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Genetic Improvement of Small Millets

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Foreword

Small millets have always been of local and regional importance and as a result have attracted little attention both at national and international level. Once an integral part of Asian and African diets, millets have been forgotten due to many demand and supply issues. The biggest factor has been absence of technological breakthrough in yield of millets similar to green revolution increasing yield of rice, wheat, and maize, thereby shrinking millets' profitability. Weak value chain in production and processing of millets, absence of advanced processing technology, lack of industrial demand for value-added millet products, and relatively shorter shelf life of the crops creates storage and spoilage-related concerns which have discouraged farmers from cultivating millets. Millets have many nutritional, health, and environmental benefits, making them more sustainable as crops. Predicted climate change scenarios indicate that climate vagaries and shorter effective growing season's lengths will be increasingly likely thus increasing the need for short-duration crops such as sorghum, pearl millet, and small millets with enhance climate resilience, without high fertilizer and pesticide needs.

The goal of the International Year of Millets—2023 easily fits into the 2030 Agenda of the United Nation for Sustainable Development and provides an opportunity to highlight how the sustainable production, processing, marketing, and consumption of millets can contribute to alleviating hunger**.** Millets can be easily integrated into an existing farming system across ages and cultures, cuisines, nations, and the dietary preferences. Millets have the potential to help us achieve SDGs like SDG 2 (Zero Hunger), SDG 3 (Good Health and Well-being), SDG 12 (Sustainable Consumption and Production), and SDG 13 (Climate Action).

There has always been low research priority on millet crops, and most landraces have already been lost due to a shift from traditional crops and landraces to cash crops and improved varieties and hybrids. Although millets have started receiving more attention in recent years, much research is needed for improving the yield and its attributing traits. Genomics-assisted improvement by utilizing various omics approaches can potentially contribute to enhanced genetic gains in small millets improvement, this knowledge is yet to be applied in millets due to the lack of a complete and annotated genome sequence for many types of millet. I am happy that the editors of this book have made an exhaustive effort to include chapters that guide us along various advanced breeding strategies adopted in different kinds of small

millets, the germplasm and genomic resources available, genomics-assisted breeding, and many more. The book will also motivate other researchers to advance agronomic and soil management options for millets. This book will be one of its kind as such type of comprehensive information is not available elsewhere particularly for small millets. I complement the editors of this book for bringing out such a book which I am sure will benefit the society.

San Jose, Costa Rica Ratan Lal

The Ohio State University
Columbus, OH, USA

Preface

The global food system faces many complex challenges, including hunger, malnutrition and diet-related diseases; an ever-growing global population that needs sufficient and healthy food; the climate emergency and the depletion of natural resources. In such a situation, we need to unlock the great potential that millets hold as an affordable nutritious food, a worthy component for global healthy diets and a crop that can withstand climate change.

The International Year of Millets 2023 is an opportunity to raise awareness of the health and nutritional benefits of millets for better production, better nutrition, a better environment and a better life. They can become a key crop within global food systems, with the potential to improve the livelihoods of smallholder farmers, suitability for cultivation under adverse and changing climatic conditions and to create sustainable market opportunities for producers and consumers. It also contributes to achieving the sustainable development goals of the United Nations (zero hunger, good health and wellbeing, economic growth, responsible consumption and production and climate action).

Millets are a diverse group of small-grained dryland cereals, climate resilient, tolerant to poor soil condition, drought and harsh growing conditions, do not need high fertilizer and pesticides, integral to ancestral traditions, cultures and indigenous knowledge are nutritious that provide dietary fibre, antioxidants, protein and minerals, including calcium, magnesium, phosphorous, zinc and iron, gluten free with a low glycaemic index to address intolerances and diabetes. By including millets in our food basket, we can prevent many lifestyle diseases like hypertension, diabetes, anaemia, osteoporosis, etc. It can be a source of income for marginal farmers, a way to create decent jobs for women and youth through innovative processing, value addition and marketing opportunities and has a huge potential to transform local agrifood systems.

Small millets are important group of coarse cereals, which have not yet been explored and exploited to its full potential. Not much attention has been drawn towards these high nutritional value crops. The objective of the breeders earlier was to develop high yielding crop varieties that made the world food sufficient; however with the passage of time, people are also concerned about the nutritional quality of the food that they eat. This calls for exploration of various different genetic resources that can complement the carbohydrate-rich diet with their high-quality nutrients.

Many food companies are coming forward to include them in their processed products. Thus, the demand of small millets has increased however their production is meagre. Many of the small millets have remained unexplored and the knowledge about the botany, genetics, diversity, breeding methods and incorporation in the existing cropping systems about small millets is still in the offing. The genetic and genomic resources of small millets need to be explored at length for some valuable insights that will eventually aid in yield and quality improvement of small millets.

This book introduces the various types of nutrient packed small millets to the readers, their genetics botany and diversity, challenges of their cultivation, breeding strategies, genomic resources available for these crops, how they can be incorporated into the existing cropping systems, advanced breeding strategies that can be adopted for genetic improvement of small millets, their processing strategies so that they can be incorporated into daily diet of humans. Not much work has been done in small millets; hence, not much literature is available in this regard. So this book will be a new resource in this field.

This book will benefit all those directly or indirectly involved in the small millet research and industry. The students, research scholars, scientists, professors and academicians can use it for small millet improvement while the industrial sector especially those involved in the nutri-food sector who prepare food products using small millets will also be benefitted by this book. This book can also be included in the postgraduate degree program in plant breeding as this book will cover all the updated technologies being used in small millet improvement. Keeping the view of current crisis during COVID-19 pandemic and ensuring food security for ten billion people by the end of 2050, alternative food source must be promoted and their genetic improvement should be done on prime focus. Therefore, the theme of the proposed book is of current interest in the global market.

Pusa, India Sweta Mishra
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About the Editors

Sweta Mishra is working as Professor and Head, Department of Genetics and Plant Breeding and is the Principal Investigator of the Small Millets scheme at Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar. She specializes in molecular plant breeding. She has 18 years of Teaching and Research experience in the capacity of Assistant Professor, Associate Professor and Professor in various organizations in different states of India. She has guided many M.Sc. and Ph.D. students. She has successfully handled two International, six National and eight State-funded research projects as PI and Co-PI. She has worked on different crops like finger millet, foxtail millet, proso millet, barnyard millet, wheat, basmati rice, pigeonpea, date palm, olives, jatropha, simarouba, noni, tomato, etc. Presently, she is the PI for AICRP small millets and is committed for reviving the lost glory of millets. She is working for the genetic improvement in small millets, area expansion, enhanced production and productivity, value addition and processing. She has developed several technologies and varieties. She has contributed tremendously to the scientific literature in the form of several books, research papers, book chapters, articles, training manuals, folders, bulletins, etc. She has also been bestowed upon with several awards.

Shailesh Kumar is Assistant Professor, is serving in the Department of Botany, Plant Physiology and Biochemistry, College of Basic Sciences and Humanities, Dr. Rajendra Prasad Central Agricultural University since 2006. Dr. Shailesh Kumar obtained his B.Sc. (Ag.) from Banaras Hindu University, Varanasi; Master degree in plant physiology and PhD in plant physiology from the Indian Agricultural Research Institute, New Delhi. He has qualified CSIR-NET and ICAR-NET. Dr. Shailesh Kumar has been teaching plant physiology and environmental science courses at undergraduate, and plant physiology courses at postgraduate and doctorate levels. He has been undertaking research in plant physiology, plant tissue culture, and medicinal and aromatic plants. He has supervised Ph.D., M.Sc. and B.Tech (Biotech) students. Dr. Kumar is a member of several scientific societies and is a member of Editorial Board of several journals. He has many research publications and books to his credit.

R C Srivastava was the former Vice Chancellor of Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar from 2016 to 2022. He specializes in rainwater management, integrated farming system, ground water management, monetization of agricultural waste and climate change. He has held the positions of Director, Central Agricultural Research Institute (ICAR), Port Blair, Andaman and Nicobar Islands. He has served the Indian Council of Agricultural Research, India in various capacities as Scientist, Senior scientist and Principal scientist. He has developed many technologies, has patents and has several research publications to his credit. He has received many awards including the Rafi Kidwai Award, Rajendra Prasad Award, etc.

Small Millets Genetic Resources Management

1

Maruthamuthu Elangovan and Karnam Venkatesh

Abstract

Plant genetic resources management is a field of science comprising mainly the activities of collection, characterization, evaluation, conservation, documentation, distribution, and utilization of diversity existing in a crop species. This genetic diversity may be in the form of cultivated varieties, wild and weedy progenitor species, any line which is an intermediate product of plant breeding activity. The small millets include finger millet, foxtail millet, proso millet, barnyard millet, kodo millet, and little millet. Small millets are gaining importance due their special adaptability to harsh and dry climatic conditions. They are also nutritionally rich. Millets contain higher amounts of protein (finger millet and proso millet), dietary fiber (barnyard millet, little millet and kodo millet), calcium (finger millet), iron (barnyard millet), and zinc (foxtail millet). Due to these facts, management of genetic resources becomes extremely important to conserve the diversity existing in these crops for future use and development. ICAR-Indian Institute of Millets Research (IIMR) is one of the National Active Germplasm Sites (NAGS) with the responsibility to collect, conserve, evaluate, document, and distribute the millets genetic resources to the bonafied user within the country. A total of 559 small millet germplasm were collected by ICAR-IIMR-Hyderabadduring 2015–2021 and conserved in the Millets Genebank. Nearly about 15,000 accessions of small millets were augmented from various National and International centers. More than 9300 small millet accessions were characterized at ICAR-IIMR and approximately 16,500 small millets accessions have been conserved at Millets Genebank.

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Keywords

Genetic resources · Conservation · Diversity · Gene bank · Characterization

1.1 Introduction

The global as well as Indian agriculture has witnessed tremendous growth in food production in the recent decades; however it is also facing challenges such as climate change and malnutrition (Sharma et al. 2015; Kumar et al. 2018). The nation has witnessed over exploitation of irrigated agriculture lands in the past. However, there is a need to change the focus towards dry and marginal lands to address the adverse effects of ongoing and future climate change scenario. Generally, the lower fertility of dry and marginal lands makes it difficult to achieve higher production from conventional crops such as rice and wheat. Millets provide a great opportunity due to their climate resilient hardy nature and can be chosen to replace the conventional crops in the dry and marginal areas. In addition to their climate resilience, millets are also rich in several vital micronutrients and vitamins necessary for reducing malnutrition (Hariprasanna et al. 2014; Elangovan et al. 2022). Millets also known as nutricereals are reported to be the treasure house of vitamins, minerals, essential fatty acids, phyto-chemicals, and antioxidants that can help to eradicate the hidden hunger. Due to the richness of millets in polyphenols and other biological active compounds, they are also considered to impart role in lowering rate of fat absorption, slow release of sugars (low glycemic index) and thus reducing risk of heart disease, diabetes, and high blood pressure (Kumar et al. 2018).

The major millets are pearl millet (*Pennisetum glaucum*, with synonyms of *P. americanum*, *P. typhoides*, and *P. typhoideum*), foxtail millet (*Setaria italica*), proso millet or white millet (*Panicum miliaceum*), and finger millet (*Eleusine coracana*). Minor millets include barnyard millet (*Echinochloa* spp.), kodo millet (*Paspalum scrobiculatum*), little millet (*Panicum sumatrense*), and browntop millet (*Urochloa ramose*/*Brachiaria ramose*/*Panicum ramosum*) (Upadhyaya et al. 2006).

Genetic resources management is a field of science comprising mainly the activities of collection, characterization, and conservation of diversity existing in a crop species for future and utilization of trait-specific germplasm in national crop improvement program (Singh et al. 2019). This genetic diversity may be in the form of cultivated varieties, wild and weedy progenitor species, any line which is an intermediate product of plant breeding activity. Plant Genetic Resources are plant genetic materials of actual or potential use available for change in genetic constitution of a plant species for the production of an improved cultivar. They are valuable natural variants in form of exotic and indigenous collections including advance cultivars, released varieties, RILs, NILs, mutants, genetic stocks, landraces, wild and weedy relatives etc. (Singh et al. 2019). Genetic resources management caters to the needs of present as well as future generations of researchers (Singh et al. 2019). Due to these facts, management of genetic resources becomes extremely important to conserve the diversity existing in these crops for future use and development.

1.2 Global Status of Small Millets Conservation

A huge number of millet germplasm collections from 92 countries are conserved at ICRISAT, ICAR-NBPGR and ICAR-IIMR. A total of 22,971 accessions of small millet germplasm have been conserved at National Genebank, ICAR-NBPGR, New Delhi, which includes finger millet (11,587 acc.), foxtail millet (4244 acc.), kodo millet (2362 acc.), little millet (1885 acc.), proso millet (1005 acc.), and barnyard millet (1888 acc.). A duplicate set of the 17,330 acc. of small millets are also conserved at Millets Genebank, ICAR-IIMR, Hyderabad. The status of other small millets genetic resources in the Genebanks is given in Table 1.1.

1.3 Species Diversity of Small Millets

Finger millet (*Eleusine coracana*): Finger millet has its origin in the highlands of Africa. The closest wild relative of finger millet is *Eleusine coracana* subsp. *africana* (Kennedy-O'Byrne) Hilu & de Wet. *Eleusine coracana* subsp. *Africana* has its origin in Africa (Upadhyaya et al. 2008). These two taxa (finger millet and subsp. africana) are tetraploids ($2n = 36$) with basic chromosome number $x = 9$. These sub-species hybridize where they are sympatric in Africa and derivatives of such crosses often occur as weeds in cultivated fields (Upadhyaya et al. 2008).

Foxtail millet (*Setaria italica*): Foxtail millet has its origin in the highlands of central China and was first domesticated some 5000 years back and later spread to Europe and India. *Setaria viridis* is the probable progenitor species with somatic chromosome number of 18 ($2n = 18$) same as the cultivated species. Foxtail millet has been classified into three races namely moharia (Europe, Russia, Afghanistan and Pakistan), maxima (grown in east China, Japan, Korea, and USA), and indica (found in India and Sri Lanka) (Upadhyaya et al. 2008).

Table 1.1 Status of millets genetic resources in the Genebank

Crop name	ICRISAT	National Genebank, ICAR-NBPGR, New Delhi	Millets Genebank, ICAR-IIMR, Hyderabad
Finger millet (IE)	7519	11,587	7806
Foxtail millet (ISe)	1542	4244	4653
Kodo millet (IPs)	665	2362	344
Little millet (IPmr)	473	1885	694
Proso millet (IPm)	849	1005	2128
Barnyard millet (IEc)	749	1888	1705
Total	11,797	22,971	17,330

Proso millet (*Panicum miliaceum*): Proso millet, also called broomcorn and common millet, was domesticated in Neolithic China as early as 10,000 years ago (Lu et al. 2009). The cultivated proso millet is classified into five races based on panicle. The first two races namely miliaceum (open inflorescences and sub-erect branches with few subdivisions), patentissimum (narrow and diffuse panicle) are common to Eurasia. The later three races, contractum, compactum, and ovatum have more compact inflorescences which are drooped, cylindrical, and curved, respectively (Reddy et al. 2007).

Little millet (*Panicum sumatrense*): It is commonly cultivated in India, Nepal, and Western Myanmar and its ancestral species is *Panicum psilopodium*. The cultivated species has two races robusta (Northwestern Andhra Pradesh and parts of Orissa) and nana.

Kodo millet (*Paspalum scrobiculatum*): It is commonly cultivated in India. Kodo millet is divided into three races (regularis, irregularis, and variabilis) based on panicle morphology (de Wet et al. 1983).

Barnyard millet (*Echinochloa crusgalli* and *E. colona*): Two species of *Echinochloa* namely *E. crusgalli* (native of Eurasia and was domesticated in Japan) and *E. colona* (domesticated in India) (Upadhyaya et al. 2008) and both species have same hexaploid chromosome number of $2n = 54$. Another report suggests that two separate species represent barnyard millet, *Echinochloa esculenta* (syn. *Echinochloa utilis*, *Echinochloa crusgalli*) is cultivated in Japan, Korea, and the northeastern part of China while *Echinochloa frumentacea* (syn. *Echinochloa colona*) is found in Pakistan, India, Nepal, and central Africa (Wanous et al. 1990).

1.4 Millets Genebank at ICAR-Indian Institute of Millets Research (IIMR)

ICAR-Indian Institute of Millets Research (IIMR) is one of the National Active Germplasm Sites (NAGS) with the responsibility to collect, conserve, evaluate, document, and distribute the millets genetic resources to the bonafied user within the country. The following progress has been made during the reporting period 2001–2022.

1.5 Millets Genetic Resources Management

1.5.1 Collection

A total of 2273 acc. collected by NRCS/DSR/IIMR from 45 explorations and 423 acc. collected by other individuals during 2000–2021. Indigenous Collection number for 1928 accessions obtained from National Genebank, NBPGR—New Delhi. Out of 45 explorations, 10 are in collaboration with NBPGR (Regional Stations). All eight millets are collected during the exploration, which includes

finger millet (244 acc.), foxtail millet (102 acc.), proso millet (19 acc.), barnyard millet (7 acc.), little millet (146 acc.), and kodo millet (41 acc.). The maximum accessions are collected from Andhra Pradesh (474 acc.) followed by Maharashtra (447 acc.), Tamil Nadu (431), Madhya Pradesh (266), Karnataka (190), Gujarat (183), Uttar Pradesh (172), Odisha (141), Rajasthan (135), Chhattisgarh (60), Jharkhand (58), Uttarakhand (30), Telangana (16), Bihar (13), and West Bengal (1).

1.5.2 Augmentation

A total of 20,000 accessions received from various national and international centers, which includes, finger millet (10,704), foxtail millet (5096), proso millet (1666), barnyard millet (1661 acc.) etc. A total of 58,572 acc. of millets are augmented from ICAR-NBPGR-New Delhi and its Regional Stations followed by 7887 acc. from CGIAR-ICRISAT-Patancheru-Hyderabad etc.

1.5.3 Characterization

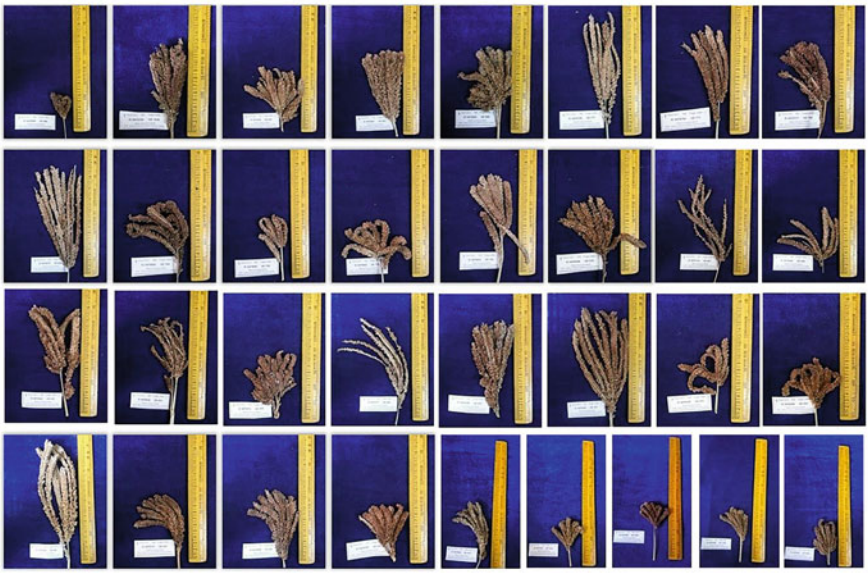
A total of 9300 acc. of small millets germplasm materials were characterized during 2003–2021 in which 7002 acc., belonged to sorghum and the remaining were of other small millets. The characterization of different small millets has been presented in detail in the following paragraphs

Finger Millet A total of 5400 acc. of finger millet germplasm characterized under CRP-AB during kharif 2017–2020 for 14 quantitative and 18 qualitative traits. The grain yield per plant is the most variable trait followed by days to 50% flowering, leaf blade length.

The maximum frequency in early plant growth was observed in poor (787 acc.) followed by good (632 acc.). Plant growth habit was observed maximum in erect (3521 acc.) followed by decumbent (1628 acc.). Leaf color was observed maximum in dark green (1541 acc.) followed by light green (1002 acc.). Plant pigmentation at leaf juncture was maximum observed as absence (3973 acc.) followed by present

(959 acc.). Leaf sheath pubescence was observed as absence (3004 acc.) followed by presence (2382 acc.). Culm branching was observed maximum as present (2645 acc.) followed by absent (2200 acc.). Ear head shape was observed in semi compact (1313 acc.) followed by fist (1259 acc.). Ear head size was observed maximum in intermediate (1065 acc.) followed by large (280 acc.). Finger branching was observed maximum as absent (3901 acc.) followed by present (878 acc.). The position of branching was observed maximum in thumb finger (336 acc.) followed by in all fingers (287 acc.). Finger multiple whorls was observed maximum as absent (2874 acc.) followed by present (1914 acc.). Gaps on finger were observed maximum as absent (1157 acc.) followed by present (338 acc.). Spikelet shattering was served maximum as present (1789 acc.) followed by absent (1023 acc.). Glume color was observed in light brown (823 acc.) followed by brown (507 acc.), Pericarp persistence on seed after threshing was observed maximum in persistent (724 acc.) followed by partially persistent (489 acc.). Seed color was maximum observed as copper brown (1569 acc.) followed by light brown (1318 acc.). Seed color (28 acc.) followed by light brown (1318 acc.), copper brown (24 acc.). Seed shape was observed maximum in round (3161 acc.) followed by reniform (1514 acc.). Seed surface was observed maximum in smooth (2622 acc.) followed by rough (651 acc.).

Ninety-five accessions were identified with more number of tillers (>12.00). Thirteen accessions were identified with early flowering (<50.00 days), 157 acc. identified with more number of leaves (>20.00), 61 acc. identified with longer leaves (>70.00 cm), 10 acc. with wider leaf (>2.00 cm), 24 acc. with shorter plant (<50.00 cm), 34 acc. identified with taller (>160.00 cm), 35 acc. identified with longer finger length (>15.00 cm), 9 acc. identified with wider ear head (>6.00 cm), 34 acc. identified with more fingers on main axis of the ear head (>13.00), 47 acc. identified with more productive tillers (>13.00), 64 acc. identified with higher grain yield (>90.00 g/plant), and 11 acc. identified with more 100-seed weight (>1.20 g).



Foxtail Millet A total of 2580 acc. of foxtail millet germplasm characterized under CRP-AB during kharif 2017–2020 for 11 quantitative and 19 qualitative traits. The plant height is the most variable trait followed by grain yield, days to 50% flowering etc.

The maximum frequency in flag leaf shape of leaf tip shape was observed in pointed (2384 acc.) followed by pointed to rounded (154 acc.). Absence or weak pigmentation of basal leaf sheath was observed maximum in 2089 acc. followed by strong (383 acc.). Dark intensity of green foliage was observed maximum in 1859 acc. followed by medium (567 acc.). Upright plant growth habit was observed maximum in 1791 acc. followed by spreading (743 acc.). Absent or weak anthocyanin coloration of the leaf collar was observed maximum in 2115 acc. followed by strong (363 acc.). Semi-erect leaf attitude of blade was observed maximum in 1813 acc. followed by slightly drooping (552 acc.). Presence of inflorescence bristles was observed maximum in 2383 acc. followed by absence (29 acc.). Long panicle length of bristles was observed maximum in 1495 acc. followed by short (478 acc.). Presence of inflorescence apical sterility was observed maximum in 1964 acc. followed by absence (447 acc.). Absence of anthocyanin coloration of the bristles was observed maximum in 2081 acc. followed by presence (320 acc.). Absence or weak anthocyanin coloration of flag leaf was observed maximum in 2374 acc. followed by medium (25 acc.). Absence of glume anthocyanin coloration was observed maximum in 2010 acc. followed by presence (387 acc.). Semi-erect panicle attitude to stem was observed maximum in 1336 acc. followed by drooping (564 acc.). Conical panicle type was observed maximum in 1135 acc. followed by

spindle (636 acc.). Oblong inflorescence shape was observed maximum in 1090 acc. followed by pyramidal (808 acc.). Absence of inflorescence lobes was observed maximum in 1259 acc. followed by presence (1059 acc.). Circular grain shape was observed maximum in 1025 acc. followed by medium ovate (777 acc.). Brown grain color was observed maximum in 1489 acc. followed by yellow (332 acc.).

Ninety-seven accessions identified with early flowering (<40.00 days), 46 acc. with longer flag leaf (>40.00 cm), 23 acc. identified with wider flag leaf (>2.90 cm), 67 acc. identified with longer peduncle (>40.00 cm), 5 acc. identified with thicker stem (>1.00 cm), 24 acc. identified with more number of basal tiller (>20.00), 60 acc. identified with longer panicle (>27.00 cm), 28 acc. identified with wider panicle (>3.00 cm), 27 acc. identified with taller plant (>150.00 cm), 34 acc. identified with shorter plant (<40.00 cm), 41 acc. identified with higher grain yield (>70.00 g/plant), and 79 acc. identified with more 100-seed weight (>0.35 g).

Proso Millet A total of 645 acc. of proso millet germplasm are characterized along with four checks viz., GPUP 8, TNAU 145, TNAU 164 and TNAU 202 in Augmented Block Design under Institute Project (IIMR/CI/2021-2026/150) at ICAR-IIMR-Hyderabad during Kharif 2021 for 23 agro-morphological traits. Which includes 11 quantitative and 12 qualitative traits. The plant height was the most variable trait followed by date of maturing, days to 50% flowering etc. In the qualitative data, erect growth habit is the most frequent in 444 acc., absence of leaf sheath pigmentation in 630 acc., sparse of leaf sheath pubescence in 333 acc., absence of ligule pubescence in 639 acc., glabrous leaf blade pubescence in 559 acc., arched inflorescence shape in 443 acc., presence of culm branching in 378 acc., absence of lodging in 677 acc., absence of seed shattering in 661 acc., intermediate panicle compactness in 303 acc., golden yellow grain color in 470 acc.,

and oval grain shape in 379 acc. There are 442 acc. of trait specific proso millet germplasm identified in which, 82 acc. are multi-trait specific germplasm for multi-trait specific germplasm for 2–6 traits.

Barnyard Millet A total of 553 acc. of barnyard millet germplasm are characterized along with four checks viz., DHBM 93-3, VL 207, VL 172 and PRJ 1 in Augmented Block Design under Institute Project (IIMR/CI/2021-2026/150) at ICAR-IIMR-Hyderabad during Kharif 2021 for 25 agro-morphological traits, which includes 12 quantitative and 13 qualitative traits. The plant height was the most variable trait followed by days to maturity, days to 50% flowering, grain yield/plant etc.

In the qualitative data, erect plant growth habit is the most frequent in 349 acc., absence of leaf sheath pigmentation in 310 acc., arched inflorescence shape in 359 acc., green inflorescence color in 376 acc., intermediate panicle compactness in 340 acc., onside spikelet arrangement on the rachis in 383 acc., curved lowest raceme shape in 340 acc., thick lowest raceme thickness in 317 acc., absence of lowest raceme branching in 494 acc., presence of culm branching in 443 acc., absence of lodging in 541 acc., light grey grain color in 267 acc., and oval grain shape in 473 acc. There are 411 acc. of trait-specific barnyard millet germplasm identified, in which 92 acc. are multi-trait specific germplasm for multi-trait specific germplasm for 2–5 traits.

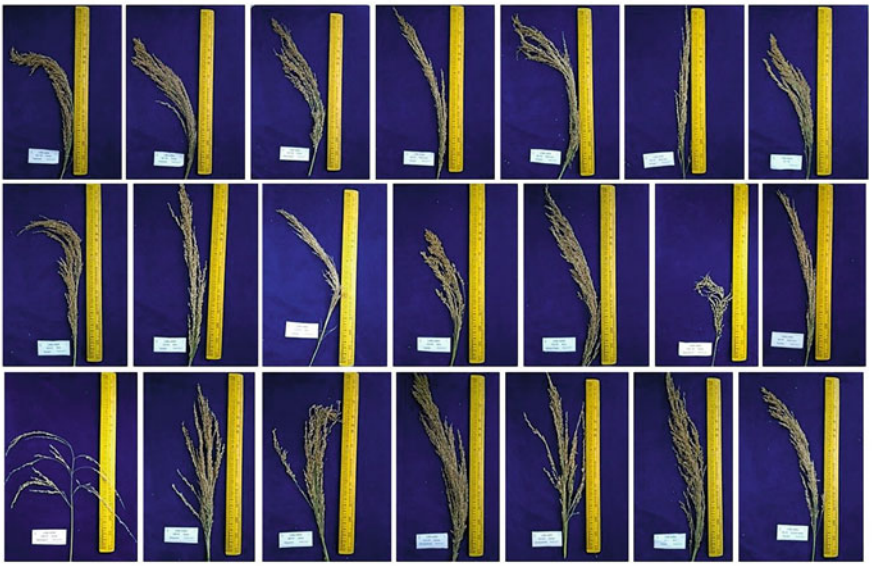
Kodo Millet A total of 69 acc. of core collections of kodo millet germplasm characterized during kharif 2017 for 13 quantitative and 15 qualitative traits. The days to 50% flowering is the most variable trait followed by plant height etc.

In growth habit, the maximum frequency was observed in erect (27 acc.) followed by decumbent (23 acc.). In leaf erectness, droopy was maximum observed (31 acc.). The leaf sheath pigmentation was present in 51 acc. The leaf juncture pigmentation was absent in 46 acc. The internode pigmentation was absent in 27 acc. The leaf blade pigmentation was present in 42 acc. In panicle compactness, the maximum frequency was observed in open (25 acc.) followed by semi-compact (22 acc.). The panicle exertion was complete in 30 acc. The spikelet arrangement on rachis was regular in 45 acc. The spike branching was present in 33 acc. the lodging was present in 40 acc. In grain color, brown was observed maximum (28 acc.) followed by dark brown (13 acc.). The grain shape was oval in 45 acc.

Eleven accessions identified with more number of basal tillers (>18.00), 10 acc. identified with early flowering (<56.00 days), 9 acc. identified with longer flag leaf blade (>35.00 cm), 11 acc. identified with wider flag leaf blade (>1.50 cm), 3 acc. identified with longer peduncle (>3.00 cm), 6 acc. identified with longer panicle (>9.00 cm), 7 acc. identified with wider panicle (>2.50 cm), 4 acc. identified with longer thumb receme (>7.00 cm), 9 acc. identified with longer receme (>10.00 cm), 4 acc. identified with more number of receme (>6.00), 8 acc. identified with taller plant (>90.00 cm), 12 acc. identified with shorter plant (<60.00 cm), 4 acc. identified with higher grain yield (>20.00 g/plant), and 17 acc. identified with more 100-seed weight (>0.40 g).

Little Millet A total of 66 acc. of new little millet germplasm characterized during kharif 2017 for 9 quantitative and 12 qualitative traits. The plant height is the most variable trait followed by days to 50% flowering, flag leaf length of blade etc.

The maximum frequency in plant growth habit was observed in erect (46 acc.), absence of leaf sheath pigmentation (52 acc.), glabrous leaf sheath pubescence (58 acc.), absence of ligule pubescence (56 acc.), glabrous leaf blade pubescence (56 acc.), diffused inflorescence shape (11 acc.), presence of culm branching (12 acc.), intermediate panicle compactness (8 acc.), presence of lodging (9 acc.), absence of seed shattering (14 acc.), light brown grain color (19 acc.) followed by brown (17 acc.), and elliptical grain shape (30 acc.). Four accessions identified for early flowering (<55.00 days), 8 acc., identified with longer flag leaf blade (>30.00 cm), 5 acc. identified with longer peduncle (>17.00 cm), 8 acc. identified with taller plant (>100.00 cm), 6 acc. with longer panicle (>25.00 cm), 7 acc. with wide panicle (>1.00 cm), and 4 acc. with higher grain yield (>10.00 g/main plant).



