leading to celiac disease (CD)-specific symptoms have been described and studied extensively (Plugis and Khosla 2015). The autoimmune disorders include gluten ataxia, CD and dermatitis herpetiformis (Anderson and Wieser 2006; Lauriere et al. 2006).

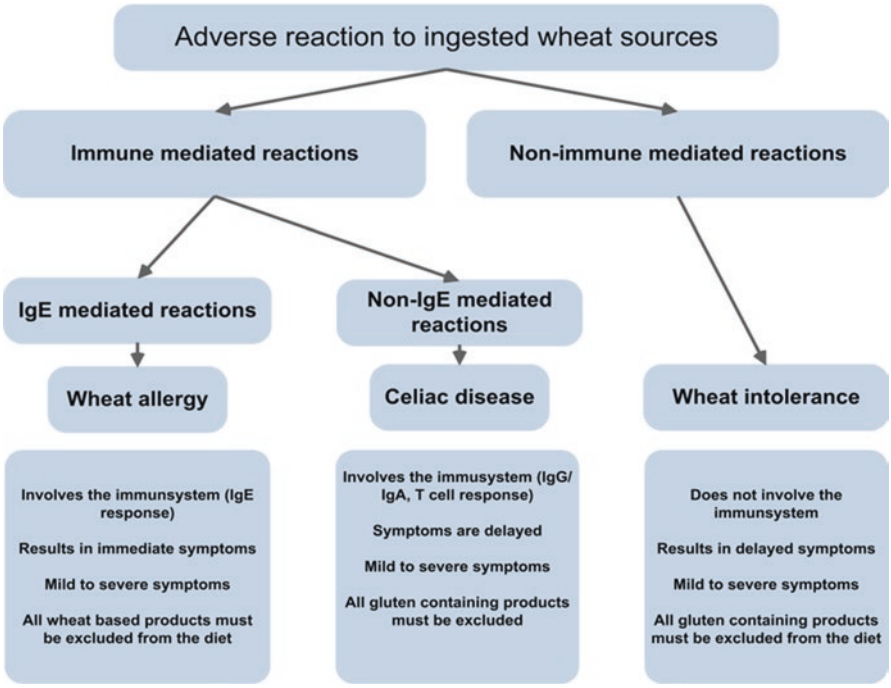

Fig. 14.1 The classification of wheat-related health disorders based on Sapone et al. (2012)

14.2.1 Celiac Disease

An abnormal response of autoantibodies causes the symptoms of celiac disease; for example, antibodies produced against glutamine- and proline-rich wheat gluten proteins, or their rye and barley homologues, or antibodies to tissue-transglutaminase (Green and Cellier 2007). There is a strong connection between celiac disease and one of the most essential genetic factors in developing detrimental symptoms, HLA-DQ alleles (Sollid et al. 2012). Heterodimers of these alleles, bound to the surface of antigen-presenting cells, function as surface-type receptor proteins. The presence of HLA-DQ compounds (DQ2.2, DQ2.5, DQ8), is a clear sign for the appearance of autoimmune symptoms, with modifying effects deriving from environmental and genetic factors (Anderson et al. 2000). The different peptides are recognized by different serotypes.

A set of criteria for the structure of an active CD epitope in a cereal protein can be described as follows: a) surrounded by amino acids with defined charge and hydrophobicity, b) the presence of a tissue transglutaminase 2 (tTG) enzyme-binding site and c) a size of nine amino acids fitting into the groove of the HLA-DQ heterodimers (Sollid et al. 2012). Patients suffering from celiac disease produce a range of autoimmune reactions to gliadins, LMW glutenins and some HMW glute-

nin subunits, just as to their analog polypeptides in the consumed cereal foods (Juhász et al. 2012).

14.2.2 Wheat Allergies

Due to specific IgE epitopes bound to mast cells, the development of wheat allergies is mediated more directly by the recognition of allergens (Catassi and Fasano 2008). Symptoms can be divided depending on the route of wheat allergen exposure into symptoms of classic food allergy: occupational asthma (bakers' asthma), wheat-dependent exercise-induced anaphylaxis (WDEIA) and urticaria (Sapone et al. 2012). Common food allergy signs may affect the respiratory or gastrointestinal tract or the skin. One of the most typical respiratory allergies is bakers' asthma, a serious symptom among adults who work with wheat flour.

Allergens presenting T-cells and B-cells have the same level of effects in a wheat allergy. Similar to other allergic reactions connected to food allergies, it is the result of cross-links between specific immunoglobulin E and short peptides that are rich in glutamine and proline and derive from the degradation of wheat grain proteins by endogenous proteases. This interaction stimulates mast cells and basophils to release chemical mediators, for instance, histamines resulting in different classes of inflammatory reactions. IgE-binding epitopes are the allergenic regions of proteins recognized by the binding sites of IgE molecules. These epitopes can be categorized into two groups: linear and conformational epitopes. Both types play a part in the development of allergic responses (Akagawa et al. 2007). In wheat, allergenic proteins are not just the prolamins (α, β, γ and ω gliadins, HMW and LMW glutenin subunits) but also proteins that are not elements of the gluten matrix {amylases, peroxidase, thioredoxin and serine proteinase inhibitors, the lipid transfer proteins (LTP)} (Tatham and Shewry 2008).

14.2.3 Non-Celiac Gluten Sensitivity

Non-celiac gluten sensitivity (NCGS) was first described about 40 years ago as mainly intestinal symptoms connected to consumption of gluten-containing cereals (Schuppan et al. 2015). There is significant proof that NCGS is induced by an innate immune response to wheat proteins (it is different from the adaptive, T cell-mediated response to gluten and non-gluten proteins in wheat allergy or to gluten in celiac disease) (Catassi et al. 2013; Sapone et al. 2012). A study by Junker et al. (2012) helped in the identification of the family of amylase-trypsin inhibitors (ATIs) as occasions of innate immunity in wheat. Nowadays NCGS has become a subject of growing interest for scientists. Research indicated a certain degree of awareness in connection with clinical relevance of gluten-related disorders among gastroenterologists (Zevallos et al. 2016).

14.2.4 Irritable Bowel Syndrome

A common gastrointestinal condition is irritable bowel syndrome (IBS) that has an impact on quality of life and can lead to significant financial costs. Generally, the efficacy of the therapeutic strategies to treat IBS, is disappointing, but has been improved significantly by the research of Gibson et al. (2007, 2015). Numerous abdominal symptoms may originate from factors which alter bowel digestion. They contribute to luminal digestion, particularly the osmotic load within the lumen, and the fermentative gas content may offer symptomatic benefit. FODMAPs (fermentable oligosaccharides, disaccharides, monosaccharides and polyols) is the collective term for candidate substrates that are highly fermentable causing an osmotic effect and are dietary, poorly absorbed and short-chain carbohydrates (Shepherd and Gibson 2006). Lactose and fructose are included in FODMAPs as there are patients in whom these are malabsorbed. Other FODMAP compounds are the polyols, for instance, sorbitol since they are poorly absorbed by humans, as well as galacto-oligosaccharides (such as raffinose) and fructo-oligosaccharides (fructans). The latter are always poorly absorbed since humans do not express suitable hydrolases (Shepherd et al. 2008). Fructans and fructose are included by major dietary FODMAPs. The most common food sources of fructans are cereals and onions; sources of fructose are fruits or honey (Biesiekierski et al. 2011; Muir et al. 2009).

A blinded placebo-controlled crossover study proved the efficiency of low FODMAP intake, confirming previous studies where about 75% of patients gain clinically-outstanding advantage (Halmos et al. 2014). As a first-line therapy, the low-FODMAP diet is progressively being applied by health specialists in patients with IBS (Halmos et al. 2014, 2015). Given the chronic nature and the high prevalence of IBS, many people may restrain the intake of FODMAPs over the short- or long-term (Catassi et al. 2017; Shepherd and Gibson 2006).

14.2.5 Prevalence of Different Disorders

In Western countries where the predisposing genes of celiac disease, *HLA DQ8* and *HLA DQ2* genes, can be found in approx. 30% of the population, celiac disease itself affect only 1% of these Western communities (Anderson and Wieser 2006).

Food allergy questionnaires were used with statistical analyses to establish relationships between individuals with clinical symptoms and immune responses (Vu 2014, Vu et al. 2014) in a study where a large number of blood samples was analyzed by the IgE RAST method against milk and wheat antigens from randomly-selected individuals (n = 1145) (Pasco et al. 2012). Outcomes indicated that the frequency of wheat allergy was 2.5% where both symptoms against wheat and a positive IgE immune response were observed. These results corroborate absolutely with similar wheat examinations where it was found that in European countries the prevalence of wheat IgE sensitization is 2.9% (Siles and Hsieh 2013; Zuidmeer

et al. 2008). In summary it is assumed that those individuals (12.8%; n = 125) who exhibit increased IgE levels without any symptoms might have a latent wheat sensitivity. They can be potential patients sooner or later as they have the chance of developing the symptoms. In the investigated population there is a large proportion (12.9%) who have symptoms connected to a wheat-consuming diet but did not show increased IgE. They may suffer from other wheat-related disorders (i.e. not IgE mediated) such as reaction to fructans (FODMAPs) for those with IBS, celiac disease or non-celiac gluten sensitivity (NCGS).

14.3 Developing Celiac-Safe Wheat

Identification of amino acid sequences of gluten proteins which are the inducing factors in sensitive individuals have shown that their distribution varies between and among the different groups of gluten proteins (Shewry and Tatham 2016). Detecting and determining the quantity of gluten proteins are crucial for two reasons: due to its direct impact on end-use quality and also for food safety reasons. There is a difference between seed composition among cereal genotypes which creates methodological problems in food allergen research and genotype selection for quality in breeding. The multi-species origin of prolamins and high sequence analogy, coupled with restrictions in the available methodologies, reviewed by Haraszi et al. (2011), make the precise identification of proteins that generate health disorders and their genotypic stability, variability and frequency, difficult to define. An accurate quantitative relationship is required between the final gluten/prolamin content and the prolamin peptide biomarkers in high-resolution methods; for instance, in mass spectrometry (MS) to connect the detection of peptide mass to their protein bases. Due to environmental and genotypic variability, these quantitative interactions, however, are difficult to detect.

To assist in protein selection, epitope mapping, peptide biomarker searches and medical studies, a database (ProPepper, https://propepper.net) was created which contains linear epitopes responsible for wheat-related food disorders, peptides obtained with single and multi-enzyme in silico digestion and the members of the prolamin superfamily proteins identified from Poaceae species (Juhász et al. 2015).

Information about the amount and composition of allergen and toxic epitopes existing in a single wheat sample can represent a substantial gap. The very soon to be completed grain proteome datasets can ensure the essential information to perform an estimation of allergen/toxicity calculation for a single cultivar. The combined use of allergen/toxic databases and genome sequence, cereal chemistry and prediction methodology results in a better understanding of the level of toxicity present from wheat flour in the end-products. Information is available about the distribution and number of epitopes for a single protein or a protein fraction based on the workflow presented in the review of Juhász et al. (2012). The most detrimental proteins and epitopes occurring in the highest frequency can be identified as

well. Researchers may obtain significant output from the analysis of large datasets such as the epitope toxicity value that can be well applied in food industry.

Two excellent reviews (Comino et al. 2013; Shewry and Tatham 2016) summarize recent research activities on the status of pseudo and alternative cereals and their derivatives, achieved by enzymatic or transgenic technology, breeding programs and natural selection, with the potential to develop products tolerated by celiac patients. Consequently, it is possible to use conventional breeding methods for selecting gluten protein fractions with lesser amounts of celiac epitopes. Molecular breeding methods can be applied to mutate celiac epitopes within individual proteins or to specifically downregulate celiac-toxic proteins. Gil-Humanes et al. (2008) provide well-known examples of this method, using RNA interference mediated gene silencing to downregulate the alpha and gamma gliadins in bread wheat (*Triticum aestivum* L.). A parallel method was applied by Altenbach et al. (2014a, b) to remove the omega-5 gliadins that are the most allergenic gluten proteins.

The combination of the above approaches may be used to develop celiac-safe wheat. However, due to the complex multigenic control of gluten protein composition, this remains a formidable challenge. Moreover, wheat must retain acceptable bread making or baking properties after any type of modification. It is not surprising that such celiac-safe wheat has not been developed, despite over a decade of research.

14.4 Gluten-Free Versus Low FODMAP Diet

According to a study of gluten-free foods (Market Research 2012), of individuals on a gluten-free diet, 65% consider it healthier, 36% do so for reasons other than sensitivity, 27% think it helps weight loss, 7% do so to lower inflammation and 4% to fight depression; only 5.7% claimed an certified medical diagnosis.

Due to the ubiquity of gluten in foods, a gluten-free diet (GFD) causes many social and economic repercussions (Comino et al. 2013). In the meantime, it cannot be considered as a healthy diet (Balakiereva and Zamyatin 2016), because gluten-free products usually are comprised of starches or refined flours containing a low amount of fiber. The fact is that consuming sufficient amounts of dietary fiber is associated with substantial health benefits, for instance, prevention of diabetes, colon cancer and cardiovascular disease. In consequence, GFD may cause different nutrient deficiencies in fiber which can lead to further subsequent health problems, as mentioned above. Numerous studies recommend using pseudo-cereal sources of fiber to maintain the required amount of fiber levels (Saturni et al. 2010). GFD can also induce a deficiency in folic acid and Vitamins B12, C and D, along with micro-elements, most importantly zinc, magnesium and calcium (Caruso et al. 2013; Hallert et al. 2002;). Besides, GFD comprises high amounts of hydrogenated fats and sugar, which can lead to an increased risk of obesity and hyperinsulinemia (Lamacchia et al. 2014). Hence, a GFD appears to be unbalanced and inadequate

with regard to both micro- and macronutrients. According to medical experts the majority of people, who may feel better consuming gluten-free foods—except for celiac disease patients—are automatically choosing a low FODMAP diet by selecting gluten-free or wheat-free products (Halmos et al. 2014, 2015).

Active research work and development in analyzing FODMAP-content, together with using food ingredients with low FODMAP content, were initiated to determine the crucial significance of FODMAP constituents related to health disorders. Establishment of a low FODMAP diet includes a much wider range of plant-origin products in contrast with the gluten-free phenomena, where the problematic food sources are a relatively small group of certain cereals. Studies by Biesiekierski et al. (2011) and Muir et al. (2007, 2009), along with computer and mobile phone applications, have helped to improve custom-designed low FODMAP diets: (http://www.med.monash.edu.au/cecs/gastro/fodmap/iphone-app.html).

Products made from traditional cereals (wheat, barley and particularly rye) are not recommended in FODMAP diets since they contain relatively high FODMAPs (mostly fructans). However, the genotypic variation within certain cereal species (Table 14.1) opens new perspectives for reducing/increasing kernel fructan

Table 14.1 Fuctan levels in different cereals

Crop	Species	Fructan concentration (% dry matter)			References
		Range	Average	No. of samples	
Rye	*Secale cereale L.*	3.6–5.0	4.2	25	Fretzdorf and Welge (2003a,b)
		4.5–6.4	5.5	25	Hansen et al. (2003)
		3.6–4.6	4.1	18	Andersson et al. (2009)
		4.6–6.6	5.6	13	Karppinen et al. (2003)
Wheat	*Triticum aestivum* L.	0.8–1.9	1.3	129	Andersson et al. (2013)
		0.9–1.8	1.4	25	Fretzdorff and Welge (2003b)
	Triticum durum L.	1.5–1.7	1.6	5	Fretzdorff and Welge (2003b)
	Triticum monococcum L.	1.6–2.3	1.9	8	Brandolini et al. (2011)
Spelt	*Triticum spelta* L.	0.9–1.3	1.1	5	Fretzdorff and Welge (2003b)
Triticale	*Triticosecale* Wittmack	1.6–2.9	2.3	16	Rakha et al. (2011)
		1.5–2.1	1.8	5	Fretzdorff and Welge (2003b)
Barley	*Hordeum vulgare* L.	Traces–1.0	0.4	92	Aman et al. (1985)
Oats	*Avena sativa* L.	Traces– 0.2	0.1	121	Aman (1987)
Maize	*Zea mays* L.	0			Fretzdorff and Welge (2003b)

Source: Based on Verspreet et al. (2015)

concentrations by breeding. In the case of wheat, it was shown that there is not a very strong genotype x environment (G x E) interaction, and that heritability is high, in a range of 0.64–0.94 (Huynh et al. 2008b).