1.5.4 Characterized/Evaluated

A total of 94,081 accessions characterized/evaluated at NRCS/DSR/IIMR, AICRP on Sorghum and Small millets centers and others. The accessions include germplasm, resistant source materials, and segregation materials. The maximum frequency of 62,789 acc. of millets germplasm materials is characterized during 2003–2021 in which 45,092 acc. belongs to sorghum followed by finger millet (7002 acc.), pearl millet (5395 acc.), followed by 17,842 acc. for evaluation in which 11,210 acc. belongs to sorghum followed by finger millet (2960 acc.), little millet (2172 acc.) etc.

The maximum frequency of 61,817 acc. of sorghum germplasm/segregating materials characterized/evaluated in which 26,271 acc. are characterized/evaluated at Hyderabad followed by Akola (5300 acc.), Rahuri (4326 acc.), etc., followed by finger millet (18,262 acc.) in which 7202 acc. characterized/evaluated at Hyderabad followed by Vizianagaram and Mandya (3157 acc. each) etc.

1.5.5 Conservation

As on 31st March 2022, a total of 48,462 accessions of millets in bulk are being conserved in the Millets Genebank (MGB). Sorghum was maximum with 27,366 acc. followed by finger millet (8057), foxtail millet, (4573) pearl millet (4094), proso millet (1463), barnyard millet (1159), little millet (670), kodo millet (333), Tef (36), browntop millet (25), Quinoa (12), and Jobs Tear (1).

1.5.6 Utilization

A total of 48 final products contributed using the ICAR-IIMR germplasm through selection/breeding by the AICRP on Sorghum trials during 2007–2021. Maximum of 22 rabi sorghum varieties followed by 6 sweet sorghum varieties, 7 kharif sorghum varieties, 1 kharif sorghum hybrid, 5 single-cut forage varieties, one each of sweet sorghum variety, sweet sorghum hybrid and dual-purpose varieties, 3 speciality sorghum contributed to the trials.

1.5.7 Distribution

A total of 121,077 accessions distributed to the bonafied users in the country. A total of 1079 MTAs signed for supplying the germplasm. The maximum of 24,827 accessions distributed to the bonafied users during 2012–2013 followed by 17,981 in 2021–2022. A maximum frequency of 17,000 acc. of millets germplasm distributed and utilized for Salinity and drought tolerant screening at Gujarat/Rajasthan followed by 7500 acc. utilized for screening leaf blast, neck blast and finger blast, 6160 acc. of germplasm evaluated for yield attributing traits etc.

1.5.8 Submission to National Genebank

A total of 29,508 accessions submitted to the National Genebank for long-term storage during 2000–2021. The maximum of 4179 accession submitted during 2016–2017 followed by 2963 during 2020–2021 etc.

1.6 Genetic Stocks Registration with ICAR-NBPGR

A total of 112 millets genetic stocks registered with ICAR-NBPGR during 2002–2022. The maximum of 84 acc. of sorghum genetic stocks registered followed by finger millet (19 acc.), barnyard millet (4 acc.), foxtail millet (3 acc.), and little millet (2 acc.).

1.6.1 Documentation

A total of 3585 accessions of sorghum genotypes have been identified based on their uniqueness of trait from the compiled information from the published annual reports from 2000–2001 to 2013–2014. A total of 1775 accessions of millets genotypes have been identified based on their uniqueness of trait from the compiled information from the published ICAR-IIMR Annual Reports from 2014–2015 to 2018–2019. A total of 17,794 sorghum genotypes have been identified based on their uniqueness of trait in the AICSIP trials from the compiled information from the published AICSIP reports from 2004–2005 to 2013–2014. A total of 19,710 accessions of pearl millet genotypes have been identified based on its uniqueness of trait in the AICRP on pearl

millet trials from the compiled information from the published AICRP on pearl millet reports from 2001–2002 to 2018–2019. Small millet genotypes were identified based on its uniqueness of trait in the AICRP on small millet trials from the compiled information from the published AICRP on small millet reports from 2007–2008 to 2017–2018 viz., 3828 accessions of finger millet, 1000 accessions of foxtail millet genotypes, 775 accessions of barnyard millet genotypes, 1032 accessions of kodo millet genotypes, 483 accessions of proso millet genotypes, and 827 accessions of little millet genotypes. Sorghum genotypes were identified based on its uniqueness of trait in the AICRP on Sorghum from the compiled information from the published AICRP on Sorghum report from 2014 to 2020. Pedigree database on Sorghum: Elite Breeding Stocks: AICRP on Sorghum (1975–2022), Pedigree database on Small millets: Elite Breeding Stocks: AICRP on Small millets (1986–2022) and Pedigree database on Pearl millet: Elite Breeding Stocks: AICRP on Pearl millet (1981–2018) are documented to know-how the germplasm as the parental lines used in their respective crop improvement program. The distribution of millets germplasm to the bonafied users is documented and published in four volumes (2000–2007; 2008–2012; 2012–2015 and 2015–2021).

1.7 Challenges to Small Millet Germplasm Resources Management

- Cross pollinated nature of few of the small millets.
- The data in the form of passport data and characterization data is in huge volume and requires special efforts and funding to handle and use such huge quantum of data.
- Mechanisms to use the characterization data for use in crop improvement need to be developed.
- Often it is difficult to track the utilization of germplasm accessions supplied by the Genebank due to poor feedback mechanism.
- Platforms for combining and analyzing the data from the different domains are also essential, and can be adapted to the needs of projects or communities (Weise et al. 2020).
- More emphasis is required to characterize the germplasm resources at molecular level and special provisions are necessary to handle the generated data.
- Application of molecular tools in curation of Genebank collections with respect to minimizing potential duplications.

References

de Wet JM, Brink D, Rao K, Mengesha M (1983) Diversity in kodo millet, Paspalum scrobiculatum. Econ Bot 37:159–163

Elangovan, M., Venkatesh, K., Pandey, S., Pandey, C.D., 2022. International Year of Millets 2023: Opportunity forEnhancing the Use of Indian Millets Germplasm. Indian Journal of Plant Genetic Resources 35, 90–94.

Hariprasanna K, Agte V, Elangovan M, Patil J (2014) Genetic variability for grain iron and zinc content in cultivars, breeding lines and selected germplasm accessions of sorghum [Sorghum bicolor (L.) Moench]. Indian J Genet Plant Breed 74:42–49

Kumar A, Tomer V, Kaur A, Kumar V, Gupta K (2018) Millets: a solution to agrarian and nutritional challenges. Agric Food Secur 7:31

Lu H, Zhang J, Liu K, Wu N, Li Y, Zhou K, Ye M, Zhang T, Zhang H, Yang X et al (2009) Earliest domestication of common millet (Panicum miliaceum) in East Asia extended to 10,000 years ago. Proc Natl Acad Sci U S A 106:7367–7372

Reddy VG, Upadhyaya H, Gowda C (2007) Morphological characterization of world's proso millet germplasm collection. J SAT Agric Res 3:4

Sharma I, Tyagi B, Singh G, Venkatesh K, Gupta O (2015) Enhancing wheat production—a global perspective. Indian J Agric Sci 85:3–13

Singh K, Kumar S, Kumar SR, Singh M, Gupta K (2019) Plant genetic resources management and pre-breeding in genomics era. Indian J Genet Plant Breed 79:117–130

Upadhyaya HD, Gowda C, Buhariwalla H, Crouch J (2006) Efficient use of crop germplasm resources: identifying useful germplasm for crop improvement through core and mini-core collections and molecular marker approaches. Plant Genet Resour 4:25–35

Upadhyaya HD, Gowda C, Reddy VG, Singh S (2008) Diversity of small millets germplasm in genebank at ICRISAT. In: 5th International symposium on new crops and uses: their role in a rapidly changing world, 3–4 September, 2007. University of Southampton, Southampton, UK

Wanous M et al (1990) Origin, taxonomy and ploidy of the millets and minor cereals. Plant Varieties Seeds 3:99–112

Weise S, Lohwasser U, Oppermann M (2020) Document or lose it—on the importance of information management for genetic resources conservation in Genebanks. Plants 9:1050

Conservation and Utilization Status of Small Millets in Nepal

2

Krishna Hari Ghimire and Ram Prasad Mainali

Abstract

Millets are small seeded cereals grown for human consumption and animal feed in various African and Asian countries. They have an excellent nutritional properties and are less vulnerable to biotic and abiotic stresses compare to other major staples including rice, wheat, and maize. Major small millets grown in Nepal include finger millet, proso millet, and foxtail millet. There are thousands of germplasm accessions of these millets in Nepal either in ex situ or at on-farm conditions yet to be explored and utilized. These millets are neglected from policy makers, researchers, development workers, and even by farming communities. Conservation and utilization of these millets for their genetic improvement could increase food and nutrition security of the country. In this chapter, we introduce the nutritional significance and climate-resilient properties of these millets, highlight their production, conservation and utilization status in Nepal, and discuss challenges and opportunities for their genetic improvement in Nepalese context.

Keywords

Finger millet · Foxtail millet · Proso millet · Small millets · Nepal

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2.1 Small Millets and Their Significance in Nepal

Millets are the group of crops belongs to grass family characterized by small seeds. They are well known for their wide adaptability to diverse but adverse agro-ecological environments in the world, particularly Africa and Asia. Among the millets, crops with smaller plants and smaller seeds such as barn yard millet, finger millet, foxtail millet, kodo millet, little millet, proso millet, etc. belong to the group of small millets. Small millets can be grown in marginal environments with minimum level of inputs. Their grain has an excellent nutraceutical value since they are rich in minerals, calories, and proteins (Devi et al. 2014). All of these crops are less vulnerable to various biotic and abiotic adversities compared to other major food crops such as rice, wheat, and maize. Developing new varieties in small millets with modern approaches is in less priority so far for any national and international research programs because of bigger attention to major staple cereals such as rice, wheat, maize, etc. This is more evident in developing countries like Nepal. In the context of climate change, small millets are the crops of the future with great potentiality to cope with food and nutrition insecurity for the global population (Goron and Raizada 2015). Small millets have multiple significance in Nepalese context.

2.1.1 Small Millets for Food Security

Finger millet is the fourth most important food crops in Nepal in terms of area and production after rice, maize, and wheat. Finger millet is consumed mainly as *Dhindo* (porridge), *Roti* (pancake), and *Khole* (millet soup) (Gaihre et al. 2021) across the hills and high mountains. Proso millet and foxtail millet are consumed as *Khir* (pudding), *Bhaat* (boiled like rice) (Ghimire et al. 2017). In the most food deficit regions of the country such as mountain districts of Karnali and Far-western provinces, proso millet and foxtail millet have significant contribution in food security together with finger millet because growing rice and wheat is not possible in those areas due to adverse climatic conditions whereas transportation of food grains from outside is very expensive due to poor access to roads.

2.1.2 Small Millets for Health and Nutrition

All crops belonging to small millets are nutrient dense as compared to major staples since they are gluten-free and rich in minerals, micronutrients, vitamins, proteins, rare amino acids, and fibers (Table 2.1). They are the most important food crops of economically suppressed but physically hard working people. Due to increasing trend of non-communicable diseases like diabetes, hypertension, cholesterol etc., inclusion of small millets in the regular meal is also increasing in urban populations since they are getting more conscious to their health and aware on the health benefits of small millets.

Table 2.1 Comparative nutrient profile of small millets and major staples grown in Nepal (DFTQC 2017)

Commodity	Protein (g)	Fat (g)	Carbohydrate (g)	Minerals (g)	Fiber (g)	Energy (kcal)	Calcium (mg)	Phosphorus (mg)	Iron (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)
Foxtail millet	12.3	4.3	60.9	3.3	8	331	31	290	12.9	0.59	0.11	3.2
Finger millet	7.3	1.3	72	2.7	3.6	328	344	283	3.9	0.42	0.19	1.1
Proso millet	11	4.22	72.9	3.25	1	378	8	28.5	3.0	–	–	–
Rice	6.8	0.5	78.2	0.6	0.2	345	10	160	0.7	0.21	0.06	1.9
Maize	11.1	3.6	66.2	1.5	2.7	342	10	348	3.3	0.42	0.1	1.8
Wheat	12.1	1.7	69.4	2.7	1.9	341	48	355	4.9	0.49	0.17	4.3

2.1.3 Small Millets for Animal Feed

Finger millet grains and fermented by-products are used as good feedstuff to animals while its green as well as dried straw is utilized as highly nutritious forage for livestock with up to 60% digestible nutrients (Gupta et al. 2017). In Nepal, small millets are used in regular feed (*Kundo*) of draft, milk, and meat animals as well as poultry feed (*Daanaa*). Green and dry straw of these crops are used by Nepalese farmers as an integral part of their animal fodder.

2.1.4 Small Millets for Climate Resilience

Small millets are grown in marginal lands with no fertilizer, pesticides, and irrigation since they are less vulnerable to various biotic and abiotic adversities arisen due to climate change compare to other major food crops such as rice, wheat, and maize. Since they are hardy crops, they can tolerate unpredictable environmental change in dry mountain areas of Nepal. Most of the small millets are short duration crops, thus can be used as catch crops or crop insurance when there is low rainfall and rice transplanting is not possible, or even when rice crop is washed out by heavy flooding.

2.1.5 Small Millets for Organic Agriculture and Agro-Tourism

Small millets are grown in remote mountain areas thus by default organic since farmers don't use any chemical fertilizers and pesticides for these crops. Millets flour is used for the preparation of modern bakery products such as pancakes, cakes, biscuits, namkins, noodles, pasta, momos, sweets, etc. are also gaining popularity in recent years. The finger millet grain is used for making high-quality traditional liquors after fermentation. These crops have been an integral component of agro-tourism in Nepal due to its *dhindo* and high quality home-made wine *raksi* in the menus of hotels and restaurants.

2.2 Production Status

Globally, foxtail millet and finger millet ranked third and fourth among millet crops after sorghum and pearl millet (Upadhyaya et al. 2007). Precise data of area and production under each small millets are not available in many countries because the production statistics of these crops had often been clubbed with other millets (Upadhyaya et al. 2010). Finger millet is the fourth most important crop of Nepal after rice, maize and wheat in terms of area and production. A total of 326,443 t of finger millet is produced in 2021 from 265,401 ha area with average productivity of 1.23 t/ha (MoALD 2022). Area of finger millet has been static over last three decades but the production and productivity is slightly increased (Fig. 2.1). Proso millet is the

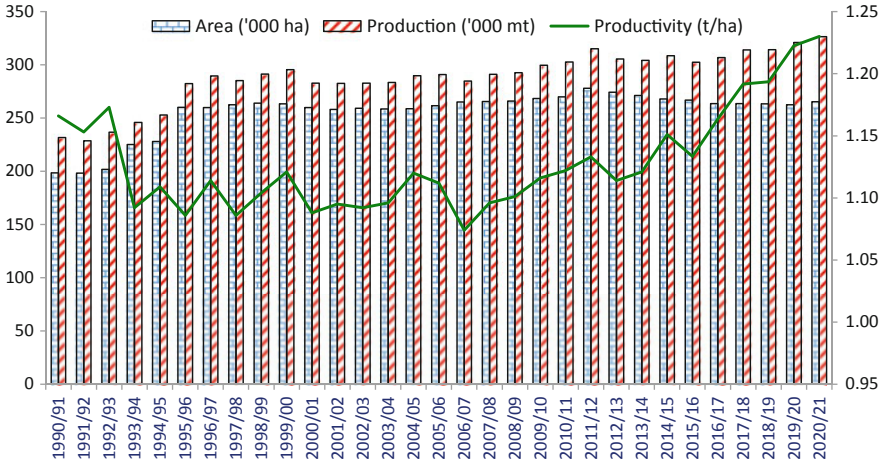

Fig. 2.1 Area, production, and productivity trend of finger millet in Nepal for the last three decades. (Source: MoALD 2022)

second important crop among millets, grown in around 2000 ha with the productivity of 0.81 t/ha whereas foxtail millet is grown in around 1500 ha with the productivity of 1.04 t/ha (DoA 2015). Production data of barn yard millet and little millet in Nepal is not available so far since they are growing in negligible area.

2.3 Conservation Status

2.3.1 Analysis of Conservation Threats

The four-cell analysis is a basic technique of understanding amount and distribution of traditional crop diversity at community level (Sthapit et al. 2006). This tool roughly estimates the risk of genetic diversity loss and the reasons why a species is in the risk zone (Joshi et al. 2020a; Dulloo et al. 2021). Based on the available national statistics and our own experience, we divided our five small millets into four cells (Fig. 2.2) to assess their abundance or conservation threats at national level. In our assessment, finger millet has very minimum threats since it is grown by many households in larger areas; however, many finger millet landraces are under threats since their cultivation is in declining trend. Proso millet is cultivated in larger areas but by less number of households or in particular niches. In contrast, foxtail millet is cultivated by more households than proso millet but in smaller areas across the country. Both proso millet and foxtail millet have moderate threat for their conservation and we need to prioritize these crops for on-farm conservation through community seed banks as well as ex situ conservation at the national and international Genebanks. The serious conservation threats are faced by little millet and barn yard millet since both of these crops are grown by very few number of households in

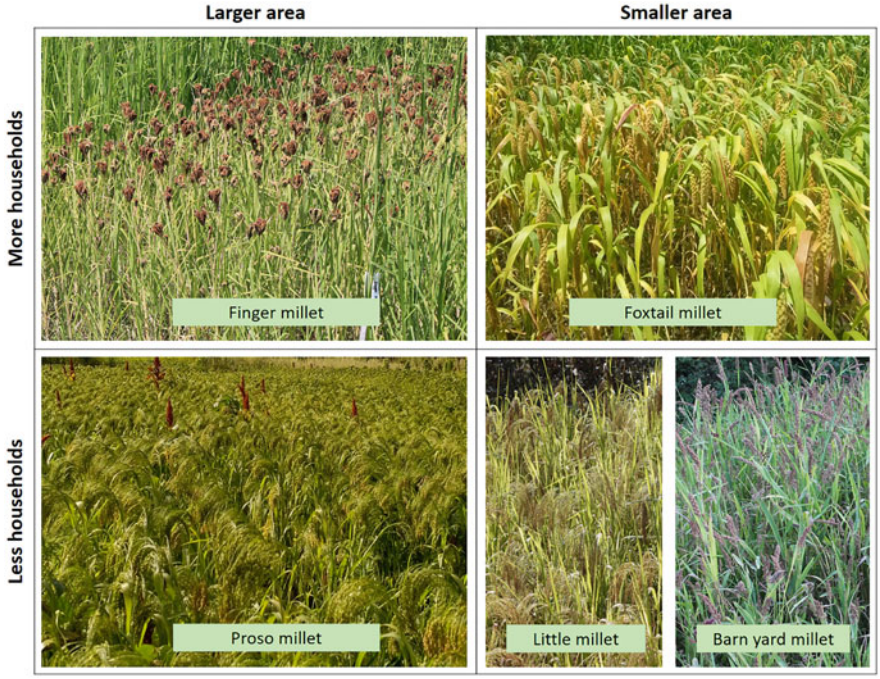

Fig. 2.2 Four-cell analysis of small millets grown in Nepal

very small parcels of their field. Our major focus for these two crops should be on exploration and collection, awareness raising and ex situ conservation. Replacement of landraces by new improved varieties is not so evident on small millets in Nepal but the threat is there since farmers are reluctant to grow either these crops or portfolio of landraces of these crops.

2.3.2 Inter and Intra-specific Diversity

Nepal has very wide ranges of altitudinal variation and millets can be grown from the lowest of 60 m up to 3500 m altitude. Similarly, the variation in land type, topography, rainfall, temperature, day length, etc. is also evident within the small boundary of the country. Due to these variations, there is high genetic diversity in small millets, especially in finger millet and foxtail millet. Among the millets, finger millet is the first important crop in Nepal in terms of area and production followed by proso millet and foxtail millet. Barn yard millet, little millet, and kodo millet are the other small millets that have been reported to be grown in parts and parcels of the country (Table 2.2). Not only species diversity, there is plenty of intra-specific diversity within finger millet and foxtail millet. Lower diversity of landrace is observed barn yard millet, little millet, and proso millet. Landraces are named based on grain color,

Table 2.2 List of small millets with their distribution and related species found in Nepal

S. No.	Common name	Nepali name	Scientific name	Distribution	Related species found in Nepal (Press et al. 2000)
1.	Finger millet	Kodo	*Eleusine coracana* (L.) Gaertn.	Widely distributed across the country including wild species *E. indica* (L.) Gaertn.	*E. indica* (L.) Gaertn.
2.	Foxtail millet	Kaaguno	*Setaria italica* (L.) P. Beauv.	Scattered in high hills of Karnali, Gandaki and Bagmati provinces	*Forbesiana*, *geniculata*, *glauca*, *pallidefusca*, *palmifolia*, *plicata*, *tomentosa*, *verticillata*, *viridis*
3.	Proso millet	Chino	*Panicum miliaceum* L.	Localized mainly in high hills of Karnali and Sudur pashchim province	*Antidotale*, *humidorum*, *notatum*, *paludosum*, *psilopodium*, *repens*, *sumatrense*, *trypheron*, *walense*
4.	Little millet	Dhaan kodo	*Panicum sumatrense* Roth. & Schult	Rarely found in small areas of Mid hills	
5.	Barn yard millet	Saamaa	*Echinochloa frumentacea* Link.	Rarely found in small areas of Mid hills	*Colona*, *crus-galli*, *crus-pavonis*, *glabrescens*, *picta*, *pyramidalis*, *stagnina*
6.	Kodo millet	Kodee	*Paspalum scrobiculatum* L.	Rarely found in small areas of Mid hills, mainly wild	*P. conjugatum* and *P. distichum*

shape and size of the heads/panicles, planting season, maturity duration, eating quality, etc. (Table 2.3).

2.3.3 Ex Situ Collection and Gaps

After the establishment of National Agriculture Genetic Resources Centre (Genebank) in 2010, collection and conservation of millets genetic resources got high priority. Among small millets, Nepal Genebank have the largest holdings of

Table 2.3 Conservation status of different millet species in Nepal Genebank and common landraces

Crop	Number of accessions	Name of common landraces
Finger millet	1175	Dalle, Jhapre, Chulthe, Laribari, Paundure, Thulo, Sano, Lurke, Lafre, Nangre, Nangkatuwa, Matyangre, Lampate, Asoje, Kattike, Mudke, Mangsire, Temase, Chaumase, Lekali, Pahenlo, Rato, Kalo, Seto, Dudhe, Jwain, Samdhi, Bhanchuwa, Chyalthe, Jhope, Tauke etc.
Foxtail millet	55	Kalo kaguno, Seto kaguno, Pahenlo kaguno, Rato kaguno, Maal kaguno, Bariyo, Aulel, Ande kaguno, Tinmase kaguno etc.
Proso millet	51	Kalo chino, Seto chino, Dudhe chino, Rato chino, Hade chino, Kaptede, Katibade, Kolte chino, Batale chino, etc.
Barn yard millet	6	Seto sama, Rato sama
Little millet	3	Dhan kodo, Suji kodo

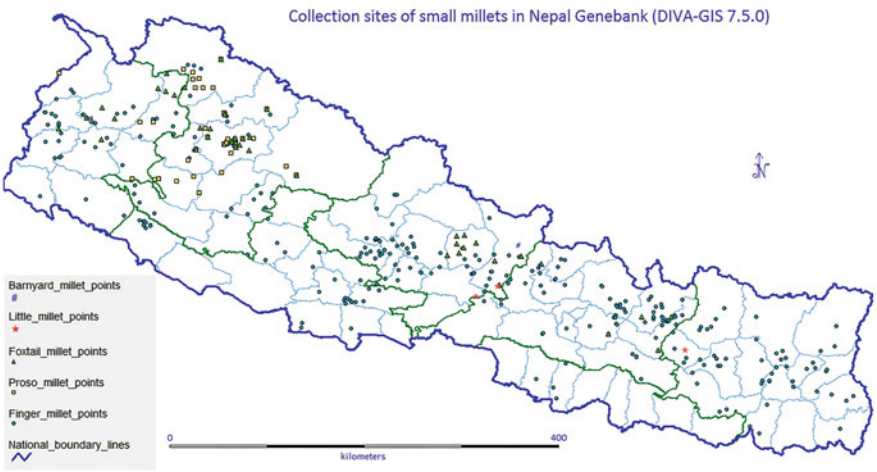

Fig. 2.3 Collection sites of finger millet accessions conserved in National Genebank, Nepal

finger millet accessions (1175 accessions) followed by foxtail millet (55 accessions) and proso millet (51 accessions) in medium and long-term conservation (Ghimire et al. 2017) (Table 2.3).

The geo-coordinates (latitudes and longitudes) of the collection sites of five small millet species have been plotted in the map of Nepal (Fig. 2.3). Finger millet accessions have been collected from far-east to far-west but we can still see the collection gaps from southern plane areas. The collections points for other four species are not distributed across the country. Future exploration and collection mission of Genebank need to be targeted in those gaps or unexplored areas.

2.3.4 In Situ and On-Farm Conservation

In situ conservation status of small millets has not been studied and documented properly but many wild relatives of *Echinochloa*, *Eleusine*, *Panicum*, *Paspalum,* and *Setaria* were reported in various in situ conservation sites (national parks, conservation areas, hunting reserves, wetlands, etc.) of Nepal as well as in semi-domesticated conditions alongside with the cultivated land (Table 2.2). However, these species are not being conserved purposefully but they are being used as forages for domestic and wild animals. Unlike wild species, cultivated species are being conserved on-farm either by individual custodian farmers (household Genebank) or with the collective efforts of the communities (community seed banks). There are more than 130 CSBs reported in the country (Joshi et al. 2018), one-third of them are actively involved in conservation of native crop diversity including landraces of small millets. However, exact number of small millets landraces under conservation in CSBs is not available.

2.4 Utilization Status

According to MoAD (2022), the average national productivity of finger millet since 31 years is roaming between 1.1 and 1.2 t/ha (Fig. 2.1). Available genetic resources have not been properly utilized in breeding programs. Research in the fourth important crop of the nation is not adequate and the situation is even far-below in other millets. Poor utilization of local landraces conserved in Genebank for the crop improvement program is due to either lack of information about the desirable accessions in the Genebank resulting from the poor characterization and evaluation data, or lack of skill manpower to use these landraces in breeding program as donors of desirable traits. In recent years, the demand of Genebank accessions is increasing slowly from scientists and students for research purpose.

2.4.1 Released Varieties

Number of released varieties is one of the indicators of utilization of any genetic resources. There are a total of eight varieties of small millets released officially by the National Seed Board for cultivation in Nepal, out of them, five are released varieties of finger millet and three are recently registered landraces, one each of finger millet, foxtail millet, and proso millet (Table 2.4). Among the eight in the table, the first two varieties Okhle-1 and Dalle-1 were released 42 years' ago.

2.4.2 Characterization and Pre-breeding

Only about 10% of genetic resources stored in Genebanks have been utilized in crop improvement program which mainly due to a lack of information about the desirable accessions in the Genebank resulting from the poor characterization and evaluation

Table 2.4 Details of finger millet varieties released in Nepal (Joshi et al. 2017; SQCC 2021)

Variety	Crop	Origin	Background	Released year	Major agro-morphological characters				Recommended domain
					Plant height (cm)	Days to maturity	Grain yield (t/ha)	Finger type	
Okhle-1	Finger millet	Nepal	Landrace from Okhaldhunga	1980	80	154–194	3.3	Compact	Mid to high hill
Dalle-1	Finger millet	India	IE-980	1980	110	125–151	3.3	Compact	Inner terai to mid hill
Kabre kodo-1	Finger millet	Nepal	Landrace from Surkhet	1990	82	147	2.3	Erect	Mid hill (900–1900 m)
Kabre kodo-2	Finger millet	India	GE-5176	2015	91	153	2.5	Open	Mid hill
Shailung kodo-1	Finger millet	India	GE-5016	2015	100	155	2.5	Compact	High hill (1500–2200 m)
Rato kodo	Finger millet	Nepal	Landrace from Jumla	2021	115	155	2.9	Compact	High hill (2000–3000 m)
Bariyo kaguno	Foxtail millet	Nepal	Landrace from Lamjung	2021	160	115–125	2.2	Compact drooping	Mid hill (900–1900 m)
Dudhe chino	Proso millet	Nepal	Landrace from Humla	2021	145	78–93	2.1	Drooping like rice	High hill (2000–3000 m)

Table 2.5 Number of small millets accessions characterized at the National Genebank, Khumaltar

Crop	Characterized accessions	Method	Diversity status	Reference
Finger millet	537	Morphological descriptors	High	Bhattarai et al. (2014)
	50	Morphological descriptors	High	Bastola et al. (2015)
	40	RAPD and SSR markers	High	Joshi et al. (2020b)
	300	Morphological descriptors	High	Ghimire et al. (2020)
Proso millet	44	Morphological descriptors	Low	Ghimire et al. (2018a)
Foxtail millet	44	Morphological descriptors	High	Ghimire et al. (2018b)

data (Nguyen and Norton 2020). A systematic study of collection, characterization, and evaluation of local millet landraces and introduced exotic varieties from Africa and India was carried out by Hill Crops Research Program (HCRP) after its establishment in 1972. During 1975–1995, more than 1000 accessions of different millet species were collected from various parts of the country and conserved at HCRP (Upreti 1995; Baniya et al. 2001). Unfortunately, those germplasm were lost due to firing of HCRP office building during political conflict period (Ghimire et al. 2017). After the establishment of Nepal Genebank in 2010, germplasm characterization of Nepalese small millet accessions has been restarted using agro-morphological descriptors. A total of 927 finger millet accessions and 44 accessions each of foxtail millet and proso millet have been characterized phenotypically (Table 2.5). A total of 300 Nepalese finger millet accessions collected from 55 districts of the country have been recently characterized at molecular level in International Crops Research Institute for Semi-Arid Tropics (ICRISAT) using genotyping by sequencing (GBS) approach but the result is not published yet.

2.4.3 Unique Genetic Resources

There are many landraces of different small millets with unique characters found in Nepal (Table 2.6). Landraces like Maal kaguno and Dhan kodo are unique and endangered but crops like barn yard millet, little millet and kodo millet etc. are endangered crops since farmers abandoned these crops to grow due to various factors such as lower yield, processing difficulty, high labor demanding, and rice food culture.