Recent research studies to obtain information about gene regulation of fructan synthesis in cereals has primarily focused on barley and wheat. In the case of wheat, two major quantitative trait loci were identified so far on chromosomes 6D and 7A, and, in case of barley, several genes were mapped which encode fructan synthesizing enzymes (Huynh et al. 2008a, 2012; Wei et al. 2000).

A total of eight QTLs with two pairs of epistatic interactions were found for grain fructan concentration. Two QTLs on chromosomes 7A and 6D explained, respectively, 17 and 27% of the total phenotypic variation. Transgressive segregation was observed, and broad-sense heritability was estimated as 0.71 (Huynh et al. 2008b). Genes encoding the enzymes of fructan biosynthesis (*1-SST*, *1FFT*, *6-SFT*) form a functional cluster which was sequenced and mapped to the major QTL on chromosome 7A (Huynh et al. 2012).

Moreover, TaMYB13, a transcriptional activator, was identified, which could control the expression of major fructosyltransferases that are involved in fructan synthesis (Xue et al. 2011). In transgenic wheat lines the overproduction of this transcriptional activator has raised the fructan concentrations in the top internode and flag leaf, but the result on grain fructans was not examined (Kooiker et al. 2013). In transgenic triticale lines, kernel fructan concentrations were increased up to 20 times by overexpressing a fructan synthesizing enzyme from wheat or rye behind an aleurone-layer specific promoter (Diedhiou et al. 2012). In addition, it is possible to enhance fructan concentrations by producing mutant lines lacking GBSSI, granule-bound starch synthase I and/or SSIIa, starch synthase IIa (Shimbata et al. 2011; Yasui and Ashida 2011). Shimbata et al. (2011) showed that in sweet wheat cultivars, where functional GBSSI and SSIIa are lacking, kernel fructan concentration are up to 6.5 times higher than in corresponding wild-type wheat lines. Due to a mutation in the SSIIa gene, Clarke et al. (2008) generated a 42-fold growth in grain fructan concentration of barley. Downregulation of FEH activity in cereal grains by RNA interference is the final strategy for increasing cereal grain fructan content. Definitely, FEH activity is high during the second phase of grain maturation (Verspreet et al. 2013) and it is essential for fructan degradation (Wardlaw and Willenbrink 2000; Yang et al. 2004). It is assumed that a reduction of FEH translation will cause higher cereal grain fructan levels.

Under stress conditions (drought, waterlogging and lack of nutrients), fructan and other water-soluble carbohydrates (WSC) accumulate temporarily—with a peak around late booting and emergence of the inflorescence—in vegetative plant tissues such as stems or roots, and can be remobilized during grain filling where fructan is again synthesized in the mature grain (Gebbing 2003). Genetic variation was reported not only for fructan contents in stems but also for its remobilization efficiency (Ehdaie et al. 2006). In the grain, net synthesis of fructan is most rapid, 5–9 days after anthesis, then reaches its maximum concentration (15–30%) and diminishes thereafter (Costantini et al. 2008).

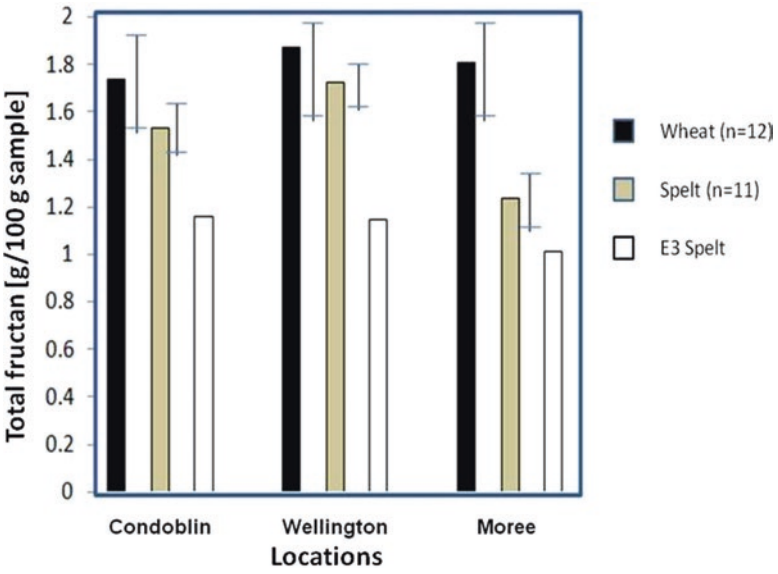

Fig. 14.2 Comparing the total fructan level found in the trade-marked cultivar, E3 spelt with those found in 12 wheat and 11 spelt samples grown at three locations in NSW, Australia. Error bars for wheat and spelt samples indicate the inter-variety variations. (Source: Békés 2015)

A significant environmental influence was observed in most fructan studies (Costantini et al. 2008); however, with no evidence of strong G x E interactions (Huynh et al. 2008a). No difference was observed between fertilization levels and management system (organic vs. conventional), only unfertilized wheat showed a slightly higher fructan concentration indicating stress due to a lack of nutrients (Langenkämper et al. 2006).

Spelt, compared to bread wheat, is comprised of considerably lower amounts of fructans and fructose. More importantly, the intervarietal differences among spelt genotypes is about five-fold larger than among bread wheat cultivars (Fig. 14.2), (Békés 2015). This provides the basis for screening spelt cultivars for low FODMAP content.

14.5 Spelt: A Nutritive Cereal

According to molecular evidence, spelt arose from hybridization between free-threshing hexaploid wheat and non-free threshing tetraploid wheat (2n = 4x = 28, genome = AABB). In the case of most hulled wild wheats and hexaploid varieties such as *Triticum aestivum* var. *vavilovii* (Tumanian) Sears, there is a recessive allele (q) at the Q locus that controls major domestication features in wheat (Faris 2014).

About 100 years ago, spelt was considered as the most important cereal in southern Germany, Austria, and Switzerland due to its hardiness and robustness concerning soil and climate, resistance to disease and nitrogen utilization efficiency. Bread wheat almost completely replaced spelt during the twentieth century because of higher yields, better baking performance and lower processing costs. However, spelt products have gained increasing popularity in the last 10 years. The major reasons are good digestibility, pleasant taste and aroma, and a better tolerance (in the case of wheat sensitivity) (Kling 1988). In addition, spelt is now being increasingly cultivated in water-protected areas and in organic farming as this cereal has fewer requirements with regard to use of herbicides or pesticides and fertilization compared to wheat (Kohajdova and Karovicova 2008).

Extensive systematic breeding and selection led to the creation of high-yielding and free-threshing cultivars of spelt, while retaining wide adaptability to different agro-climatic conditions. These new spelt cultivars have replaced most of the low-yielding traditional landraces and subspecies. However, in most parts of the world spelt has been superseded by bread wheat, leaving spelt to be grown as a marginal or niche crop with low inputs.

Recent interest in the use of spelt for ecologically-grown food has led to a revival in its cultivation after many years of marginal cultivation. As a matter of fact, in Europe spelt is used traditionally in the everyday diet, especially in Germany where numerous grain-based foods are produced using spelt (Cubadda and Marconi 2002). Several excellent works have been published in the last 10–15 years in connection with the evolution, physiology, genetics and the G x E effects on quality of spelt (Blatter et al. 2004; Förster et al. 2013, 2014).

The genetic diversity of major crops has suffered an overall reduction over time. Such genetic erosion can cause serious difficulties in the maintenance of the biodiversity of crop plants, as well as in crop improvement (Caballero et al. 2008). As a consequence of the reduced genetic variation, the stability of the crops in response to various stress factors has decreased in their local environments. In order to compensate for the loss of variation, breeders have turned to crops that were neglected in the recent past.

Spelt represents a useful gene reservoir for breeding programs, as it has the same ploidy level (2n = 6x = 42, AABBDD) as bread wheat. It has valuable nutritional quality due to its protein content and composition, which differ from those of wheat (Abdel-Aal and Huck 2002; Bonafaccia et al. 2002). The main difference is the variation in the amount and type of grain proteins, especially prolamins. Spelt dough is softer and stickier after kneading and is characterized by lower stability, less elasticity and higher extensibility than bread wheat dough (Schober et al. 2002).

In many modern breeding programs, spelt has intentionally been crossed with wheat cultivars for the improvement of yield, baking quality, and resistance to lodging and disease. It is essential to distinguish between spelt and bread wheat cultivars. The genetic diversity and chemical composition of spelt and modern wheat cultivars have been assessed and discussed from many perspectives. The gliadin protein patterns obtained with acidic polyacrylamide gel electrophoresis (A-PAGE)

were reported to be an excellent tool for qualitative cultivar identification (Ng et al. 1988). Schober et al. (2006) used size-exclusion high-performance liquid chromatography to classify spelt cultivars by their gluten proteins. Furthermore, a spelt-specific gamma-gliadin gene was discovered (Büren and Lüthy 2000) and then utilized in a simple polymerase chain reaction (PCR) method to detect the wheat adulteration of spelt flour and products (Büren et al. 2001). In addition, capillary zone electrophoresis can detect not only differences between the gliadin patterns of closely related spelt cultivars, but also the presence of wheat elements in the gliadin patterns of wheat/spelt crosses. In a study by Schober and Kuhn (2003), the presence of bread wheat was revealed in a number of spelt cultivars using capillary zone electrophoresis (CZE). The genetic diversity of spelt has been extensively examined on the basis of prolamin composition using reversed-phase HPLC (Wieser 2000), acid polyacrylamide gel electrophoresis (A-PAGE) (Caballero et al. 2004), one- and two-dimensional polyacrylamide gel electrophoresis (An et al. 2005) and capillary zone electrophoresis (Schober and Kuhn 2003). Other methods were also used to characterize genetic variation in spelt, by analyzing the rheological and viscoelastic properties of spelt gluten (Pruska-Kedzior et al. 2008; Schober et al. 2002) or its fiber content (Escarnot et al. 2010).

The molecular characterization of cultivars is also a useful way of evaluating genetic diversity during the breeding process. The genetic diversity of the Triticeae has been explored using a range of molecular markers (Manifesto et al. 2001) and with diversity arrays technology (DArT) markers (White et al. 2008). A high level of genetic diversity was revealed in spelt germplasm by microsatellite markers (Bertin et al. 2004). Gulyás et al. (2012) reviewed the milling, technological, compositional and bread making characteristics of European spelt cultivars and breeding lines, and estimated the phylogenetic relationships among spelt accessions using AFLP markers and tried to establish relationships between the presence of wheat genes and the technological and nutritional quality characteristics. The extent of genetic diversity in spelt germplasm, characterized by polymorphism information content (PIC) (Anderson et al. 1993), was far greater than found by Martos et al. (2005), for durum, or by Lelley (2000), for bread wheat. It was concluded that the diversity could be due in part to the extent of crossing between pure spelt cultivars and bread wheat. This suggests that spelt cultivars cannot be characterized without considering their origin. In view of the fact that it is mostly genotypes with bread wheat in their pedigree which achieve the quality required for bread making, spelt could be used as a source of lodging and disease resistance (Campbell 1997) and a source of genetic diversity for grain protein and mineral nutrients (Gomez-Becerra et al. 2010). Both the quality assessment and molecular analysis revealed a great diversity in spelt accessions that can be further utilized by breeders to incorporate them into specific breeding programs aiming to improve not only resistance but quality traits.

Spelt can be cultivated in harsh ecological conditions, without the use of pesticides because it shows a higher tolerance of environmental factors than bread wheat (Raman et al. 2008). Furthermore, spelt is a proper crop for organic farming as it can grow on low-fertility and poorly drained soils (Bonafacci et al. 2002). Spelt was not

among the targeted cereals in systematic breeding in the past; the objectives for improvement have only been identified recently (Neeson et al. 2008).

Spelt has been emerging as a worthy cereal for the past decade because of its repute as a healthy food not just for celiacs, but also for people suffering from wheat allergy (Elia et al. 2004; Galova and Knoblochova 2001). Spelt flour is also regarded to be more nutritious than bread wheat flour and to possess a unique flavor (Campbell 1997). Generally, spelt products, especially organic products, such as bread command a higher price in the marketplace.

In the last decade, the chemical, functional and nutritive properties of spelt compared to bread wheats have been described in numerous publications, recently reviewed by Escarnot et al. (2012). According to their results, on average, the spelt milling fractions and whole meal flour were higher in unsaturated fatty acids and lipids, but contained lower tocopherol content than in wheat samples. This suggests that there is no close relationship between high lipid content and high germ proportion in spelt. Although the proportion of bran and flour in milling fractionation is quite similar in wheat and spelt, there can be significant differences in mineral content. It was found that copper, zinc, phosphorus iron, magnesium and ash contents were higher in spelt samples, particularly in coarse bran and in aleurone-rich fine bran. Even spelt bran has the higher phosphorus content than wheat; the opposite trend was shown by phytic acid content, as it was 40% lower in spelt versus wheat fine bran. This suggest that spelt has either a lower phytic acid content or a higher endogenous phytase activity than wheat. Ruibal-Menieta et al. (2005) provide important suggestions about the true nutritional value of spelt compared to wheat. Furthermore, Gomez-Becerra et al. (2010) showed that the oleate/palmitate rate together with the Ca/Fe proportion provide a highly discriminating tool to face the growing issue of spelt-flour adulteration and to authenticate spelt from wheat flours. The results showed that spelt is a highly promising source of genetic diversity for mineral nutrients, particularly Zn and Fe, and for grain protein. Similar results have been obtained comparing wheat and spelt samples grown in Australia (Muir et al. 2014), (Table 14.2).

Spelt-based products have a slightly higher protein content and are more easily digestible than wheat. The variation in the amount and type of grain proteins, especially prolamins, is the main difference in the nutritive value of spelt flours (Galli et al. 2000; Shewry 2002).

Systematic crossbreeding of wheat and spelt was started at the beginning of the twentieth century to compensate, not for the detrimental properties of spelt in agriculture and processing, but to preserve its desirable properties. Not only pure spelt but also wheat/spelt crossbreeds have been cultivated and used in food processing since that time. The assignment of crossbreeds to sp. *spelt* is exclusively done based on morphological properties, for instance, shape of the ears and the fact that after threshing the husks are still attached to the seeds. Typical components of the seeds that assure the characteristics and quality of spelt are not accounted for at all and information on the rate of wheat crossing has not been obtainable to both consumers and producers of spelt products. To classify spelt cultivars, according to crossbreeding with bread wheat, is of special interest for persons tolerant towards spelt and sensitive to wheat (Catassi et al. 2013; Ludvigsson et al. 2013).

Table 14.2 Comparison of the levels of some important nutritive components in bread wheat and spelt samples, grown at the same location in NSW, Australia

		Nutritive components								
		Protein	Total fat	Mono unsaturated fatty acids	Poly unsaturated fatty acids	Total dietary fiber	Ca	Mg	Zn	Fe
Species	Parameter	%	%	g/100 g	g/100 g	g/100 g	mg/kg	mg/kg	mg/kg	mg/kg
Wheat (n = 12)	Mean	11.8	1.325	0.200	0.850	11.1	264.3	940.3	22.7	30.7
	Std. Dev.	1.1	0.096	0.082	0.058	5.0	287.9	338.2	7.9	9.4
	cv%	9.4	7.226	40.825	6.792	45.4	124.6	36.0	34.9	30.8
Spelt (n = 12)	Mean	13.8	1.843	0.457	1.086	10.6	287.9	1071.3	28.1	62.4
	Std. Dev.	1.9	0.310	0.053	0.318	4.1	124.6	364.8	8.5	28.9
	cv%	13.9	16.830	11.693	29.334	38.3	43.3	34.1	30.1	46.3

Source: Muir et al. (2014)

Several studies showed that reliable distinction between wheat and spelt can be provided by the differences in the protein patterns (Schober and Kuhn 2003; Wieser 2006); however, wheat and spelt seeds, due to their close botanical relationship, contain similar endosperm proteins.

Wheat proteins can be classified into the Osborne fractions of glutenins, GLUT; gliadins, GLIA and albumins/globulins ALGL. These can further be subgrouped into different types of gluten protein (α/β-,γ-, ω1,2-, ω5-, gliadins; low- and high-molecular-weight glutenin subunits, LMW-GS and HMW-GS (Wieser 2000). The main protein fractions of both cereals are the gliadins. Their patterns are determined by several components. For decades, the differentiation and identification of genotypes was based effectively on the variations of these patterns. SDS gel and acid gel electrophoresis, reversed-phase high-performance liquid chromatography (RP-HPLC) and gel isoelectric focusing have been applied, in particular, to the differentiation of wheat cultivars worldwide (Cornell and Hoveling 1998; Radic et al. 1997; Radic-Miehle et al. 1998; Wrigley and Bietz 1988).

Capillary zone electrophoresis (CE) of GLIA from 27 European spelt samples showed that differences were detected in the patterns of some wheat/spelt crossbreeds, even of closely-related cultivars and wheat elements (Schober and Kuhn 2003). Similarly, RPHPLC of GLIA allowed determination of the degree of wheat crossing in spelt and the differentiation of spelt and wheat cultivars (Wieser 2006). A total of 23 spelt cultivars were categorized into 5 groups ranging from pure spelts to spelts similar to wheat. Different degrees of wheat crossing and different GLIA patterns were detected in crossbreeds with identical parents (wheat and spelt). Therefore, no conclusion can be drawn from the pedigree on the real content of wheat elements. Until recently in spelt products the wheat contaminations could not be determined for authenticity and quality control of cereal grains and pure genotypes. It was demonstrated by Mayer et al. (2012) that a fast and simple observation of wheat in spelt flours can be accomplished by the combination of lab-on-a-chip

capillary gel electrophoresis and the polymerase chain reaction-restriction fragment length (PCR-RFLP) analysis.

The quantitative protein compositions of the Osborne fractions of whole meal flours from 62 spelt and 13 wheat cultivars were determined by Koenig et al. (2015) using reversed-phase high-performance liquid chromatography. The result shows that the chromatograms of the reduced gliadin fractions could be applied best for spelt classification and to distinguish spelt from wheat. Composition of the reduced spelt gliadins revealed one to three markers that cannot be found in wheat. Spelt cultivars were categorized into three groups based on these markers ranging from *typical spelt* to *similar to bread wheat*. Marker 1 was referred as ω1,2-gliadin and markers 2, 3a and 3b were identified as γ-gliadins by the determination of the relative molecular mass by mass spectrometry and the means of N-terminal sequence analysis. Glutenin-bound ω-gliadins may be used to quantitate and detect even small amounts of wheat in spelt products because this type of protein is absent in spelt but present in wheat.

There have been anecdotal clinical observations for a long time that a high percentage of non-celiac disease patients who suffer from wheat-related health problems can consume goods made from some spelt cultivars. The first research paper with robust statistical and experimental results also proved this important discovery (Armentia et al. 2012).

Since the endosperm of the kernels contain the major amount of FODMAP components, producing less bran in the flour or reducing the milling yield do not increase the FODMAP level. Baking and milling technology has an important effect on FODMAP levels in the products because during fermentation a certain amount of soluble carbohydrates are metabolized by yeast. Thus, FODMAP content in the end-products can be altered both by the length of fermentation and/or by the amount of yeast applied in the bread formulation. Sourdough technology has an even more substantial effect compared to yeasted dough products in reducing the FODMAP level (Fig. 14.3).

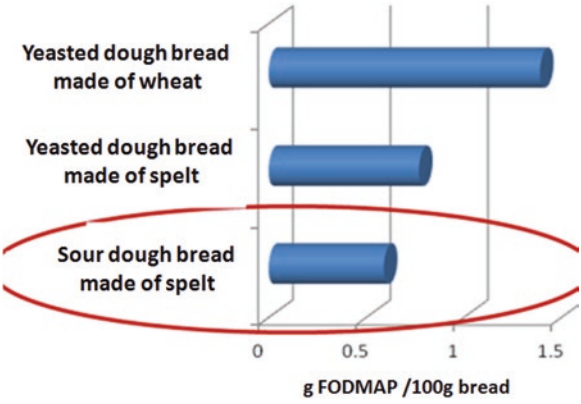

Fig. 14.3 FODMAP content of yeasted and sour dough breads made from wheat and spelt. (Source: based on the data of Muir et al. 2014)

14.6 Breeding less Allergenic Spelt with Low FODMAP Content

Australia grown spelt cultivar GWF was successfully selected with considerably lower FODMAP content. According to Muir et al. (2014), in the baked goods produced from this line, the FODMAP content—using an optimized technology and formulation—was significantly lower than the threshold defined for low FODMAP products.

Intriguingly, this same line was observed to have considerably less immune reactivity than any other spelt and wheat cultivars to wheat sensitive individuals (Vu 2014, Vu et al. 2014). It was also observed that the albumin-globulin protein composition of GWF spelt is different from other wheat-related and wheat species (Fig. 14.4). One of these discrepancies has been identified as a mutation in a CACTA retrotransposon region tightly connected to a gene coding a beta-expansin protein in many spelt and wheat germplasm (Fig. 14.5) (Breen et al. 2010).

A protein family closely connected to nonenzymatic proteins, the beta-expansins, located in plant cell walls, are known as pollen allergens. This alteration in the soluble protein composition was stated to be one of the reasons for the considerably lower immune reactivity of GWF spelt for wheat sensitive people (Vu 2014, Vu et al. 2014). The mutation in the expansin gene alone cannot account for the lower immune reactivity; however, it could be applied as an indicator for the uncommon

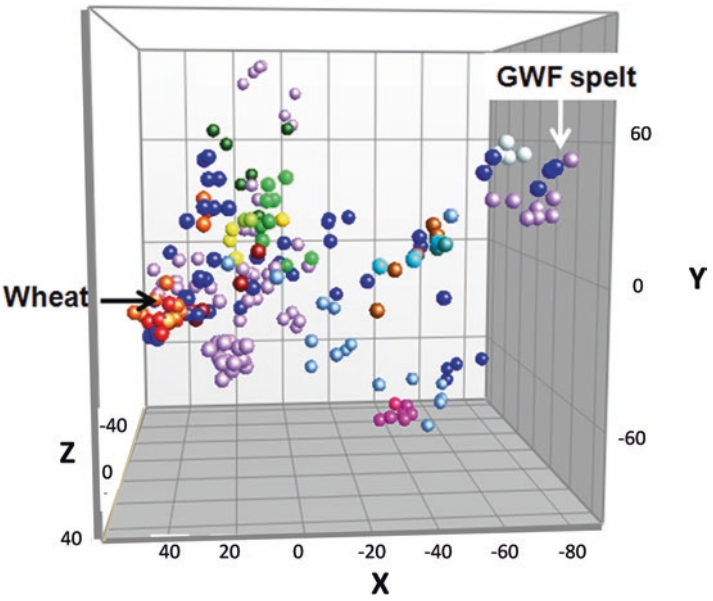

Fig. 14.4 Principle component analysis based comparison of soluble protein composition (albumins and globulins) of 127 wheats and wheat relatives. (Source: Vu 2014)

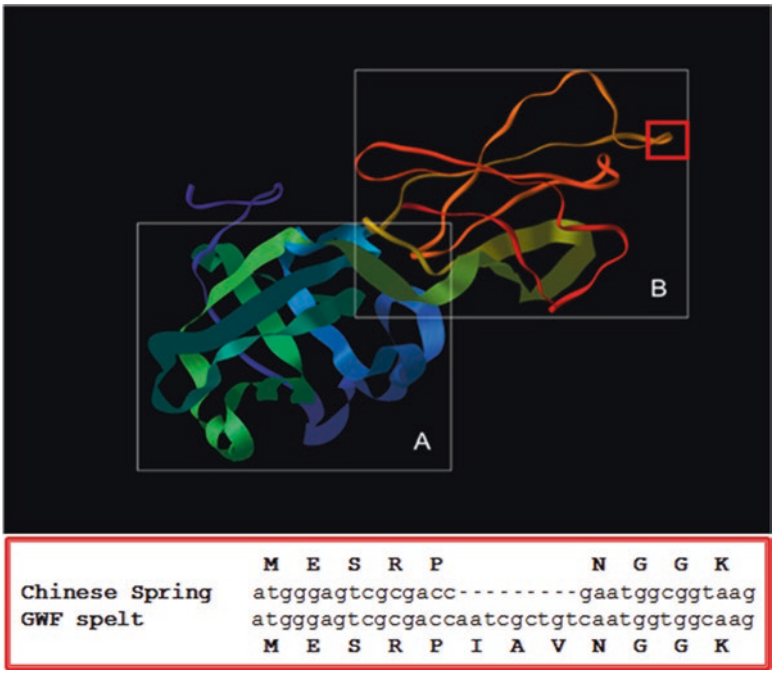

Fig. 14.5 Mutation in the expansin coding gene based on Breen et al. (2010)

soluble protein composition observed in GWF spelt. This modified protein configuration could be linked to the lower immune reactivity detected (Fig. 14.6).

Several bakeries in Australia have recognized the potential candidate source for producing cereal products with dual health benefits. A closed production line has been created, incorporating contract growing of GWF spelt with strict rules to avoid any contamination from other grains. A small milling capacity has been developed and special care has been devoted to clean the all milling equipment before GWF spelt processing. A PCR-based procedure has been established to reveal the above-mentioned mutation in the expansin gene (Suter and Békés 2012) to screen for any contamination in the flour or grain samples induced by any other cereals with the wild type expansin protein (Fig. 14.7). This procedure is used for quality guarantee purposes. A dedicated laboratory monitors the FODMAP content of flour and their goods (sourdough bread with unique formulation). Flour from this unique spelt source, characterized by lower immune reactivity and low FODMAP, is currently being supplied to Australian artisan bakeries. The name of this special flour is Hildegard spelt flour, after St Hildegard von Bingen, an eleventh century nun who first observed the health advantages of spelt.

In connection with this spelt line, there are prior experiences which provide some evidence to support the dual health benefits. The question is whether this observation is connected to the apparent lower allergenicity of spelt for those with wheat allergy. The answer needs a double-blind, placebo-controlled randomized clinical

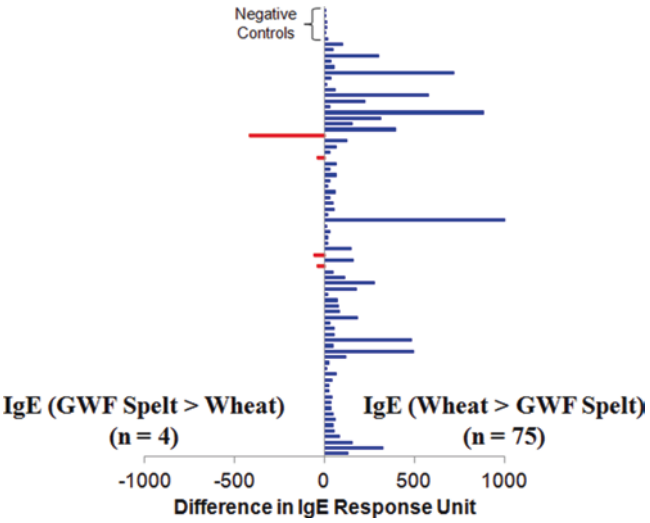

Fig. 14.6 The comparison of immuno-reactivity of wheat and GWF spelt, determined by RAST measurements. (Source: Based on the data from Vu 2014, Vu et al. 2014)

trial, compared to the allergic signs and IgE levels generated by consuming GWF spelt and wheat products, involving those who eat chiefly wheat and those who have replaced spelt for wheat in their diet.

A pre-breeding activity has been initiated in Hungary based on the positive experiences on evaluating GWF spelt to screen large spelt populations, selecting suitable processing quality and lines with low FODMAP content. The chosen lines are further examined by searching for genotypes containing the expansin-mutation, applying the ELISA based techniques (Bodinier et al. 2008) and PCR-based test of Suter and Békés (2012), respectively. As described in an initial survey carried out on 105 spelt lines (Pauk et al. unpublished results), the FODMAP content of spelt lines grown in the Carpathian Basin show large variation (0.7–1.8 g fructan in 100 g grain). More than 10% of the lines comprise lower or equal level of fructan than the Australian control, found to be helpful in low FODMAP diet (Fig. 14.8).

Selection for quality in this program is based on three independent attributes: low FODMAP content, satisfactory bread making properties and the presence of the expansin mutation to be able to monitor the purity of the samples on grain, flour, and even the end-product stage.

In the early stages of a breeding program, when the sample size is extremely limited for traditional dough testing methodologies, a set of small-scale testing methods is applied. The FQC2000 micro mill (MeteFEM Budapest, Hungary) is used to mill flour for the functional studies (Békés et al. 2000; Tömösközi et al. 2001). This equipment is capable of making good quality flour from only 2–5 g of grain. Mixing properties of the breeders' lines are monitored on a micro-doughLAB (Perten Instruments) mixer (Dang and Bason 2013; Haraszi et al. 2004). The Kieffer rig methodology and equipment (Stable MicroSystems) is applied for the determi-

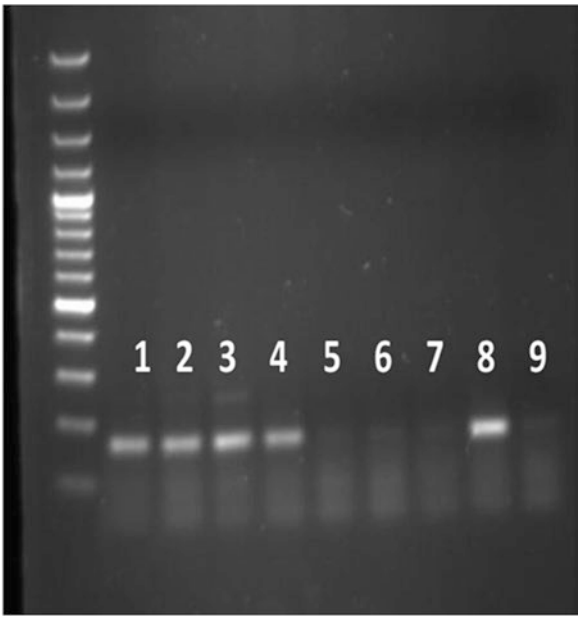

Fig. 14.7 Monitoring the presence of mutation in the expansin gene, using a molecular marker, developed by Suter and Békés (2012). 1 - wheat; 2 - kamut; 3 - emmer; 4 - normal spelt; 5,6,7 - GWF spelt - 9 negative control. (Source: Békés et al. unpublished results)

nation of extensional parameters (Rmax and extensibility) (Kieffer et al. 1998), while a prototype SediCom System micro Zeleny equipment (Lab-Intern Ltd., Budapest, Hungary) was used for determination of Zeleny values. The measurements are carried out according to the modified ICC Standard No. 116/1. (Tömösközi et al. 2010).

14.7 Spelt Breeding Via in Vitro Androgenesis Using Anther and Isolated Microspore Culture Methods

Classical breeding is a time-consuming process. It takes about 10–12 years to release a new cultivar, because the selection of segregated population and growing and testing of different generations requires much time. To accelerate the generation changing in plant breeding and to achieve the homogeneity of selected lines within one generation, an efficient plant biotechnology method—based on in vitro androgenesis—was developed in spelt (Lantos et al. 2016, 2018).