Table 2.6 Some unique landraces of small millets with their characteristics (Ghimire et al. 2017)

Crop	Landrace name	Distribution	Unique/ endangered	Characteristic features
Finger millet	Samdhi kodo	Scattered in small areas of lower hills	Endangered	White color seeds, white color porridge, prestigious due to color
Finger millet	Paaundur kodo	Rarely found in lower hills	Endangered	Adapted to spring season cultivation, drought tolerant, good in back pain for human and animals
Finger millet	Raato kodo	Localized in Jumla	Unique	Red color seeds, early maturing, adapted to high altitudes (>2000 m)
Finger millet	Asoje kodo	Scattered in eastern hills	Unique	High yielding, early maturity
Finger millet	Nangkatuwaa kodo	Scattered in central and western hills	Unique	High yielding, easy picking with nails
Foxtail millet	Aule kaaguno	Localized in Jumla	Unique	Typical finger-like branching at the tip of panicles
Foxtail millet	Bariyo kaaguno	Localized in Lamjung	Unique	High yielding, attractive panicles, early maturing
Foxtail millet	Maal kaaguno	Localized in Gorkha	Endangered	Medicinal value, good for lactating animals, effective in mastitis control
Foxtail millet	Kaalo kaaguno	Localized in Humla	Unique	Black color grains, medicinal value, drought tolerant
Proso millet	Dudhe chino	Localized in Humla	Unique	High yielding, drought tolerant, white color seeds
Proso millet	Haade chino	Localized in Humla	Unique	Good taste, drought tolerant, red color seeds
Barn yard millet	Saamaa	Rarely found in mid hills	Endangered	Considered as holy grains, consumed in fasting as fruits, difficult for processing
Little millet	Dhaan kodo	Rarely found in mid hills	Endangered	Drought tolerant, small oval seeds with shiny brown color

2.5 Promotional Initiatives

We have discussed that small millets are in low priority of the nation compared to major food crops. However, finger millet is getting significant attention from both government and non-government sectors since five decades. Some initiatives from public and private sectors for the promotion of small millets in Nepal have been briefly discussed in this section.

2.5.1 Hill Crops Research Programme (HCRP)

Ministry of Agriculture Development established HCRP in Dolakha of eastern Nepal in 1972 with the research mandate of finger millet, buckwheat, barley, grain amaranth. Despite of inadequate human as well as financial resources, HCRP under the umbrella of Nepal Agricultural Research Council (NARC) is working as lead public institution for small millets research and released five finger millet varieties together with their quality seeds and package of practices.

2.5.2 National Agriculture Genetic Resources Centre (National Genebank)

Ministry of Agriculture Development established the National Genebank in Lalitpur in 2010 with the mandate of promoting conservation and use of agricultural biodiversity including small millets. Besides ex situ repository, the National Genebank has been working for characterization and pre-breeding, support farming communities for on-farm conservation and support the Government of Nepal for the development of enabling policy environment for agro-biodiversity conservation. The National Genebank has been collaborating with national non-government organization like Local Initiatives for Biodiversity Research and Development (LI-BIRD) and international organizations like Bioversity International, ICRISAT, etc. for the promotion of native crops including small millets and facilitate CSBs to register three landraces of small millets.

2.5.3 Millet Mission Program

The Department of Agriculture (DoA) launched Millet Mission Program in 24 hill districts from 2013 to promote small millet crops for food security of hill farmers. Collection of germplasm from project districts and submission in the National Genebank, distribution of quality seeds and processing machines to the farmers in subsidized rate, awareness creation programs including training on value chain and product diversification were the key activities of the program. Unfortunately, this mission is terminated due to the inadequacy of improved quality seeds and machines.

2.5.4 Native Crops and Organic Agriculture Promotion Program

The Centre for Crop Development and Agro-biodiversity Conservation (CCDABC) of DoA is running Native Crops Promotion Program and Organic Agriculture Promotion Program since 2018 in hill districts in collaboration with provincial and local governments. Both of these programs have small millets in their mandate crops.

2.5.5 Global Collaborative Projects (In-Situ, NUS, LCP)

The National Genebank-NARC, LI-BIRD, and Bioversity International have successfully implemented three important global projects namely In-Situ Conservation Project (1998–2006), Neglected and Underutilized Species (NUS) (2005–2009) and Local Crops Project (LCP) (2014–2019). All these projects prioritized small millets mainly finger millet, foxtail millet, and proso millet as their mandate crop. These projects were able to create awareness at local and national level, enhance potential landraces, deploy portfolio of varieties from Genebank to farming community, empower farmers and CSBs on variety and seed selection, processing, value addition, and product diversification.

2.6 Problems and Challenges

Although small millets are resilient to adverse environments, there are some biotic factors (neck and finger blast diseases in finger millet and stem borers and birds in proso and foxtail millet) and abiotic factors (drought, cold, etc.). There are some socio-cultural factors affecting small millets production such as rice-based food habit, detraction of youth population from small millets cultivation and consumption, youth migration, etc. Farmers are reluctant to grow small millets due to their low productivity per unit area, less profit, high labor demanding (finger millet), difficulties in processing (proso millet, barn yard millet, little millet), etc. Inadequate research on these crops to develop high yielding and disease resistant varieties leaving less varietal options to the millet growing farmers. Research on processing equipment, value addition, and product diversification is also limited. Lack of policy environments such as subsidy to farmers growing these crops also hindering the production of small millets in the country. Besides these challenges, there are great opportunities to promote these small millets as future smart foods.

2.7 Way Forward

- The National Genebank is holding thousands of accessions of different small millet species; however, there are still unexplored areas and landraces exists on-farm. Exploration of such areas with close collaboration with farmers, community seed banks, and local governments needs to be continued.
- There is an urgent need of safety duplication of collected accessions in international Genebanks like Global Seed Vault-Norway, ICRISAT-India, etc. so that we can repatriate them as and when needed.
- Most of the millet genetic resources conserved so far have not characterized so far at molecular level. There is an urgent need to characterize and sequence those accessions using high throughput DNA technology in collaboration with international institutes such as ICRISAT.

- The research mandate of small millets in Nepal is for HCRP of NARC. Since this station is not suitable for foxtail millet and proso millet, Agriculture Research Station (ARS) Jumla should be mandated for foxtail and proso millets while HCRP should be dedicated for finger millet.
- Increased research funding is necessary for institutional as well as researcher's capacity enhancement. Moreover, there is an urgency for the deployment of multidisciplinary team of scientists in HCRP and ARS Jumla without further delay.
- Nepalese farmers are facing the problem of improved and quality seed of small millets. Seed multiplication of promising and locally adapted landraces should be started through Community Seed Banks or farmers groups and mainstreamed as in major cereal crops.
- Conservation through utilization is the key for any genetic resources. To enhance the use of small millets in Nepal, awareness creation and promotional activities should be launched with improved processing technology, value addition, product diversification, and inclusion of small millets in national food and nutrition programs.

References

Baniya BK, Sharma DR, Mandal DN (2001) Local germplasm collection, characterization, evaluation and documentation of small millets. Agriculture Botany Division and Hill Crops Research Program, NARC, Nepal

Bastola BR, Pandey MP, Ojha BR, Ghimire SK, Baral K (2015) Phenotypic diversity of Nepalese finger millet (*Eleusine coracana* (L.) Gaertn) accessions at IAAS, Rampur, Nepal. Int J Appl Sci Biotechnol 3(2):285–290. https://doi.org/10.3126/ijasbt.v3i2.12413

Bhattarai M, Ghimire KH, Joshi BK, Bhatta MR (2014) Characterization of finger millet (*Eleusine coracana* Gaertn.) germplasm with agro-morphological markers. In: Proceedings of the 27th National Summer Crops Workshop, vol II. Nepal Agricultural Research Council, pp 184–189. https://opac.narc.gov.np/opac_css/index.php?lvl=notice_display&id=13547&seule=1

Devi PB, Bharathi VR, Sathyabama S, Malleshi NG, Priyadarisini VB (2014) Health benefits of finger millet (*Eleusine coracana* L.) polyphenols and dietary fiber: a review. J Food Sci Technol 51:1021–1040. https://doi.org/10.1007/s13197-011-0584-9

DFTQC (2017) Nepalese food composition table 2017. Department of Food Technology and Quality Control, Ministry of Agricultural Development, Kathmandu, Nepal. http://www.dftqc.gov.np/downloadsdetail/6/2020/45564816/

DoA (2015) Annual Report 2070/071. Millet Mission Program, Regional Agriculture Directorate, Surkhet, Department of Agriculture, Nepal

Dulloo ME, Carmona NE, Rana JC, Yadav R, Grazioli F (2021) Varietal threat index for monitoring crop diversity on farms in five agro-ecological regions in India. Diversity 13:514. https://doi.org/10.3390/d13110514

Gaihre S, Gauchan D, Timsina KP (2021) Prospect and potentiality of finger millet in Nepal: nutritional security and trade perspective. J Agric Nat Resour 4(2):63–74. https://doi.org/10.3126/janr.v4i2.33657

Ghimire KH, Bhandari B, Gurung SB, Dhami NB, Baniya BK (2017) Diversity and utilization status of millets genetic resources in Nepal. In: Joshi BK, Khatri-Chhetri HB, Acharya AK (eds) Proceedings of 2nd national workshop on conservation and utilization of agricultural plant genetic resources in Nepal, 22–23 May 2017, Dhulikhel. NAGRC, FDD, DoA and MoAD,

Nepal. https://scholar.google.com/scholar?hl=en&as_sdt=0,5&cluster=15818782615603068207

Ghimire KH, Joshi BK, Dhakal R, Sthapit BR (2018a) Diversity in proso millet (*Panicum miliaceum* L.) landraces collected from Himalayan Mountains of Nepal. Genet Resour Crop Evol 65:503–512. https://doi.org/10.1007/s10722-017-0548-7

Ghimire KH, Joshi BK, Gurung R, Sthapit BR (2018b) Nepalese foxtail millet genetic diversity revealed by morphological markers. Genet Resour Crop Evol 65:1147–1157. https://doi.org/10.1007/s10722-017-0602-5

Ghimire KH, Gurung SB, Mahat PM, Karkee A, Gauchan D, Joshi BK, Ghimire SK, Manandhar HK, Pandey MP (2020) Agro-morphological diversity in Nepalese finger millet landraces. Traditional crop biodiversity for mountain food and nutrition security in Nepal. In: Gauchan D, Joshi BK, Bhandari B, Manandhar HK, Jarvis DI (eds) Tools and research results of the UNEP/GEF Local Crop Project, Nepal. NAGRC, LI-BIRD and the Alliance of Bioversity International and CIAT, pp 55–64. http://himalayancrops.org/project/traditional-crop-biodiversity-for-mountain-food-and-nutrition-security-in-nepal/

Goron TL, Raizada MN (2015) Genetic diversity and genomic resources available for the small millet crops to accelerate a new green revolution. Front Plant Sci 6:1–17. https://doi.org/10.3389/fpls.2015.00157

Gupta SM, Arora S, Mirza N, Pande A, Lata C, Puranik S, Kumar J, Kumar A (2017) Finger millet: a "certain" crop for an "uncertain" future and a solution to food insecurity and hidden hunger under stressful environments. Front Plant Sci 8:643. https://doi.org/10.3389/fpls.2017.00643

Joshi BK, Bhatta MR, Ghimire KH, Khanal M, Gurung SB, Dhakal R, Sthapit BR (2017) Released and promising crop varieties of mountain agriculture in Nepal (1959–2016). LI-BIRD, NARC and Bioversity International, Nepal. https://cgspace.cgiar.org/bitstream/handle/10568/80892/

Joshi BK, Ghimire KH, Shrestha DS (2018) The National Genebank's promotion of community seed banks: status and strategy. In: Joshi BK, Shrestha P, Gauchan D, Vernooy R (eds) Community seed banks in Nepal. Proceedings of the 2nd national workshop on CSB, Kathmandu. NAGRC, LI-BIRD and Bioversity International. https://libird.org/wp-content/uploads/2022/04/Community-Seed-Banks-in-Nepal.pdf

Joshi BK, Gauchan D, Sapkota S, Poudyal K, Ghimire KH, Dongol DMS (2020a) Germplasm rescue and rebuilding local seed systems in red zone areas. J Agric Nat Resour 3(2):9–20. https://doi.org/10.3126/janr.v3i2.32294

Joshi BK, Joshi D, Ghimire SK (2020b) Genetic diversity in finger millet landraces revealed by RAPD and SSR markers. Nepal J Biotechnol 8(1):1–11. https://doi.org/10.3126/njb.v8i1.30204

MOALD (2022) Statistical information on Nepalese agriculture, 2077/78 (2020/2021). Government of Nepal, Ministry of Agriculture and Livestock Development, Kathmandu, Nepal. https://www.moald.gov.np/publication-types/agriculture-statistics/

Nguyen GN, Norton SL (2020) Genebank phenomics: a strategic approach to enhance value and utilization of crop germplasm. Plants 9:817. https://doi.org/10.3390/plants9070817

Press JR, Shrestha KK, Sutton DA (2000) Annotated checklist of the flowering plants of Nepal. The Natural History Museum, London. http://www.efloras.org/flora_page.aspx?flora_id=110

SQCC (2021) Notified and denotified varieties of different crops in Nepal (till July 2021). Seed Quality Control Center, Ministry of Agriculture and Livestock Development, Government of Nepal, Lalitpur. https://www.sqcc.gov.np/pages/publications

Sthapit BR, Rana RB, Subedi A, Gyawali S, Bajracharya J, Chaudhary P, Joshi BK, Sthapit S, Joshi KD, Shrestha P (2006) Participatory four-cell analysis (FCA) for understanding local crop diversity. In: Sthapit BR, Shrestha P, Upadhyay MP (eds) On-farm management of agrobiodiversity in Nepal: good practices. NARC, LIBIRD and Bioversity International, Nepal, pp 13–16. http://www.nuscommunity.org/fileadmin/templates/nuscommunity.org/upload/documents/Publications/2011-2014/2012_Sthapit_et_al_Bioversity_International.pdf

Upadhyaya HD, Gowda CLL, Reddy GV (2007) Morphological diversity in finger millet germplasm introduced from southern and eastern Africa. J SAT Agric Res 3(1):1–3. http://oar.icrisat.org/2656/1/Morphological_diversity_in_finger.pdf

Upadhyaya HD, Sarma NDRK, Ravishankar CR, Albrecht T, Narasimhudu Y, Singh SK, Varshney SK, Reddy VG, Singh S, Dwivedi SL, Wanyera N, Oduori COA, Mgonja MA, Kisandu DB, Parzies HK, Gowda CLL (2010) Developing a mini-core collection in finger millet using multilocation data. Crop Sci 50:1924–1931. https://doi.org/10.2135/cropsci2009.11.0689

Upreti RP (1995) Status of millet genetic resources in Nepal. Plant genetic resources, Nepalese perspective. NARC/IPGRI

Quality Seed Production of Small Millets

3

R. Siddaraju, Parashivamurthy, and M. S. Harish

Small-grained grains called millets are a mainstay in sections of Europe, Africa, and Asia's dry and semi-arid areas. The smallest of them are finger millet, kodo, foxtail, proso, tiny, and barnyard millets (Fig. 3.1). These tiny grains have great nutrition and offer low-cost sources of protein, minerals, and vitamins to all societies. They have an endless storage life and are nearly pest-free in storage. Small millets have the potential to develop into future food crops because of their high nutritional value and untapped seed output potential, especially in rainfed regions.

Small millets are mostly cultivated in diverse soils, climates, and harsh environments. The cultivation of these millets is also done with minimum inputs. By minimum interventions, it is possible to increase the seed yields substantially. One of the important intervention in millet cultivation is to provide good quality seeds. This section of the book provides quality seed production techniques in small millet crops.

3.1 Seed Production in Finger Millet

More than 25 nations in Asia and Africa plant finger millet, often known as ragi in India. With over 12% of the world's millet area under its cultivation, it is one of the most significant millet crops in the tropics. Its cultivation begins at sea level and continues up to higher altitudes, such as the Himalayas. Its botanical name is *Eleusine coracana* (L.) Gaertn is a member of the Poaceae family. The inflorescence of the crop is a panicle with 2–11 digitate whorls that are either straight or slightly

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Fig. 3.1 Different small millets

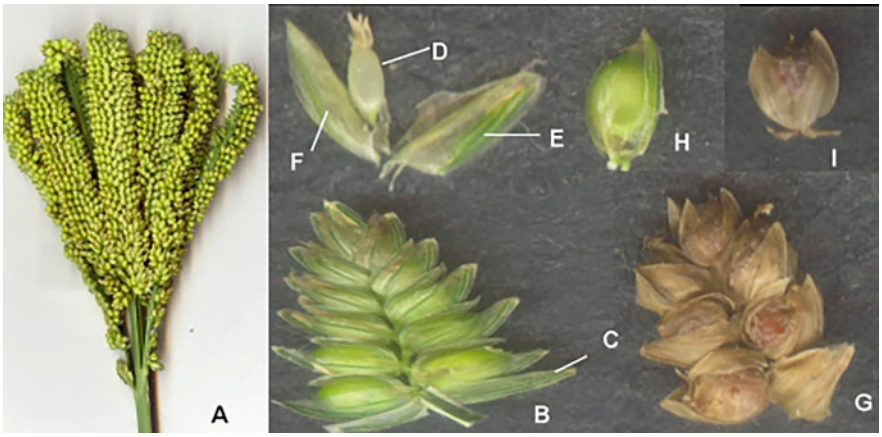

Fig. 3.2 Finger millet inflorescence and spikeleta. (**a**) Inflorescence; (**b**) Spikelet; (**c**) Outer glume; (**d**) Ovary; (**e**) Lemma; (**f**) Palea; (**g**) Matured spikelet; (**h**) Grain with in lemma and palea; (**i**) Matured grain with in lemma and palea

bent. On one side of the rachis, each spike has 50–70 alternately arranged spikelets. Three to 13 florets are seen in each spikelet. The anthers are larger than the filaments, which are quite short. The seed is small and light brown to brick red in color (Fig. 3.2).

3.1.1 Floral Biology

In contrast to spikelets, which open from the top down, individual florets inside them open from the bottom up. The third day after flowering starts is when the majority of flowers bloom, and the process takes 5–7 days to finish. Between 1:00 and 5:00 a.m., anthesis takes place. At the same moment as the lemma and palea start to gape, the stigma and anthers appear. Before the florets open, the anthers divide lengthwise. The anther pollinates its own stigma, and during dehiscence, both the sticky stigma and anther attain the same height. Pollens last for 20 min; however, the stigma is still open for 5 h. In finger millet, out-crossing is limited to 1–2%.

3.1.2 Stages of Seed Production

Breeder seed–Foundation seed–Certified seed

3.1.3 Seed Production Techniques

3.1.3.1 Land Requirement

The area needed to grow finger millet seeds should be clear of stray plants. It should not have been the same crop or a different variety of the same crop in the previous season. Choose fertile soil and avoid problematic soil.

3.1.3.2 Cultural Practices

Main Field Preparation

The main field is prepared by 2–3 plowings to make it fine tilth. During land preparation, apply the compost or farmyard manure at 5 t/acre (12.5 t/ha) and incorporate into the soil, 15 day before sowing or transplanting of the crop.

Time of Sowing

Finger millet is a season bound crop and the best season for sowing is June–July and December–January to harvest the higher seed yield and to maintain quality of seeds.

Seed Material

The seed used for raising a seed crop should be of known its genetic purity, appropriate class, and obtained only from an authorized agency. During seed purchasing, carefully examine the following factors.

- For raising a foundation seed crop, a breeder's seed is required and for raising a certified seed crop, a foundation seed is required and seed should be purchased from authentic source.
- That the tag and seal of breeders/foundation seed bags purchased are should be intact.

- That the validity period has not expired.
- That all the bags are of the same variety.

Seed Rate and Pre-sowing Seed Treatment

Recommendedseed rate is at 2 kg/acre (5 kg/ha). Selected seeds should be treated with *Azospirillum* at 125 g/kg of seeds.

Nursery Preparation

Nursery plots for planting 1 hectare of field require at least 500 m^2. The soil should be plowed two to three times to get fine tilth. Seeds should be broadcasted and covered with a thin layer of 500 kg/ha (200 kg/acre) farmyard manure. Watering should be done immediately after sowing.

Method of Sowing

Twenty- to 25-day-old seedlings should be moved to the main field. Per hill, two seedlings should be sown. The transplants should be gently taken out of the nursery and placed on a damp surface. They should then be tightly covered after being positioned immediately in a damp furrow. The transplants must not be allowed to dry up and must be seeded as soon as they are removed from the nursery. If the soil hasn't been watered before, it has to be watered right away and frequently for a few days until the plants start to take root.

Spacing

Optimum spacing for finger millet should be 30 cm between the rows and plant to plant is 15 cm.

Fertilization

Generally, fertilizer recommended to get a good crop in rain fed condition is at 40: 20:20 kg NPK/ha, and for irrigated is 100:50:50 kg NPK/ha.

3.1.3.3 Irrigation

Adequate moisture must be ensured at tillering and flowering, both are critical stages of the crop growth and crop should not allow wilting. During heavy rains the excess water from the field should be drained out.

3.1.3.4 Weed Control

Weeding must be done on schedule, and cross-cultural interactions must take place. While plants are still young, particularly 35–40 days after sowing, weeds must be suppressed. After planting, the first weeding should be done 15 days following, and the second one 30 days afterwards. In rainfed and irrigated areas, it was suggested to employ isoproturon at 0.5 kg a.i./ha and oxyflurofen at 0.1 L a.i./ha, respectively, for pre-emergence soil treatment.

3.1.3.5 Plant Protection

Control measures as per the following schedule should be adopted in seed fields.

Disease control	
Name of the disease	Control measures
Blast	Since seeds are the main vehicle for transmission of the illness, treating seeds with tricyclazole (8 g/kg seed) and applying sprays made from two plant extracts, notchi and *Prosopohis* sp., is beneficial. Two sprays of ediphenphos, kitazin, propiconazole (0.1%), carbendazim, or tricyclazole (0.05%) are sufficient during the late stages of the crop. The first should be administered when the ear emerges, and the second should be applied 10 days later, or with a 0.05% Carbendazim or tricyclazole initial spray, followed 10 days later by a 0.2% Mancozeb spray
Seedling blight	Pre-emergence damping off seedling blight can be completely controlled by seed treatment with Agrosan G.N. Mancozeb can be sprayed at a dosage of 0.2% to control the illness and minimize infection

Insect control	
Name of the insect	Control measures
Pink stem borer of Ragi	Plow deeply soon after harvest to destroy the eggs and pupae, apply neem cake at 42–50 kg/ha as basal manure, use pheromone traps to attract and destroy male adult moths, and use *Trichograma* cards
Aphids of ragi (*Rhopalosiphum maidis*), Root aphid of Ragi	Spray garlic extract (100 g crushed and mixed with 50 L of water) or apply manure prepared using *Adhatoda vasica*
Ear head caterpillar of ragi	Managed by planting crops in early *kharif* season

3.1.3.6 Field Inspection

Minimum of two inspections shall be made to maintain quality of seeds, the first field inspection should be during flower and the second at maturity and prior to harvesting to estimate the yield.

3.1.3.7 Rouging

It may be necessary to perform two or three rouging sessions to bring the seed plot up to seed certification standards. The first rouging session should be carried out just prior to or during the flowering stage. It is crucial that this rouging is done promptly to eliminate any off-type plants, including plants of different colors, objectionable weed plants, and designated diseased plants. The seed field must meet specific requirements.

Specific Requirements

	Maximum permitted (%)[a]	
Factor	Foundation	Certified
Off-types	0.05	0.10

[a] Maximum permitted at final inspection

3.1.3.8 Harvesting and Threshing

Harvesting should be done in two pickings to ensure that only physiologically mature ear heads are harvested. These mature ear heads should then be threshed with bamboo sticks and the grains further cleaned by winnowing.

3.1.3.9 Drying and Storage

The seeds should be sun-dried until they reach a safe moisture level of 12%. Care should be taken to avoid mechanical damage and contamination during the seed drying and seed process. The seeds can be stored for up to 13 months under ambient storage conditions.

3.1.3.10 Seed Standards

	Standards for each class	
Factor	Foundation	Certified
Pure seed (minimum)	97.0%	97.0%
Inert matter (maximum)	3.0%	3.0%
Other crop seeds (maximum)	10/kg	20/kg
Weed seed (maximum)	10/kg	20/kg
Germination (minimum)	75%	75%
Moisture (optimum)	12.0%	12.0%
Vapor proof containers (maximum)	8.0%	8.0%

3.2 Seed Production in Foxtail Millet or Italian Millet

The second-most produced millet in the world is foxtail millet. Due to its adaptability to different altitudes and drought tolerance, it may thrive in poor soils. It is also planted on dry ground and as a short-term catch crop. *Setaria italica* (L.) Beauv is the botanical name for it.

3.2.1 Floral Morphology

The foxtail millet inflorescence is a terminal spike that droops and is made up of a main stalk, shorter side branches, spikes, and bristles. Each spikelet bears a pair of glumes that encircle two small flowers, the top one of which is fertile or bisexual and has three stamens, an oval, smooth ovary, two long styles, and feathery tips. The lower flower is sterile. The thickened lemma and palea surround the oval, glossy, and tightly confined grain, which ranges in color from cream to orange, golden brown to black (Fig. 3.3).

Fig. 3.3 Foxtail millet inflorescence and its parts. (**a**) Foxtail inflorescence; (**b**) Spikelet's cluster; (**c**) Subtended spikelet; (**d**) Opened spikelet; (**e**) Outer glume; (**f**) Grain enclosed in lemma and palea; (**g**) Grain

3.2.2 Floral Biology

Foxtail millet, which barely makes up 1.4–4% of all crops, is a highly self-pollinating, non-crossing crop. The flowers below the apex of a head open when three-fourths of the head emerge from the sheath. Flowering in a head happens from top to bottom. A head will fully blossom between 8 and 16 days. Foxtail millet's anthesis happens at night and in the morning; however the exact time depends on the environment.

3.2.3 Stages of Seed Production

Breeder seed–Foundation seed–Certified seed

3.2.4 Seed Production Techniques

3.2.4.1 Land Requirement

Any field that wasn't previously used to cultivate foxtail millet of any kind, and it should be clear of stray plants. The chosen field should have good drainage and aeration of the soil.

3.2.4.2 Cultural Practices

Main Field Preparation

After plowing with MB plow once, the land should be harrowed once, or it should be plowed twice with local plow. When you are plowing, add compost or farmyard manure at a rate of 5 t/acre (12.5 t/ha), then work it into the soil 15 days before planting.

Time of Sowing

States have different seasons because of their unique environments. July is the season in Tamil Nadu, July to August in Karnataka, the first 2 weeks of July in Andhra Pradesh and Telangana, and the third week of July in Maharashtra. In Tamil Nadu, summer crops should be produced as irrigated crops in January, while kharif crops should be planted between the beginning of June and the end of July.

Source of Seed

Use a proper class of seed and only receive seed from sources that may be trusted (Foundation seed for certified seed production). High-quality, healthy seeds with a high germination rate are ideal.

Seed Rate and Pre-sowing Seed Treatment

It is advised to use 2 kg/acre (5 kg/ha) of seed. Azospirillum should be applied to selected seeds at a rate of 125 g/kg of seeds. To avoid soil and seed borne illnesses during the initial establishment of the crop, seed should be treated with Ceresan at 3 g/kg of seed.

Sowing Method and Spacing

Treated seeds should be sown at 30 cm of row spacing and at 10 spacing between the plants in 3–4 cm depth.

Fertilization

Generally, 40 kg N, 20 kg P_2O_5, and 20 kg K_2O/ha of fertilizer are advised for healthy crops. The full amount of phosphorus and potash and half of the nitrogen at planting and the other half 30 days later is applied. The fertilizer recommended for Karnataka is 30:15:0 NPK (kg/ha).

3.2.4.3 Irrigation

Although crops during the Kharif season are rain-fed, 1–2 irrigations might increase production if there is a dry spell. Depending on the soil and climate, summer crops require 2–5 irrigations. After significant rainfall, extra water should be drained out.

3.2.4.4 Weed Control

In line-sown crops, two rounds of inter-cultivation and one round of manual weeding are advised for higher seed yields. Two hand weeding sessions are recommended for broadcast crops, as well as the post-emergence treatment of 2,4-D sodium salt (80%) at 1.0 kg a.i./ha at 20–25 DAS. Isoproturon pre-emergence spray at 1.0 kg a.i./ha is also effective in controlling weeds.

3.2.4.5 Plant Protection

Control measures as per the following schedule should be adopted in seed fields.

Disease control	
Name of the disease	Control measures
(a) Downy mildew	Removal of crop residues from the field. Fungicidal seed treatment or spray of Ridomil-MZ at 3 g/L water control the disease
(b) Rust	A foliar spray of Mancozeb at 0.2% effectively controls the rust

Insect control	
Name of the insect	Control measures
Shoot fly and its control	Apply *Carbofuran* 3G granules at 20 kg/ha in furrows or as broadcast before sowing in the soil at the time of field preparation

3.2.4.6 Field Inspection

A Seed Certification Officer should conduct two inspections between the flowering and maturity stages. The first inspection checks isolation and off-types during flowering, while the second inspection estimates yield and checks off-types prior to harvest.

3.2.4.7 Rouging

Rouging should be done regularly to remove off-types, volunteer plants, and diseased plants from the seed production plots in order to prevent genetic contamination. Critical roguing should be done until the flowering stage, and the seed field must meet specific requirements as detailed below.

Specific Requirements

	Maximum permitted (%)[a]	
Factor	Foundation	Certified
Off-types	0.05	0.10

[a] Maximum permitted at field inspection

3.2.4.8 Harvesting and Threshing

Harvesting occurs when ear heads are physiologically mature, typically 80–100 days after sowing. The heads can be harvested with the plant or alone and must be dried before threshing. Threshing is done by stone roller or trampling, and the grains are cleaned by winnowing.

3.2.4.9 Drying and Storage

The seeds must be dried under the sun until they reach a safe moisture level of 12%. It is important to handle them with care during the seed drying and seed processing to prevent mechanical mixture or contamination.

3.2.4.10 Seed Standards

Factor	Standards for each class	
	Foundation	Certified
Pure seed (minimum)	97.0%	97.0%
Inert matter (maximum)	3.0%	3.0%
OCS (maximum)	10/kg	20/kg
Weed seed (maximum)	10/kg	20/kg
Germination (minimum)	75%	75%
Moisture (maximum)	12.0%	12.0%
Vapor-proof container (maximum)	8.0%	8.0%

3.3 Seed Production in Kodo Millet

Kodo millet, botanically known as *Paspalum scrobiculatum* L., is a long-duration cereal crop that thrives in shallow and deep soils of India.

3.3.1 Floral Morphology

The inflorescence of kodo millet consists of two to six racemes that spread widely along a sub-digitate or short axis. The spikelets are often sessile or on a short pedicel, and the racemes are 3–15 cm long. Some spikelets are paired in the center of the raceme, and they are arranged in two rows on a flattened rachis (Fig. 3.4). The rachis has scabrous borders and is ribbon-like, measuring 1.5–3 mm broad. The tall and short pedicelled series of spikelets are alternately placed. There is no glume I, and glume II is the same length as the spikelet. Lemma II encloses both florets, but lemma I is essentially identical to glume II. The upper floret in the spikelet is a hermaphrodite flower, whereas the lower one is sterile and reduced to the valve. Grain is protected by tough, horny, persistent husks.

3.3.2 Floral Biology

Kodo millet is cleistogamous, with less than 20% of open flowers, leading to self-pollination. Spikelets open from the middle of the raceme and gradually spread to both ends, between 2:30 a.m. to early morning. Artificially manipulating the tight lemma damages the flower. Protogyny has been observed in some cultures of Kodo millet like “IPS 147,” “IPS 197,” “IPS 427,” but apomixis has not been reported.

3.3.3 Stages of Seed Production

Breeder seed–Foundation seed–Certified seed

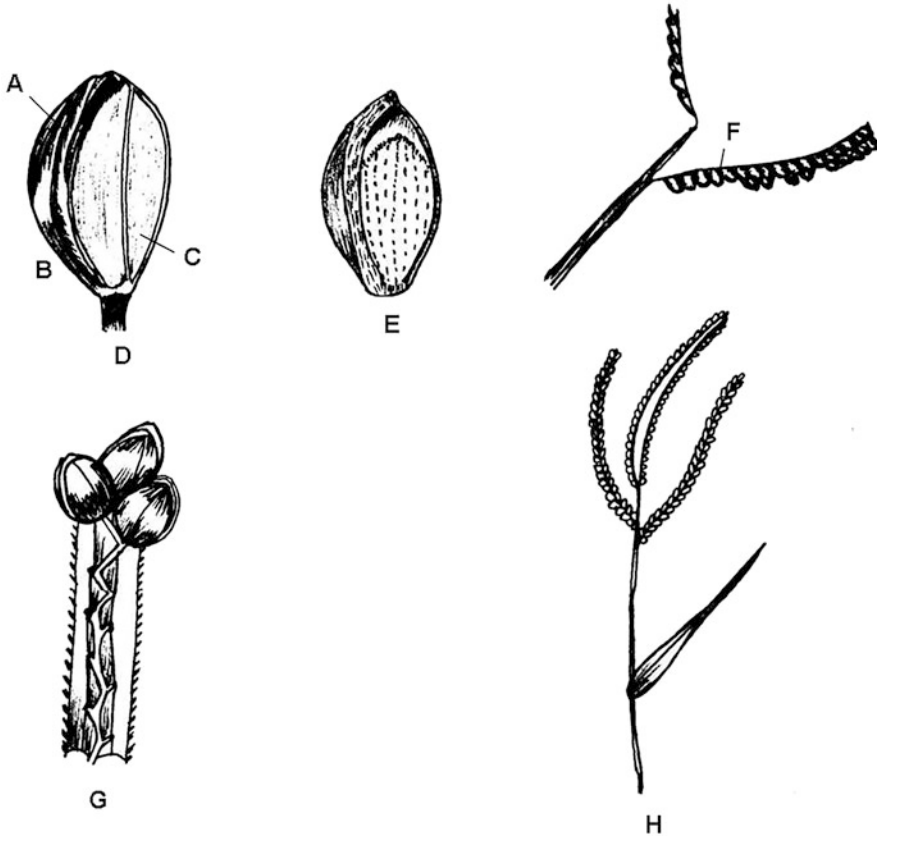

Fig. 3.4 Kodo millet inflorescence and its parts. (**a**) Upper floret; (**b**) Second glume; (**c**) Lemma; (**d**) Spikelet; (**e**) Floret; (**f**) Rachis; (**g**) Rachis in spikelet; (**h**) Inflorescence

3.3.4 Seed Production Techniques

3.3.4.1 Land Requirement

The quality of the seed set is affected by problem soils and the repetition of previous crops. Certified same-variety crops can be accepted, but volunteer plants must be avoided.

Main Field Preparation

The main field should be plowed twice before the onset of monsoon to enable the soil to hold moisture. At the onset of the monsoon, the field should be plowed three times to get fine tilth and form ridges and furrows. At the time of final plow, apply compost or farmyard manure at 5 t/acre (12.5 t/ha) and incorporate it into the soil.

Time of Sowing

The crop is normally sown during second fort night of June to first fort night of July. In Andhra Pradesh and Tamil Nadu, it is sown in September–October also.

Source of Seed

Seeds for certified production must purchase from authenticated sources and should meet the required germination percentages.

Seed Rate and Pre-sowing Seed Treatment

For optimal seed production, it is recommended to use 10–15 kg of quality seeds per hectare. If the seeds haven't been previously treated, they should be treated with an organo-mercurial fungicide. Additionally, treating the seeds with nitrogen-fixing bacteria *Azospirullum brasilense* and phosphate-solubilizing fungus *Aspergillus awamori* at 25 g/kg is beneficial.

Method of Sowing and Spacing

The seed crop is sown in rows 25–30 cm apart. The depth of seeding should not be more than 3 cm.

Fertilization

Seed crop should be applied with nitrogen at 60 kg, 40 kg phosphorous, and 20 kg potash per hectare.

3.3.4.2 Irrigation

The rainy season crop does not needs much irrigation. In case of prolonged drought conditions, one to two irrigations may however be given. The crop is more prune for water logged condition; hence, water should not be allowed to stagnate in the field.

3.3.4.3 Weed Control

Weeds must be eradicated when still in the early stages of development. Usually, two weeding separated by 15 days are adequate. For seeded plants, weeding can be done manually using a hand hoe or a cycle weeder. It is advised to manually weed twice, between 20 and 35 days after sowing, and to intercrop two to three different crops. Pre-germination of isoproturon at 0.5 kg ai/ha in Madhya Pradesh regions where rainfall is guaranteed is also efficient at controlling weeds. Broadleaf weeds can be controlled by using 2,4-D (80%) sodium salt at 1.0 kg ai/ha at DAS 20–25 following germination.

3.3.4.4 Plant Protection

Control measures as per the following schedule should be adopted in seed fields.

Disease control	
Name of the disease	Control measures
Rust	Spraying Mancozeb 75 WP at a concentration of 0.2% helped to some extent in controlling

(continued)

Disease control	
Name of the disease	Control measures
Head smut	It is advised to grow a cultivar that is resistant to disease, such GPUK 3. Additionally, it is advised to soak seeds in hot water at 55 °C for 7–12 min after treating them with Thiram at a rate of 2.5 g/kg

Insect control	
Name of the insect	Control measures
Shoot fly and its control	Before planting, sprinkle 20 kg/ha of carbofuran 3G granules in the furrows. Shoot fly incidence rises as sowing is delayed. It is advantageous to sow when the monsoon season begins. Plant the crop before the second week of July. If planting is delayed, use more seed (1 1/2 times the suggested seed rate) and pesticides must be used based on need
Termites and stem borer	These two insects are the main pests of the kodo crop. Applying 20–25 kg of Malathion 5% dust per acre into the soil before planting can help reduce termites

3.3.4.5 Field Inspection

The Seed Certification Officer shall conduct a minimum of two quality inspections between the blooming and maturity stages in order to preserve the better seed quality. The first inspection is carried out at the time of blooming to look for isolation and off-types, and the second is carried out just before harvest to look for off-types and gauge the output.

3.3.4.6 Rouging

Rogue out off-types at flowering and maturity. Remove smutted plants before harvest. Seed fields must meet specific requirements. Rogue out off-types at flowering and maturity. Remove smutted plants before harvest. Seed fields must meet specific requirements.

Specific Requirements

	Maximum permitted (%)[a]	
Factor	Foundation	Certified
Off-types	0.050	0.10

[a] Maximum permitted at final inspection

3.3.4.7 Harvesting and Threshing

Crop harvest is done once the ear heads are physiologically mature. The crop is ready for harvest in around 100 days. When crop is ready for harvest, the ears turn

from brown to green. After crop being cut close to the ground, the plants are bundled and stacked for a week before the grains are threshed and cleaned by winnowing.

3.3.4.8 Drying and Storage

The seeds should be cleaned and dried in the sun to reach a safe moisture level of 12%. Care must be taken during the seed drying and processing stages to prevent mechanical damage and contamination. Proper storage conditions can keep the seeds fresh for up to 13 months.

3.3.4.9 Seed Standards

	Standards for each class	
Factor	Foundation	Certified
Pure seed (minimum)	97.0%	97.0%
Inert matter (maximum)	3.0%	3.0%
Other crop seeds (maximum)	10/kg	20/kg
Weed seeds (maximum)	10/kg	20/kg
Germination (minimum)	75%	75%
Moisture (maximum)	12.0%	12.0%
For vapor-proof containers (maximum)	8.0%	8.0%

3.4 Seed Production in Little Millet

Little millet, botanically known as *Panicum sumatrense* Roth. Ex. Roem. & Schult (syn. *P. miliare* Lam.), is an important crop for food and feed in the tribal belt of Madhya Pradesh, Chhattisgarh, and Andhra Pradesh in India. It is a quick-growing, short-duration cereal that can withstand both drought and waterlogging. It belongs to the family *Gramineae*.

3.4.1 Floral Morphology

Little millet inflorescence is a panicle, contracted or thyrsiform, 15–45 cm long, and 1–5 cm wide. The spikelet is persistent, 2–3 mm long. Panicle branches droop and are scabrous at maturity. Spikelets produce on unequal pedicels but solitary at the end of branches, each consisting of two-minute flowers. The lower one is sterile, and the upper one is fertile or bisexual without rachilla extension. The lemma I and its palea enclose the staminate or sterile flower; lemma II and its palea enclose the fertile flower. Spikelets are elliptical, dorsally compressed, and acute. It has three anthers about 1.5 mm in length. The glume reaches the apex of florets, thinner than fertile lemma; the lower glume is ovate, 0.7–1.2 mm long, membranous, without keels, 1–3 veined. The lateral vein is absent in the lower glume, and its apex is acute. The upper glume is also ovate and without keel but larger than the lower glume. It has 11–15 veins (Fig. 3.5).

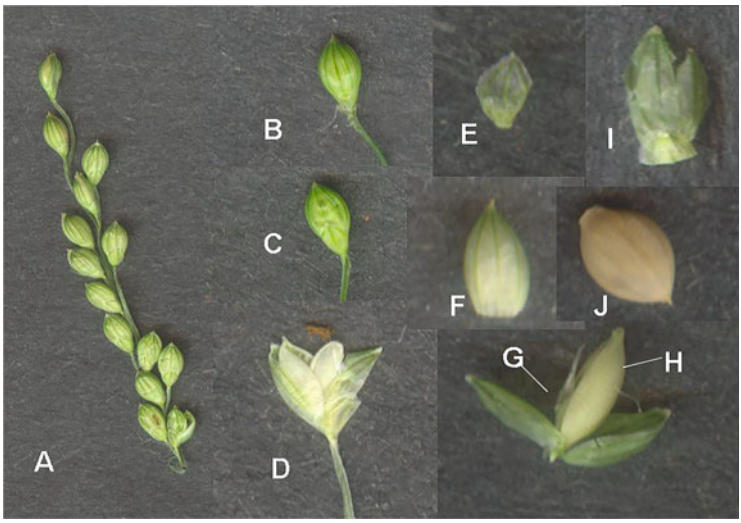

Fig. 3.5 Little millet inflorescence and its parts. (**a**) Inflorescence; (**b**) Spikelet; (**c**) Side view of spikelet; (**d**) Opened spikelet; (**e**) Outer glumes; (**f**) First lemma; (**g**) Sterile floret; (**h**) Fertile floret; (**i**) Upper glumes; (**j**) Grain enclosed in lemma and palea

3.4.2 Floral Biology

The panicle begins to produce spikelets on the second or third day after its appearance. Flowering occurs from the top to the bottom, with the largest number of flowers opening on the sixth or seventh day. It takes about 2 weeks for the panicle to complete flowering, with anthesis occurring between 9:30 and 10:30 a.m. The glumes open briefly for self-pollination, and the entire anthesis process is rapid, taking 2–5 min.

3.4.3 Stages of Seed Production

Breeder seed–Foundation seed–Certified seed

3.4.4 Seed Production Techniques for Certified Seed

3.4.4.1 Land Requirement

Little millet can thrive in nutrient-rich or poor soil, but it thrives best in well-drained loam or sandy loam soils that are rich in organic matter. The cultivation site should be selected carefully, it should be free from volunteer plants, and the land should not have been cultivated with the same crop in the previous season.

3.4.4.2 Cultural Practices

Field Preparation

The field is prepared by plowing or harrowing two times and then leveling it to make it a fine tilth. Afterward, the leveled field is formed into ridges and furrows. During the final plow, 5 t/acre (12.5 t/ha) of compost or farmyard manure should be applied and incorporated into the soil.

Time of Sowing

Optimum season for the higher seed yield and quality, seeds should be sown in June–July at the onset of monsoon rains. Summer crop should be sown in the month of February–March wherever irrigation facility is available.

Source of Seed

Seeds must obtained from an authenticated source and meet the necessary standards for certified seed production, including seed quality viz., seed health with the required germination standards.

Seed Rate and Pre-sowing Seed Treatment

Optimum seed rate required for sowing is 4 kg/acre (10 kg/ha). Selected seeds should be treated with *Azospirillum* at 60 g/kg of seeds.

Method of Sowing and Spacing

Treated seeds should be sown with row spacing of 30 cm and plant to plant is 10 cm. The depth of seeding should not be more than 3 cm.

Fertilization

For a successful seed crop, it is recommended to use 50–60 kg of nitrogen, 30–40 kg of phosphorus, and 20–30 kg of potash per hectare.

3.4.4.3 Irrigation

Kharif crops do not require irrigation, but one irrigation should be given at the tillering stage if there is long dry spells exist. The first irrigation should be given 25–30 days after sowing, followed by the second one at 40–45 days after sowing. Summer crops require two to four irrigations depending on soil type and climatic conditions. During heavy rains, excess water should be drained out.

3.4.4.4 Weed Control

Effective weed control in line sown crops requires two inter-cultivations and one hand weeding, while broadcast crops need two hand weeding. Additionally, post-emergence application of 2,4-D sodium salt (80%) at 1.0 kg a.i./ha at 20–25 DAS and pre-emergence spray of Isoproturon at 1.0 kg a.i./ha are effective weed control measures.

3.4.4.5 Plant Protection

Control measures as per the following schedule should be adopted in seed fields.

Disease control	
Name of the disease	Control measures
Smut	Soaking seeds in hot water at 55 °C for 7–12 min, followed by drying, can kill seed-borne pathogens. Alternatively, treating the seeds with Thiram at 2.5 g/kg seed or Carboxin at 2 g/kg seeds is also effective

Insect control	
Name of the insect	Control measures
Shoot fly and its control	Apply Carbofuran 3G at 20 kg/ha in the soil at the time of field preparation
Termites	Use Methyl parathion (2%) dust at 20–25 kg/ha before sowing
Stem borer	Apply Carbofuran 3G at 20 kg/ha in the soil at the time of field preparation

3.4.4.6 Field Inspection

Minimum of two inspections should be done between flowering and maturity stages by the Seed Certification Officer for effective quality control. The first inspection is done at the time of flowering to check the isolation and off-types and the second inspection is done during the maturity stage prior to harvest to check the off-types and to estimate the seed yield.

3.4.4.7 Rouging

Rouging should be done often to remove the off types, volunteer plants and diseased plants from the seed production field to avoid genetic contamination. Rogueing should be done up to the flowering stage. The seed field should meet specific requirements as detailed below.

Specific Requirements

	Maximum permitted (%)[a]	
Factor	Foundation	Certified
Off-types	0.050	0.10

[a] Maximum permitted at final inspection

3.4.4.8 Harvesting and Threshing

Harvest is done once the ear heads are physiologically mature. Normally crop is ready for harvest in 80–85 days after sowing. The crop should be harvested when two thirds of the seeds are ripe. The harvested ear heads are threshed by hand or

trampling under the feet of bullocks. The threshed grains are further cleaned by winnowing.

3.4.4.9 Drying and Storage

The cleaned seeds should be sun dried to attain a safe moisture level of 12%.

3.4.4.10 Seed Standards

	Standards for each class	
Factor	Foundation	Certified
Pure seed (minimum)	97.0%	97.0%
Inert matter (maximum)	3.0%	3.0%
Other crop seeds (maximum)	10/kg	20/kg
Weed seeds (maximum)	10/kg	20/kg
Germination (minimum)	75%	75%
Moisture (maximum)	12.0%	12.0%
Vapor-proof containers (maximum)	8.0%	8.0%

3.5 Seed Production in Proso Millet

The ubiquitous and significant minor millet known as prosomillet (*Panicum miliaceum*) is a member of the *Gramineae* family. In India, a lot of this short-lived millet type is farmed. The crop's fast maturation allows it to avoid drought. *Panicum miliaceum* is its botanical name. The land is a member of the *Gramineae* family.

3.5.1 Floral Morphology

The inflorescence of proso millet is a drooping panicle that is 10–45 cm long, may be open or compact, and has primary branches that spread, rise or apprises before ending in a spikelet (Fig. 3.6). There are no bristles below the spikelets. The average spikelet is solitary and is 0.5 cm in length. There are two glumes and two lemmas in each spikelet. The outer and inner glumes differ in length; the outer glume is shorter than the spikelet. Each lemma contains one floret. The lower lemma's floret lacks a stamen and is sterile; the upper lemma is fertile and shorter than the lower lemma. While the palea of the upper lemma (fertile floret) is prominent, the palea of the lower lemma (sterile floret) is greatly diminished. It contains three stamens, and the anthers are tan, amber, dark brown, or blackish in color. The ovary features plumose stigmas and a bifid style.

Fig. 3.6 Proso millet inflorescence and its parts. (**a**) Inflorescence; (**b**) Opened spikelet; (**c**) Outer glume; (**d**) Inner glume; (**e**) Inner lemma; (**f**) Palea; (**g**) Inner glume; (**h**) Outer glume; (**i**) Upper lemma; (**j**) Anther; (**k**) Grain enclosed in lemma and palea; (**l**) Grain

3.5.2 Floral Biology

Proso millet begins to bloom from the top of the panicle all the way to the bottom. The period between 10 a.m. and 12 p.m. when proso millet goes through anthesis. From the beginning of the first flower's anthesis to the end of the last floret on the panicle, it takes 12–15 days. In proso millet, anther pollen loss and stigma receptivity occur simultaneously. Nelson (1984) noted that the anthers were sticky and the pollen was not shed when the florets were open. The anthers dry out and start to release pollen shortly after the florets emerge. The florets remain open for 10–15 min. The factors such as high temperatures, low humidity, and bright sunlight promote the flowering. Flowering gets reduced on cloudy days. It can be stimulated by heating a panicle with lens. Proso millet ($2n = 36$) is a self-pollinated crop, but natural cross-pollination may exceed 10%.

3.5.3 Stages of Seed Production

Breeder seed–Foundation seed–Certified seed

3.5.4 Seed Production Techniques

3.5.4.1 Land Requirement

Proso millet can be cultivated in both rich and marginal soils. Well drained loam or sandy loam soils rich in organic matter are ideal for cultivation. The selected land should be free from volunteer plants. The land should not be cultivated with same crop in the previous season.

Field Standards

General Requirements
Isolation Distance
Seed fields of foxtail millet shall be isolated from the contaminants detailed below table

Contaminants	Minimum distance (m)	
	Foundation	Certified
Fields of other varieties	3	3
Fields of the same variety not conforming to varietal purity requirements for certification	3	3

3.5.4.2 Brief Cultural Practices

Field Preparation

The main field should be harrowed 2–3 times to make it a fine powder and levelled. The leveled field is formed into ridges and furrows. During the final plow apply compost or farmyard manure at 5 t/acre (12.5 t/ha) and incorporate it into the soil.

Time of Sowing

Seeds should be sown in June–July onset of monsoon rains to harvest quality of seed. Summer crop should be sown in the month of February–March wherever irrigation facilities are available.

Source of Seed

Must be from authenticated source and use a suitable class of seed (Foundation seed for certified seed production). Seeds should be healthy with the required germination percentage.

Seed Rate and Pre-sowing Seed Treatment

Recommended seed rate is 4 kg/acre (10 kg/ha). Selected seeds should be treated with *Azospirillum* at 60 g/kg of seeds.

Method of Sowing and Spacing

Treated seeds should be sown in rows of 30 cm with 10 cm plant to plant spacing. The depth of seeding should not be more than 3 cm.

Fertilization

Proso millet is a short-duration crop, which requires relatively less amount of nutrients compared to other cereals. To get good crop, general fertilizer recommendations under irrigated conditions are 40–60 kg N, 30 kg P_2O_5, and 20 kg K_2O/ha. Apply half of the N and the whole amount of phosphorus and potash as a basal dose at the time of sowing. The remaining half of N should be applied at the time of the first irrigation. Under rain fed conditions, the fertilizer dose is reduced to half of the irrigated crop.

3.5.4.3 Irrigation

Cultivation of the *Kharif* season does not require watering. However, if the drought period is prolonged, it should be watered at least once during tillering to increase yield. The first watering should be done 25–30 days after sowing, followed by 40–45 days after sowing. The summer crop needs to be watered two to four irrigations depending on the type of soil and climatic conditions. When there is heavy rain, the excess water in the field must be drained.

3.5.4.4 Weed Control

Hand weeding may be done for the removal of broad-leaf weeds.

3.5.4.5 Plant Protection

Control measures as per the following schedule should be adopted in seed fields.

Disease control	
Name of the disease	Control measures
Head Smut	Seed treatment with an organic mercury compound such as Ceresan at the rate of 3 g/kg seed or treatment with hot water (soaking seeds in hot water at 55 °C for 7–12 min) will reduce the effect

Insect control	
Name of the insect	Control measures
Shoot fly and its control	Early sowing with the onset of monsoon is an effective and cheapest method of control. Use seeds treatment with thiamethoxam 25 WDG at 4 g/kg of seed. Apply Carbofuran (Furadon) 3G granules at 20 kg/ha in furrows before sowing

3.5.4.6 Field Inspection

The certified seed must perform a minimum of two checks between flowering and maturation. The first check was performed at flowering time to check for isolates and type errors and the second check was performed at the mature stage before harvest to check for errors and estimate yield.

3.5.4.7 Rouging

Field inspections should be performed regularly to remove stray, voluntary, and diseased plants from seed production fields to avoid genetic contamination. Care should be carried out until the flowering stage. Seed field should meet specific requirements as detailed below.

Specific Requirements

	Maximum permitted (%)[a]	
Factor	Foundation	Certified
Off-types	0.05	0.10

[a] Maximum permitted at final inspection

3.5.4.8 Harvesting and Threshing

Crop harvesting is done when the cob is physiologically mature. Normally, crops will be harvested after 65–75 days from sowing. Crop should be harvested when 2/3 of the seeds are ripe. The harvested ear heads are threshed either by hand or trampled with cows or running of stone rolls. The pulverized particles are then cleaned by sieving.

3.5.4.9 Drying and Storage

The cleaned seeds should be sun-dried to attain a safe moisture level of 12%.

3.5.4.10 Seed Standards

	Standards for each class	
Factor	Foundation	Certified
Pure seed (minimum)	97.0%	97.0%
Inert matter (maximum)	3.0%	3.0%
Other crop seeds (maximum)	10/kg	20/kg
Weed seeds (maximum)	10/kg	20/kg
Germination (minimum)	75%	75%
Moisture (maximum)	12.0%	12.0%
For vapor-proof containers (maximum)	8.0%	8.0%

3.6 Seed Production in Barnyard Millet

Barnyard millet is an important small millet crop grown in India. Botanically, it is called *Echinochloa frumentacea* and belongs to the family Gramineae. Crops can escape drought due to rapid maturation. The best sowing time is September–October and February–March. Pollination should not coincide with the rains so that the seeds are of good quality and effective.

3.6.1 Floral Morphology

The inflorescences of barnyard millet are usually erect, rarely at the apex drooping. The inflorescences are few to numerous, and dense at the apex with the florets arranged in four irregular rows on tricuspid axes (Fig. 3.7). The florets are two-flowered, 2–3 mm long, ovate to elliptical, with a hairless but pointed lower part, near the stalk and set on short coarse stalks supported by double eyelids. The lower eyelid is about 1/3 cotton, while the upper eyelid is slightly shorter than cotton. The lower notes and lemma are slightly pubescent. The lower flowers are neutral (sterile) with a lemma and a small pale spot, while the upper flowers are hermaphroditic. The sterile lemma has five veins. The fertile lemma is convex, elliptical, smooth and shiny, pointed or apex, the edges of the leaf sheath rolled downward, and the top of the sheath unbound. Palea is flat and the surface texture is similar to

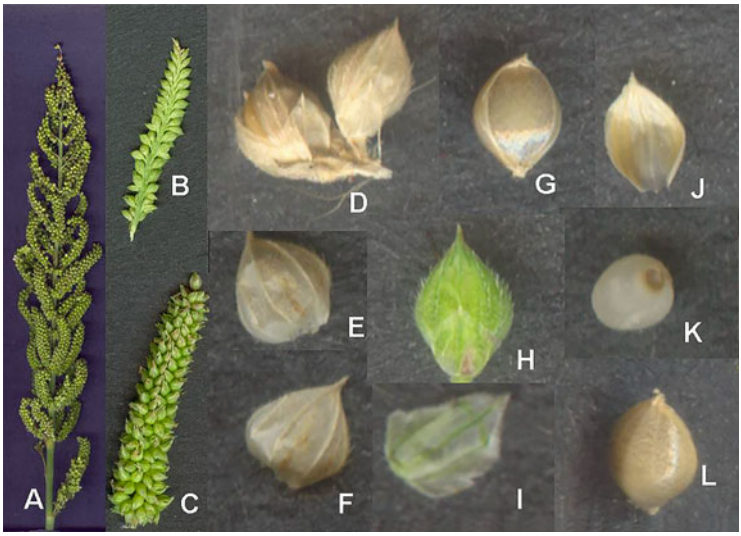

Fig. 3.7 Barnyard millet inflorescence and its parts (**a**) Inflorescence; (**b**) Arrangement of spikelet in raceme; (**c**) Raceme; (**d**) Spikelet; (**e**) Lower lemma; (**f**) Upper glume; (**g**) Side view of fertile lemma enclosing grain; (**h**) View of spikelet from lower glume; (**i**) Lower glumes; (**j**) Fertile lemma; (**k**) Grain; (**l**) Grain enclosed in lemma and palea

that of fertile lemma. Stamens come in three numbers; the upper ovary contains two distinct types with a hairy stigma. The seed is tightly enclosed in a lemma and is shiny white, hardened.

3.6.2 Floral Biology

Flowers first bloom in the upper inflorescence and moves downward. Crop takes 10–14 days to appear and 10–15 days to fully flower in hilly conditions. The maximum number of flowers opens in 6–8 days after flowering. Flowers bloom from 5 to 10 a.m., the maximum number of flowers bloom at 6–7 a.m. In an individual cluster, flowering begins at both fringe ends first, and then continues to the middle of the cluster. Before the anther splits, the stigmas open and the flower opens. The flowers will close in half an hour. It is a highly self-mating species. Treatment of inflorescences with hot water at 48 °C for 4–5 min (observed) was also effective in inducing male infertility under hilly conditions in barnyard millet on the farm.

3.6.3 Stages of Seed Production

Breeder seed–Foundation seed–Certified seed

3.6.4 Seed Production Techniques

3.6.4.1 Land Requirement

Millet can be grown in both rich and marginal soils with different textures. A sandy loam with well-drained, organic-rich loam soils is ideal for seed production. Land required for barnyard millet production must be free from voluntary crops. The land should not be cultivated with the previously with same crop.

3.6.4.2 Cultural Practices

Field Preparation

The main field must be plowed and harrowed for two to three times in order to get smooth and fine tilth. Main fields are formed by ridges and furrows. In the final plowing, apply organic fertilizer or manures at 5 t/acre (12.5 t/ha) and mix well into the soil 15 days prior to sowing.

Time of Sowing

Seeds should be sown in September–October for harvest of higher seed yield with quality. Summer crops should be sown in the month of February–March with proper irrigated facilities.

Source of Seed

Must have an authentic origin and use the right kind of seed (basic seed to produce certified seed). Seeds must be healthy with the standard germination.

Seed Rate and Pre-sowing Seed Treatment

Seed rate is require per acre is 4 kg and it is 10 kg per ha. Seeds should be treated with *Azospirillum* at 60 g/kg of seeds before its sowing.

Method of Sowing and Spacing

Treated seeds should be sown with 30 cm of row spacing and with 10 cm apart from plant to plant and seed should not be sown more than 3–4 cm depth in soil.

Fertilization

Recommended fertilizer dose under irrigation conditions is 40 kg N, 20 kg P_2O_5 and 20 kg K_2O per hectare. Apply half the amount of nitrogen and the entire amount of phosphorus and potassium at the time of sowing. The remaining half of N should be applied after the first irrigation. In open-air farming, the dose of fertilizer is reduced to half of that of irrigated cultivation.

3.6.4.3 Irrigation

Crop cultivated during Kharif season does not require irrigation; however, if the drought condition is prevail for long period, it should be irrigated at least once at the tillering stage to increase seed yield. The first irrigation should be done at 25–30 days, followed by 40–45 days after sowing. The summer crop needs to be irrigated for two to four times, it depends on the type of soil and climatic conditions. This crop requires proper drainage system, whenever heavy rain is received.

3.6.4.4 Weed Control

The crop should be inter-cropped and manual hand weeding is required. Two manual hand weedings should be done in broadcast culture. The use of 2,4-D (80%) sodium salts after germination at 1.0 kg ai/ha at 20–25 days after sowing and Isoproturon at 1.0 kg ai/ha as a pre-spray spray appear also effective in weed control.

3.6.4.5 Plant Protection

Control measures as per the following schedule should be adopted in seed fields.

Disease control	
Name of the disease	Control measures
Smut	Seed treated with thiram at 2.5 g/kg of seed and soaking seeds in hot water at 55 °C for 7–12 min is done

Insect control	
Name of the insect	Control measures
Shoot fly and its control	Early sowing with the onset of monsoon is an effective and cheapest method of control
Stem borer	Apply Carbofuran 3G at 20 kg/ha in the soil at the time of field preparation
Termites	When sowing should mix soil with Chlorphyriphos 5D at 35 kg/ha. When detecting pests on standing plants, dilute Chlorphyriphos 20EC in 5 L of water and mix with 50 kg of soil, spread over 1 hectare, then water lightly. Use methyl parathion powder (2%) in the amount of 20–25 kg/ha before sowing

3.6.4.6 Field Inspection

Seed crop requires minimum of two field inspections between flowering and maturity. The first field inspection should be done at flowering time to check for isolation and type and the second field inspection should be done at the mature stage before harvest to check the type and estimate the seed yield.

3.6.4.7 Rouging

Disinfection should be performed regularly to remove varietal, voluntary, and diseased plants from seed production fields to avoid genetic contamination. Rouging should be done until the flowering stage. Seed field should meet specific requirements as detailed below.

Specific Requirements

	Maximum permitted (%)[a]	
Factor	Foundation	Certified
Off-types	0.05	0.10

[a] Maximum permitted at final inspection

3.6.4.8 Harvesting and Threshing

Crop harvesting will be done when the cob is attains physiologically mature. Normally, crops will be harvested after 75–90 days from sowing and it should be harvested when 2/3 of the seeds are ripe. The harvested ear heads are pounded by manually or trampled with cows and threshed particles are then cleaned by sieving.

3.6.4.9 Drying and Storage

The cleaned seeds should be sun dried to attain a safe moisture level of 12%.

3.6.4.10 Seed Standards

	Standards for each class	
Factor	Foundation	Certified
Pure seed (minimum)	97.0%	97.0%

(continued)

Factor	Standards for each class	
	Foundation	Certified
Inert matter (maximum)	3.0%	3.0%
Other crop seeds (maximum)	10/kg	20/kg
Weed seeds (maximum)	10/kg	20/kg
Germination (minimum)	75%	75%
Moisture (maximum)	12.0%	12.0%
For vapor-proof containers (maximum)	8.0%	8.0%

Millet Based Cropping Systems for Enhanced Productivity

4

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Abstract

Cropping systems are generally planned and accomplished to realize social particularly human objectives consequently, to become a decisive systems. The cropping systems have been intended to maximize the yield, but modern agriculture is progressively concerned with endorsing environmental sustainability. The crop and cropping pattern vary under different agroecosystems. The cropping system necessity is to provide sufficient nourishment for the household, feed for livestock, and make satisfactory cash income for domestic and cultivation incidentals. Having the advantage of short duration, millets very easily fit into cropping systems like multiple cropping, mixed farming, intercropping, sequence

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cropping, and relay cropping systems. Integration of crop production, diverse farming systems with best conservation measures lead to sustainable production which eventually increase the revenue and towards better livelihoods. Millets are reliably are the hardiest, resilient, and climate adaptable crops for adverse, hot and comparatively dry environments. The droughts are becoming more recurrent in most prominent agro-ecological regions of the globe, here exists a farmer's necessity to accept farming practices, which have the tiniest influence on the environment although producing adequate quantity to withstand their living. Millet's cultivation would be the right choice under these circumstances and thorough information about the millet-based cropping systems, prominent millet cropping systems followed in India, and their prospect under alternate land use and fallow system is described in this chapter. Year 2023 is been celebrated as "International Year of Millets" and the importance of millet-based cropping system for enhancement of productivity is more relevant and hence furnished in the chapter.