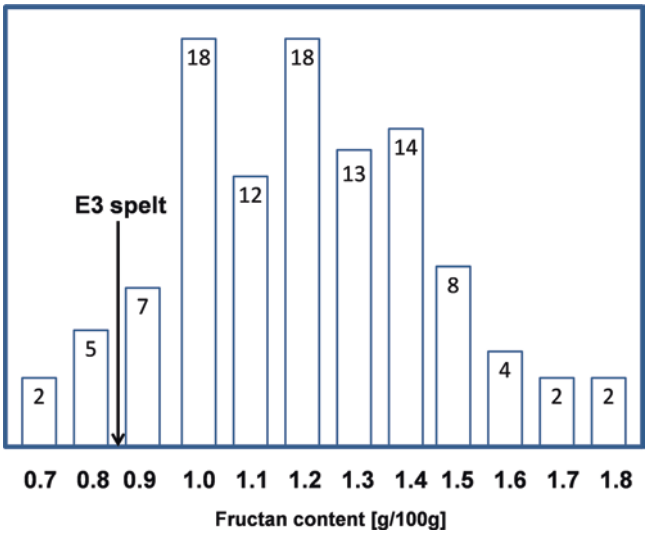

Fig. 14.8 Variation of fructan levels in 105 spelt cultivars grown in Hungary at the same location. (Source: Ács et al. 2017)

14.7.1 Induction of in Vitro Androgenesis in Anther Culture

In spelt, in vitro anther culture was first tested with Hungarian spelt cv. GK Fehér. Later the protocol was also implemented with three other spelt cultivars. The number of produced embrioids and embryo-like structures (ELS) was 62/100 anthers. These structures regenerated dominantly green plantlets in vitro. The green plantlets production was 89.0% among the regenerated plantlets while the number of albino plantlets was low, 3.8/100 anthers. More than 1000 in vitro green plantlets were produced from anther culture of different spelt cultivars. After ploidy-level analyses (measurement of stomata length and flow cytometric analyses), using the haploid plants, doubled haploid plants were produced via colchicine treatment (Pauk et al. 2003) and produced seeds after rediploidization. The colchicine induced and spontaneous DH spelt plants were propagated in the greenhouse. The in vitro doubled haploid plant production method was integrated into our spelt breeding program in the similar way as was done with bread wheat (Pauk et al. 2003).

In our study, the first anther culture-derived ELS were observed after 3 weeks of androgenesis induction. The microspore-derived ELS with 1–2 mm size (Fig. 14.9a) were transferred to a Petri dish containing regeneration medium. The ELS produced significantly more green plantlets than albinos (Fig. 14.9b). Green plantlets were rooted in individual culture tubes (Fig. 14.9c) and regenerated plantlets acclimatized and grown in the greenhouse (Fig. 14.9d).

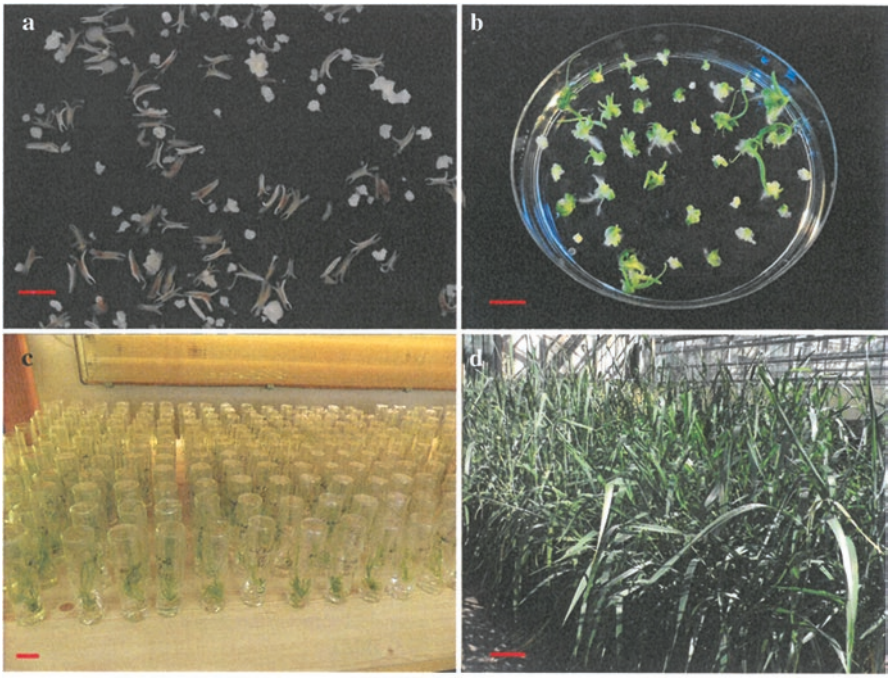

Fig. 14.9 Important steps of anther culture in spelt: (**a**) Microspore-derived ELS, (**b**) Green plantlets in regeneration, (**c**) Rooting of individual plantlets, (**d**) Acclimatized plantlets in greenhouse. Red bars 5 mm for **a**; 10 mm for **b** and **c**; 50 mm for **d**. (Source: Lantos et al. 2018)

14.7.2 Effect of Genotype and Cold Pre-Treatment in Anther Culture

ELS could be produced with or without cold pre-treatment of the donor tillers (0 and 12 day cold pre-treatment) in anther culture. However, cold pre-treatment significantly improved the production of ELS and the number of regenerated green and albino plantlets in anther culture of each tested genotype (Table 14.3). The number of albinos was limited in anther culture (3.48 albinos/100 anthers), these values had a range of 0.5–7.47 albino plantlets/100 anthers depending on the cultivar. However, the production of green plantlets achieved high values, on average 41.45 in vitro green plantlets were regenerated from 100 anthers. The number of produced in vitro green plantlets had a range of 20.93–83.07 green plantlets/100 anthers. The most green plantlets (83.07/100 anthers) was regenerated from anther culture-derived ELS of cv. Franckenkorn. Altogether, more than 1000 green plantlets were produced from the anther culture of the 4 cultivars.

In a two-way analysis of variance (Lantos et al. 2018), the effect of pre-treatment and genotype was tested in anther culture of four spelt cultivars (data are not shown here). Based on the ANOVA test, genotype and cold pre-treatment of donor tillers

Table 14.3 The effect of genotype and cold pre-treatment on the parameters of anther culture in spelt

	Embryo-like structures /100 anthers		Regenerated plantlets/100 anthers		Green plantlets/100 anthers		Albinos/100 anthers	
Genotype	0 day	12 days	0 day	12 days	0 day	12 days	0 day	12 days
Franckenkorn	9.17 b	134.80 a A	8.67 b	90.53 a A	5.50 b	83.07 a A	3.17 b	7.47 a A
GK Fehér	2.33 a	46.33 a B	1.33 b	43.33 a AB	1.00 b	38.33 a AB	0.33 b	5.00 a B
Mv Martongold	4.33 a	34.80 a B	3.00 a	21.87 a B	2.67 a	20.93 a B	0.33 a	0.93 a C
Oberkulmer Rotkern	3.60 a	27.07 a B	1.07 a	25.47 a B	0.67 a	23.47 a B	0.40 a	0.50 a C
Mean	4.86	60.75	3.52	45.3	2.46	41.45	1.06	3.48

Values followed by the same capital letters (A, B, C) are not significantly (P = 0.05) different for the genotypes. Values followed by the same letters (a, b) are not significantly (P = 0.05) different for different treatment within genotype
Source: Lantos et al. (2018)

significantly influenced the parameters of androgenesis (number of ELS, regenerated, green and albino plantlets). The effect of genotype × pre-treatment interaction was significant in the number of ELS, regenerated and green plantlets, too.

On the greenhouse grown plants, the fertile and partial fertile spikes were harvested. In the case of four tested spelt cvs. (Frankenkorn, Oberkulmer Rotkorn, GK Fehér, Mv Martongold) the values of seed setting were 11.8, 19.35, 44.44 and 21.47%, respectively. These data show that the anther culture method of spelt is ready for integration into the breeding process.

14.7.3 Induction of Androgenesis in Isolated Microspore Culture of Spelt

Because the anther culture technique needs significant manual work in isolation, to reduce this manual effort, we began to study the isolation microspore culture in spelt wheat as well. The microspore isolation was started, when in donor spikes, the stage of microspores was in uni- and binucleate stages (Fig. 14.10a, b). The ovary coculture was crucial in isolated microspore culture. Multicellular structures and ELS were not observed in isolated microspore culture without ovaries. The ovaries supported the development of multicellular structures and ELS (Fig. 14.10c, d). The transferred ELS produced both green and albino plantlets (Fig 14.10e) on the regeneration medium (Lantos et al. 2018).

In isolated microspore culture, cv. Franckenkorn produced the most ELS and regenerated plantlets, while the production of ELS was the lowest in case of cv. Oberkulmer Rotkorn. In isolated microspore culture, the growth regulators were not

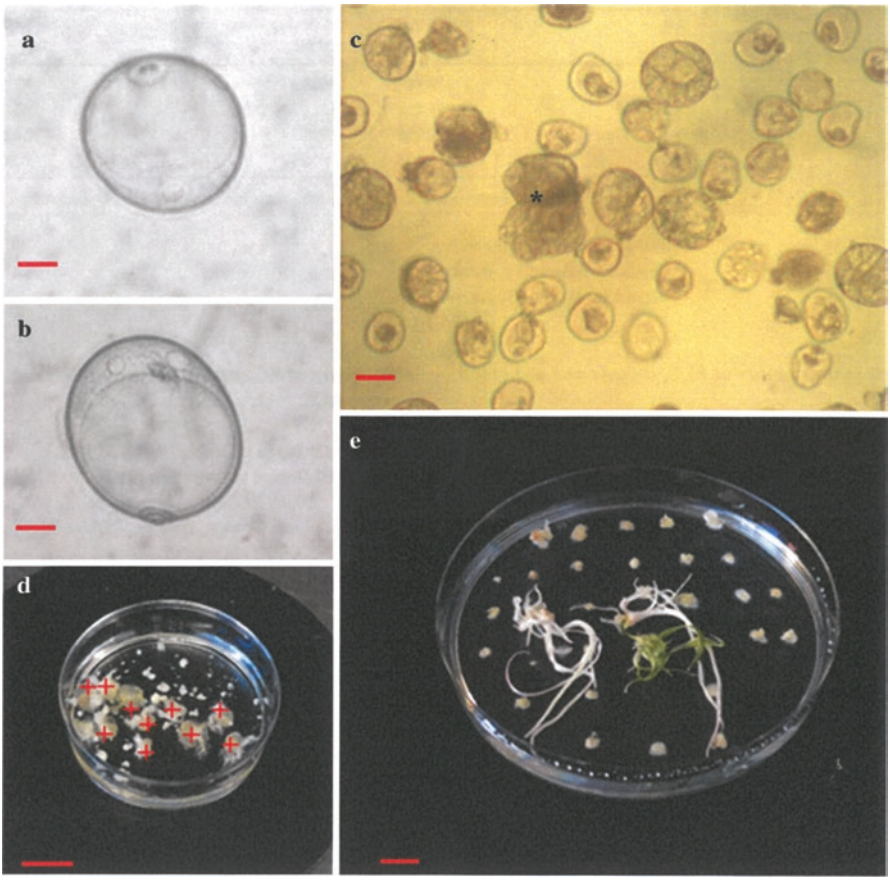

Fig. 14.10 Critical steps of isolated microspore culture: (**a**) Late uninucleate microspore, (**b**) Early binucleate microspore, (**c**) Multicellular structures (∗) and divided microspores, (**d**) Obtained ELS in ovary-supported (+) microspore culture, **e** Regenerated green and albino plantlets. Red bars 10 μm for (**a**) and (**b**); 50 μm for (**c**); 10 mm for (**d**) and (**e**). (Source: Lantos et al. 2018)

essential in induction of androgenesis, but growth regulators increased the efficiency of the method (ELS, green and albino plantlets). The ELS production was high in isolated microspore culture. In practical terms, it was a big problem that most of the regenerants were albino plantlets. Altogether, three isolated microspore culture-derived in vitro green plantlets were regenerated. Two from cv. Franckenkorn and one from GK Fehér, respectively.

Androgenesis was induced in isolated microspore culture of each cultivar. The cultivars influenced the number of produced ELS, in vitro regenerated plantlets and albinos, while the exogenous growth regulators had significant effect on the tested parameter.

14.8 Conclusions and Prospects

The abovementioned method of producing a cereal line which is beneficial to human health can be used as a model, demonstrating how to protect baked goods from contamination by any other cereal material from the field to the bakery and how to accomplish (protect, monitor, check) the quality of the final product. These kinds of issues will hopefully emerge soon because of selecting and producing germplasm with proven reduced allergenicity and altered gluten and/or non-gluten protein composition.

Developing cereals with largely reduced allergenicity and with low FODMAP content is a much more realistic approach, compared to the ultimate task, developing celiac-safe cereals. Most wheat sensitive individuals—except for celiac patients—can consume these kinds of products.

Further detailed basic and applied research is required for the development, manufacturing and commercial availability of such products. This must include developing cost-effective, reliable and high-throughput analytical tools to monitor the quantity and the presence/absence of certain key components in the source material, the intermediates and the final product through the whole production chain, from the farm to the supermarket. Nevertheless, developing the necessary infrastructure for the production and commercialization of a multipurpose, healthier cereal product basis is only one of numerous requirements needing attention in the complex matter of providing appropriate food for people suffering from cereal-based health problems. It is essential to alter the legislative environment in order to permit the production/commercialization of products with altered/low gluten content, giving instructions on analytical procedures, defining thresholds of certain key components, determining food safety control systems, labelling, policing and regulating the very often misleading media-based marketing. However, the ultimate purpose is a more effective collaboration and communication among plant science, the grain industry as well as medical researchers and practitioners, together with up-to-date, accurate and permanent information of customers.

An effective in vitro androgenesis method was developed in spelt to develop the homogeneity of newly-selected spelt lines and to accelerate the pace of generation changes in breeding (Lantos et al. 2016). In this activity we were able to apply successfully those small and micro scale dough testing methods which have been developed earlier for characterizing bread wheat samples. With Hungarian cv. GK Fehér the anther culture method was carried out; however, in three other registered spelt cultivars tested using the protocol, 62 ELS/100 anthers produced embryo-like structures (ELS), from which we were able to regenerate green plantlets. A total of 89% of green plantlets production was achieved among the regenerated plantlets whereas the number of albinos was limited (3.8/100 anthers). All in all, from an anther culture of different spelt genotypes over 1000 in vitro green plantlets were created. The doubled haploid plantlets were produced via colchicine treatment and obtaining seed after spontaneous diploidization, based on ploidy-level analyses

from the haploid plantlets (Pauk et al. 2003). In the greenhouse the colchicine treated and spontaneous DH plants were grown out. The in vitro haploid induction system was incorporated into the breeding program of spelt in the same way as was done with bread wheat (Pauk et al. 2003, 2004).

Acknowledgement This study was supported within project OTKA-K_16-119835, funded by the National Research, Development and Innovation Office. The experiments were interlocked with GINOP project (GINOP-2.2.1.-15-2016-00026). This project was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences and by the ÚNKP-18-4-BME-393 National Excellence Program of the Ministry of Human Capacities. The work is connected to fulfil of the scientific goals of BME-Biotechnology FIKP grant of EMMI (BME FIKP-BIO).

Appendices

Appendix I: Research Institutes Relevant to Spelt

Institution	Specialization and research activities	Contact information and website
Cereal research non-profit ltd., Szeged, Hungary	Research and breeding	https://www.gabonakutato.hu/en/contact
Centre for Agricultural Research, agricultural institute, Martonvásár, Hungary	Breeding and research (FODMAP)	http://www.agrar.mta.hu/en

Appendix II: Spelt Genetic Resources

Cultivar	Important traits	Cultivation location
Center for Plant Diversity, Tápiószele, Hungary	Gene bank, genetic resources, germplasm collection	http://www.nodik.hu/english/?page_id=2348

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Chapter 15
Recent Advances in Wheat (*Triticum* spp.) Breeding

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Abstract Wheat (*Triticum* spp. L; Gramineae), a self-pollinating crop, is one of the most important cereal crops. Globally, wheat is an economic crop, utilized as food, feed, seed and industrial uses. Gene banks have conserved a large genetic resource collection of wheat germplasm including wild *Triticum* species. There are numerous species of *Triticum* with different genomes and chromosome numbers. *Triticum* harbors significant diversity based on ploidy level, biological status, geographical regions and morpho-agronomic traits. Introgression of novel alleles through crossing between various wheat genetic resources, e.g. modern varieties with locally-adapted varieties, enhances genetic diversity and preselection for traits of interest, which is required to ensure meaningful natural variation at the phenotype level. Improving wheat for biotic and abiotic stress tolerance traits, quality traits and yield attributes are the main objectives of wheat breeders and geneticists. Achieving these objectives can be facilitated by the application the modern genomics tools to augment traditional breeding programs. This chapter presents an overview of wheat germplasm biodiversity and conservation, objectives and stages of wheat breeding programs, cultivation and traditional breeding methods, in addition to modern plant breeding tools including marker-assisted breeding, genetic engineering and genome editing.