Keywords

Millet · Productivity · Soil · Climate · Resilience · Cropping system

Cropping system denotes to crops, arrangements, and its management practices applied on a precise cultivated field over a period of years. This comprises spatial and temporal features of managing an agricultural system. Millets play a vital role towards achieving the nutritional security specifically under areas of rainfed. In Indian agriculture, three cropping systems are majorly followed and these are mono-cropping, inter-cropping, and multiple cropping. Millets are the special crops that even can cultivate in arid-soil, wanting only 350–400 mm yearly rain and can withstand higher temperatures. However, Sood et al. (2019) stated that tribal communities in Odisha, Rajasthan, Jharkhand, Karnataka, Uttarakhand, and Madhya Pradesh take millets as integral part of their diet. Owed to appreciable nutraceutical properties are gaining popularities among urbanites too. The irrigated area under millets is less than 10% indicating their mere suitability for rainfed agriculture. Remunerative cropping systems involving different pulse/cereal/oil seed crops in millets for diverse regions have been evolved. The detailed information on the basic concept of cropping system, millet-based cropping systems, prominent millet cropping systems in India, and other related facets are described in this chapter.

4.1 Importance of Millets

Indian agriculture is highly dependent on monsoon rainfall. Millets are also gaining acceptance among farmers as climate-friendly, drought-resistant crops which can thrive even on barren soil. Millets are free from gluten further glycemic index is low, resulting into a balanced and healthy diet for people suffering from diabetics. Millets

are also anti-carcinogenic and anti-hypertensive foods, as well as aiding in the prevention of obesity and heart disease. These are also called as miracle crops or nutricereals because they combine all these characteristics so well. Millets have an excellent nutritional value and grow well under diverse situations, but they aren't utilized to their full potential. Pearl millet and sorghum are known to be major, while finger millet and other small millets, i.e., foxtail millet, proso millet, little millet, barnyard millet, kodo millet, and browntop millets, are named as minor millets. Millets are more affluent in minerals and vitamins than rice and wheat, and have a significant potential to supply food, fodder, fiber, health, livelihood, and environmental security. Millets are the primary grain to be produced for native use. These are branded for climate-resilient characteristics due to their ready acclimatization under an extensive array of agro-ecological situations, improved growth and efficiency in poor fertile situations, less dependence on synthetic manures, and least susceptibility to various stresses (Kole et al. 2015). As droughts are common in most prominent agrarian sections, the farmers necessity to implement farming practices that take the minimum environmental impact though producing a large enough quantity of yields to withstand their living and the nation's food mandate. They also have an extremely low water requirement, require around 80% lesser than rice, wheat, and sugarcane. The other encouraging fact is that these are exceptional for preserving soil. Finger millet is cultivated on 10.48 lakh (2016–2020) hectares with 16.37 lakh tonnes production in India. Whereas, other small millets are cultivated over 5.45 lakh hectares (2016–2020) with 3.95 lakh tonnes production. Despite a drastic decline in the area in last six decades, production is upheld owing to enhanced productivity (Fig. 4.1).

4.2 Cropping System and Importance of Millet-Based Cropping System

Millets are probably the finest alternative for farmers who would like to achieve triple aims of farming versatility, sustainability, and profitability. The millets-based farming techniques advantages are many as millets are awfully resistant to harsh temperatures, droughts, and floods. In India, cropping system varies owed to physical multiplicities and cultural differences. This perception is as deep-rooted as agriculture. Farmers preferred mixed cropping specially under dryland circumstances. This cropping is to curtail the risk of total failure of crop. Cropping system is the concept of growing one or more crops in the same season and or different in an agricultural year. System of cropping is also the system under which diverse crops are grown. On all the whole, cropping is to be progressed based on soil, water, and climate to afford adequate food, fodder, and satisfactory cash. Hence, cropping system encompasses all components required for the crop production and interrelationships between them and environment. An intensive attempt to include millet crops in cropping systems, especially in vulnerable environments, is a positive step toward long-term sustainability. The cropping system is mostly determined by

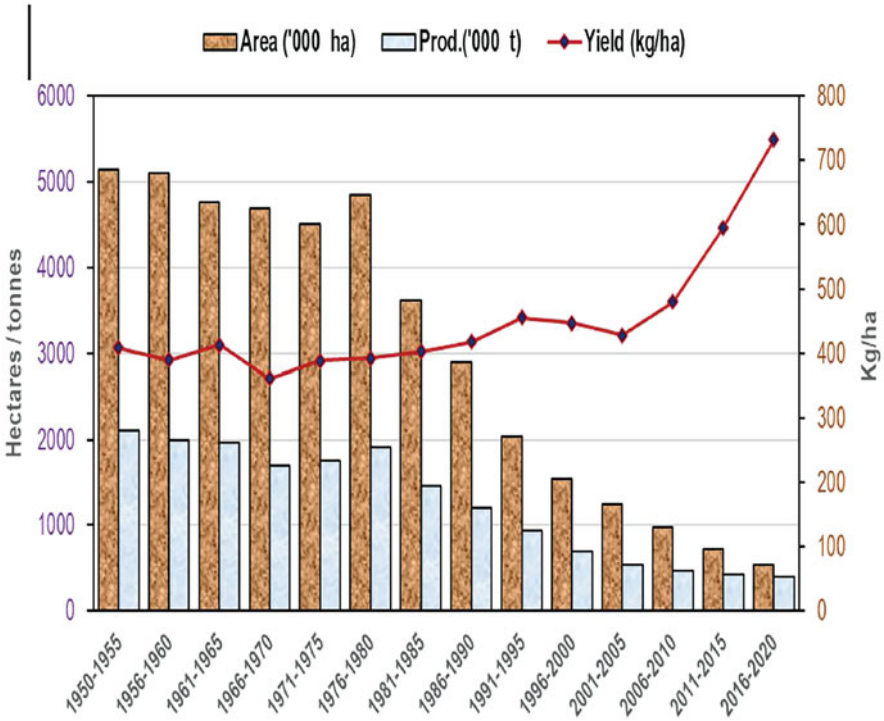

Fig. 4.1 Status of small millets in India (1950–2020). (Source: Ministry of Agriculture and Farmers Welfare, Govt. of India, 2020)

type of soil, rainfall, climate, temperature, and the technology. For determining the cropping pattern, the factors are the following:

Factors	Determinants
Natural	Soil, rainfall, climate, etc.
Historical	Land category, type of possession, system land tenure
Social	Social environment, traditions and customs
Economic	Size of holdings, input prices and incomes
Government policies	MSP, subsidies, export policy and taxes

Hence the cropping systems are decided mainly by soil moisture or rainfall received, soil type, and length of growing period. Rainfall being the important factor which influences the choice and success of crop through deciding the length of growing period. Soil that stores moisture also decides the cropping systems again through deciding the length of growing period (LGP; Table 4.1). Millets can survive in areas with as little as 350 mm of seasonal rainfall and in different soils (Tables 4.2 and 4.3). The multiple cropping denotes to cropping intensification in both space and time dimensions.

Table 4.1 Choice of cropping system based on length of growing period

LGP (days)	Cropping system
<75	Perennial vegetation, mono-cropping with short duration pulses/millets
75–140	Mono-cropping
140–180	Intercropping
>180	Double cropping

Table 4.2 Choice of cropping system based on rainfall received

Rainfall(mm)	Cropping system
350-650	Single crop(*kharif*)
650-750	Intercropping(can be attempted)
750-900	Sequential cropping is possible
>900	Sequential cropping is assured

Table 4.3 Potential cropping systems in relation to rainfall and soil type

Rainfall (mm)	Soil type	LGP (weeks)	Cropping system
350–650	Alfisol and shallow vertisol	20	Single crop (*kharif*)
	Deep aridsols, entisols	20	Single crop (*kharif*/*rabi*)
	Deep entisols	20	Single crop (*rabi*)
650–750	Alfisol, entisol, vertisol	20–30	Inter cropping
750–900	Deep vertisols and alfisols, entisols, inceptisols	30	Double cropping
>900		>30	Sequential and double cropping assured

4.3 The Forms of Multiple Cropping

1. Mixed intercropping: Rising crops of two or more numbers simultaneously without distinct row arrangement. This term is also stated as mixed cropping, e.g., The most seen mixed cropping under rainfed situation is Sorghum, pearl millet, and cowpea whereas in Southern Karnataka, finger millet, and mustard.
2. Row inter cropping: Rising of two or more than two crops concurrently where these crops are established in rows and often referred as intercropping, e.g., Finger millet + Red gram (8:2).
3. Strip intercropping: Cultivation of crops in two or more numbers simultaneously under strips wide abundant to permit their cultivation but sufficient for the crops to interrelate agronomically, e.g., Ground nut and Redgram in 6:4.
4. Relay intercropping: Cultivation of two or more crops instantaneously in which only a fragment of the life cycle is overlapped. Next crop is sown only later the first crop touched its reproductive stage of growth, but, earlier to its physiological maturity and it is habitually referred as relay cropping, e.g., Rice fallow pulse/sorghum/small millets (Plate 4.1).

Plate 4.1 Mixed cropping of finger millet with niger/amaranthus/soyabean

4.4 Existing and Recommended Millet-Based Cropping Systems Observed at Farmers' Field

Under dryland conditions, small millets grains are usually sown through broadcasting the seeds at 20–25 kg/ha. Thinning of plants needs to be done after 2 weeks once they are about 2–3 cm height to lessen the plant density. The plants which are thinned could be used for feed for animal. Optimum space can be achieved if crops are sown in rows and for best yields. Monocropping of millets is not much lucrative in the existing agricultural situation for the fulfilment of the consumers demand and increasing population. Major millets, i.e., sorghum and pearl millet, are important component of cropping system in rainfed areas. Being a widely spaced crop and short to medium crop duration, it provides chances for growing intercrops for better exploitation of natural resources. In assured rainfall areas, intercropping of sorghum with red gram at 2:1 or 2:2 ratios or soybean at 2:4 or 3:6 ratios not only give higher productivity but also expands soil health and provides nutritional security to rural people in semi-arid regions. Sorghum + chickpea and sorghum + safflower is the important cropping system in *rabi* sorghum growing regions of Maharashtra and Karnataka in India. Dimes et al. (2008) observed that, intercropping of pigeon pea with sorghum was more climate resilient compared to maize and groundnut under semiarid regions of Zimbabwe through simulation studies. In western Uttar Pradesh, Madhya Pradesh, Maharashtra, and Haryana, pearl millet-barley/wheat/rapeseed etc. as sequential cropping system are practised. As promising intercrop with pearl millet, leguminous crops like green gram, black gram, ground nut, cow pea, cluster bean and oilseed like castor, sesame exploit the environment better than sole cropping. Intercropping in pearl millet generally increases land equivalent ratio and more specifically monetary returns per unit area (Table 4.4).

In India viz., Andhra Pradesh, Karnataka, Odisha, and Tamil Nadu, finger millet + red gram in 8:2 ratio proportions gave higher monetary returns (Gowda et al. 2004). Pradhan et al. (2014) reported from 2-year experiment in Madhya

Table 4.4 Prominent millet intercropping systems observed in different states

Millet-based intercropping	Region	References
Finger millet + Red gram (6:1) Little millet + Red gram (6:1)	Andhra Pradesh	Kiranmai et al. (2021)
Finger millet + Soybean (9:1) Finger millet + Ricebean (9:1)	Uttarakhand	Singh and Arya (1999)
Finger Millet + Frenchbean (3:1)		Yadav (2010)
Little millet + Pegion pea (6:2)	Karnataka	Kumar et al. (2008)
Finger Millet + black gram (8:2) Finger millet + Mothbean (8:2)	Maharashtra	Nigade et al. (2012)
Finger millet + Pegion pea (4:1)	Chhattisgarh	Pradhan et al. (2014)
Finger Millet + Pegion pea (8:2)	Bengaluru	Mallareddy et al. (2016)
Finger Millet + Pegion pea (6:2)	Southern Odisha	Anchal Dass (2010)
Finger millet + Okra (4:2)	Dapoli	Jadhav et al. (1992)

Pradesh, intercropping finger millet with sesame, soybean, black gram, horse gram, or red gram resulted in sustainable higher yield and net monetary return. Intercropping foxtail millet with green gram, cow pea, or field beans in 4:2 row ratio has been found ideal for enhancing productivity of both the crops (Anonymous 2002). Kodo millet with soybean with 4:1 row ratio established as an ideal for sustainable yield (Anonymous 2007).

Similarly, soybean and pigeon pea can successfully grow as intercrop with little millet and barnyard millet under rainfed situation. Normally, in southern parts of India, intercropping of finger millet with crops like sorghum (fodder), field beans, castor, niger, and pigeon pea and in some regions with bajra (pearl millet). Finger millet is also alternated with other crops of dryland like horse gram, groundnut, other small millets, tobacco, cotton, and sesame. In some of the finger millet growing regions, monocropping is followed. It may be by a fallow or mixed with other crops in the same season. In most situations, however, it is rotated with sorghum, millet, cotton, and tobacco. In other parts of India, the dry crop is sometimes rotated with groundnut, cotton, sorghum, or millets. In the better-fertilized areas with supplementary irrigation, finger millet is grown either before or after crops of gingelly, onion, sweet potato, chillies, tobacco, wheat, gram, or cotton.

Crop diversification over intercropping has shown the productivity improvement, profitability, resource utilization, and offer a kind of biological insurance counter to risk and anomalous rainfall (Dutta and Bandyopadhyay 2006). The finger millet yield attributes were improved once black gram intercropped with it. The black grams fix atmospheric nitrogen and made available to finger millet along with moisture conservation, weed suppression (Dass and Sudhishri 2010).

The use of short duration legume as intercrop helps in providing sufficient ground cover which condense erosion by stopping rain drop from striking the bare soil. The

Table 4.5 Finger millet-based cropping systems observed in country

	Ratio	States
Inter/mixed cropping		
Finger millet + pigeon pea	8–10:2	Karnataka, Tamil Nadu
	6:2	Bihar
Transplanting of pigeon pea as intercrops with Finger millet	2:8	Chhattisgarh
Finger millet + field bean	8:1	Karnataka, Tamil Nadu
	6:2	Bihar
Finger + soybean	4:1	Karnataka
Finger millet + black gram/moth bean	4:1	Maharashtra
Finger millet + soybean (mixed cropping system)	90:10	Uttarakhand
Finger millet + Ground nut strip cropping	6:9	Karnataka
Sequential cropping		
Finger millet + soybean (Kharif)-Oat	Northern hilly region	
Potato–paddy–finger millet	Northern Bihar	
Finger millet–potato–maize Finger millet–onion–finger millet Cowpea/sesamum/black gram/green gram-finger millet Finger millet–horse gram	Southern Karnataka or Deccan plateau	
Finger millet–Mustard/Barley/Linseed/Tobacco/Gram	North India	
Finger millet–Potato–finger millet/Maize Finger millet–Groundnut/Sugarcane/Tobacco	South India	

inter cropping finger millet + pegion pea (6:2) registered twice higher net return than mixed sown finger millet + pigeon pea (Dass and Sudhishri 2010). Intercropping with other crops is also common. It is frequently grown in association with sorghum, pigeonpea, cotton, and gram. Proportion of component crops in intercropping depends on the smothering effect of the crops. Results of the experiments at Bengaluru (Karnataka) have revealed the finger millet and soybean sown in alternate rows 22.5 cm apart did not depress the finger millet yield (2.5 t/ha) with a bonus yield (200 kg/ha) of soybean (Anonymous 2014). Staggered planting of pigeon pea 3.3 m apart in May followed by planting finger millet in July in the interspaces between sowing of the two crops for improving the yield advantage. To minimize the weed problem in the interspaces, cowpea can be planted and plowed back as green manure after 45–50 days of vegetative growth (Table 4.5).

Small millet intercropping systems most commonly seen with pulses in Southern India. Fingermillet + dolichos, millet/pigeonpea, millet + black gram, millet + castor are the few examples of intercropping with pulses, whereas with other cereals, finger millet + maize, finger millet + foxtail millet, finger millet + jowar, finger millet + little millet are found common. Millets are also intercropped with other species, i.e., finger millet + mustard and millet + brassicas. Ramachandrappa et al. (2016) deliberated on the result of conservation furrow on finger millet and groundnut-based intercropping system. Finger millet (MR-1) + pigeonpea (BRG-2) (8:2) with paired rows of pigeon pea having conservation furrow recorded the highest finger millet grain equivalent yield and economic profitability (3774 kg/ha, Rs. 44,940/ha and 2.56, respectively) in comparison with the with the farmers practice of sole cropping of finger millet with a yield increase about 45%. The conservation furrow will store the moisture and help the crop to attain higher yield (Table 4.6).

Table 4.6 Influence of conservation furrow on finger millet-based intercropping system

Cropping system	Crop equivalent yield (kg/ha)	Net returns (Rs./ha)	B:C Ratio	Rain water use efficiency (kg/ha mm)
Finger millet based				
Finger millet + pigeonpea (8:2)	3774	44,940	2.56	5.79
Finger millet	1869	23,618	1.68	3.50

4.4.1 Rotation/Sequential Cropping

A single crop system is not successful system for long run to maintain sustainability. The successive cultivation of two or more crop in a year from a unit field is called crop rotation or sequence cropping. It would be the best option to utilize soil fertility and labor. The crop rotation can be able to generate employment throughout the year. The small millet-based cropping systems are prevalent only tribal and hilly

Table 4.7 Sequence cropping systems observed in country

Crop rotations/sequence cropping	Region	References
Maize–finger millet (relay/sequential cropping)	Nepal	Pilbeam et al. (2002)
Potato–finger millet	Karnataka	Saravanane et al. (2011)
Sunflower–proso millet	Pacific Northwest, U.S.	Habiyaremye et al. (2017)
Finger millet–groundnut	Karnataka	Pavankumar et al. (2016)
Finger millet and redgram	Karnataka/Andhra Pradesh	Shankar et al. (2018)

Table 4.8 Millet sequence cropping in different parts of country

Sequence cropping	State
Sorghum–chickpea	Karnataka, Maharashtra, Madhya Pradesh, Rajasthan
Sorghum–wheat	Punjab
Sorghum–safflower	Karnataka
Black gram–sorghum	Karnataka, Maharashtra
Ground nut–sorghum	Karnataka
Pearl millet–wheat	Haryana
Pearl millet–chickpea	Haryana, Uttar Pradesh
Pearl millet cluster bean	Haryana
Pearl millet–moth bean/mung bean/cluster bean	Rajasthan
Pearl millet–black gram/barley	Jammu
Pearl millet–mustard	Rajasthan, Haryana
Groundnut–potato–pearl millet	Gujarat
Paddy–finger millet	Odisha, Madhya Pradesh
Foxtail millet–safflower	Karnataka

areas of India. The small millets rotation or relays cropping with legumes or oilseeds are able to attain sustainability of crop production. The key finger millet-based crop rotations or sequential cropping are as follows (Tables 4.7 and 4.8).

4.4.2 Mixed Cropping

Mixed cropping is the traditional practice to get coverage against the total failure of the crop. Most of small and marginal farmers are adopting this system to fulfil their basic needs of cereal, legume, and oil seeds. In Garhwal region of Uttarakhand, the mix cropping of finger millet + soybean (90%:10% crop mixture) was registered more profitable than the sole cropping (Singh and Arya 1999). The mixed cropping of lupine and finger millet (50:100 and 75:10 seeding proportion) had a greater yield

advantage compare to sole of lupine or finger millet. In Ethiopia as reported by Bitew (2014). Finger millet with black gram or pigeon pea mixed cropping is playing important role in reducing soil loss through runoff (Dass and Sudhishri 2010).

4.4.3 Strip Cropping

Strip cropping is the necessary method to attain augmented productivity on sloppy land to guard the soil from erosion. Strip cropping is also an intercropping includes growing of soil-depletion and soil-conserving crops in alternate strips running perpendicular to the land grade for reducing erosion. Jakhari et al. (2015) reported that six rows of finger millet with groundnut in four rows progressively gave more millet equivalent yield than sole finger millet. The land equivalent ratio of 1.38 was calculated in 6:4 ratios representing 38% area advantage compared to sole cropping.

4.4.4 Relay Cropping Systems Observed in South Asia

Maize–millet in Nepal (Pilbeam et al. 2002; Sherchan et al. 1999), potato–millet in Karnataka, India (Saravanane et al. 2011) and groundnut–millet were established as feasible cropping system.

4.4.5 Cropping System Interactions

The proceeding crop in sequential cropping has substantial effect on the succeeding crop. In soil, sorghum leave toxic chemicals which restrict the subsequent crops germination. However, the previous leguminous crop leaves substantial amount of nitrogen for succeeding crop. A short span of overlapping under relay cropping happens in sequence of two crops.

- For deep plowing, the deep-rooted crops respond while shallow tillage is sufficient for most cereals.
- Millets require finer seedbed preparation due to seeds smaller in size and all millet crops are grown on flat seedbed.
- Short duration and varieties of photo insensitive nature are important for effective sequence cropping. In general, genotypes of determinate growth habit and show lesser response to population changes.
- The varieties of constituent crops should be less contending and for the peak demand of nutrient period must be unlike from base crop.
- The base crop sowing and intercrop is to be completed in the fixed ratios.
- The component crops under traditional cropping systems are grown with sub-optimum plant population.
- When legumes are involved, a share in the requirement of nitrogen of millet is augmented by legume.

- The nitrogen required for base crop as the pure crop is adequate for whole intercropping with millet + legume. For phosphorus and potassium, one-fourth to one-eighth of the recommendation of intercrop dose is required.
- The nitrogen basal dose is applied to both components rows in millets + legume intercrop system while, the top dressing is only done for cereal rows. However, potassium and phosphorus are applied as basal to both crops.
- The total water used in intercropping is nearly the same as for sole crops and hence the intercropping water-use efficiency is higher than sole crops.
- The intensive cropping diminishes weed hitches in addition, residual toxicity, and herbicide selectivity are perilous under intensive cropping.
- Chemical weed control is problematic because the herbicide may be selective.
- Recommendation of herbicides under sequence cropping must consider the following crop, e.g., more dose of atrazine applied to sorghum distresses the germination of successive pulse crop.

4.5 Pests and Diseases

Plant density plays a vital role and affects the disease incidence. The wider the separation between individual hosts plants—less infection to new plants. Crop rotation lowers the level of inoculum of many pathogens. Crops in sequence or as intercrops is effective in lowering populations of soil borne diseases and cover crops reduced the movement or spread of pathogens. Pest outbreaks are to be less common in mixed stands due to crop multiplicity than in sole cropping system. Adarsh et al. (2019) reported that the cropping system with pulses under intercropping, mixed cropping, sequential cropping, paira cropping, and relay cropping improved soil properties and have reduced the disease and pests incidence, while Dharam Singh Meena et al. (2017) revealed that in diverse finger mill-based cropping systems, finger millet + legumes noted more sustainable harvest and lesser weeds, disease, and insects infestation for the crop.

4.5.1 Bajra

Over laborious ecological selection concluded an extensive period, a few crops like pearl millet, mungbean, cluster bean, moth bean, sesame, and castorare grown in many proportions. Pearl millet is the vital crop, sole pearl millet, or a pearl millet-fallow alternation is common. Pearl millet is assorted with legumes only when sowings are late beyond July third week. Most of the farmers in Rajasthan desire mixed cropping over sole cropping and mixing of pearl millet, mungbean, mothbean, and sesame is followed. A pearl millet-rotated with wheat bid better projections in irrigated areas. Table 4.9 shows the most commonly practiced cropping patterns in Rajasthan, India. For varied regions, most appropriate cropping systems are worked out and conservation of moisture through numerous techniques formulate a significant commendation in dry areas.

Table 4.9 Prevailing cropping pattern in agroclimatic zone in Western Rajasthan

First year		Second year	
Rainy season	Post rainy season	Rainy season	Post rainy season
Pearl millet	Fallow	Fallow	Barley
Moth bean	Fallow	Pearl millet	Fallow
Pearl millet	Chickpea	Fallow	Fallow
Cluster bean	Fallow	Pearl millet	Fallow
Sesame	Fallow	Fallow	Fallow
Pearl millet + grain legumes	Fallow	Sesame	Fallow
Fallow	Fallow	Grain legume	Wheat
Fallow	Mustard	Cluster bean	Fallow

Table 4.10 Suitable pearl millet-based intercropping

Rajasthan	Pearl millet + clusterbeanor moth bean/sesame
Haryana	Pearl millet + Green gram or sesame
Gujarat	Pearl millet + Green gramor sesame
Utttar Pradesh	Pearl millet + Green gram or sesame
Madhya Pradesh	Pearl millet + Black gram/soybean
Delhi	Pearl millet + Pigeon pea/groundnut/castor
Karnataka	Pearl millet + Pigeon pea
Maharashtra	Pearl millet + Moth bean or Pigeon pea
Tamil Nadu	Pearl millet + cowpea/sunflower

The techniques contain widespread spaced crop and usage of mulch moreover through manipulating topsoil or by organic resources and to cultivate short duration cultivars to evade moisture stress condition. Though, adoptions of agronomic approvals are largely limited to better rainfall/guaranteed moisture available areas but essential to accept improved agronomic practices more in drier pearl millet growing areas to withstand the efficiency.

In legume followed by pearl millet cropping sequences, cluster bean prior pearl millet found more fruitful compared to mung bean or moth bean. Table 4.10 describes the important and generally adopted cropping patterns in different states.

Mixed systems: Varying proportions (2–25%) of crops like sorghum, green gram, pigeon pea, cowpea, field bean, horse gram, and sesame with bajra or pearl millet (75–90%) are practiced in mixed cropping systems during *kharif* season. If the component crops are traditional long duration crops, pearl millet is harvested early.

Replacement series of intercropping with crops like pigeon pea, groundnut, and cowpea is adopted in many bajra growing regions during *kharif* season (8:2, 10:2; 10:4; 2:1). To safeguard more production, higher land equivalent ratio and economic returns under deviant weather environments, diverse crops are recognized for intercropping with pearl millet in the nation. Due to its short duration and its suitability to all three seasons, it fits into many rotational systems with crops like rice, cotton, sorghum, and groundnut under irrigation. More common irrigated

system is pearl millet–wheat. Under rainfed conditions, it is rotated in 2-year rotation with crops like cotton (Rajasthan), Chick pea (Punjab), and tobacco (Gujarat).

4.5.2 Sorghum

Sorghum is grown in sequence with crops like wheat, pea, Bengal gram, potato (in north India) and cotton, tobacco, or finger millet (in south India). In *rabi* dominant area, systems like groundnut–sorghum and pulses–sorghum are practiced. Sorghum fits into three crop sequences also. Some three crop sequences are Sorghum–wheat–moong, sorghum–wheat–lobia (in north India) as well as sorghum–ragi–groundnut (south India). Sorghum is also grown with soybean, pigeonpea, moong, and blackgram in intercropping systems. Some hybrids are considered more apt for intercropping systems. *Rabi* sorghum is generally intercropped with Bengal gram, safflower or even sunflower.

4.5.3 Kodo Millet

Kodo millet + pigeonpea (2:1) or Kodo millet + green gram or black gram (2:1) are profitable in Madhya Pradesh and the other alternative inter cropping systems are kodo millet + soybean in 2:1 proportion and kodo millet + horse gram in 4: 2 proportion. Kodo–niger–kodo or kodo–soybean–kodo crop rotation was found to be sustainable system. On the source of 9 years yield information at Dindori, India, niger–soybean–kodo millet crop rotation and kodomillet–soybean–kodo millet were emerged as the most remunerative crop rotation (Sukanya et al. 2022).

4.5.4 Foxtail Millet

The yield advantage in intercropping foxtail millet with pigeon pea in 6:1 proportion was reported by the experiments at AICRP on small millets (Sukanya et al. 2022). Foxtail millet with pigeon pea in 4:1 or with cotton in 5:1 under heavy soils is an ideal and remunerative cropping systems for royal seema regions. In Andhra Pradesh, foxtail millet + groundnut/pigeonpea in 5:1 proportion is recommended. The other intercropping system is foxtail millet is with castor in 7:1 proportion. If monsoon is early in Andhra Pradesh, the practice of sowing foxtail millet with 45 cm spacing at row and *rabi* sorghum as a relay crop when first crop foxtail millet is approaching maturity is well practiced.

In medium deep black soils, growing two crops, foxtail millet–mustard or foxtail millet–green gram in sequence is gainful than taking one crop of foxtail millet. Among the *rabi* crops tested, after *kharif* foxtail millet growing Jowar found with higher returns and the following best stood Foxtail millet–Bengal gram cropping system which provided higher foxtail millet grain equivalent yield as against fallow–Bengal gram. Foxtail millet as irrigated crop in garden lands during summer is

Plate 4.2 Foxtail millet and pigeon pea intercropping system in Andhra Pradesh

usually preceded by rice during both *kharif* and *rabi* or by rice in *kharif* and groundnut in *rabi*. Summer irrigated foxtail millet can fit into many intensive cropping systems owing to its shorter duration (Plate 4.2).

4.5.5 Foxtail Millet–Chickpea Sequence

The results (2010 and 2011) at Nandyal, India, showed the improvement in harvest under cropping systems when in situ soil preservation measures were accepted and was reported 45%, 34% and 10% more soil moisture storage during development stages at 25, 60, and 75 DAS, correspondingly as related to cropping system deprived of soil preservation practices. Enhancing the cropping intensity by 200% with the insertion of foxtail millet at early *kharif* followed by chick pea either with or without in situ soil moisture preservation provided yield benefit of 42% and 35%, correspondingly (Table 4.11) (Sukanya et al. 2022) (Plate 4.3).

4.6 Nutrient Management Crop Sequence

The 2 years consolidated data (2008 and 2009) at Berhampur in India instituted that submission of RDF to both crops (rice and finger millet) in order along with an added dose of FYM at 5 t/ha for finger millet provided more FMGEY (Fig. 4.2), while at Mandya, India, at irrigated condition, the submission of 75% RDF to rice–sunhemp–

Table 4.11 Impact of moisture conservation practices in cropping system

Crop	Moisture conservation practice (without)			Moisture conservation practice (with)			% rise in moisture		
	25 DAS	60 DAS	75 DAS	25 DAS	60 DAS	75 DAS	25 DAS	60 DAS	75 DAS
Foxtail millet–Bengal gram	20.4	18.4	14.5	29.6	24.6	16.0	45	34	10
Fallow–Bengal gram	27.7	19.6	14.6	30.5	26.4	15.6	10	35	7
Treatment details									
T_1: Rice–Finger millet (75% RDF)									
T_2: Rice–Finger millet (100% RDF)									
T_3: Rice–Finger millet (125% RDF)									
T_4: Rice–Sunhemp–finger millet (75% RDF)									
T_5: Rice–Sunhemp–finger millet (100% RDF)									
T_6: Rice–Sunhemp–finger millet (125% RDF)									

Plate 4.3 Foxtail millet and niger intercropping system

ragi system provided more grain yield of finger millet as related to rice–finger millet system with 75% RDF (Table 4.12).

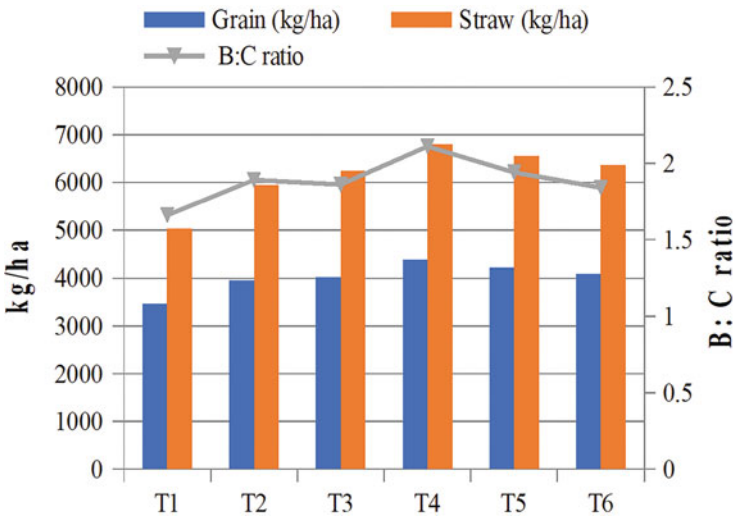

Fig. 4.2 Rice–finger millet sequence as influenced by different nutrient managing practice at Berhampur

Table 4.12 Finger millet affected by inorganic nutrient management under irrigated situations

Treatments	Grain yield (kg/ha)	Straw yield (kg/ha)	Benefit cost ratio
T_1: Rice–Finger millet (75% RDF)	3461	5031	1.66
T_2: Rice–Finger millet (100% RDF)	3951	5940	1.89
T_3: Rice–Finger millet (125% RDF)	4017	6243	1.86
T_4: Rice–Sunhemp–finger millet (75% RDF)	4376	6797	2.11
T_5: Rice–Sunhemp–finger millet (100% RDF)	4216	6549	1.94
T_6: Rice–Sunhemp–finger millet (125% RDF)	4096	6366	1.84

4.6.1 Proso Millet

Proso millet intercropping with black gram or greengram at 2:1 proportion is suggested for Bihar and Uttar Pradesh. Potato–proso millet rotation is lucrative in western Bihar. Many sequential systems include proso millet either before or after *kharif* or *rabi* crop. Some such systems are proso millet–wheat/barley; proso millet–chick pea, maize–potato–proso millet or Maize–wheat–proso millet. In India, it is rarely grown as intercrop, but in the USA, it is successfully grown as inter crop with crops in high intensity cropping systems. Milenkovic et al. (2019) stated that 1:1 proportion of soybean and proso millet resulted higher biomass yield in Belgrade, Serbia, while, Chapke et al. (2018) also reported a similar result with proso millet + green gram with 2:1 row ratio in Bihar and Uttar Pradesh, India.

4.6.2 Barnyard Millet

Barnyard millet and rice bean/niger at 4:1 proportion is endorsed for Uttaranchal. Mixed cropping with cotton, pigeon pea, or short duration pulse crops is also practiced. Mixtures of barnyard millet (90%) and soybean (10%) were found as feasible system. The next better cropping system is the mixed cropping of barnyard millet with amaranths (90:10) by weight. Sukanya et al. (2022) reported that the barnyard millet higher grain yield was seen in the mixed cropping of barnyard millet and Amaranthus (90:10) by weight followed by barnyard millet and Amaranthus (4:1) (Fig. 4.3).

Treatment
T1: Barnyard millet sole crop
T2: Amaranthus sole crop
T3: Intercropping of Barnyard millet + Amaranthus (4:1)
T4: Barnyard millet and Amaranthus mixed cropping (90:10) by weight
T5: Barnyard millet and Amaranthus mixed cropping (85:15) by weight
T6: Barnyard millet and Amaranthus mixed cropping (80:20) by weight
T7: Farmers practice (60:40) by weight

4.6.3 Little Millet

Little millet + black gram in 2:1 proportion in Odisha; little millet + soybean or sesamum or Pigeonpea at 2:1 association in Madhya Pradesh; little millet + pigeonpea

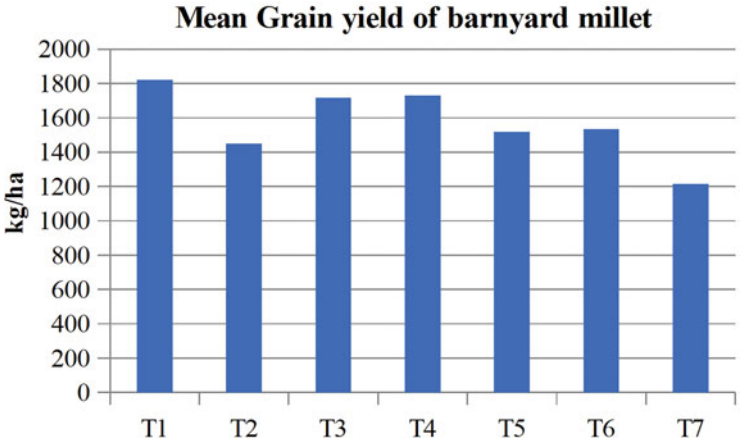

Fig. 4.3 Barnyard millet grain yield (kg/ha) under different cropping systems at Ranichauri under rainfed situations

in 2:1 proportion in South Bihar are found profitable. Little millet and pigeon pea in 8:2 ratio and a conservation furrow between pigeon pea paired row is instituted to be a better practice to be followed (Sukanya et al. 2022). Little millet–Niger and little millet–toria are found to be the promising sequence cropping. The experiment conducted in Tamil Nadu, India, during rainy period discovered that little millet equivalent yield in little millet + pigeon pea in 6:1 proportion and later horse gram sequence (Sharmili et al. 2019). Thesiya and his coworkers in (2019) also reported the similar results with little millet followed by green gram cropping.

4.7 Performance of Millets in Alternate Land Use System

Custard + finger millet gave higher custard apple equivalent yield compared to sole custard apple, while Amla + finger millet intercropping gave higher amla equivalent yield and B:C (1810 kg/ha and 2.71, respectively) compared to sole amla (667 kg/ha and 2.48, respectively) as (Anonymous 2018). Among different intercrops tested under amla-based intercropping (finger millet, fodder maize, field bean, grain amaranth, cowpea, horse gram), higher crop equivalent yield, net returns and rain water use efficiency were observed with amla + finger millet, amla + field bean and amla + cowpea (Anonymous 2013–2017).

4.8 Promising Cropping System: Rice Fallow System

Fallow period refers to the duration when there are no crops are grown between the two successive crop or one crop is taken in a season and in another season, land become vacant or without crop. Due to this, the system productivity and net returns of the farmers decreases. The effective utilization of fallow lands enhances the crop productivity that led to sustainable system productivity of the cropping system (Chowdhury et al. 2020; Khan et al. 2018). The rice fallows provide good room for area extension of millets and intensification of crop, i.e., integrating millets in existing agricultural practices. Millets are the model crop for cultivation in fallow stages, i.e., between cultivation of one group of crops and sowing the next. Their prolific utilization can overwhelm many social and economic problems like labor migration, low income, and unemployment. Subsequently the harvest of rice during *kharif*, climatic conditions in most of the areas found suitable for growing pulses is profitable due to cool and warm season. The study by Chapke et al. (2011) communicated that land holding, sorghum area, cost of fertilizers, total inputs cost, labor cost, cost of irrigations and insecticides used, and yield had substantial association with returns attained from the sorghum cultivation. The farmers in Andhra Pradesh are cultivating sorghum after rice on the remaining soil moisture with zero tillage. Finger millet under rice fallows is likewise a common practice in few patches of rice regions in Southern part of India. In similar lines, extensive research on growing other small millets in rice fallow is required for out spreading the millet area.

4.9 Conclusion

The intensive planning in agriculture is lacking as we are living in a country which has diversified agro-climatic zones resulting into insufficient production. Under these circumstances, climate resilient nutricereals are imminent and might cater to nutritive demands of cumulative population. It would advocate that climate change impacts would be overcome by multiplicity and the climate change is not exclusive in nature as it is complicated steady deviations over time. The best combination of cropping practices must be determined for each soil and ecosystem and that are socially acceptable, economically profitable, and environmentally compatible must be designed for each ecosystem. The agronomic crop and cropping pattern vary from situation to situation. Systematic efforts on socio-economic, political, genetic, and technological developments enabling the acceptance of millets would certainly progress the food and nutritional safety under the background of climate variation. The millet cropping systems provide plentiful food, fodder, and produce satisfactory cash income for domestic and farming expenditures. Cropping design plays a vigorous role in defining the level of agricultural production and differs from one situation to another. Conserving water, soil, and maintaining yield depend mainly on managing cropping systems.

References

Adarsh S, John J, Thomas G (2019) Role of pulses in cropping systems—a review. Agric Rev 40(3). https://doi.org/10.18805/ag.R-1888

Anonymous (2002) Annual progress report. Indian Council of Agricultural Research—All India Coordinated Small Millets Improvement Project, Bengaluru

Anonymous (2007) Annual progress report. Indian Council of Agricultural Research—All India Coordinated Small Millets Improvement Project, Bengaluru

Anonymous (2013–2017) Annual progress report. Indian Council of Agricultural Research—All India Coordinated Small Millets Improvement Project, Bengaluru

Anonymous (2014) Annual progress report. Indian Council of Agricultural Research—All India Coordinated Research Project on Dryland Agriculture, ICAR, GKVK, Bengaluru

Anonymous (2018) Annual progress report. Indian Council of Agricultural Research-All India Coordinated Dryland Agriculture Project, Bengaluru

Bitew Y (2014) Influence of small cereal intercropping and additive series of seed proportion on the yield and yield component of lupine (*Lupinus* spp.) in north western Ethiopia. Agric For Fisheries 3:133–140

Chapke RR, Rakshit S, Mishra JS, Patil JV (2011) Factors associated with Sorghum cultivation under rice fallows. Indian Res J Ext Educ 11(3):67–70

Chapke RR, Prabhakar, Shyamprasad G, Das IK, Tonapi VA (2018) Improved millets production technologies and their impact. Technology Bulletin. ICAR-Indian Institute of Millets Research, Hyderabad, India, p 84

Chowdhury R, Subhaprada D, Koushik S, Gulati JML (2020) Pulses in rice fallow: a way towards achieving nutritional security: a review. Agric Rev 41:264–271

Dass A, Sudhishri S (2010) Intercropping in fingermillet (*Eleusine coracana*) with pulses for enhanced productivity, resource conservation and soil fertility in uplands of Southern Orissa. Indian J Agron 55(2):89–94

Dimes J, Cooper P, Rao KPC (2008) Climate change impact on crop productivity in the semi-arid tropics of Zimbabwe in the 21st century. In: Proceedings of the workshop on increasing the productivity and sustainability of rainfed cropping system of poor, smallholder farmers, Tamale, Ghana, 22–25 September 2008, pp 189–198

Dutta D, Bandyopadhyay P (2006) Production potential of groundnut (*Arachis hypogaea*) with pigeonpea (*Cajanus cajan*) and maize (*Zea mays*) under various row proportions in rainfed Alfisols of West Bengal. Indian J Agron 51(2):103–106

Gowda KTK, Jena BK, Ramamoorthy K, Dubey OP, Rao TV, Shankarlingappa BC, Ashok EG (2004) Augmenting legumes production in small millet based cropping system. All India Coordinated Research Project on Small Millets, Indian Council of Agricultural Research, Bangalore, p 144

Habiyaremye C, Matanguihan JB, D'Alpoim Guedes J, Ganjyal GM, Whiteman MR, Kidwell KK, Murphy KM (2017) Proso millet (*Panicum miliaceum* L.) and its potential for cultivation in the Pacific Northwest, U.S.: a review. Front Plant Sci 7:1961. https://doi.org/10.3389/fpls.2016.01961

Jadhav SN, Bal AS, Gadre UA (1992) Intercropping urdbean and okra in finger millet is remunerative in Konkan. Indian Farming

Jakhari P, Ashikary PP, Naik BS, Madhu M (2015) Finger millet (*Eleusine coracana*)—groundnut (*Arachis hypogea*) strip cropping for enhanced productivity and resource conservation in uplands of Eastern Ghats of Odisha. Indian J Agron 60(3):365–371

Khan MAH, Sultana N, Akter N, Zaman MS, Choudhury AK (2018) Increasing cropping intensity and productivity through mung bean inclusion in wheat-fallow-T. Aman rice cropping pattern. Bangladesh. J Agric Res 43(2):333–343

Kiranmai J, Saralamma S, Reddy CVCM (2021) Enhancing the millet system productivity with intercrops. Biol Forum 13(3b):81–83

Kole C, Muthamilarasan M, Henry R, Edwards D, Sharma R, Abberton M (2015) Application of genomics-assisted breeding for generation of climate resilient crops: progress and prospects. Front Plant Sci 6:563

Kumar BHP, Halikatti SI, Hiremath SM, Chittapur BM (2008) Effect of intercropping system and row proportions on the growth and yield of little millet and pigeonpea. Karnataka J Agric Sci 21(4):479–481

Mallareddy, Thimmegowda MN, Ramachandrappa BK, Hebbal N (2016) Influence of moisture conservation practices on productivity and economics of finger millet + pigeonpea intercropping system in eastern dry zone of Karnataka. Adv Life Sci 5(8):3256–3260

Meena DS, Gautam C, Patidar OP, Singh R, Meena HM, Prakash VJ (2017) Management of finger millet based cropping systems for sustainable production. Int J Curr Microbiol Appl Sci 6:676–686

Milenkovic M, Simic M, Brankov M, Milojkovic-Opsenica D, Kresovic B, Dragicevic V (2019) Intercropping of soybean and proso millet for biomass production. J Process Energy Agric 23:38–40

Nigade RD, Karad SR, More SM (2012) Agronomic manipulations for enhancing productivity of finger millet based on intercropping system. Adv Res J crop 3(1):8–10

Pavankumar G, Ramachandrappa BK, Thimmegowda MN (2016) Influence of rotation, use of organic and inorganic sources of nutrients on yield, nutrient uptake and economics of finger millet (Eleusine coracana (L.) Gaertn). Ecol Environ Conserv 22:39–46

Pilbeam CJ, Gregory PJ, Tripathi BP, Munankarmy RC (2002) Fate of nitrogen-15-labelled fertilizer applied to maize-millet cropping systems in the mid-hills of Nepal. Biol Fertil Soils 35:27–34. https://doi.org/10.1007/s00374-001-0436-2

Pradhan A, Thakur A, Sao A, Patel DP (2014) Biological efficiency of intercropping in finger millet (*Eleusine coracana* (L.) Gaertn) under rainfed condition. Int J Curr Microbiol App Sci 3(1):719–723

Ramachandrappa BK, Krishnamurthy R, Thimmegowda MN, Savitha MS, Srikanth PN, Manjunatha BN, Bhavitha NC, Ravindra Chary G, Gopinathan KA, Srinivasarao C (2016)

Long term integrated nutrient management—soil and crop. AICRP on dryland agriculture. University of Agricultural Sciences, Bengaluru

Saravanane P, Nanjappa HV, Ramachandrappa BK, Soumya TM (2011) Effect of residual fertility of preceding potato crop on yield and nutrient uptake of finger millet. Karnataka J Agric Sci 24(2):234–236

Shankar MA, Thimmegowda MN, Bhavitha NC, Manjunatha BN (2018) Yield and economics of finger millet and redgram rotation as influenced by zinc, boron and biofertilizer nutrition. Mysore J Agric Sci 52(1):43–48

Sharmili K, Parasuraman P, Sivagamy K (2019) Studies on intercropping in rainfed littlemillet (*Panicum sumatrense*). Int J Curr Microbiol Appl Sci 8:299. https://doi.org/10.20546/ijcmas.2019.803.037

Sherchan DP, Pilbeam CJ, Gregory PJ (1999) Response of wheat- rice and maize/millet systems to fertilizer and manure applications in the mid-hills of Nepal. Exp Agric 35:1–13

Singh RV, Arya MPS (1999) Nitrogen requirement of finger millet + pulse intercropping system. Indian J Agron 44(13):47–50

Sood S, Joshi DC, Chandra AK, Kumar A (2019) Phenomics and genomics of finger millet: current status and future prospects. Planta 250:731–751

Sukanya TS, Prabhakar, Krishne Gowda KT, Swarna R, Hariprasanna K, Tonapi VA (2022) Good agronomic practices for higher yield in small millets. ICAR-Indian Institute of Millets Research, Hyderabad

Thesiya NM, Damasia DM, Bambharolia RP (2019) Effect of integrated nutrient management on grain yield, quality and nutrient content and uptake of little millet under little millet-greengram cropping sequence. Crop Res 54:70–74

Yadav R (2010) Production potential of finger millet and French bean intercropping under rainfed conditions of Uttarakhand. J Food Legumes 23(2):121–123

Major Diseases of Small Millets and Their Management Strategies

5

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Abstract

Small millets, also known as coarse cereals comprises finger millet, foxtail millet, proso millet, kodo millet, barnyard millet, little millet and browntop millet etc. Small millets are considered as super grains since ancient times owing to their excellent attributes viz., climate resilience, richness of minerals (Ca, Zn and Fe etc.), fibers and vitamins etc. Superior nutritional and agroecological traits of millets are capturing global importance as smart foods and smart crops which can serve as potential alternative for staple food grains and contributing to global food and nutritional security. Small millets are being widely cultivated in semi-arid regions like India as rainfed crops. Despite their admirable characteristics, small millets production accounts only 2% of worlds cereal production. This is due to the genetic potential of small millets is hindered by various biotic and abiotic stresses resulting in substantial yield and economic losses. Among the biotic stresses, diseases caused by fungal pathogens such as blast (*Magnaporthe* spp.), leaf spot or leaf blight (*Helminthosporium* spp.), rusts, and smuts are the predominant constraints in cultivation of small millets in major millet growing areas. Addressing these challenges on global scale needs better understanding of the disease, causal organism along with their symptomatology and epidemiology for

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devising effective management strategies. This book chapter accentuates on predominant diseases of small millets, their symptoms, modes of survival, and spread along with integrated mitigation practices as well as novel approaches which can be exploited for managing diseases in millets resulting in increased production and productivity of millets.

Keywords

Diseases · Small millets · Management · Host plant resistance · Epidemiology

5.1 Introduction

Millets are the coarse cereal crops with small seeded grains that are relatively less cultivated globally than commercial cereal crops like rice, wheat, and maize. Though millets are known to cultivating since ages majorly by the subsistence farmers under rainfed conditions in Asian and African countries, they lagging behind to receive attention from the breeders, consumers, and growers compare to other food crops. Millets are categorized into two groups viz., major millets (include sorghum and pearl millet) and minor or small millets comprised of finger millet (*Eleusine coracana* L. Gaertn), foxtail or Italian millet (*Setaria italica* L.), barnyard millet (*Echinochloa frumentacea* L.), Proso millet (*Panicum miliaceum* L.), kodo millet (*Paspalum scrobiculatum* L.), little millet (*Panicum sumatrens* L.), and browntop millet (*Brachiaria ramosa* L. Stapf). Small millets are mostly grown in semiarid tropics of Asia and Africa and these can be easily grown in diverse agro-ecological climates where cereals couldn't able to produce substantial yield. With the growing health concerns and to sustain in the world of increasing population, agricultural sustainability is of prime importance which consists of food and nutritional security for which small millets will serve as an ideal solution. Small millets are regarded as nutri-cereals owing to their significant nutritional and agro-ecological characteristics. They are fat-rich, good source of minerals viz., iron (Fe), zinc (Zn), magnesium (Mg) and calcium (Ca), vitamins, dietary fiber content and richness of essential amino acids like cysteine and methionine etc. Owing to their remarkable nutraceutical attributes and climate resilience, millets are capturing global attention over cereals in recent times which were hitherto predominant (Anju Jr and Sarita 2010). Small millets as smart foods and smart crops can serve as potential alternative for staple food grains and has capability for contributing to global food and nutritional security.

Despite their admirable significance, the small millets production accounts only 2% of worlds cereal production where Asia holds 40% of global millet production which is majorly contributed by India and China. In India, millets occupy 13.8 m ha (138 lakh ha) of area with production of 173 lakh ton which accounts 80% of Asia's and 20% of global millet production (FAOSTAT 2021). Millets are known for their climate resilience and enduring capacity towards drought and are comparatively less prone to pest and diseases. However, changing climate scenario has increased the

exposure to various biotic and abiotic deterrents that limit millet production as well as productivity. Among the various biotic constraints, diseases caused by fungal pathogens are wide spread and destructive. Fungal disease recorded on small millets includes blast, leaf blight/brown spots, smuts, rusts, downy mildew, leaf spot etc. In addition to the fungal diseases, small millets are also occasionally/sporadically attacked certain bacterial (viz., *Xanthomonas* spp., *Pseudomonas* spp., etc.) and viral pathogens (like *ragi mottle streak virus*, *sugarcane mosaic virus*, etc.) which are of low economic importance to the millet cultivation under natural conditions.

The genetic potential of small millets is affected by the attack of these diseases under varying climatic conditions resulting in substantial yield losses. Albeit many pathogens attack small millets, not all cause significant losses, only some diseases on specific millets occur in severe form while others show negligible effect unless prevalence of vulnerable conditions. Addressing these challenges on global scale needs better understanding of the disease, causal organism along with their symptomatology and epidemiology for devising effective management strategies. Hence, attempts have been made in this chapter to compile the available information by accentuating on major diseases of small millets which are of regular occurrence, their symptoms, modes of survival, and spread along with revised mitigation practices helping in disease control and improving millets production and nutritional quality.

5.2 Diseases of Small Millets

Small millets are known to cope up against abiotic and biotic stresses; nevertheless, under favorable conditions, some of the diseases cause heavy losses and can damage entire crop (Kumar and Singh 2010). All the small millets don't exhibit same level of resistance to biotic factors which varies with genus to genus and also depends on prevailing environmental conditions. Among the biotic constraints, a variety of diseases caused by fungal, bacterial, and viral pathogens became huge economic importance over the time which can devour the crop if left uncontrolled. Various diseases possibly occur on all (seven) small millets are enlisted in the Table 5.1 along with their causal organisms and nature of disease. Although enlisted table comprised of many fungal, bacterial, and viral diseases, the number and intensity of fungal diseases are predominant over the other pathogens which comes about frequently in severe form.

Here, fungal diseases have been categorized into three groups based on their nature of infection or survival and spread of pathogen which includes foliar/airborne diseases (diseases that affect foliar parts, i.e., leaves and stems etc. and spread inoculum through air), soilborne diseases (diseases that affect stem near soil line, root, sheath etc. and survival inoculum in soil) and complex or mixed carrier disease (includes the diseases that affect different (reproductive) plant parts like flowers, grains, and dispersal of inoculum occurs by various agents like seed or air or insects etc.). Among the three categories, foliar diseases are abundant and increasing over the time with emergence of new pathotypes/races or species under diverse environmental conditions. Potential fungal diseases of millets are blast, banded sheath

Table 5.1 List of small millet diseases along with their causal organisms and host range

S. No.	Disease	Causal organism	Host(s)	Reference
Fungal diseases				
(1)	*Foliar/air borne diseases*			
1.	Blast	*Pyricularia grisea* (Cooke.) Sacc. (PS: *Magnaporthe grisea* (Herbert) Barr)	Finger, Barnyard, Proso and Little millet	Viji et al. (2000)
		Pyricularia setariae Y. Nisik. (PS: *Magnaporthe setariae*)	Foxtail millet	Richardson (1990)
2.	Leaf spot/Leaf blight (*Helminthosporium* spp.)	*Drechslera nodulosum* (Sacc.) Subram & Jain (PS: *Cochliobolus nodulosus*)	Finger and Little millet	Sivaesan (1987)
		Bipolaris setariae (PS: *Cochliobolus setariae*)	Foxtail and Browntop millet	Ramesh et al. (2021a, b)
		Bipolaris panici-miliacei	Proso millet	Hyung (1997)
		Exserohilum crusgalli	Barnyard millet	Nagaraja et al. (2007a, b)
	Alternaria leaf blight	*Alternaria alternata* (Fr.) Keissl.	Little and Kodo millet	Praveen et al. (2021)
	Cercospora leaf spot	*Cercospora eleusinis*	Finger millet	Munjal et al. (1961)
3.	Rust	*Puccinia substriata* Ellis & Barthol. (Syn: *Uredo paspali-scrobiculati* Syd.)	Kodo millet	Sydow and Butler (1906)
		Uromyces eragrostidis	Finger millet	Channamma et al. (1996)
		Uromyces setariae-italicae Yoshino	Foxtail millet	Lu et al. (2000)
		Uromyces linearis Berk. & Broome	Little and Proso millet	Cummins (1971)
4.	Smut			
	Grain smut	*Melanopsichium eleusinis* (Kulk.) Mundk. & Thirum. (Syn: *Ustilago eleusines* Kulk.)	Finger millet	Mundkur and Thirumalachar (1946)
		Ustilago crameri	Foxtail and Proso millet	Pall et al. (1980)
		Ustilago panici-frumentacei Bref.	Barnyard millet	Vasudeva (1954)
		Macalpinomyces sharmae Vanky (Syn: *Tolyposporium sharmae*)	Little millet	Jain et al. (2006)

(continued)

Table 5.1 (continued)

S. No.	Disease	Causal organism	Host(s)	Reference
	Head smut	*Sorosporium paspali-thunbergii* (Henn.) S.Ito (Syn: *Sorosporium paspali* Mc Alp.)	Kodo millet	Viswanath and Seetharam (1989)
		Sporisorium destruens (Schltdl.) Vánky (Syn: *Sphacelotheca destruens*)	Proso millet	Sinha and Upadhyay (1997)
		Ustilago crus-galli Tracy & Earle	Barnyard millet	Pall et al. (1980)
		Ustilago crameri	Foxtail millet	Kumar (2011)
	Kernel smut	*Ustilago paradoxa*	Barnyard millet	Viswanath and Seetharam (1989)
5.	Downy mildews	*Sclerophthora macrospora* (Sacc.) Thirum. (Syn: *Sclerospora macrospora* Sacc.)	Finger millet	Venkatarayan (1947)
		Sclerospora graminicola (Sacc.) J. Schröt.	Foxtail and Proso millet	Sinha and Upadhyay (1997)
6.	Udbatta disease	*Ephelis oryzae* Syd. (PS: *Balansia oryzae* (Syd.) Naras. & Thirum.)	Foxtail, Kodo, Proso and Little millet	Das et al. (2016)
7.	Sheath rot	*Sarocladium oryzae* (Saw.) Gams & Hawksw.	Kodo millet	Das et al. (2016)
(2)	*Soil borne diseases*			
8.	Banded sheath blight	*Rhizoctonia solani* Kuhn. (PS: *Thanatephorus cucumeris*)	Finger, Foxtail, Barnyard, Proso, Kodo and Little millet	Nagaraja et al. (2007a, b)
9.	Foot rot	*Sclerotium rolfsii* (PS: *Pellicularia rolfsii*)	Finger millet	Coleman (1920)
(3)	*Complex/mixed carrier diseases*			
10.	Grain mold	*Fusarium moniliforme*, *Curvularia lunata*, *Alternaria alternata*, *Phoma sorghina*, *Aspergillus* spp.	Finger millet	Das et al. (2016)
11.	Ergot or Sugary disease	*Claviceps paspalis*	Kodo millet	Ramakrishnan and Sundaram (1950)
Bacterial diseases				
1.	Bacterial leaf spot	*Xanthomonas eleusinae*	Finger millet	Rangaswami et al. (1961)
		Pseudomonas albo-precepitans Rosen.	Foxtail millet	–

(continued)

Table 5.1 (continued)

S. No.	Disease	Causal organism	Host(s)	Reference
2.	Bacterial leaf stripe	*Pseudomonas eleusinae*	Finger millet	Billimoria and Hegde (1971)
		Pseudomonas syringae Van Hall pv. *panici*	Proso millet	Ramakrishnan (1971)
3.	Bacterial leaf streak	*Xanthomonas axonopodis* pv. *coracanae*	Finger millet	–
		Pseudomonas avenae	Foxtail, Barnyard and Proso millet	Nagaraja et al. (2007a, b)
		Xanthomonas spp.	Kodo millet	Nema et al. (1978)
4.	Bacterial leaf blight	*Xanthomonas axonopodis* pv. *coracanae*	Finger millet	Desai et al. (1965)
Viral diseases				
1.	Ragi mottle streak disease: *Ragi mottle streak virus*		Finger millet	Mariappan et al. (1973)
2.	Ragi severe mosaic: *Sugarcane mosaic virus*			Subbayya and Raychaudhuri (1970)
3.	Ragi streak disease: *Eleusine* strain of *Maize streak virus*			Anonymous (1975)
4.	*Wheat streak virus*, *Sugarcane mosaic virus* and *Eleusine virus 2*		Barnyard millet	Sill and Agusiobo (1955)
5.	*Wheat streak virus*, *Rice dwarf or stunt virus* and *Maize leaf streak virus*		Proso millet	

Note: *PS* perfect stage, *Syn* synonym(s)

blight, grain mold, ergot, rust, smut, anthracnose, downy mildew, foot rot, and sheath rot etc. Fungal pathogens incite various plant parts like root, stem, leaves, peduncle or grain and adversely affect yield and quality of the produce. Hence, in this chapter more importance was given to the fungal diseases which were presented with detailed information while the bacterial and viral diseases were included in a context to describe their role and symptomatology by respective pathogens under favorable conditions along with the revised management strategies framed in sustainable manner.

5.2.1 Major Fungal Diseases of Small Millets

5.2.1.1 Blast

Blast caused by *Pyricularia* spp. is one of the serious threats and most destructive disease that occur widely in major millet growing regions of world. Blast of millets is the major production constraints under natural conditions especially in finger and foxtail millet cultivation causing considerable economic losses with varying

damage. In India, the finger millet blast was first reported from Tanjore delta of Tamil Nadu by McRae (1920). While foxtail millet blast was recorded by Nishikado from Japan in 1917, but in India, it was reported from Tamil Nadu in 1919, latter it has also been recorded from Maharashtra, Andhra Pradesh (Sinha and Upadhyay 1997), and Uttarakhand (Kumar 2013). The disease is prevalent in all the major millet growing areas and spreading to new location as well with emerging pathotypes showing varying intensities depending on the cultivar, favorable conditions, and production practices.

Causal Organisms

Pyricularia grisea (Cooke.) Sacc. [Perfect stage: *Magnaporthe grisea* (Herbert) Barr] causing blast in finger and proso millet whereas *Pyricularia setariae* Y. Nisik. infects foxtail millet.

Kulkarni and Patel (1956) grouped *P. setariae* into four physiological races on the basis of pathogenicity, cultural, physiological, and morphological characters of the fungus. However, Gaikwad and D'Souza (1987) concluded that *P. setariae* that infects foxtail millet is different from the isolates that infect rice, pearl millet, and finger millet.

Host Range

Finger millet, proso millet, foxtail millet, pearl millet, rice and wheat etc., Nagaraja et al. (2016) described that *P. grisea* isolated from finger millet possess the potential to infects rice crops but not vice-versa. Likewise, *P. setariae* isolated from foxtail millet shows the ability to infect finger millet, pearl millet, wheat, and *Dactyloctaenium aegyptium* (Viswanath and Seetharam 1989).