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J. M. Al-Khayri et al. (eds.), *Advances in Plant Breeding Strategies: Cereals*,
https://doi.org/10.1007/978-3-030-23108-8_15

Keywords Biodiversity · Genetic improvement · Genetic map · Modern wheat breeding · Traditional breeding · *Triticum* spp. · Wheat

15.1 Introduction

Wheat (*Triticum* spp. L.), is the oldest cereal crop which is grown under a wide range of climate and soil conditions. It is adapted to the temperate regions with 30–90 cm annual rainfall. South Asia is the center of origin of wheat. There are many species of *Triticum* with different genome and chromosome numbers. Wheat is a self-pollinated crop, taxonomically, belonging to the genus *Triticum* (Linnaeus 1753), tribe Triticeae, family Poaceae (Gramineae) and order Cyperales (Briggle and Reitz 1963). Inflorescences consisting of one to several flowered spikelets which are sessile and alternate on both sides of the rachis and form a spike. Wheat has economic value as food, feed, seed and industrial uses (Nachit 1992). It is cultivated by seed under rainfed and irrigated conditions. Traditional plant breeding plays an important role in introgression of novel alleles through crossing genotypes from various plant genetic resources e.g. modern varieties with locally-adapted varieties, to enhance the genetic diversity and selection for the traits of interest such as high grain yield, early maturing, improved grain quality as well as resistance to lodging, biotic and abiotic stresses. Although new wheat biotechnology approaches using advanced DNA sequences and molecular methods have attracted plant breeders and geneticist, traditional plant breeding methods are still the key and first points to develop new wheat cultivars with desirable traits. In the present chapter, we present an overview of the center of origin, objectives and stages of a breeding program, traditional plant breeding methods, germplasm diversity and conservation, modern plant breeding tools for developing new wheat cultivars with desirable traits.

15.1.1 Origin and Distribution

Understanding the origin of wheat is one of the most important steps to improve it through breeding programs. South Asia is the center of origin of wheat. High genetic variability is found in the Fertile Crescent and bordering countries. There are many species of *Triticum* with different genomes and chromosome numbers (Table 15.1).

Triticum aestivum is the most common wheat species and represents the most widely grown of all crops including other cereals. It is allohexaploid wheat including three different genomes (A, B, D) with 42 chromosomes. It was developed by crossing *T. monococum* (2n = 14, AA) with an unknown wheat (2n = 14, BB). The F_1 (AB, 2n = 14) was spontaneously doubled and became tetraploid wheat (2n = 28,

Table 15.1 Different wheat species and their genome types

Species name	Number of chromosomes	Ploidy level	Genome type
Triticum monococum	14	Diploid	AA
Aegilops speltoids	14	Diploid	BB
Aegilops caudate	14	Diploid	CC
Aegilops speltoids	14	Diploid	DD
Triticum squrrosa	14	Diploid	EE
Triticum durum	28	Tetraploid	AABB
Triticum aestivum	42	Hexaploid	AABBDD
Triticum compactum	42	Hexaploid	AABBDD

AABB). The later was crossed with *Aegilops squarrosa* or *T. tauschii* (Coss.) Schmalh. (2n = 14, DD) and the F_1 (2n = 21, ABD) was spontaneously doubled to produce the hexaploid wheat (2n = 42, AABBDD). This evolutionary process has great impact in wheat breeding; (i) it increased the genetic diversity within wheat and it's relatives and (ii) it increased the genetic redundancy (defined as possessing many genes that code for similar proteins) within wheat. The first impact was very important for wheat plant breeding to improve target traits and has made wheat the most important cereal crop in the world, while, the second impact had a negative effect on diploid species because it makes for difficult chromosomal manipulations and breeding strategies.

15.1.2 Economic Importance

Wheat (*Triticum aestivum*) is a strategic and important cereal crop for a significant proportion of the world's population. It is the main food source of carbohydrates for one-third of the world's population, or more than two billion people worldwide (36%). Wheat (*Triticum* spp.) provide about 55% of carbohydrates and 20% of the world's consumed food calories (Breiman and Graur 1995). Wheat is the third crop in terms of cultivated area and production following rice and maize, which are considered the most important grain crops in the world (FAO 2017). Wheat is grown under a wide range of climatic conditions. The Poaceae family also includes several other major crops, such as barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.), maize (*Zea mays* L.) and rice (*Oryza sativa* L.). Triticeae is one of the tribes that contains more than 15 genera and 300 species including wheat. Wheat (*Triticum*) and rye (*Secale*), *Aegilops*, *Agropyron*, *Eremopyron* and *Haynalidia* form the subtribe Triticineae (Simmonds 1976). Linnaeus (1753) first classified wheat; Sakamura (1918) reported the number of chromosomes of each species. The latter was a turning point in the *Triticum* classification. Wheat was separated into three groups. The binary diodes are 14 (n = 7), tetraploids 28 (n = 14) and hexaploids 42 (n = 21) chromosomes. Wheat bread is *T. aestivum*; *T. durum* and *T. compactum* are the other two main types. These three are natural hybridization products among ancestors no longer grown commercially (Briggle 1967).

15.2 Cultivation and Traditional Breeding

15.2.1 Cultivation and Use of the Wheat Crop

Wheat is one of the oldest cereal crops. It is grown under a wide range of climates and soils and adapted to temperate regions with annual rainfall of 30–90 cm. There are two major types of wheat: winter and spring wheat. Always, the winter wheat is sown in the fall; however, the spring wheat is sown in the spring. In 2017, the ten leading wheat producing countries were China, India, Russia, USA, France, Australia, Canada, Pakistan, Ukraine and Germany (FAO 2017). Bread wheat cultivars belong to hexaploid wheat (*Triticum aestivum*). Wheat genotypes which grown in dry zones are generally considered to be hardened, containing 11–15% protein and strong gluten. The strong gluten of bread wheat entraps carbon dioxide (CO_2) formed during the process of fermentation of the dough and the fermented dough can rise. Wheat cultivars grown in humid areas are soft, with 8–10% protein content and weak gluten. Soft wheat flour is used in making cakes, biscuits, pastries and flour. Durum wheat (*T. durum* Desf.) is considered to be one of the best sources of semolina production and is suitable for pasta and other products (Nachit 1992). On the other hand, diploid wheat is not cultivated because it has no economic importance as a crop anywhere in the world. Most wheat is grown for human nutrition and about 10% of the resulting grains are used industrially to produce starch, paste, dextrose and gluten). Chemical analysis of wheat grains shows they contain all the essential nutrients; 12% water, carbohydrates in the form of starch (60–80%), proteins (8–15%) contain sufficient amounts of all essential amino acids (excluding lysine, tryptophan. methionine), minerals (1.5–2%), vitamins (such as complex B, vitamin E) and crude fiber 2.2%.

15.2.2 Traditional Breeding Methodologies and Limitations

Although modern plant breeding utilizing the advances in DNA technology has attracted wheat breeders and geneticist, traditional plant breeding methods are still the key and first points to develop new wheat cultivars with desirable traits. The primary objective of conventional wheat breeding is to have a plant that can grow and thrive in a wide range of different environment easily. Changes in arable land, harsh cropping systems and food security should be highly considered along with emerging global issues (Davis et al. 2004). These global issues can be addressed in wheat by utilizing methods of plant breeding which provide the ability to select genotypes having desirable genes/QTLs (quantitative trait loci) controlling important traits.

15.2.3 Objectives and Stages of Wheat Breeding Programs

The main challenges for wheat breeders and geneticists are to genetically improve high grain yield, resistance to main diseases (rust, smut, bunt, *Fusarium*), tolerance to abiotic stresses (drought, salt, heat), early flowering and maturity, response to high doses of fertilizers, dwarf and lodging resistance, etc. (Mohammadi et al. 2012; Mwadzingeni et al. 2016; Salem 2015; Salem et al. 2007; Sallam et al. 2014, 2018b). Wheat can grow in many different environments ranging from temperate irrigated to dry and high rainfall areas and from warm humid to dry cold conditions (Bowne et al. 2012; Sallam et al. 2015). Hence, addressing the problem and setting the objectives are the key points for success in any wheat breeding program. Presence of genetic diversity plays a vital role in improving wheat crop for target traits in breeding programs. Baenziger (2016) determined five main stages for a successful breeding program (Fig. 15.1). Each stage is important and critical to a successful breeding program.

15.2.3.1 Addressing the Problem and Determining the Objectives

Although wheat can be grown in many different environments, wheat breeders work hard to genetically improve wheat crop to solve serious problems that limit wheat production and productivity. Each environment has a specific problem such as drought stress, heat stress, salt stress, diseases, low input environments, insects, etc. Therefore, identifying the problem is very important to determine the appropriate breeding program to improve the target traits. Moreover, the breeding program can also differ based on growth stage. For example, improving drought tolerance in wheat depends on the growth stage of wheat which is exposed to drought (Sallam et al. 2018b). Drought can occur during the seedling stage or grain filling stage (Salem et al. 2004, 2007). Some studies report that there was no correlation between

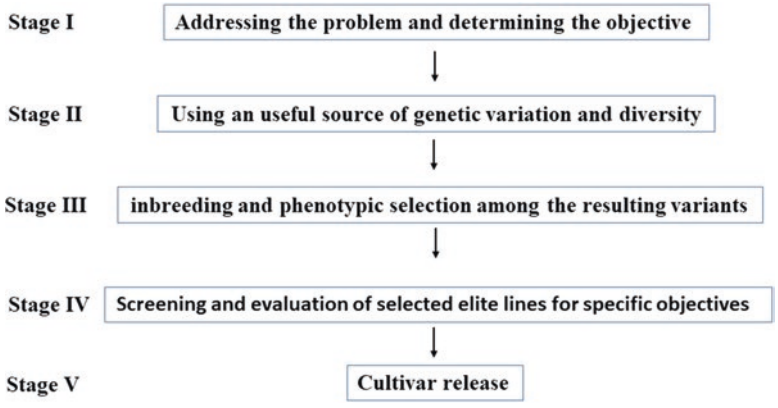

Fig. 15.1 Stages of a plant-breeding program

drought tolerance in grain filling stage and drought tolerance in the seedling stage because genotypes can respond differently for their drought tolerance according to the growth stage (Salem et al. 2007; Sallam et al. 2018a).

15.2.3.2 Using a Useful Source of Genetic Variation and Diversity

The next stage after defining the problem and determining the objective is to look for appropriate germplasm that has variation in the target traits to establish how they are inherited. Plant germplasm includes an agronomic description of the material for traits that are useful for breeders and research in crop improvement. There are different types of germplasm including the following:

(a) Landraces which are primitive cultivars selected and cultivated by the farmers for many generations. Landraces have a high level of genetic diversity which is an important source of resistance to various biotic factors and tolerance to abiotic stresses.
(b) Obsolete cultivars which can be defined as earlier-popular varieties that have been changed by new varieties.
(c) Modern cultivars are cultivated high-yielding varieties. They are normally used as parents in a breeding program to improve yield and its attributes.
(d) Advanced breeding lines are highly homozygous lines. They are developed by plant breeders for improving targets traits through plant breeding programs.
(e) Wild forms of cultivated species.
(f) Wild relatives are very important and useful for genetic diversity and they are considered an interesting source of resistance to biotic factors and tolerance to abiotic stresses. They are wild plant species that are genetically related to cultivated crops
(g) Mutants can be obtained from mutation breeding.

In wheat, crossing two or more different genes is the main method of introducing genetic variation. Most of the crosses are between highly homozygous lines (pure lines) to produce a F_1 generation. Many studies have focused on the F_1 generation to understand the inheritance of important traits using different types of diallele analysis. Others try to achieve offspring with a genetic identity for one or more traits (e.g. early flowering) which is closer to that of the parents by crossing the F_1 with the target parent. The F_2 population could also be a very interesting target to observe the segmentation of target traits. Recently, most studies have focused on the biparental populations in which highly homozygous lines derived from crossing between parents can be obtained by single seed descent (SSD). These biparental lines are normally used for identifying important QTLs controlling target traits. More interestingly, diverse populations are widely used these days for genome-wide association study (GWAS). This diverse population constitutes different genotypes from different parts on the world. To achieve the goals of any breeding program,

germplasm should have a high degree of genetic diversity among plant materials (Eltaher et al. 2018; Salem and Sallam 2016; Salem et al. 2015).

15.2.3.3 Inbreeding and Phenotypic Selection Among the Resulting Variants

The next stage for wheat breeders is to choose which selection and inbreeding methods they will use. Inbreeding transforms genotypes from heterozygosity to homozygous lines. Then, selection chooses a very few of the superior homozygous lines to be integrated into the next stages of the breeding program. Unselected lines are discarded from the breeding program.

Selection is one of the basic methods of traditional plant breeding. It can be artificial (made by human) or natural (by the power of nature). Selection in breeding programs differs by the environment in which the plant will be grown, hence the wheat breeder should be very careful with the plant material tested (Baenziger 2016). For example, if the objective is to select wheat genotypes for winter hardiness, selection should be in environments that allow breeders to have a variation in the traits of interest among the tested genotypes.

15.2.3.4 Screening and Evaluation of Selected Elite Lines for Specific Objectives

Although all the five stages are important for a successful breeding program, screening and evaluating germplasm for the specific objective is the most crucial step before releasing a cultivar. Collecting precise phenotypic data is critical to the selection process. Plant material should be evaluated and screened over years or/and replications or/and locations or/and environments. This step is entirely based on the objectives of the breeding program. To have a fruitful selection and genetic improvement of the wheat crop, a selected trait should have a high heritability probability. The evaluation could also be performed at any growth stage or at many growth stages (Sallam et al. 2016). For example, a biparental population was evaluated for seedling and grain filling stages under drought tolerance to select genotypes having high tolerance at both growth stages (Salem et al. 2004, 2007; Sallam et al. 2018b). The genotype × environment interaction should be highly considered in the selection.

15.2.3.5 Cultivar Release

The last stage of a breeding program is the decision to release a cultivar if it is superior for at least one trait important target trait. In most cases, wheat grain yield should be one of among target traits for each breeding program. The procedures of releasing cultivar differ by country.

15.3 Germplasm Diversity and Conservation

Natural variation including phenotypic and genotypic variation is the fundamental concept of plant breeding, which aims to select useful variation for future generations. The variation can be introduced by crossing, mutation and/or present in nature due to historical recombination of alleles. In wheat, wild relatives, landraces, modern cultivars, breeding materials in addition to gene bank accessions are sources of variation.

15.3.1 Germplasm Diversity

The allopolyploid nature and origin of wheat undoubtedly contribute to its diversity that allowed wheat to grow and adapt to a wide range of environments. Wheat as an allohexaploid crop has different genome levels, e.g. *Triticum* as diploid 2n = 2x = 14 (*T. urartu* Than. ex Gand. and *T. monococcum*, AA genome) and *Aegilops* (*A. speltoides*, BB genome) (Marcussen et al. 2014; Rasheed et al. 2018). Due to the hybridization between *Triticum* and *Aegilops*, the tetraploid (durum) wheat became in nature (*T. turgidum* ssp. *durum*, AABB genome; 2n = 4x = 28). While hexaploid (bread) wheat developed by the hybridization of tetraploid wheat (AABB) with diploid *Aegilops* species, (*A. tauschii* Coss., DD genome; 2n = 2x = 14) to form (*T. aestivum*, AABBDD genome; 2n = 6x = 42) (International Wheat Genome Sequencing 2014; Rasheed et al. 2018).

Wheat crop improvement relies on genetic diversity and utilizing natural variation for selection using landraces and wild relatives, thus increased the rate of genetic gain in breeding programs. Old wheat germplasm including landraces and wild relatives are an important genetic resource for enhancing modern wheat by capturing new alleles. Wheat diversity bottlenecks that reduced the genetic variation was influenced by many biological processes that started at domestication, which is considered as the first bottleneck in reducing genetic variation. Wheat dissemination from the domesticated Fertile Crescent area to Europe and Asia has slowly adapted to local environments; therefore, the genetic diversity shrank. Further, the genetic diversity in wheat was reduced by depletion of certainly desired alleles from a gene pool as a result of crosses between diploid and tetraploid to produce hexaploid wheat, which occurred naturally a few times in addition to early selection by farmers. Then the diversity within bread wheat was reduced due to breeding procedures by separating environmental from genetic effects and replacing local landraces with newly-improved cultivars. Narrowing the genetic variation is a major concern for plant breeders in wheat genetic improvement progress; a significant decrease in wheat genetic diversity during the last century has been detected (Novoselović et al. 2016). Therefore, protecting wheat genetic diversity is essential for improving yield and adaptation. Introgression of novel alleles through crossing plants from various plant genetic resources e.g. modern varieties with locally

adapted varieties, enhances the genetic diversity and preselection for traits of interest which is required to ensure that meaningful natural variation at the phenotypic level. Salem et al. (2015) studied genetic diversity in Egyptian wheat. A dendrogram derived from UPGMA cluster analysis based on the genetic similarity (gs) matrix coefficient for 33 Egyptian wheat genotypes was constructed (Fig. 15.2).

15.3.2 Cultivar Characterization and Phylogeny

Characterization and evaluation of wheat gene bank collections represent a powerful means for the classification of old and new materials to understand natural variation and its application in breeding. Using the recent advances in technology for characterizing collections at a large scale by applying high-throughput genotyping is applicable. The development of DNA markers makes the genetic diversity in the wheat germplasms, including old collections, highly attractive (Börner et al. 2000a; Salem et al. 2015; Sehgal et al. 2015). Population structure using molecular markers in large diverse collections including wild, landrace and modern varieties can help

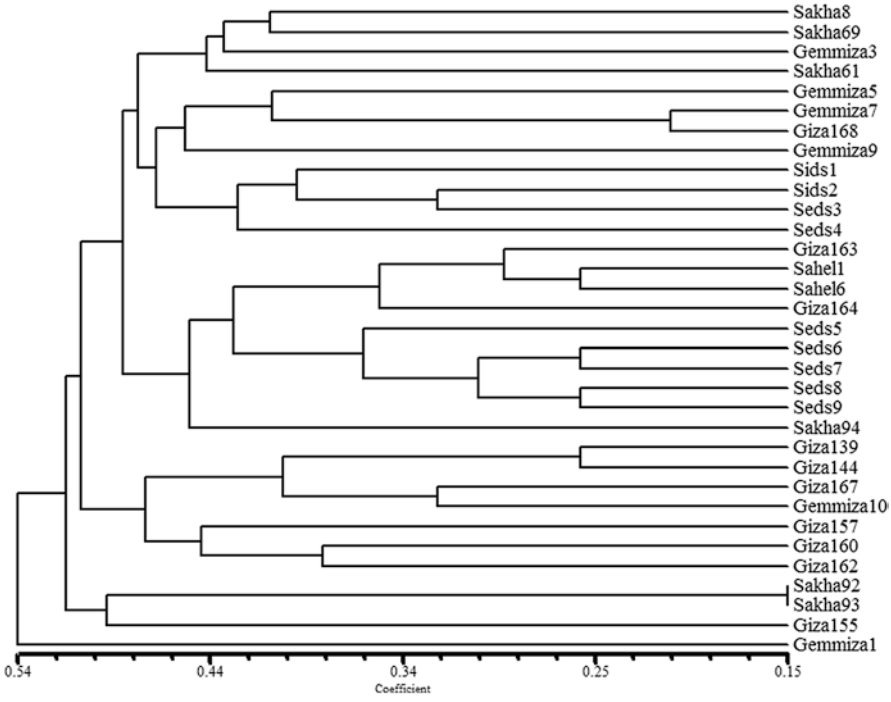

Fig. 15.2 UPGMA cluster analysis-based dendrogram depicting genetic relationships among 33 Egyptian hexaploid wheat genotypes and based on data of 17 microsatellite markers. (Source: Salem et al. 2015)

in understanding and monitoring genetic diversity. By applying the analysis that accurately calculates the relatedness among the individuals and clusters them based on their genetic information is commonly used in association mapping studies. Phylogeny analysis helps in understanding the complex history of wheat dissemination and genetic diversity. Many phylogeny studies have been conducted which were able to characterize wheat collections based on the ploidy level, biological status and geographical regions (Demir et al. 2015; Golovnina et al. 2007; Goncharov et al. 2009). Allelic variation and its distribution over historical time using wild relatives, landraces and cultivars, originating from different geographical regions, and genotyped by high-density single nucleotide polymorphism (SNP) arrays, has long been a main goal of phylogeny studies. Genetic diversity characterization of more than a half million wheat genetic accessions at the level of a collection is a huge challenge that aims to redesign the exploitation of wheat genetic resources. An ex situ gene bank should begin strategies for the exploitation and valorization of wheat genetic resources to unlock their hidden favorable genetic diversity for breeding as a pivotal step for enhancing yield.

15.3.3 Genetic Resources Conservation Approaches

Ex situ collections of wheat held in gene banks are important genetic resources that need to be managed securely in a cost-effective manner and easily accessed by a broad range of users. To maintain plant genetic resource with natural genetic variation, a number of ex situ seed gene banks have been established worldwide. Hundreds of thousands of wheat accessions have been collected since N. I. Vavilov and H. Harlan began seed collection at the beginning of the last century (Börner 2006). Globally, there are over 80 wheat germplasm collections, holding more than 800,000 accessions. The larger wheat collections are maintained at CIMMYT-Mexico (>100,000 accessions); USDA-NSGC, Aberdeen, Idaho, USA (nearly 40,000); Vavilov Research Institute (VIR), Russia; IPK-Gatersleben, Germany; ICARDA, Syria; NBPGR, India and Instituto del Germoplasma, Bari, Italy (each holding approximately 30,000 accessions). These collections represent worldwide geographical regions, biological status and other features like growth habit that need to be phenotypically and genetically characterized appropriately to reveal their potential value in crop improvement and to provide a wider basis for breeding purposes. A list of important world gene banks for plant genetic resources conservation is given in Table 15.2.

The purpose of an ex situ gene bank is to cultivate the accession in a garden or store it in a seed bank; so-called ex situ conservation. In general, the goal of ex situ conservation is to prevent the local, regional or global extinction of a species and to represent, as much as possible, the genetic diversity. Maintaining genetic integrity is one of the major challenges for ex situ conservation, due to contamination by foreign pollen or incorrect handling during multiplication (Börner 2006). Since most of the wheat collections are evolved and delivered by breeders or farmers they

Table 15.2 List of important wheat gene banks for plant genetic resources

Gene Bank	Country	Website
International Maize and Wheat Improvement Center (CIMMYT) Gene Bank	Mexico	https://www.cimmyt.org/seed-request/
National Small Grains Collection Gene Bank	USA	https://www.ars.usda.gov/paci
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben Gene Bank	Germany	https://www.ipk-gatersleben.de/en/gbisipk-gaterslebendegbis-i/
Winter Cereal Collection Gene Bank	Australia	www.dpi.nsw.gov.au/about-us/research-development/centres/
Genetics Resources for Wheat Sciences Gene Bank	Japan	https://shigen.nig.ac.jp/wheat/komugi/
National Gene Bank	China	http://www.cgris.net/cgris_english.html
International Center for Agricultural Research in the Dry Areas (ICARDA), Gene Bank	Syria	http://www.icarda.org/
Czech Republic Gene Bank	Czechia	http://genbank.vurv.cz/wheat/pedigree/
ICAR-National Bureau of Plant Genetic Resources (NBPGR)	India	http://www.nbpgr.ernet.in/
Instituto del Germoplasma	Italy	https://biodiversitapuglia.it/la-banca-del-germoplasma-dellibbr-cnr-bari/

consist of predominantly local or regional materials that are most likely duplicate accessions within and between collections. Therefore, characterization and exploitation of the natural variation in wheat germplasms are maintained at the plant genetic resource centers and are essential for future research.

15.3.4 Cytogenetics

Cytogenetic methods such as chromosome banding and in situ hybridization remain relevant in the post-genomic era for molecular characterization of allopolyploid plants such as wheat, where the combination of the different genomes in some cases makes it difficult to assess the reorganization of chromosomes during evolution. The first wheat cytogenetic study was conducted by Sakamura (1918) who discovered polyploid series of the diploid, tetraploid and hexaploid in wheat, with a basic chromosome number of x = 7. The cytogenetic analyses could split the chromosomes of polyploid wheat and their progenitors into tetraploid and hexaploid genomes (Shcherban et al. 2016). Chromosome banding techniques allow for the identification of the chromosome duplication and chromosome polymorphism, as well to understand the evolutionary processes (Friebe and Gill 1996). Hybridization in situ is another technique that directly localizing DNA sequences

on chromosomes of which fluorescence in situ hybridization (FISH) is used to show the DNA sequence distribution on chromosomes, whereas genomic in situ hybridization (GISH) is commonly used to identify the genomic composition of wheat amphiploids and hybrids (Cuadrado et al. 2008). The translocations of chromosome segments in wheat varieties have been detected by a cytogenetic method that allowed the breeders to use marker-assisted breeding for selection of the desired genotypes. Such an approach was found useful in breeding aspects to determine the resistance of phytopathogens and increased productivity e.g. Russian wheat varieties which carried intact wheatgrass chromosomes had high resistance to fungal diseases and high grain quality (Salina et al. 2015). There is clear evidence that despite extensive development of high-throughput molecular markers, cytogenetic methods are still imperative for characterizing the genetic diversity and application in breeding research.

15.4 Molecular Breeding

The first step toward the creation and release of a new cultivar is to identify the sources of genetic variation by evaluating a large number of genotypes. After identifying the available genetic variation, superior genotypes can be used as parents in breeding programs. In a highly diverse crop like wheat, the number of evaluated genotypes could reach into the hundreds of thousands. Evaluating such a large number of genotypes in the field is very expensive and time-consuming. Molecular markers are a possible technique which can help to reduce the number of evaluated genotypes and hence save time and expense. For example, molecular markers are typically used during backcrossing programs to track a small number of loci, which helps the breeder identify the germplasm close to the recurrent parent and reduce the required efforts, as compared to the traditional backcrossing programs (Langridge 2003).

15.4.1 Molecular Markers

Due to the size and complexity of the wheat genome, the application of molecular markers is quite complicated compared to other crop genomes. However, many efforts have been made to understand the wheat genome using different types of molecular markers. These efforts led to publication of the first wheat genome map in 1998 using simple sequence repeat (SSR) markers (Röder et al. 1998). That study was followed by many others which mapped many quantitative loci associated with important traits using different types of molecular markers (Bhusal et al. 2017; Börner et al. 2000b, 2002; Echeverry-Solarte et al. 2015; Salem et al. 2007). The different types of molecular breeding tools can be classified into two major types, molecular markers and molecular maps.

15.4.1.1 Molecular Markers in Wheat

Molecular markers have been used routinely in wheat breeding over the last 50 years. Due to continuous advances in biotechnology, new types of molecular markers are typically appearing which are usually faster than the previously developed types. Generally, molecular markers used in wheat can be classified into three general types: hybridization-based DNA markers, polymerase chain reaction (PCR)-based markers and DNA chip and sequencing-based DNA markers.

Hybridization-Based DNA Markers These marker types are considered as first-generation markers. Developing this type of molecular markers was based on the variation in the DNA fragment lengths which are produced by a specific restriction enzyme. These are commonly used to differentiate between two or more individuals or for fingerprinting purpose. One example is restriction fragment length polymorphism (RFLP). However, using this type of marker in wheat is not very effective due to the low level of polymorphism identified by this marker resulting from the high frequency of monotonous DNA in the wheat genome (Khan et al. 2014).

PCR-Based/Markers These molecular markers are considered as second-generation markers which were developed to reduce time, effort and cost required for molecular mapping and genotyping. They function depending on developing a primer which can be hybridized to a part of the DNA and produce a new DNA strand. Due to the development of PCRs, many copies of the DNA can be obtained. PCR products are then separated by gel electrophoresis. This type of marker can be used for two main different purposes; to identify the existence of a specific gene and the diversity between the studied genotypes. Different kinds of markers following this type are random amplified polymorphic DNA (RAPD), simple sequence repeats (SSRs), inter-simple sequence repeat (ISSR), amplified fragment length polymorphism (AFLP) and diversity arrays technology (DArT). SSR markers are considered the most common type of PCR-based markers used in wheat. SSR is a very short sequence (1–6 nucleotide) repeated randomly in wheat (Figs. 15.3 and 15.4). Due to the repetition of the SSR in different parts of the genome, it becomes a useful tool to predict a high level of polymorphism (Gupta et al. 1999; Röder et al. 1998). Due to this advantage, SSR markers have been widely used to study the diversity in the wheat genome. Many SSR markers are now identified as specific markers for important genes in wheat. Two of the databases which present specific wheat SSR markers and mapped SSR markers are Grain Genes (https://wheat.pw.usda.gov/ggpages/SSRclub/) and Integrated Breeding Platform (https://www.integratedbreeding.net/104/communities/genomics-crop-info/agricultural-genomics/markers/ssr-markers/wheat).

DNA Chip and Sequencing-Based DNA Markers SNP is a single base change in the DNA sequence. Due to the advances in sequencing methods which produce numerous SNPs, SNP markers are now widely used in genetic diversity, population structure, linkage mapping, linkage disequilibrium, whole genome association

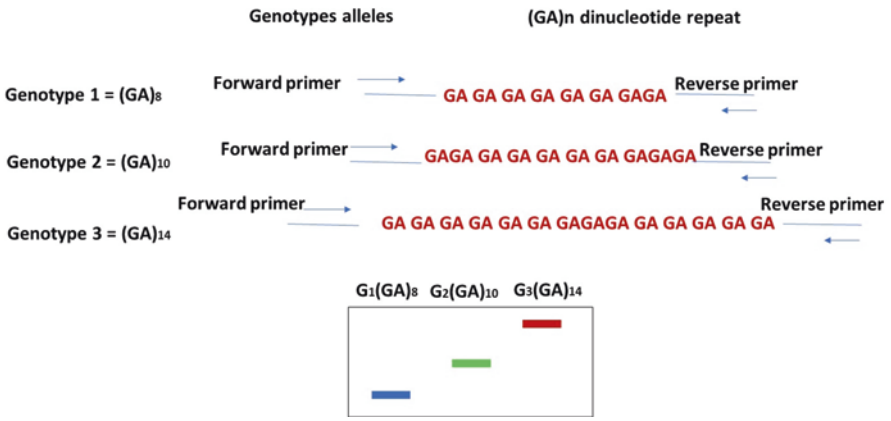

Fig. 15.3 The simple sequence repeats (SSRs) or microsatellite principle based on a (GA)n motif in three different genotypes. Prepared by K.F.M. Salem

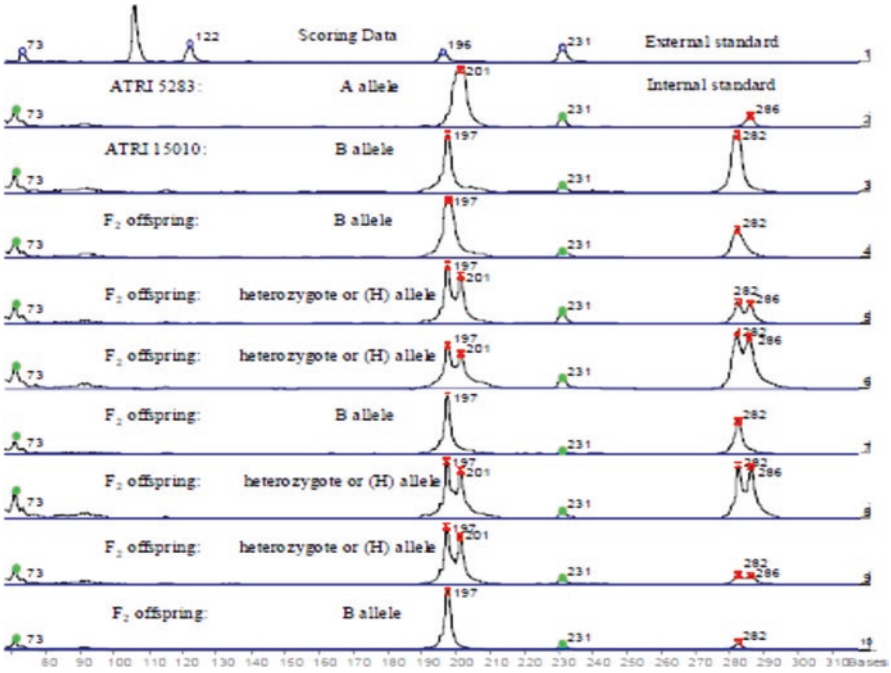

Fig. 15.4 Eropherogram analysis using fragment analyser software of polymorphic SSR marker in mapping population of two wheat parental lines ATRI 5283 x ATRI 15010 and F_2 generation amplified with the fluorescence labels SSR marker locus *Xgwm429-2BS*. Source: Salem (2004). Dissertation under supervision of Dr. Marion Röder Laboratory, Gene and Genome Mapping Group, IPK, Gatersleben, Germany

study, genomic selection and marker-assisted selection. SNPs replaced SSR markers in many plant species because they have the following attributes: low cost, high genomic abundance, codominance inheritance, easy documentation, locus specify and low genotyping error rates. Identifying SNP markers and their availability lead to the development of different SNP genotyping platforms such as Kompetitive Allele Specific PCR (KASP).