Economic Importance

Ragi blast is economically one of the most important diseases, while blast of proso and foxtail millet are relatively of minor occurrence. The disease occurs almost every year in finger millet during rainy season and losses varies with the time of onset of the disease, severity, cultivar, and climatic conditions. During late 1970s to 1980s, incidence of finger and neck blast by *M. grisea* was increased 1% which resulted in a corresponding enhancement of yield losses by 0.32% and 0.084% for neck and finger blast, respectively. However, grain yield losses in finger millet ranged from 6.75% to 87.5% (Rao 1990). In its severe form, foxtail millet blast can lead up to 30–40% loss of economic yield (Nagaraja et al. 2007b) while mean yield loss of ragi blast ranged from 28% to 36% and may go up to 90% in endemic areas with frequent disease (Ramappa et al. 2002).

Epidemiology

The crop is susceptible to the blast disease during all stages of its growth, i.e., seedling (vegetative) to grain formation (reproductive) stage. Especially, young seedlings more prone to the blast both in the nursery and field conditions with favorable weather (Kumar and Singh 2010). Moderate temperature (25–30 °C) with high relative humidity (>90%) and cloudy days in following days coupled

with intermittent rainfall creating continuous leaf wetness for more than 10 h are congenial for rapid development and spread of the disease. Continuous rains at the time of heading may lead to development of finger blast causing huge yield losses in both finger and foxtail millet. Also, excessive application of nitrogen fertilizers observed to enhance the blast incidence (Prakash et al. 2007).

Diagnostic Symptoms

Blast pathogen can infect all the stages of plant in both finger and foxtail millet in which young seedlings/germlings are more prone for the attack resulted in formation of dark patches with burnt appearance in nursery under severe infection (Rachie and Peters 1977). In finger millet, *P. grisea* attack at different stages of the crop lead to formation of symptoms like leaf blast, neck blast, and finger blast while in foxtail millet, *P. setariae* attacks the leaf lamina producing leaf blast symptoms (Nagaraja et al. 2007a, b).

On leaf lamina, pathogen produce typical symptoms of water-soaked, spindle, or diamond shaped lesions which are initially surrounded by chlorotic halo. Typical leaf blast symptoms are the formation of elliptical or diamond-shaped lesions containing greyish center with dark brown margins (Plate 5.1). Under severe infection, adjacent lesions enlarge and may coalesce to form large necrotic areas which gives the crop burnt appearance from far. The pathogen infects and develops lesions on the leaf, peduncle, and finger depending on the stage of the crop. The most damaging stage of finger millet blast is neck blast where the pathogen attacks the neck region, which significantly reduces number and weight of grain per spikelet that leads to spikelet sterility (Rath and Mishra 1975). In this, neck portion of 2–4 in. below the ear immediately turns initially brown and later to black, where olive grey fungal growth can be observed in the blackened portion under high humid climate. In finger blast where the pathogen attacks fingers, i.e., attacks usually the apical portions running towards the base (Plate 5.1a). Infection of finger blast result in shriveled and blackened seeds which makes unfit for seed purpose and human consumption because of loss of minerals and vitamins.

Disease Cycle

The pathogen harbors in glumes, straw as well as on some graminaceous weeds. Anitha et al. (2005) described that the blast pathogen is seed-borne with presence of inoculum in the pericarp and endosperm (Viswanath and Seetharam 1989). Blast fungal life cycle is complex due to its nature of disease which show sensitivity to the weather conditions, survival, and spread inoculum in different ways. During off-season, i.e., in the absence main host, it survives on the graminaceous weeds as collateral hosts who provides the primary inoculum for onset of infection. Further, the fungus spreads mainly by airborne conidia and occasionally through seeds.

Characterization of the Pathogen

For proper diagnosis of the disease, the understanding of the pathogenic characteristics is needed as much of knowing symptomatology and disease cycle. Blast caused by the *Pyricularia* spp. is identified based on its above-described

a. Finger millet blast (Leaf, neck and finger blast, respectively)

b . Leaf spot/blight

c. Rusts

Plate 5.1 Diagnostic symptoms of major diseases of small millets (compiled from Nagaraja et al. 2016; Das et al. 2016; Kumar and Singh 2010). (**a**) Finger millet blast (leaf, neck and finger blast, respectively), (**b**) leaf spot/blight, (**c**) rusts, (**d**) grain smut, (**e**) head smut, (**f**) downy mildews, (**g**) foot rot or wilt of ragi, (**h**) udbatta disease of kodo millet, (**i**) banded sheath blight, (**j**) sheath rot of kodo millet

d. Grain smut

e. Head smut

f. Downy mildews

Plate 5.1 (continued)

g. Foot rot or wilt of ragi

h. Udbatta disease of kodo millet

i. Banded sheath blight

j. Sheath rot of kodo millet

Plate 5.1 (continued)

symptoms in the field while in vitro, pathogen characterized based on morphological and molecular attributes. Morphological characterization includes studying mycelial characteristics on agar plates, viz., appearance, color, and amount of melanin pigment produced as well as the microscopic conidial characters. Molecular characterization of pathogen includes amplification of targeted genomic regions such as *ITS*, *beta tubulin*, *TEF*, *LSU* etc. and also by studying the DNA polymorphism using various molecular markers (Longya et al. 2020).

5.2.1.2 Leaf Spot/Leaf Blight

Leaf blights are also known as leaf spots/brown spots/seedling blights are among the common diseases occurred on small millets after the blast. Leaf blights in small millets are caused by different pathogens like *Helminthosporium* spp., *Alternaria alternata,* and *Cercospora eleusinis* in different millets respective to their host range. Among them, leaf blights caused by *Helminthosporium* spp. are gaining importance because of their increasing severity, distribution, and change in virulence to the new hosts by adapting to different agro-ecological regions. Leaf blight is a serious threat

in all the small millet crops yet it is more damaging in the finger millet which was first noticed by Butler (1918) in India. Ramesh et al. (2021a, b) stated that, leaf blight caused by *Bipolaris setariae* was found to be most important and destructive disease in browntop millet.

Causal Organisms

Drechslera nodulosum (finger millet and little millet), *Bipolaris setariae* (foxtail and browntop millet), *B. panici-milliacei* (proso millet), *Exserohilum crusgalli* (barnyard millet).

The genus *Helminthosporium* belongs to phylum *Ascomycota*, class *Dothidiomycetes*, subclass *Pleosporomycetidae*, order *Pleosporales* and family *Pleosporaceae*. It produces hyphae, conidiophores, and conidia. Hyphae are septate, conidiophores are brown to dark brown, erect, parallel-walled, and ceasing to elongate when the terminal conidium is formed. Conidia are multicellular (six or more-celled), large (9–40 μm), solitary, club-shaped and pale to dark brown in color, and are placed along the sides of the conidiophores and their wider end is towards the conidiophore. *Helminthosporium* differs from *Bipolaris*, *Drechslera,* and *Exserohilum* by forming parallel walled, erect conidiophores (Alcorn 1988).

Host Range

Leaf spots/blight pathogens attack all the small millets at all stages of the crop. Several other hosts are infected by the leaf blight pathogen includes *Setaria italica*, *Eleusine indica*, *Dactyloctenium aegyptium*, *Echinochloa frumentacea*, *Panicum miliaceum*, *Pennisetum typhoides*, *Sorghum vulgare,* and *Zea mays* (Mitra 1931; Pall et al. 1980).

Epidemiology

Warm humid regions having temperature ranges from 15 to 30 °C during cropping season are congenial for the development of symptoms. Under humid conditions, growth of pathogen on the older spots can be observed. Moderately high temperature and high relative humidity (R.H) are favorable for leaf blight disease (Nagaraja et al. 2007a, b).

Symptomatology

The characteristics symptom on leaf lamina is appearance of brown to dark brown spots. These spots are generally oval in shape and measure 8–10 mm in length and 1–1.5 mm in breadth. Later, these spots coalesce to give the brightening appearance of leaf, especially towards tip which would ultimately be killed prematurely. The disease also affects culm especially at nodal joints. Symptoms are also seen on leaf sheath, especially in older plants, where, in the center of the lesion, the wooly growth of the fungus may be observed under high humid conditions. The area at the juncture of leaf sheath and leaf blade is usually affected resulting in dark brownish discoloration. In neck infection dark tan lesions are seen initially, which may enlarge and extend up and down (Plate 5.1). In severe conditions, the neck may break and hang on to the plant. The pathogen may attack ear head, fingers as well as grains. The

affected grains may not develop fully and shrivel, resulting in heavy crop losses (Kumar and Singh 2010).

Disease Cycle

The pathogen survives in soil for over 18 months and the spores on grains are reported to be viable for a year (Vidhyasekaran 1971; Nagaraja et al. 2007a, b). The fungus also remains viable on the stubbles and plant debris. Secondary spread is through air borne conidia. The optimum temperature of 30–32 °C congenial for initiation of infection, however disease can occur in 10–37 °C (McRae 1922). High humidity and intermittent rains during the period of ear emergence and before grain formation causes heavy ear infection results in substantial yield loss. Under severe infections, young seedlings/germlings may get killed within 3 days after infection while elder plants get killed 15 days after infection.

Characterization of Pathogen

Diagnosis of the disease done primarily based on the typical leaf blight symptoms produced in the field on the leaf sheath or by the observation of fungal growth in vitro based on the morphological characteristics viz., mycelial color, texture, pigmentation on reverse side of the Petri plate, conidial morphology includes shape, size, and hilar end structure. Molecular characterization of *Helminthosporium* spp. carried using molecular regions like *ITS*, *LSU*, *SSU* and *gpdh* (glyceraldehyde-3-phosphate dehydrogenase) gene sequences and DNA profiling etc. (Ramesh et al. 2021a).

5.2.1.3 Rusts

Rusts caused by diverse species of *Puccinia* and *Uromyces* in small millets are of minor economic importance unless disease triangle is fulfilled which is the rare possibility. The disease occurs every season and in most of millet growing areas prevailing congenial conditions yet they don't cause considerable economic losses (Butler 1918). Out of seven small millets, rusts are economically important diseases on foxtail millet which is caused by *Uromyces setariae-italica*, which was first recorded by Yoshino from Japan. On foxtail millet, it occurs regularly and in severe form but doesn't result in heavy yield losses. In India, foxtail millet rust is prevalent in predominant millet growing states like Karnataka, Maharashtra, Tamil Nadu, Madhya Pradesh, Bihar, and Andhra Pradesh.

Causal Organism

U. setariae-italica (foxtail millet), *Uromyces eragrostidis* (finger millet), *Puccinia substriata* (kodo millet), and *U. linearis* (little and proso millet). The rust fungus produced both uredial and telial stages. Uredospores are yellowish-brown, stalked, oval to globose, echinulate with 3–4 germ pores, while teliospores are dark brown colored, single celled, pedicellate, oblong to globose by possessing smooth and thick walls which gets more concentrated towards apex rather on its base.

Host Range

Finger millet, foxtail millet, kodo millet, pearl millet and little millet etc. Various pathogens infecting small millets are harboring on graminaceous hosts as collateral hosts.

Epidemiology

Low temperature and high relative humidity are favorable for initiation of rust disease. With the congenial weather conditions prevailing during December and January, rust appears early in the season, i.e., within 20–25 days after sowing and the severity of rust increases with the plant ages (Viswanath and Seetharam 1989).

Symptomatology

The disease has potential to affects the crop at all growth stages; however, the damage is severe only when infection starts before flowering. Disease starts as small, brown uredosori on both the sides of the leaf. Mature and broken pustules arranged linearly on the upper surface of the leaves; however, pustules cover entire leaf blade rather than linear form. Infection can be observed on the most foliar plant parts like leaf sheath, culms, and stem. The disease is relatively more severe on the upper leaves in contrast to middle and lower leaves. Under severe infection, premature drying of leaves and poor grain set are commonly observed in field. Morphology of spore production is different in all the rusts which includes production of light yellow black teliospores in *U. setariae-italica* and *U. linearis*, respectively. *P. substriata* develops small brown, oval spots on upper surfaces of leaf. The teliospores are dark brown colored, single celled, stalked, oblong to globose shaped with smooth and thick walls (Kumar and Singh 2010).

Disease Cycle

The uredospores perpetuate, spread, and infect the host and reproduce by forming uredia in 7–10 days. Collateral hosts such as most graminaceous weed species possibly play an important role in its disease cycle by aids in perpetuation. Early infection of rust appears within 20–25 days of sowing depending on prevalence of inoculum and favorable weather while the intensity increases as the plants grow older. The dark telial pustules develop at the time of crop maturity (Nagaraja et al. 2007a, b).

Characterization of Pathogen

Genus level characterization of rust pathogens can be done based on the microscopic analysis of the uredospores and teliospores based on their color, size, shape, echinulations, presence or absence of stalk, septations, etc.; however, the species demarcation is difficult based on morphological characteristics. Hence, molecular features are of increasing importance owing to their consistency, easy application, and conserved throughout the genus. Molecular regions targeted for species differentiation are majorly based on *ITS*, *LSU*, *SSU* regions, etc.; nevertheless the extraction of genomic DNA is challenging which need to be taken care (Bai et al. 2021).

5.2.1.4 Smuts

Small millets possess potential to cope up with biotic factors; however many pathogens have emerged as threat to the millet cultivation. Among them, smut pathogens are one of the large groups causing varying damaging symptoms at different intensities depending on the genotype and favorable conditions. Smut pathogens belonging to different genus viz., *Ustilago* spp., *Melanopsichium* spp., *Sporisorium* spp., etc. are known to attack all the small millets and produce different kinds of symptoms like grain smut, head smut, and kernel smut. Among the smuts, the grain smut is common on finger, foxtail, and barnyard millet, while head smut is common in kodo, barnyard and proso millet (Das et al. 2017). Generally, smuts of small millets are of minor importance owing to their sporadic nature of occurrence and low economic losses, these smuts can be controlled by practicing clean cultivation as well as seed treatment with fungicides.

Grain Smut

Grain smuts are the most commonly occurring smut diseases compared to the other smuts in small millets. These are common in finger, little, barnyard, foxtail, and proso millet inciting by different genus. Ragi grain smut was first reported by Kulkarni (1922) from Kolhapur. Later, its occurrence was recorded by many scientists such as Coleman (1920), McRae (1924), Narasimhan (1934), Mundkur (1939), and Venkatarayan (1947) in Mysore state province.

Causal Organism

Melanopsichium eleusinis (finger millet), *Macalpinomyces sharmae* (little millet), *Ustilago panici-frumentacei* (barnyard millet), and *Ustilago crameri* (foxtail and proso millet).

Economic Importance

Although the grain smut is negligible important, Mantur (1994) stated that they possess the ability to appear in epidemic form with an infection of 200 grains per ear. Recent studies showed the change of virulence in grain smut pathogens, known summer diseases have appeared to infect during *Kharif* also. Losses caused by *U. crameri* ranges 8–50% of the grain yield (Nagaraja et al. 2007a, b) and 75% of grain infection (Sundararaman 1921). Jain and Tripathi (2002) investigated yield loss due grain smut in little millet showed 9.80–53.55% reduction in grain yield and 6.40–38.90% of panicle length.

Epidemiology

Low temperature and high relative humidity are congenial conditions for rapid development and spread of the disease. The fungus attack majority of the grains in an ear by producing sori in flowers and basal parts of palea; however, terminal portion of the spike may escape the infection. The sori are pale greyish and measure 2–4 mm in diameter. On maturation, the sori rupture and produce dark powdery (dusty) mass of spores. The spores are dark brown colored, angular to round with smooth walls measuring 7–10 μm diameter (Das et al. 2017).

Symptomatology

The symptoms of grain smut can be observed at grain formation stage, i.e., within few days after flowering. The affected ovaries initially turn into velvety greenish smut sori without increase in size than the normal grain. Eventually, the glumes are pushed apart by the transformed spore balls (sori) which are several times bigger in size. The sorus remains enclosed by thin and delicate membrane, which later easily get ruptured exposing the sorus. The greenish outer tunica of the sorus gradually turns pinkish green and finally to dirty black on maturation (Thirumalachar and Mundkur 1947). With the wind, loosened spores are easily blown away leaving nothing inside the glumes. Occasionally, infected grains of some crops develop late in season, remain greenish, and slowly increase in size which release spores upon pressing (Sharma and Khare 1987).

Disease Cycle

Grain smuts are majorly externally seed and airborne pathogens. Infection of flowers occurs through secondary inoculum, i.e., air borne spores. Very limited systemic research has been done on grain and head smuts. Soil borne infection has also been observed. Fungus serves are dormant dikaryotic mycelium in the seed tissues which serves as primary inoculum to the following season (Wang 1943).

Head Smut

Head smut in small millets are relatively minor importance with low economic losses unless left uncontrolled under conditions of fulfilling disease triangle. It is more common in countries like Europe, Australia, Eastern Asia etc. In India, it is known to occur widespread on kodo, barnyard and proso millet which have been consistently reported from Karnataka, Madhya Pradesh (Pall et al. 1980), Tamil Nadu and Uttarakhand (Kumar et al. 2007).

Causal Organism

Sorosporium paspali-thunbergii (Henn.) S.Ito [Syn: *Sorosporium paspali* Mc Alp.] infecting kodo millet, *Sporisorium destruens* (Schltdl.) Vánky [Syn: *Sphacelotheca destruens*] infecting proso millet and *Ustilago crus-galli* Tracy & Earle causing head smut of barnyard millet.

Teliospores of *S. paspali* produced on loose spore ball like masses. Gradually, spore ball split into individual spores with little pressure. The individual spores are globose, angular to roughly pear shaped, dark to yellowish brown with thick smooth wall. The spore germinates by producing septate, single or branched hyphae constricted at septum, which bears lateral and terminal sporidia.

Economic Importance

S. paspali became endemic but its distribution and severity vary with environment and cultivar. Viswanath (1992) reported 30–40% loss in grain yield due to *S. paspali*. Jain and Yadava (1997) observed that loss in yield ranges 13.15–32.98% vary with smut incidence.

Epidemiology

Head smut is externally seed borne which appear sporadically late in the crop season when the crop is about to mature. Temperature range 20–25 °C is optimum for initiation and colonization of infection.

Symptomatology

Diagnostic symptom of head smut is the transformation of entire panicle into single sorus in which the inflorescence is deformed and turn to smut ball. Head smut causing pathogen infects only ovaries of the plant. In addition, head smut also produces some of characteristic symptoms viz., gall-like swellings on stem, nodes of young shoots and in the axils of the older leaves. Sometimes, twisted, deformed clusters of leafy shoots with aborted ears may also develop. The affected ovaries manipulated into hairy, round and grey sac which initially does not increase in size. The gall-like swellings are covered by a hairy rough membrane of host tissue (Mundkur 1943) which on rupturing expose the spore mass carried by wind.

Disease Cycle

The disease is mainly externally seed borne; hence the initial seedling infection starts by penetration of germ tube from seedborne teliospores through the cell wall. After entering into the seedling tissue, the hyphae spread inter and intra-cellularly and become systemic infection which eventually enters the meristematic tissues and finally infects the ear by the time of crop maturation (Kumar and Singh 2010).

Kernel Smut

Kernel smut is a minor disease affecting barnyard millet occasionally in unprotected cultivation. It was first noticed in Italy while in India, Viswanath and Seetharam (1989) reported the incidence of kernel smut from Bihar, Maharashtra, and Tamil Nadu.

Causal Organism

Ustilago paradoxa Syd., P. Syd. & E.J. Butler

Chlamydospores produced by *U. paradoxa* are smooth, olive brown in color and round which measures up to 7–11 μm in diameter.

Symptomatology

Fungi cause infection at the time of flowering which further convert floral parts into fungal bodies resembles greenish swollen bodies. Infection occurs in scattered manner with only few grains gets affected in an ear, i.e., up to 25 grains/ear by forming smut sori of 1.5–4 × 1–2 mm (Nagaraja et al. 2016).

Characterization of Pathogen

Diagnosis of the smut diseases under field conditions can be easily done because of their unique dusty appearance of reproductive parts but the characterization to the genus level is only possible with the microscopic studies of teliospore bodies based on their size, color, shape, septation, arrangement in sori, echinulations etc.

However, the proper species distinction is possibly through molecular analysis using molecular markers like RAPD, RFLP, SSR, SNP etc. Another challenging aspect of smut characterization is the genomic DNA isolation from spores which is different from regular CTAB protocols and needed to be taken care of during the characterization (Ladhalakshmi et al. 2012; Goswami et al. 2022).

5.2.1.5 Downy Mildews

This disease is also referred as crazy top/green ear disease. Downy mildew is a one of the major threats of finger and foxtail millet occurring widely in predominant millet growing regions of world. In India, green ear of ragi was first reported by Venkatarayan (1947) in erstwhile Mysore state, followed by Tamil Nadu and Uttarakhand (Kumar et al. 2007), whereas downy mildew of foxtail millet was reported from many parts of India such as Karnataka, Tamil Nadu, Maharashtra, Bihar, Andhra Pradesh, and Kashmir (Rangaswami and Mahadevan 1999). The disease has potential to be destructive leading to total crop failure owing to underdevelopment of the affected ears result in substantially yield losses ranges up to 50% (Pall et al. 1980).

Causal Organism

Sclerophthora macrospora (Sacc.) Thirum. [Syn. *Sclerospora macrospora* Sacc.] infecting finger millet whereas, *Sclerospora graminicola* (Sacc.) J. Schröt. infects foxtail millet.

Host Range

Finger millet, foxtail millet, pearl millet, maize, *Eleusina indica* (Ulstrup 1955), wheat, oat, *Eragrostis pectinacea,* and *Digitaria marginata*.

Epidemiology

Moderately high temperature (25–30 °C) and high relative humidity is highly favorable for disease development. Temperature around 22–25 °C during night enhance the production of sporangia and facilitates the release of zoospore from sporangia. Raghavendra and Safeeulla (1973) reported the internal and external seed borne nature of the pathogen.

Symptomatology

Early symptom of downy mildew affected plant is the chlorosis of seedling leaves. Characteristic symptoms of downy mildew affected plants are stunting with shortened internodes and profuse tillering. Also, pale yellow translucent lesions are observed on infected leaves. Whitish bloom of sporangiophores and sporangia are prominently noticed on the leaf surface under humid conditions. Plants with mild infection may develop ears, but malformed into green leafy structures giving "green ear" symptom. While the fungus usually invades the entire ear, sometimes only a portion of the ear is involved, the remainder producing normal grains. On maturation, chlorotic patches can be seen on the upper surface of leaf with corresponding

downy fungus growth on the lower surface (Kumar and Singh 2010; Nagaraja et al. 2016).

The downy mildew of finger millet generally doesn't show the characteristic symptom, i.e., white cottony growth under-side of leaves. Hence, the disease can only be identified after the formation of ears (reproductive stage of plant). Eventually, partial/whole ear including palea, lemma, and glumes change into leafy-like structures. The proliferation takes place first in the basal spikelet and spreads towards tip. On maturation, the whole ear shows a bush-like appearance with typical "green ear" symptom (Thirumalachar and Narasimhan 1949).

Disease Cycle

The downy mildew fungus is an obligate parasite and found to be both internally and externally seed borne (Raghavendra and Safeeulla 1973) with broad host range. Pathogen life cycle in small millets was elaborated by Safeeulla (1955). Here, the primary inoculum is mainly soil or seed borne oospores. Disease severity is influenced majorly by temperature and soil moisture content and time of sowing. It requires an optimum soil temperature of 20–21 °C with minimum of 12–13 °C and maximum of 30 °C for initiation of infection by the pathogen. Early sown crop in contrast to late/timely sown crop is more prone to attack by downy mildew pathogens. High relative humidity and high soil moisture content favors rapid development of the disease (Nagaraja et al. 2007a, b). Recently high incidence of downy mildew was observed in two popular finger millet varieties viz., GPU 28 (blast resistant) and PR 202 (blast susceptible) in the farmers field of Tumkur district in Karnataka.

Characterization of Pathogen

Diagnosis of the diseases under field conditions is easy due to their unique above-briefed symptoms of downy fungal growth on lower side of leaves and corresponding chlorotic patches on upper leaf surface as well as formation of green ear symptom. Being an obligate pathogen, downy mildew fungus can't be cultured on artificial media to study their morphological characteristics. However, microscopic examination of the pathogen can be done by observing the conidia, conidiophores, oogonia and oospore etc. Morphological characterization is carried for the genus-level identification based on the conidiophore branching pattern, kind of germination, production of sporangia/conidia etc.; morphological classification is further confirmed and extended to species-level distinction by genomic DNA isolation and amplification of conserved molecular regions viz., *ITS*, *LSU,* and *cox2* genes (Kara et al. 2020). Nevertheless, the challenges in characterization of downy mildew pathogen are not scarce in which genomic DNA isolation is the primary concern that needs to be carried from the fungal filaments or spores scraped from infected leaves.

5.2.1.6 Foot Rot or Wilt of Ragi

Earliest report of the foot rot/wilt disease was done by Coleman in 1920 from Mysore state. Afterwards, it was reported from different parts of India where finger

millet is cultivated. In ragi, losses may reach up to 50% under field conditions with congenial weather (Basta and Tamang (1983). The disease has been reported mostly from Karnataka, Tamil Nadu, Gujarat, and Odisha. Due to the sporadic nature, it was considered of as minor disease but recent studies have shown increased incidence of the disease under irrigated conditions.

Causal Organism

Sclerotium rolfsii Sacc. [Perfect Stage: *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr.] or *S. rolfsii* (Perfect state: *Pellicularia rolfsii*). Imperfect stage is prevalent mostly in both the field and laboratory conditions while perfect stage can be seen rarely under favorable situation where fertile sporophores are also produced.

Host Range

S. rolfsii has broad host range (Aycock 1966) but among the small millets, finger millet is the only host reported so far.

Epidemiology

Fungus overwinters as sclerotia in soil which acts as the primary source of inoculum. Sandy loam soils with low moisture upsurge the disease incidence. The disease development is favored by warm temperature and high relative humidity which exists during monsoon season.

Symptomatology

Generally, plants are attacked at inflorescence or seed formation stage. Infection occurs just above the soil line in the collar region of the plant. The affected area is water-soaked initially which late turns brown or dark brown which finally shrinks which cause wilting of the entire plant. The cottony white growth of the fungus is evident on different plant parts which become roundish ultimately turning into velvety mustard seed like sclerotial bodies.

Disease Cycle

The fungus survives as sclerotia in soil which is the source of primary infection. The sclerotia germinates with the on the onset of rainy season to produce basidia and each basidia produce four haploid basidiospores. The fungus attacks the collar region of the plant which becomes infected leading to the death of the entire plant. At the end of the cropping season due to lack of available nutrients and onset of unfavorable climatic conditions, fungus forms as mass of tightly packed mycelia called sclerotia. These bodies can be seen easily on the plant which are old and about to die. These sclerotia get accumulated in soil with plant debris and move through rain water from field to field.

Characterization of the Pathogen

Diagnosis of finger millet foot rot can be done based on their symptoms which appear initially at soil line as water-soaked lesions and observation of sclerotial bodies on sheath with coalesced patches. Systemic studies on characterization of

S. rolfsii are vast in various food crops based on morphological attributes like mycelial morphology, pattern of sclerotial production while molecular confirmation is based on analysis of molecular gene regions like *ITS*, *LSU*, *SSU*, *beta-tubulin*, *TEF*, *rpb2*, etc. (Mahadevakumar et al. 2016).

5.2.1.7 Banded Sheath Blight (BSB)

Earliest report of banded sheath blight (BSB) was on finger millet at Vellayani, Kerala, India (Das and Girija 1989). Thereafter, reports on prevalence of disease in severe forms were also reported from experimental plots of Birsa Agricultural University, Ranchi (Dubey 1995). Since then, the disease has been observed commonly on all small millet growing areas of hot and humid climatic regions.

Causal Organism

Rhizoctonia solani (Basidial state: *Thanatephorus cucumeris*)

The fungus produces dull (cream) white mycelial colony which later turn light brown in color. *R. solani* is identified by its characteristic branching at right angle and constrictions on the point of branching.

Host Range

Similar to *S. rolfsii*, *R. solani* has the wide host range among agriculture and horticultural crops. All the small millets viz. finger, foxtail, barnyard, proso, kodo, and little millets are the host for the pathogen.

Epidemiology

Pathogen survives the weather extremities in soil as sclerotia which will act as the primary source of inoculum for next season. Disease is favored by moderate temperature (26 ± 2 °C) and high relative humidity (>80%) (Dubey 1995). Low disease severity is observed on the late maturing varieties due to disease escapes in autumn as the low temperature prevails (Patro 2008).

Economic Importance

BSB generally does not cause high economic losses. However, upon completing disease triangle, disease has the potential to cause huge economic losses. Infection on peduncle results neck rot which deteriorate the grain production due to poor grain filling result in formation of small and shrivelled grains.

Symptomatology

The disease symptoms appear on the lower leaves and leaf sheath as oval to irregular spots. Initially the lesions are light grey to dark brown in color whose center turns white with narrow reddish-brown border. The lesions are distributed throughout the leaf lamina. Under congenial environment, all the lesions may also enlarge and coalesce to cover the entire leaf sheath and lamina appearing as brown colored characteristic bands across the plant leaf. Hence, the entire leaf becomes blighted and dries up. Symptoms are also observed on peduncles, fingers, and glumes. Mycelial

growth and sclerotia can also be observed on the lesions under humid conditions (Das et al. 2016).

Disease Cycle

R. solani survives as dormant mycelium which is germinates to produce infective mycelium under prevailing favorable weather (high relative humidity and moderate temperature). Symptoms are visible as small irregular or oval brown spots on lower leaf lamina which later enlarge and coalesce to form bands. Numerous sclerotia are observed on the blighted leaves which subsequently dies. The fungus also spreads from field to field through irrigation water, infected soil and plant debris.

Characterization of the Pathogen

Banded sheath blight can be well diagnosed under favorable field conditions due to their appearance of blight on sheath and presence of sclerotia in severe infections. *R. solani* is well studied pathogens regarding its characterization based on morphological and genomic attributes. Owing to their broad host range, cross-infectivity studies among crops are gaining importance which confirms the anastomosis grouping of *R. solani* and their specificity to the host species (Al-Fadhal et al. 2019; Pralhad et al. 2019).

5.2.2 Minor Fungal Diseases of Small Millets

Apart from the major diseases affecting the production potential of small millets throughout the millet growing areas of world, there are some diseases which are of course not negligible but of relatively minor importance owing to their irregular occurrence and damage to the crop and its produce. Such diseases were also listed in Table 5.1 includes cercospora leaf spot of ragi (*Cercospora eleusinis*), Udbatta diseases (*Ephelis oryzae*) affecting kodo, foxtail, proso and little millet, sheath rot (*Sarocladium oryzae*) of kodo millet, and grain mold in finger millet etc. Albeit, these diseases are less important in small millet cultivation which can be eliminated by protective cultivation, some of them are most destructive in other graminaceous crops like major millets (viz., sorghum, pearl millet) and maize, where it showed to cause huge economic losses especially the grain mold caused by different deuteromycetes and ergot caused by *Claviceps* spp. Among these minor diseases, Udbatta caused by *E. oryzae* are of relative importance due to its effect on kodo and little millet in which affected panicles are transformed into a compact *agarbatti* like shape, hence the name "Udbatta" (Kumar and Singh 2010; Das et al. 2016; Nagaraja et al. 2016).

5.2.3 Bacterial Diseases of Small Millets

Along with fungal diseases, a variety of bacterial diseases has been an inconsistent constraint to the small millet cultivation. Diseases caused by bacterial pathogens are

of lesser importance compared to the fungal pathogens unless favored by the prevailing environmental conditions, presence of susceptible cultivar and ample amount of inoculum during cropping season. They have been reported on all the small millets in some millet growing corners yet they failed to establish as major factors under natural conditions. Some of the important bacterial diseases are bacterial leaf spot caused by *Xanthomonas eleusinae* and *Pseudomonas albo-precepitans* in finger and foxtail millet, respectively, bacterial leaf stripe (*Pseudomonas* spp.) in finger and proso millet, bacterial leaf streak (*Xanthomonas* spp. and *Pseudomonas* spp.) in most of small millets and bacterial blight (*Xanthomonas axonopodis* pv. *coracanae*) in finger millet etc.