KASP is a homogenous, non-gel based, fluorescence-based genotyping technology. Genotyping technology in KASP is based on allele-specific oligo extension and fluorescence resonance energy transfer (FRET) for signal generation. KASP genotyping can be carried using 96-, 384- and 1536-well plate formats. This enables the breeder to determine many genotypes in a very short time as no gel is required. Despite the fact that KASP markers are a recent development, many types of research have identified KASP markers for important genes in the wheat genome. Some of the identified markers are listed in Rasheed et al. (2016).

15.4.1.2 Molecular Maps in Wheat

Advances in genome sequencing methods and the appearance of next, third and fourth sequencing techniques, make it possible to identify the sequence of numerous genotypes in a short time. Genotyping-by-sequencing (GBS) is a good example of the revolution in sequencing methods which is used broadly. GBS produces an enormous number of SNP markers distributed on the whole genome. These SNPs can be used in GWAS, genetic diversity, genetic linkage analysis and molecular marker discovery (He et al. 2014). This progress enables wheat breeders to identify the location of QTLs associated with important genes using different analyses such as genome-wide association study and quantitative trait loci.

By applying these new sequence techniques to different wheat populations, the sequence of the 21 chromosomes is available and can be used to assign gene sequence to individual chromosomes, develop physical maps, and identify gene models and the annotation of these models. One of the available databases for this information is International Wheat Genome Sequencing Consortium (IWGSC) (https://www.wheatgenome.org/). This database was first available at 2014 with one milestone chromosome-based genome sequence and will be updated to reach four milestones. The data of this update will be available in 2019.

Genome-Wide Association Study (GWAS) This type of analysis uses diverse genotypes with known genetic information such as single nucleotide (SNPs), SSRs or DArT markers. Any number of genotypes can be used to conduct GWAS analysis; however, a minimum of 100 genotypes is required (Kumar et al. 2011). Using the genomic and morphological available data, GWAS detects QTLs responsible for the studied traits (Chang et al. 2018). By studying the linkage disequilibrium (LD) between the identified loci, a number of candidate genes responsible for the studied

trait can be identified. Many GWAS studies were done in wheat to detect genes controlling biotic stress resistance. For example, Mourad et al. (2018b) identified SNPs associated with *Sr6* stem rust resistance gene. Juliana et al. (2018) identified candidate genes associated with wheat resistance to leaf rust, stripe rust and tan spot. Pariyar et al. (2016) identified candidate genes controlling nematode resistance in wheat using GWAS. Ando et al. (2018) detected the candidate genes controlling the resistance of stripe rust, *Septoria* blotch and Hessian fly in spring wheat. Mourad et al. (2018a) identified candidate genes controlling common bunt resistance in winter bread wheat. Combining the results of the different GWAS studies will enable wheat breeders to develop maps containing the chromosomal location of the resistance genes. For example, maps of wheat stem rust and stripe rust resistance genes are available (https://maswheat.ucdavis.edu/).

GWAS played an important role to identify candidate genes controlling abiotic stresses. For example, Sukumaran et al. (2018) identified candidate genes controlling drought and heat tolerance in durum wheat. Ayalew et al. (2018) identified 5 candidate genes located on 4 different chromosomes controlling root length under water stress conditions. Liu et al. (2018b) detected 24 candidate genes on 17 chromosomes controlling salt tolerance using SSR markers.

In addition, many studies detected candidate genes controlling important agronomic traits such as spike-related traits (Liu et al. 2018a), grain yield and its related traits (Garcia et al. 2019; Wang et al. 2017) and plant height and 1000-kernel weight (Daba et al. 2018). Identifying the candidate genes which control important traits in wheat will improve breeding, especially if GWAS is followed by deep analysis of the detected loci using haplotype-block analysis. However, the accuracy of GWAS is affected by the accuracy of the available phenotypic data. In this case, high phenotyping platforms and skilled researchers are required. With the advances in sequencing methods, bioinformatics and statistics, the future of GWAS will be very promising in wheat improvements.

QTL Mapping To apply QTL mapping for a specific trait, a biparental mapping population (Fig. 15.5) such as double haploid lines (DHLs), backcross mapping population (BC), $F_{2:3}$ mapping population, $F_{6:8}$ or recombinant inbred lines (RILs) should be used. Parents used to produce any type of these populations should be different in their alleles which affect the phenotypic value of the target trait. QTLs are mapped based on the distance between it and the genetic marker. Based on the number of markers used in genotyping the studied population, a different type of QTL mapping can be used such as single-marker, double-marker or multiple-marker mappings. Different statistics could be applied to map the QTL such as: single interval mapping, multiple interval mapping, QTL-composite interval mapping (CIM), multi-interval mapping (MIM), multiple QTL mapping and multi-trait mapping (MTM) (Tian et al. 2015). A number of genotypes in the studied population vary based on the purpose of the QTL study and the type of population. However, the more genotypes studied, the higher the mapping precision.

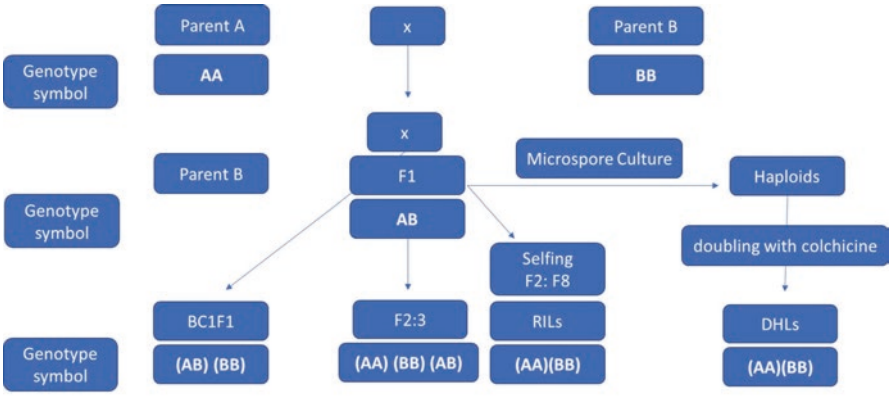

Fig. 15.5 Construction of the biparent mapping populations and its genotypes symbols. Prepared by K.F.M. Salem

Many QTL mappings have been done in wheat using different types of markers. Examples of QTL mapping for different wheat traits are listed in Table 15.3. Applying QTL mapping on the different traits will accelerate plant breeding as it helps the breeder in i) identifying a number of genes controlling the trait, ii) understanding the effect of the genes controlling the trait, iii) determining the location of the gene/s and iv) studying the linkage between the different genes of interest. All of these help in pyramiding many target genes in one genotype to understand the diversity of the studied germplasm (Khan 2015).

15.4.2 Genomic Selection in Wheat

Genomic selection (GS) is used broadly in both animal and plant breeding programs. The main object of GS is to shorten breeding time by predicting the performance of genotypes based on genomic data and evaluating a few genotypes to predict the performance of the rest of the population. GBS produces abundant numbers of SNP markers distributed all over the genome. These SNPs can be used to develop accurate genomic prediction (GP) methods, even for complex genomes like wheat. However, before applying GP, many factors which affect its accuracy should be taken into account. Some of these factors are population size, population structure and marker density. It is reported that the accuracy of GP improved when the tested set is highly diverse, used markers of high density and a maximum of 2000 genotypes are used (Norman et al. 2018).

Many efforts have been made to identify the best percentage of genomic selection in wheat. Belamkar et al. (2018) reported that 50% genomic prediction could be applied in preliminary yield trials in winter bread wheat. For grain yield, it was reported that genomic prediction accuracy reached 0.28–0.45

Table 15.3 List of QTL mapping for some important traits in wheat using different molecular markers

Trait	QTLs	Population	Chromosomal location of the detected QTL	Markers linked to the QTL	References
Drought-induced abscisic acid production	One QTL	DHLs Chinese spring × SQ1	5AL	RFLP	Quarrie et al. (1994)
Preharvest sprouting tolerance	One major QTL	RIL SPR8198 × HD2329	3A	SSRs, AFLP, SAPML	Kulwal et al. (2005)
Stem reserve mobilization	3 QTLs	RILs ITMI W7984 × Opata 85	2D, 5D, 7D	RFLP, SSRs	Salem et al. (2007)
Growth response to exogenously applied stress-induced hormones	9 QTLs	DHR Chinese spring (CS) × Synthetic 6A (S6A)	6AS	SSRs	Castro et al. (2008)
Aluminum toxicity	2 major QTLs	DHLs CS x CS (synthetic 3B)	4D and 3B	SSRs	Navakode et al. (2009)
Senescence-related traits under high temperature	16 QTLs	RILs Ventnor × Karl 92	2A, 6A, 6B, 3A, 3B, 7A	SSRs, AFLP	Vijayalakshmi et al. (2010)
Terminal heat tolerance	3 major *QTLs*	RILs NW1014XHUW468	2B, 7B, 7D	SSRs	Paliwal et al. (2012)
Grain yield under multi-environments	One major QTL	DHLs RAC875 × Kukri RILs RAC875 × Kukri	3B	SSRs, ISBP	Bonneau et al. (2013)
Powdery mildew resistance	4 QTLs	DHLs Pingyuan 50 × Mingxian169	2BS, 3BS, 5AL, 3BL	SSRs	Asad et al. (2014)
Common bunt resistance	10 QTLs	DHLs Carberry x AC Cadillac	1B, 4B, 4D, 6D, 7D	SSRs, DArT	Singh et al. (2016)
Stem rust resistance	9 QTLs	RILs Kristal x Sebatel	1AL, 2AS, 3BS, 4BL, 5BL, 6AL 7A, 7AL, 7BL	SSRs, STS	Haile et al. (2012)
Stripe rust resistance	140 QTLs	Many populations	–	–	Rosearne et al. (2013)
Root penetration ability	13 QTLs	DHLs Cranbrook × Halberd	2D, 4A, 6B, 7B	AFLP, DArT	Acuna et al. (2014)

(Poland et al. 2012). GP accuracy showed a range of 50–80% for grain yield, protein content, gluten index and alveograph measures using different prediction models (Haile et al. 2018).

Fusarium head blight (FHB) was found to be controlled by a limited number of loci with low effects based on GWAS study. The accuracy of GS for this trait was

reported to reach 60% (Dong et al. 2018). It seems that using GS in wheat breeding programs will increase breeding progress and lead to new eras in breeding and functional genomics.

15.5 Genetic Engineering

Genetic engineering, or genetic modification, is carried out to manipulate wheat genes directly. It is expected to support conventional breeding for further wheat production by increasing wheat production efficiency and avoid losses due to biotic and abiotic stresses through producing high tolerance lines under diverse conditions.

15.5.1 Methodologies

Genetic engineering offers the opportunity to improve the performance and yield of wheat by using the advances in wheat genome sequencing and molecular breeding. It leads to highly efficient and robust transformation systems targeting sequence-specific nucleases, such as zinc fingers (ZFNs), transcription activator-like effector nucleases (TALENs) and RNA-guided engineered nucleases such as clustered regularly interspaced short palindromic repeats associated protein 9 (Cas9). Transformation systems e.g. *Agrobacterium tumefaciens* have been successfully applied in wheat using genes of agronomic importance. Since the first report of wheat transformation using the *A. tumefaciens* approach (Cheng et al. 1997), it has become a worldwide technique that successfully produced transgenic wheat plants with desired gene(s) of agronomic traits (Habib et al. 2014). Such a technique is routinely applied but using the recent advances in engineered nucleases e.g. ZFNs, TALENs and Case9 have been emerging for understanding gene function of valuable traits. Cas9 has shown its applicability and accuracy in producing a wheat line with a trait of interest (Gil-Humanes et al. 2017; Liang et al. 2017). The successful progress in genome editing using technologies such as Cas9 is promising for performance and yield-boosting; therefore, wheat genetic engineering needs to overcome the traditional transformation approaches.

15.5.2 Enhanced Traits

Producing plants carrying multiple desired traits with stable inheritance and expression in the following generations is the aim of genetic engineering. Thus, large increases in yield would be expected if new gene editing e.g. Cas9 were applied to wheat agronomic important traits. There are many agronomic traits that have been

improved using transformation and gene editing techniques for the development of stable wheat transgenics, reviewed by (Ishida et al. 2015; Shrawat and Armstrong 2018). Knockout *TaGW2*, *TaLpx-1* and *TaMLO* genes using Cas9-based multiplexed gene editing (MGE) showed high improvement in 1000-grain weight, grain area, grain width, and grain length, resistance to *Fusarium graminearum* and powdery mildew, *Blumeria graminis* f. sp. *tritici* (Wang et al. 2018). Also, it has been shown that Cas9 in bread wheat improved resistance to infection of powdery mildew by mutated *TaMLO* genes (Wang et al. 2014). Wheat nutrient value, especially Fe content, has been improved through editing TaVIT2 using Cas9 (Connorton et al. 2017). Kim et al. (2018) showed that Cas9 in wheat helps to improve abiotic stress-related traits through knockout genes, namely wheat dehydration responsive element binding protein 2 (TaDREB2) and wheat ethylene responsive factor 3 (TaERF3). Gene editing techniques face a challenge in wheat due to ploidy levels; for example, in hexaploid wheat. The aforementioned findings show the feasibility of using Cas9 in wheat improvement.

15.5.3 Variation from in Vitro Tissue Culture

Tissue culture techniques are used for many purposes to: i) study the general combining ability (GCA), determine specific combining ability (SCA) and heterosis (Nawara et al. 2017); ii) rescue embryos from wide crosses made to transfer genes from wild relatives to wheat (Tyankova 2000); iii) screen for biotic and abiotic stress in vitro (Nawara et al. 2017); iv) create haploid plants (Santra et al. 2017); v) use as source material for wheat transformation (Waheed et al. 2016); vi) create doubled haploid lines (DHLs) (Srivastava and Bains 2018) and vii) create somaclonal variation (Danci et al. 2010). In order to improve wheat productivity, one of the most significant steps in a breeding program is the select of the right parents. To reach gains in plant biotechnology of wheat using immature embryo culture system, GCA and SCA for in vitro traits is necessary under biotic and abiotic stresses (Nawara et al. 2017). Numerous studies of the genetic control of in vitro traits using immature embryos were also reported in wheat (Barakat and Shehab El-Din 1993; Nawara et al. 2017). Mating designs such as diallel, line x tester have been widely used in genetic investigation to study the inheritance of in vitro traits among some genotypes (Nawara et al. 2017). Analysis of diallel data is usually conducted according to Griffing (1956), who partitioned the total variation of diallel data into GCA of the parents and SCA of the crosses, according to Barakat and Shehab El-Din (1993), Torres and Geraldi (2007) and Nawara et al. (2017). Biotechnology offers several valuable techniques such as cell, tissue and organ culture, which develop the breeding methods to improve the genetic characters including salt tolerance in economic crops. Tissue culture generates a wide range of genetic variation in plant species, which can be incorporated into plant breeding programs. By in vitro

selection, mutants with useful agronomic traits, i.e. salt or drought tolerance or disease resistance can be isolated in a short duration. However, the successful use of somaclonal variation is very much dependent on its genetic stability in the subsequent generations (El-Aref 2002; Jain 2001; Mercado et al. 2000). Embryo rescue is more important when it benefits the introduction of genetic material from wild relatives to cultivated species or to produce DHLs. Friebe et al. (1996) reported the importance of genes from wild relatives in wheat breeding programs.

15.5.4 Transgenic Wheat Lines

Transformation systems and gene editing in wheat have produced several transgenic lines showing strong and constitutive transgene expression with improvement in growth performance, yield and stress tolerance. For instance, wheat streak mosaic virus (WSMV), considered as the most destructive viral disease, was engineered and produced independent wheat transgenic lines that showed high resistance through overexpressing the viral coat protein gene (Sivamani et al. 2002). Mackintosh et al. (2007) showed that transgenic wheat lines exhibited resistance against *Fusarium graminearum* under greenhouse and field trials. It was also confirmed that the transgenic lines showed high tolerance to salt and drought stresses by TaERF3-overexpression (Shavrukov et al. 2016) and significantly higher yield under water stress conditions by transforming TaDREB3 from Bobwhite, a high transformation efficiency cultivar (Rong et al. 2014). In wheat, there are many model cultivars with high transformation efficiency such as Bobwhite, Florida, Chinese Spring, Lunxuan 987, Yumai 66, Kontesa and Fielder, which have been extensively used in genetic studies (Shrawat and Armstrong 2018). Therefore, using targeted genome editing for the development of wheat cultivars with stable performance will potentially revolutionize crop breeding.

15.5.5 Genome Editing

The main objective of plant breeding programs is to produce a new high-yielding cultivar that is also resistant to biotic and abiotic stresses. To achieve this goal, breeders have tried to edit the plant genome by induced random mutations or editing specific gene(s) in the genome using different approaches like ethyl methanosulfonate (EMS) mutagenesis, transfer, T-DNA insertions, zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). However, many difficulties have arisen in applying the previous methods, including protracted time requirements and high costs due to the need for protein engineering (Bortesi and Fischer 2015; Gaj et al. 2013). The new genome-editing technique, CRISPR/CRISPR-associated protein 9 (Cas 9), has been developed to overcome

the difficulties of the previous methods. CRISPER/Cas 9 was first discovered as a component of bacterial adaptative immunity (Ishino et al. 1987). Its function was first identified as it when it was observed that *Streptococcus thermophilus* resists bacteriophage by integrating part of the virus genome into its CRISPR locus (Barrangou et al. 2007). Appropriate restriction enzymes should be used to confirm the targeted gene editing. CRISPR was applied to edit the genome of different plant types like tobacco, *Nicotiana tobaccum* (Gao et al. 2015); thale cress, *Arabidopsis thaliana* (Li et al. 2014); rice, *Oryza sativa* (Shan et al. 2013) and sorghum, *Sorghum bicolor* (Jiang et al. 2013). Despite, the low cost and time-saving of the CRISPR technique, it is very complicated, especially in dealing with polyploidy germplasm like the wheat genome, which requires editing of each copy of the targeted gene. However, many studies report success in CRISPR editing of some wheat genes. For example, Kim et al. (2018) reported the success of CRISPR/Cas 9 technique in editing some protoplasts like TaDREB2 and TaERF3 which control the stress response of the wheat plant.

15.6 Wheat Breeding Methods

Several traditional breeding methods are still in use by plant breeders for crop improvement; the most common methods for wheat are pedigree breeding, pure-line breeding, bulk breeding, mass selection, single seed descent and backcross breeding (Baenziger 2016). The differences in breeding methods depend on two important points: i) the inbreeding of the population and ii) the selection process.

15.6.1 Pedigree Breeding

In this method, wheat breeders evaluate F_2 plant for target traits and select the best plants in this generation. The selected seeds are grown in a next generation in a progeny rows. Selection of best plants is done among the grown plants. The selection is performed each generation until some segregation is observed within the progeny row. Segregation within a row is normally based on many factors including the number of segregating traits in the population of study, number of inbred generations and phenotypic data.

15.6.2 Pure-Line Breeding

In this breeding method, a number of selected individual plants from a cultivar are grown in rows and the best rows selected. Finally, the best rows are grown in replicates to make a decision of which selection in most promising (Baenziger 2016).

15.6.3 Bulk Breeding

The wheat breeders grow a bulk of the progeny derived from a specific cross. Then, after harvesting the bulk, a part of the bulk seed is planted again in the next year. The population is considered again when it contains a mixture of predominantly homozygous lines and this takes place after many plantings and harvestings of the bulk (Tee and Qualset 1975).

15.6.4 Mass Selection

Mass selection is the simplest method used for crop improvement. Breeders select large numbers of plants that present a similar phenotype. Seeds of selected plants are mixed together to constitute the new cultivar. The cultivar developed using this method has high genetic variation. Thus, mass selection could be performed again in such a cultivar (Marais and Botes 2009).

15.6.5 Single Seed Descent

The main goal of this method is to rapidly inbreed without artificial or natural selection. Wheat breeders begin with a large number of plants in an F_2 population. Then, from each plant a single seed is sown. A single seed is harvested again and sown next year. Wheat breeders continue harvesting and sowing the single seed each year until all plants are predominantly homozygous. At this stage, no selection is normally performed (Pignone et al. 2015).

15.6.6 Backcross Breeding

The main objective of using backcross methods is to incorporate simply target inherited traits from unadapted donor genotypes into recipient genotypes. Backcross methods include repeated cycles of crossing to the recipient genotype (recurrent parent), followed by selection of the target trait of interest being transferred (Kenaschuk 1975). Breeders repeat crossing the F_1 to a single cultivar which has a good character that could be added. This method was widely used for transferring many favorable characters such as resistance to disease. For example, many stem, leaf, and stripe rust resistant genes were transferred from *Triticum* species to common wheat (Marais et al. 2003). Resistance to *Fusarium* head blight was improved in winter wheat through the backcross method (Clark et al. 2016)

15.7 Mutation Breeding

Mutations or sudden heritable changes in the phenotype and genetic material (DNA bases) can occur by repetition errors during cell division or exposure to mutant chemicals or radiation, such as ultraviolet radiation, ionizing radiation or even the viruses of an individual. The change caused by the mutation is not targeted and may lead to the generation of new materials. Mutations are the novel source of difference of all genes. Mutation breeding induces desirable mutations to exploit for crop improvement. It is commonly used in self-pollinated crops such as wheat to produce traits in crops such as larger seeds. Mutations are the method of generating variations. If a mutation cannot be transmitted across the germ line, it cannot be inherited, it has little value in improving the variety in seed-bearing crops such as wheat. If it is not possible to transfer a mutation to the next generation through a pure line, it cannot be inherited and transmitted from one generation to the next, and therefore has little value in the development of the wheat variety, which multiplies by seed. The mutation line resulting from the mutation can be registered directly and released as a cultivar or benefit from the serious line in the breeding programs of the background and the production of new cultivars.

15.7.1 Mutation Breeding Program

A mutation breeding program begins with the M_0 generation in which wheat seed is treated with a mutagen. The M_1 generation in grown in the first year of treated seeds and the individual plants are harvested separately. M_2 generation (second year), individual plants are grown in a line and the plants that contain the mutations or phenotype changes or the mutant allele are selected and harvested separately. M_3 (third year), individual plants are grown and stable lines selected from each row. M_4 (fourth year), comparative yield trial in the preliminary yield trial with a suitable check and selected superior lines. $M_{5–7}$ (5th–7th years), in these generations replicated yield trials at several locations with the outstanding line released as a new cultivar. M_8 (8th year) seed multiplication for distribution to farmers. A generalized scheme for a wheat mutation breeding program for high grain yield and its component traits is shown in Fig. 15.6.

As of 2013, 286 cultivars of *Triticum* ssp. reportedly feature a mutation (https://mvd.iaea.org/#!Search?Criteria[0][val] = wheat/). The first use of mutations in wheat breeding programs began in Japan to introduce dwarfism (Kihara 1984; Nonaka 1984). There were spontaneous mutations, *Rht8* on 2D chromosome (Salem 2015) and *Rht9* on 7BS chromosome, in the Japanese genotype Akakomugi (Gale and Youssefian 1985). As one feature, induced mutations can be used to understand the role of many genes by applying of targeting induced local lesions in genomes (TILLING) (McCallum et al. 2000).

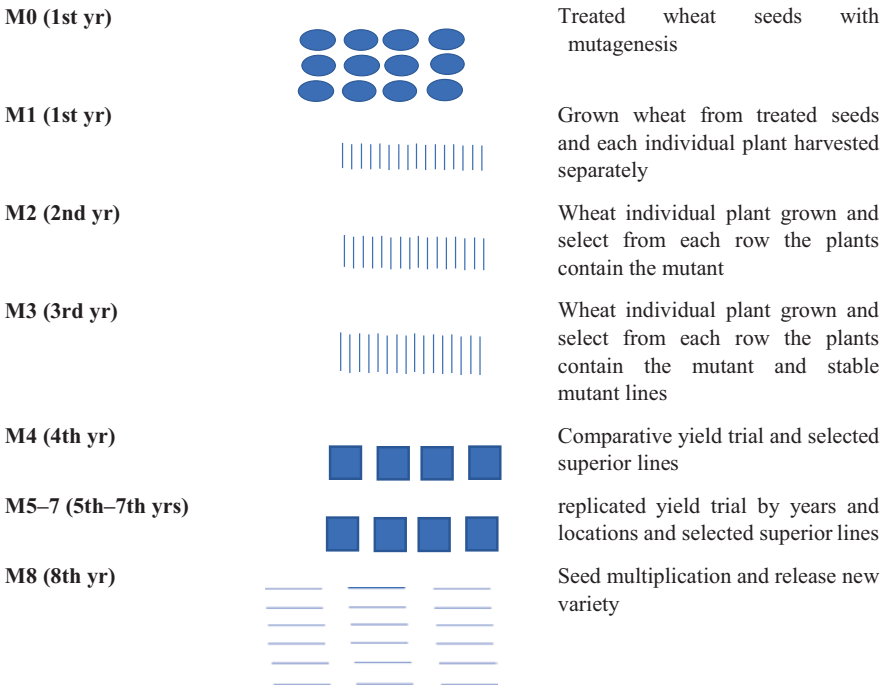

Fig. 15.6 Generalized scheme for wheat mutation breeding program for grain yield and its components trait. Prepared by K.F.M. Salem

15.8 Conclusion and Prospects

15.8.1 An Overview of the Current Status

In view of the great economic importance of wheat due to its uses as food, feed, and seed and for industrial uses, a significant body of scientific research has already been carried out worldwide. Since classical breeding methods are laborious and time-consuming, the introgression of novel alleles through crossing plants from various plant genetic resources e.g. modern varieties with locally adapted varieties, enhance genetic diversity and preselection for traits of interest, which is required to ensure that meaningful natural variation at phenotype level. Although new wheat biotechnology approaches that use the advances in DNA sequences and molecular methods have attracted plant breeders and geneticist, traditional plant breeding methods are still the key and first points to developing new wheat cultivars with desirable traits. However, many promising cultivars adapted to climate change and biotic and abiotic stress conditions have been developed. For that, new breeding approaches in wheat for high grain yield, biotic and abiotic stresses to develop new cultivars are needed.

15.8.2 Current Research Initiatives to Combat Global Climate Change

Overpopulation, and biotic and abiotic stress are the most important challenges facing a wheat breeding program. Pre- and post-flowering stresses are the most important effect in wheat growing. Global climate change i.e. frost or high temperature are the greatest important climatic changes attracting considerable wheat breeders' attention worldwide. Also, diseases such as Ug99, a new stem rust (*Puccinia graminis* f. sp. *tritici*), present in some regions of Africa and the Middle East, is predicted to spread rapidly through these regions and may affect the food security in those counties and worldwide (Singh et al. 2011). This requires more investment in breeding programs and the training of new plant pathologists and breeders. Also, more efforts must be made to breed new cultivars with wide adaptability, to extend wheat cultivation under abiotic stress i.e. drought and salt resistance to diminish the effect of global warming. Recent biotechnology tools have been used to develop promising new wheat cultivars with desirable traits. Also, new wheat genomes have been sequenced and innovative molecular methods developed that have inspired plant breeders and geneticist to develop new wheat cultivars with desirable agronomic traits, along with resistance to biotic and abiotic stress.

15.8.3 Recommendations for Future Research

Because there are no genetically distinct pure lines in wheat of most crop traits that are economically important, it is necessary to obtain pure lines through the production of haploids and doubled-haploid lines (DHLs) that can be exploited in breeding programs. Also, DNA markers must be developed which are closely linked to important biotic and abiotic stresses, physiological and anatomical characters, as well as grain yield and its components traits. Genes or QTLs should be identified for qualitative and quantitative attributes to improve these traits. Furthermore, germplasm and biotechnology should be improved to speed up and facilitate the improvement of new promising lines with high yield and enhanced grain quality.

Appendices

Appendix I: Research Institutes Relevant to Wheat

Institution	Specialization and research activities	Contact information	Website
International Maize and Wheat Improvement Center (CIMMYT)	Breeding and molecular breeding program	Wheat Program, The International Maize and Wheat Improvement Center, Mexico	https://www.cimmyt.org
International Center for Agricultural Research in the Dry Areas (ICARDA)	Breeding and molecular breeding program	Department of Wheat Breeding/ Genetics, International Center for Agricultural Research in the Dry Areas, Rabat, Morocco	http://www.icarda.org/
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben	Breeding and molecular breeding research	Genbank Department, Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany	https://www.ipk-gatersleben.de/
Wheat Department, Agriculture Research Center (ARC)	Breeding program	Department of Wheat, Agriculture Research Center, Giza, Egypt	http://www.arc.sci.eg/

Appendix II: Wheat Genetic Resources

Cultivars	Year of release	Pedigree	Cultivation location / Breeding sites	Important traits
Mazha	1940	Landrace	Shaanxi Province, China	None dwarf genes
Bima1	1951	Mazha/Biyu	Shaanxi Province, China	None dwarf
Fengchan3	1964	Danmai 1/Xinong 6028 × Bima1	Shaanxi Province, China	None dwarf
Xiaoyan6	1981	(ST2422 × 464)/ Xiaoyan96	Shaanxi Province, China	*Rht-B1b* + *Rht8*
Changhan58	2004	Changwu112/PH 82–2	Shaanxi Province, China	*Rht-B1b*
Yaqui 50	–	–	CIMMYT	4.5 mt/ha yield, none *Rht* gene

(continued)

Cultivars	Year of release	Pedigree	Cultivation location / Breeding sites	Important traits
Pitic 62	–	–	CIMMYT	6.5 mt/ha yield, *Rht2* *Vrn1* + *Vrn2*
Siete Cerros	–	–	CIMMYT	6.5 T/ha yield, *Rht1* *Vm1* + *Vrn2*
Yecora 70	–	–	CIMMYT	7 T/ha, *Rht1* + *Rht2* *Vrn1* + *Vrn3*
Seri 82	–	–	CIMMYT	8 T/ha, *Rht1* + *Vrn3*
Opata 85	–	–	CIMMYT	8 T/ha, *Rht1*
Baviacora 92	–	–	CIMMYT	9 T/ha, *Rht1*
Croc_1/*Aegilops tauschii* (224)// Opata	–	–	CIMMYT	Primary synthetic wheat, resistance to *Pratyulenchus thornei*
Iraq 48	–	–	Iraq	Possibly identical genetic location as *Cre1*; also resistant to *P. thornei*
AUS4926	–	–	Australia	Resistance to *P. thornei*
Seds 9	1994	Maya“s”/ Mon“S”/4// CMH72.428/MRC// jip/3/ CMH74A582/5/ Giza157∗2SD10003	Egypt	High yield, susceptible to rust, resistance to smut, long spike
Giza 168	1999	Mil/Buc//Seri	Egypt	High yield, resistance to rust and smut
Sakha 94	2004	Opata/Rayon//Kauz	Egypt	High yield, resistance to rust and smut
Gemmiza 10	2004	Maya74”s”/ On//1160147/3/ Bb/4/Chat s /5/Ctow	Egypt	High yield, resistance to rust and smut

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