Systemic studies on bacterial diseases of small millets are very much limited due to which the literature available is scarce. Generally, bacterial entry to the plant tissues takes place mainly through natural opening like stomata or through wounds (physical injury) during inter-cultivation operations or insect damage on leaves, sheath, and roots. In the absence of main host, bacteria survive in infected crop debris/residues left in the soil and also on graminaceous weeds and other crop hosts. Disease development for bacterial pathogens is favored by warm temperature and high humidity (Nagaraja et al. 2007a, b; Das et al. 2017).

5.2.4 Viral Diseases of Small Millets

Unlike other food crops, small millets are less affected by the viral pathogens which might be due to their inherent capacity to sustain in moderately extreme agro-ecological conditions and potential against virus and their transmitting agents. Diseases caused by the viral pathogens are of mere important in the small millet cultivation which occurs once in a while but couldn't able to show consistent infection to the crop. Of all the small millets, viral diseases majorly prevalent in finger, proso, and barnyard millet and has been reported from many parts of the world with less virulent infection. In general, viral pathogens cause symptoms like chlorotic stripe, streak, mosaic or mottling symptoms on leaves while the early infection results sterility of the plant where the plant bears no ear. However, finger millet is reported to be affected by many viral pathogens and cause notable diseases viz., ragi mottle streak disease (*Ragi mottle streak virus*), ragi severe mosaic (*Sugarcane mosaic virus*), and ragi streak disease (*Eleusine* strain of *Maize streak virus*). Likewise, barnyard millet (*Wheat streak virus*, *Sugarcane mosaic virus* and *Eleusine virus 2*) and proso millet (*Wheat streak virus*, *Rice dwarf or stunt virus* and *Maize leaf streak virus*) are also reported to be attacked by viral pathogens in recent past. Transmission of viruses through biotic agents like insect and non-insect vectors serves as inoculum which spread to the healthy fields (Nagaraja et al. 2016; Das et al. 2017).

Despite of their minor effect on small millets, many instances have shown that viruses are potential agents that can lead to enormous crop losses. One such instance in finger millet was reported from Chitradurga and Bangalore districts of Karnataka during *Kharif* 1966 where the farmers have to abandoned their ragi crop due to the

severe infection by *sugarcane mosaic virus* which results in infected plants failed to set seed (Joshi et al. 1966).

5.2.5 Mitigation Strategies of Prominent Diseases of Small Millets

5.2.5.1 Cultural Practices

Several agricultural practices such as timely sowing, maintaining optimum plant populations and spacing, timely weeding, balanced use of fertilizers, crop rotation, deep ploughing during summer season, removal of crop residues from the field, cleaning of field bunds after crop season, uprooting the diseased plant from the field and burning, regulating irrigation water from entering into other field etc. will help in reducing chances of disease occurrence. Some of the important cultural practices which helps in millet disease management are as follows:

Methods	Disease managed
Crop rotation	Majorly controls seed and soil borne pathogens viz., downy mildew, ergot, smut, banded blight and sheath rot
Deep summer ploughing	Expose resting spores of the pathogen and controls disease like downy mildew, smut, and a few fungal and bacterial leaf diseases
Adjustment of date of sowing	Early sowing reduces blast and rust severity
Optimization of plant population	High plant population favors disease development, so maintaining optimal plant population helps in managing disease like downy mildew, blast, rust, and grain mold in millets
Use of disease free seeds	Most eco-friendly method for controlling any kind of disease. In millets downy mildew, banded sheath blight, foot rot, ergot and blast can be managed using disease free seeds
Sanitation	This helps in the reduction of primary inoculum and surviving structures of the pathogen. Downy mildew, banded sheath blight, foot rot, ergot, and blast can be controlled by this method
Eradication of alternate and collateral hosts	Their timely removal helps to control diseases like ergot, downy mildew, rust, blast, leaf spots, and bacterial and viral diseases
Fertilizer management	Nutrient can affect the relationship between crop and pathogen in many ways. Regulating the amount of nitrogenous fertilizer reduces incidence of blast and downy mildew
Vector management	A number of viral, bacterial and some of the fungal diseases may get introduced through the visits of their respective vectors to the hosts. For this reason, eradication of such pathogens should include this measure to get the optimum result
Clean cultivation	Practice of clean cultivation like collecting smutted heads in cloth bags and dipping in boiling water to kill the pathogen will reduce the inoculum for the next year and minimize incidence

5.2.5.2 Host Plant Resistance

Exploiting host resistance to control disease is not only economical but also a practical necessity in a low value crop like millets where there is a limitation for

Table 5.2 Disease resistant varieties identified and released for the different sorghum growing areas of India (2000–2018)

Crop	Disease	Resistant or tolerant cultivars
Finger millet	Blast	GPU 26, GPU 45, Chilika (OEB 10), VL 315, GPU 48, PRM 1, Bharathi (VR 762), Srichaitanya, KMR 301, KOPN 235, OEB 526, OEB 532, PPR 2700 (Vakula), VL 352, GNN-6, GN-5, VL Mandua-348, KMR 340, Dapoli-2 (SCN-6), CO 15
Foxtail millet	Downy mildew	Meera (SR 16), SiA 3085, RAU (Rajendra Kauni 1–2)
	Rust	TNAU 196, RAU (Rajendra Kauni 1–2)
	Blast	RAU (Rajendra Kauni 1–2), SiA 3085
	Brown spot, smut and leaf blight	RAU (Rajendra Kauni 1–2)
Barnyard millet	Grain smut	VL Madira
	Blast	Tarini (OLM 203), GNV-3
	Grain smut	Tarini (OLM 203), OLM 217 and GNV-3
	Rust	OLM 217
	Brown spot	Kolab (OLM 36)
	Sheath blight	Kolab (OLM 36), GNV-3
	Head smut	Jawahar Kutki 4 (JK 4)
Proso millet	Brown spot	GPUP 8
	Leaf blight	PRC 1
Kodo millet	Head smut	Jawaharkodo 155 (RBK 155), Jawaharkodo 48 (JK 48), JK 106, JK 65, JK 98, Jawaharkodo 137, JK 13

any additional cash inputs such as fungicides etc. Development of resistance varieties is the best means of combating the disease, which is predominantly grown by resource-poor and marginal farmers. Disease resistant varieties identified and released for the different millets growing areas of India are tabulated in Table 5.2 (www.aicrpsm.res.in).

5.2.5.3 Biological Control

Biological control is an alternative to synthetic chemical pesticides and having several benefits to human beings and ecosystem; they can ensure the protection of plants against biotic and abiotic stresses, production of good quality grains, improve soil fertility, sustainable and safety of environment. The demand for development and application of indigenous bioinoculant products has increased among researchers because of their role in plant growth promotion and crop protection in sustainable farming systems and also for their economic value. Soil-borne diseases of millets (e.g., foot rot in finger millet and Banded sheath blight in small millets), for which adequate host resistance is lacking, use of biocontrol agents are useful. Bio-control agents especially strains of *Trichoderma* and *Pseudomonas* are useful for seed and soil borne diseases of millets and can be applied as seed treatment and soil application.

5.2.5.4 Chemical Control

Disease	Chemical control
Downy mildew	Seed treatment with Ridomyl-MZ at 6 g/kg seed followed by one or two need based spray of Ridomyl-MZ at 3 g/L reduces incidence
Blast	Seed treatment with carbendazim at 1 g/kg of seed. Spray any one of the fungicides viz., Carbendazim (0.2%) or Iprobenphos (IBP) (0.1%) or premixture fungicide (Carbendazim + Mancozeb) (0.1%), Ediphenphos (0.1%) or propiconazole (0.1%) or Tricyclazole (0.1%). First spray immediately after noticing the symptoms. Need based second and third sprays at flowering stage at 15 days interval to control neck and finger infection in finger millet
Grain mold	Spray any one of the following fungicides in case of intermittent rainfall during earhead emergence, a week later and during milky stage. Mancozeb + Captan at 0.2%, thiram + carbendazim at 0.2%, Propiconazole at 0.1%. Two to three sprays should be taken up to reduce the grain mold
Smuts	Seed dressing with sulfur at 4 g/kg seed
Banded sheath blight	Seed treatment with Mancozeb at 2–3 g/kg. Seed and need based spray with the fungicides viz., propiconazole at 1 mL/L orhexaconazole at 2 mL/L or Validamycin at 2 mL/L is highly effective
Rust	Foliar spray of Mancozeb (0.2%), hexaconazole (0.1%), difenconazole (0.1%) and propiconazole (0.1%) for control of disease. Two sprays at 15 days interval immediately after appearance of symptoms is recommend better management of the disease
Leaf spot/leaf blight	Seed treatment with carbendazim at 2 g/kg or mancozeb 0.2% and need based spray

5.2.6 Novel Strategies for Enhanced Control of Small Millet Diseases

Changing climate scenario following the cultivation practices of small millets has resulted in increasing reports of new diseases as well as the enhanced aggressiveness of established pathogens. Besides the unique advantages offered by various existing management strategies, they do carry few limitations under current perspective. Management of small millet diseases under current situation needs not only the conventional approach but also the innovative, cost effective, feasible, efficient mitigation strategies which can be included in the integrated disease management (IDM). By keeping in the view of enhanced cost of cultivation due to agrochemicals, novel ecofriendly strategies need to be explored for controlling the diseases which will serve as best alternative rather than replacement. Such novel green technologies include nanomolecule formulations, endophytes biopriming, use of rhizosphere, phyllosphere-derived bioagents, employing biofumigation techniques, plant immunization approaches (Singh and Gopala 2021), genome assisted breeding methods, genome editing techniques and multi-omics approach etc.

- Germplasm exploitation: Achieving durable plant resistance to diseases is ultimate goal of plant disease management which minimizes the use of chemicals. Analyze the new germplasm and other possible wild relatives for the source of major gene resistance which can be used for breeding into elite cultivars with molecular marker-based breeding methods and development of resistant varieties in relatively short time.
- Use of Microbiome concept: In recent years, use of microbiome in plant disease management has revolutionized by the ways how microbiome interacting with plants and in the environment are perceived which may lead to a switch away from the conventional-driven research and implementation. The potential relevance of microbiome usage in disease management is clear but needs to be exploited by further research (Jeger et al. 2021).
- Use of genomic tools (CRISPR/Cas9) for improved resistance/control: CRISPR/Cas9 system has been utilized vastly in the field of plant pathology and plant breeding for various approaches. This system facilitates genome editing of various organisms precisely using RNA-guided DNA endonuclease activity. It has been exploited to enhance disease resistance in different commercial crops such as rice, wheat, tomato, and grape. Apart from the genome editing of crops, genome editing of fungal and fungi-like pathogens can also provide new insights for plant disease management in eco-friendly manner. Durable management of plant diseases perceives the targeting of multiple plant disease resistance mechanisms with CRISPR/Cas9. The insights gained by probing fungal and oomycete genomes with this system will be powerful approaches (Paul et al. 2021) and will be dual purpose, i.e., for deep understanding the pathogen as well as framing management strategies. CRISPR/Cas9 system has facilitated targeted mutagenesis efficiently and precisely in plants to enhance resistance to fungal diseases.
- Multi-omics approach: It facilitates the comprehensive understanding of the mechanisms underlying ability and nature of plant-mediated effects during interactions of plant tissue and the microbial communities. Multi-omics approach allows to study different plant defense pathways as well as the pathogen response to counteract them by producing various toxic metabolites. There are many pathways apparent in plants needs to be explored for a better understanding and control such as salicylic acid (SA) signaling pathway as prime importance followed by other critical plant hormones such as abscisic acid and jasmonic acid which regulates acquired resistance in plants and mediates the interactions between members of plant microbial communities (Crandall et al. 2020).

5.2.7 Looking into the Future of Small Millet Diseases and Conclusion

With the everyday increasing population of world demands, not only the food security but also the nutritional security are combined to form agricultural sustainability. Updated reports show that the agricultural production needs to be

increased 50% by 2050 to meet the growing food and nutritional demand (FAO 2020). Nutritional security is as much important as food security for better quality of life which can be fulfilled by the cultivation of millets on large scale. Erstwhile commercial crops like rice, wheat, sugarcane, maize, etc. have been given more importance owing to their wide distribution and acceptance as daily food. In recent times, millets especially small millets gain huge attention with growing health concerns which are fulfilling by their nutraceutical properties. This results in increasing area of cultivation followed by bringing new problems, i.e., new reports of pests and diseases throughout the predominant millet growing areas which were unseen before on particular crop.

However, systemic research in small millets on many aspects like breeding of new varieties, sequencing of genome, etc. are still underway. With the advent of advanced genomic approaches like next generation sequencing (NGS), genome editing techniques, etc. made identification, cloning, and transfer of resistant (R) genes easy. Using of such approaches in small millets aids in better understanding of the crops and paves way for possible manipulation of crop genome to generate disease-resistant crops which is an ecofriendly perspective. This will make the small millets possibly the ecofriendly alternative for nutraceutical supplement, cost effective due to no use of pesticides, farmer friendly. Also, surveillance of the established diseases and regular monitoring of new diseases aid in achieving the food and nutritional security.

It is concluded that the fungal diseases pose a significant challenge to the small millets production, now and in the future. In this chapter, attempts have been made to briefly summarize the key aspects of some of the most significant diseases of small millets which are threatening its production potential. We acknowledge that, along with major diseases which detailed in chapter, there are many other minor diseases viz., bacterial and viral diseases that also threaten production; however, keeping the space limitations in mind, we have concentrated more onto fungal diseases which are major threat in reality and considered to have the greatest impact on yield. This chapter serves as a reference point for pathologists accompanied in field study in non-exhaustive manner to comprehend the complexity of diseases and to contemplate them in a more holistic manner.

References

Al-Fadhal FA, Al-Abedy AN, Alkhafije DA (2019) Isolation and molecular identification of *Rhizoctonia solani* and *Fusarium solani* isolated from cucumber (*Cucumis sativus* L.) and their control feasibility by *Pseudomonas fluorescens* and *Bacillus subtilis*. Egypt J Biol Pest Control 29:47. https://doi.org/10.1186/s41938-019-0145-5

Alcorn JL (1988) The taxonomy of helminthosporium species. Annu Rev Phytopathol 26:37–56. https://doi.org/10.1146/annurev.py.26.090188.000345

Anitha K, Chakrabarty SK, Rao RDVJ, Babu P, Sarath B, Babu A, Varaprasad KS, Khetarpal RK (2005) Quarantine processing of exotic cereals and millets germplasm during 1986–2003. Int J Plant Prot 33:105–110

Anju T Jr, Sarita S (2010) Suitability of foxtail millet (*Setaria italica*) and barnyard millet (*Echinochloa frumentacea*) for development of low glycemic index biscuits. Malays J Nutr 16(3):361–368

Anonymous (1975) Annual report of the virologist AICRP on small millets 1974–75. UAS, Bangalore

Aycock R (1966) Stem rot and other diseases caused by *Sclerotium rolfsii*. North Carolina Agric Expt Station Bull. No. 174

Bai Q, Wan A, Wang M et al (2021) Molecular characterization of wheat stripe rust pathogen (*Puccinia striiformis* f. sp. *tritici*) collections from nine countries. Int J Mol Sci 22(17):9457. https://doi.org/10.3390/ijms22179457

Basta BK, Tamang DB (1983) Preliminary report on the study of millet diseases in Nepal. In: Maize and finger millet. 10th Summer workshop 23–28 January 1983, held at Rampur, Chitwan, Mysore

Billimoria KN, Hegde RK (1971) A new bacterial disease of ragi, *Eleusine coracana* (Linn) Gaertn in Mysore state. Curr Sci 40:611–612

Butler EJ (1918) Fungi and diseases in plants, vol 547. Thaker Spinck and Co., Calcutta

Channamma KAL, Viswanath S, Mathur SG (1996) New record of *Uromyces* sp. on ragi from Karnataka. Curr Res 25:97

Coleman LC (1920) The cultivation of ragi in Mysore. Bull Dep Agric Mysore Gen Ser:11–12

Crandall SG, Gold KM, Jiménez-Gasco MDM et al (2020) A multi-omics approach to solving problems in plant disease ecology. PLoS One 15(9):e0237975. https://doi.org/10.1371/journal.pone.0237975

Cummins GB (1971) The rust of cereals, grasses and bamboos. Springer, Berlin

Das IK (2017) Millet diseases: current status and their management. In: Patil JV (ed) Millets and sorghum: biology and genetic improvement. John Wiley & Sons Ltd. https://doi.org/10.1002/9781119130765.ch11

Das L, Girija VK (1989) Sheath blight of ragi. Curr Sci 58:681–682

Das IK, Nagaraja A, Tonapi VA (2016) Diseases of millets—a ready reckoner. Indian Institute of Millets Research, Hyderabad, Telangana, p 67

Desai SG, Thirumalachar MJ, Patel MK (1965) Bacterial blight disease of *Eleusine caracana* Gaertn. Indian Phytopathol 28:384–386

Dubey SC (1995) Banded blight of finger millet caused by *Thanetephorus cucumeris*. Indian J Mycol Plant Pathol 25:315–316

FAOSTAT (2020) Rome. http://www.fao.org

FAOSTAT (2021) Rome. http://www.fao.org

Gaikwad AP, D'Souza TF (1987) A comparative study on *Pyricularia* spp. J Maharashtra Agric Univ 12:134–135

Goswami SK, Thakur C, Choudhary P et al (2022) Characterization of *Ustilaginoidea virens* causing rice false smut and its bio-control in north India. Indian Phytopathol 75:565–571. https://doi.org/10.1007/s42360-022-00460-5

Hyung L-D (1997) Morphological characters and seed transmission of *Bipolaris panicimiliacei* causing leaf spot of common millet. Korean J Plant Pathol 13(1):18–21

Jain AK, Tripathi SK (2002) Pathogenic variability in the isolates of *Sorosporium paspali-thunbergii* causing head smut of kodo millet. In: Rodrigues BF, Gour HN, Bhatt DJ, Kamat N (eds) Advances in fungal diversity and host pathogen interactions. Goa, Department of Botany, Goa University, pp 85–89

Jain AK, Yadava HS (1997) Recent approaches in disease management of Small Millets. In: Proc. Nat. Semi. on Small millets—current research trends and future priorities as food, feed and in processing for value addition, held at TNAU, Coimbatore (T.N.) from 23–24 April 1997, pp 31–33

Jain AK, Tripathi SK, Singh RP (2006) *Macalpinomyces sharmae*—a new threat for the cultivation of little millet in Madhya Pradesh. In: Proc. Nat. Symp. on emerging plant diseases, their diagnosis and management. N.B.U., Siliguri, W.B., pp 31–32

Jeger M, Beresford R, Bock C et al (2021) Global challenges facing plant pathology: multidisciplinary approaches to meet the food security and environmental challenges in the mid-twenty-first century. CABI Agric Biosci 2:20. https://doi.org/10.1186/s43170-021-00042-x

Joshi LM, Raychaudhuri SP, Batra SK, Renfro BL, Ghosh A (1966) Preliminary investigations on a serious disease of *Eleusine coracana* in the states of Mysore and Andhra Pradesh. Indian Phytopathol 19:324–325

Kara M, Soylu EM, Uysal A et al (2020) Morphological and molecular characterization of downy mildew disease caused by *Peronospora variabilis* on *Chenopodium album* in Turkey. Australas Plant Dis Notes 15:10. https://doi.org/10.1007/s13314-020-0381-2

Kulkarni GS (1922) The smut of nachani or ragi (*Eleusine coracana*). Ann Appl Biol 9:184–186

Kulkarni S, Patel MK (1956) Study of the effect of nutrition and temperature on the size of spores in *Pyriculria setariae* Nishikado. Indian Phytopathol 9:31–38

Kumar B (2011) First record of smut disease of foxtail millet caused by *Ustilago crameri* Korn. J Mycol Plant Pathol 41(3):459–461

Kumar B (2013) Diseases of small millets in Uttarakhand and their management. In: Singh KP, Prajapati CR, Gupta AK (eds) Innovative approaches in plant disease management-crop diseases and their management. LAP Lambert Academic Publishing, Germany, pp 257–287

Kumar B, Singh KP (2010) Important small millets diseases in India and their management. Plant Pathology Section, College of Forestry and Hill agriculture, Ranichauri, Tehri Garhwal, Uttarkhand

Kumar VBS, Amruta Bhat S, Nagaraju (2007) Virulence analysis of *Pyricularia grisea* (Cke.) Sac. on different finger millet genotypes and cultivars to determine race differential lines. Environ Ecol 25(1):190–192

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

Longya A, Talumphai S, Jantasuriyarat C (2020) Morphological characterization and genetic diversity of rice blast fungus, *Pyricularia oryzae*, from Thailand using ISSR and SRAP markers. J Fungi 6:38. https://doi.org/10.3390/jof6010038

Lu B, Hyde KD, Ho WH et al (2000) Checklist of Hong Kong fungi. Fungal Diversity Press, Hong Kong, p 207

Mahadevakumar S, Yadav V, Tejaswini G, Janardhana G (2016) Morphological and molecular characterization of *Sclerotium rolfsii* associated with fruit rot of Cucurbita maxima. Euro J Plant Pathol 145(1):215–219. https://doi.org/10.1007/s10658-015-0818-1

Mantur SG (1994) Studies on the management of the smut disease of finger millet caused by Melanopsichium eleusinis (Kulk) Mundk. and Thirum, M.Sc. (Agri) thesis. UAS, Bangalore, p 55

Mariappan V, Natarjan C, Kandaswamy TK (1973) Ragi streak disease in Tamil Nadu. Madras Agric J 60:451–453

McRae W (1920) Detailed administration report of the government mycologist for the year 1919–20

McRae W (1922) Report of the imperial mycologist. Agric Res Inst Pusa Sci Rep 1921–1922:44–50

McRae W (1924) Economic botany, Part III—Mycology annual report Bot Sci Advice, India (1922–23). pp 31–35

Mitra M (1931) Report of the imperial mycologist. Sci Rep Agric Res Int Pusa. pp 58–71

Mundkur BB (1939) A contribution towards a knowledge of Indian Ustilaginales. Trans Br Mycol Soc 23:105

Mundkur BB (1943) Studies in Indian cereal smuts. VI. The smuts on sawan, *Echinochloa frumentacea*. Indian J Agric Sci 13:631–633

Mundkur BB, Thirumalachar MJ (1946) Revisions and addition to Indian fungi. Mycol Paper 16. CMI, Kew

Munjal RL, Lall G, Chona BL (1961) Some Cercospora species from India VI. Indian Phytopathol 14:179–190

Nagaraja A, Kumar J, Jain AK, Narasimhudu Y, Raghuchander T, Kumar B, Hanumanthe Gowda B (2007a) Compendium of small millets diseases. Project coordination cell, All India Coordinated Small Millets Improvement Project, UAS, Bangalore, p 80

Nagaraja A, Jagadish PS, Ashok EG, Krishne Gowda KT (2007b) Avoidance of finger millet blast by ideal sowing time and assessment of varietal performance under rainfed production situations in Karnataka. J Mycopathol Res 45(2):237–240

Nagaraja A, Kumar B, Jain AK, Patro TS, Nageswar Rao TG (2016) Diseases of small millets. In: Diseases of field crops and their management. Indian Phytopathological Society, New Delhi, pp 295–371

Narasimhan MJ (1934) Report of the work done in Mycology section. Department of Agriculture, Mysore, pp 1932–1933

Nema AG, Kulkarni SN, Pall BS (1978) Bacterial leaf streak of kodo (*Paspalum scrobiculatum* L.). Sci Cult 45:365–366

Pall BS, Jain AC, Singh SP (1980) Diseases of lesser millets. JNKVV, Jabalpur, pp 53–54

Patro TSSK (2008) Survey for important diseases in finger millet in Andhra Pradesh. Annual Report of AICRP on smallmillets 3

Paul NC, Park SW, Liu H et al (2021) Plant and fungal genome editing to enhance plant disease resistance using the CRISPR/Cas9 system. Front Plant Sci 12:700925. https://doi.org/10.3389/fpls.2021.700925

Prakash P, Ravishankar CR, Prasad N (2007) Blast incidence as affected by nitrogen levels in finger millet (*Eleusine coracana*) genotypes. J Mycol Plant Pathol 37:590

Pralhad SP, Krishnaraj PU, Prashanthi SK (2019) Morphological and molecular characterization of *Rhizoctonia solani* causing sheath blight in rice. Int J Curr Microbiol App Sci 8(1):1714–1721. https://doi.org/10.20546/ijcmas.2019.801.182

Praveen B, Nagaraja A, Prasanna Kumar MK et al (2021) First report of *Alternaria alternata* causing leaf blight on little millet (*Panicum sumatrense*) in India. Plant Dis 105(4):1202

Rachie KO, Peters LV (1977) The *Eleusines*—a review of world literature. ICRISAT, Hyderabad, p 179

Raghavendra S, Safeeulla KM (1973) Investigation on the ragi downy mildew. J Mysore Univ 26: 138–155

Ramakrishnan TS (1971) Diseases of millets. Indian council of Agricultural Research, New Delhi, pp 83–100

Ramakrishnan TS, Sundaram NV (1950) Ergot on two grasses from South India. Sci Cult 16:214

Ramappa HK, Ravishankar CR, Prakash P (2002) Estimation of yield loss and management of blast in finger millet (*ragi*). In: Proceedings of Asian Congress of Mycology and Plant Pathology, 1–4 October 2002. University of Mysore, Mysore, India, p 195

Ramesh GV, Palanna KB, Vinaykumar HD, Koti PS, Mahesha HS, Nagaraja TE, Tonapi VA, Jeevan B (2021a) Occurrence and characterization of *Bipolaris setariae* associated with leaf blight of browntop millet (*Brachiaria ramosa*) in India. J Phytopathol 169(10):613–622

Ramesh VG, Palanna KB, Praveen B, Vinaykumar HD, Koti PS, Sonavane P, Tonapi VA (2021b) First confirmed report of leaf blight on browntop millet caused by *Bipolaris setariae* in Southern Peninsular India. Plant Dis. https://doi.org/10.1094/PDIS-11-20-2445-PDN. (NAAS-9.53)

Rangaswami G, Mahadevan A (1999) Diseases of cereals. In: Diseases of crop plants in India, 4th edn. Prentice Hall of India, Pvt. Ltd, New Delhi, pp 160–264

Rangaswami G, Prasad NN, Eswaran KSS (1961) Two new bacterial diseases of sorghum. Andhra Agric J 8(6):269–272

Rao ANS (1990) Estimates of losses in finger millet (*Eleusine coracana*) due to blast disease (*Pyricularia grisea*). J Agric Sci 24:57–60

Rath GC, Mishra D (1975) Nature of losses due to neck blast infection in ragi. Sci Cult 41:322

Richardson MJ (1990) An annotated list of seed-borne diseases, 4th edn. International Seed Testing Association, Zurich. 387+ pages

Safeeulla KM (1955) Comparative morphological and cytological studies in some species of the genera *Albugo*, *Sclerophthora* and *Sclerospora*. PhD Thesis, University of Mysore, India. p 179

Sharma PN, Khare MN (1987) Two new smut diseases in little millet (*Panicum sumatrense*) from India. Acta Bot Indica 15:143–144
Sill WH, Agusiobo PC (1955) Host range studies of the wheat streak-mosaic virus. Plant Dis Reporter 39:633–642
Singh RK, Gopala (eds) (2021) Innovative approaches in diagnosis and management of crop diseases: volume 2: field and horticultural crops, 1st edn. Apple Academic Press. https://doi.org/10.1201/9781003187837
Sinha AP, Upadhyay JP (1997) Millet ke rog. Directorate of Publication, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, p 180
Sivaesan A (1987) Graminicolous species of *Bipolaris*, *Curvularia*, *Drechslera*, *Exserohilum* and their teleomorphs. Mycol Pap 158:1–261
Subbayya J, Raychaudhuri SP (1970) A note on a mosaic disease of ragi (*Eleusine coracana*) in Mysore, India. Indian Phytopathol 23:144–148
Sundararaman S (1921) Ustilago crameri Koern on Seatria italica. Bull Agric Res Inst 97:11
Sydow H, Butler EJ (1906) Fungi Indiae Orientalis Pars I. Ann Mycol 4:424–445
Thirumalachar MJ, Mundkur BB (1947) Morphology and the mode of transmission of the ragi blight. Phytopathology 37:481–486
Thirumalachar MJ, Narasimhan MJ (1949) Downy mildew of *Eleusine coaracana* and *Iseilema laxum* in Mysore. Indian Phytopathol 2:47–51
Ulstrup AJ (1955) Crazy top of some wild grasses and the occurrence of the sporangial stage of the pathogen. Plant Dis Reporter 39:839–841
Vasudeva RS (1954) The fungi of India, Sci Monogr. ICAR, p 12
Venkatarayan SV (1947) Diseases of ragi (*Eleusine coracana*). Mysore Agric J 24:50–57
Vidhyasekaran P (1971) Saprophytic survival of *Helminthosporium nodolosum* and *H. tetramera* in soil. Indian Phytopathol 24:347–353
Viji G, Gnanamanickam SS, Levy M (2000) DNA polymorphisms of isolates of *Magnaporthe grisea* from India that are pathogenic to finger millet and rice. Mycol Res 104:161–167
Viswanath S (1992) Management of biotic factors (diseases). In: 6th Annual small millets workshop at BAU, Ranchi-Kanke (Bihar) from 30 April–2nd May, 1992
Viswanath S, Seetharam A (1989) Diseases of small millets and their management in India. In: Seetharam A, Riley KW, Harinarayana G (eds) Small millets in global agriculture. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, pp 237–253
Wang CS (1943) Studies on the cytology of *Ustilago crameri*. Phytopathology 33:1122–1133

Conventional and Advanced Methods in Small Millet Processing

6

Anupam Amitabh, Ankit Kumar, and Vishal Kumar

Abstract

Small millets, which are mostly grown in rainfed environments, are a forgotten subclass of millets. Sorghum and pearl millets are referred to as "big millets," while finger, kodo, little, foxtail, proso, barnyard, and browntop millets are considered as "small millets" in the Indian context. Small millets are high-valued crops with excessive nutritional attributes and climate resilience. They can serve as an essential crop for nutritional security in present world. The present scenario calls for better utilization of small millets through innovative postharvest processing. The conventional processing of millets is also important to study as they are more adaptable to local processors. Hence, a detailed study of conventional and advanced methods in millet processing can bring new opportunities for efficient utilization of millets, along with new product development which can help in sustainable agricultural growth and income generation for the farmers as well as processors. This chapter discuss in detail various conventional and modern techniques of small millet processing together with a comparative analysis among them to find a solution for better adoption of these methods.

Keywords

Millet processing · Mechanical · Bioprocessing · Primary processing · Secondary processing

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6.1 Millet Processing: A Brief Introduction

Millets are more nutrient-rich than refined cereals, although they have gained less attention. Millets are among the first foods that humans have grown. Depending on the size of the grain, these are categorized as major millets and minor/small millets. They require less input than other grains since they can withstand challenging agroclimatic conditions. Additionally, numerous studies have shown that small millets are superior to commonly consumed grains like wheat and rice in most nutritional categories. They contribute to a well-balanced diet and, if regularly taken, play a significant part in sustaining nutritional security. Additionally, they are crucial to the economies and food security of developing nations on the continents of Asia and Africa. Small millets play a crucial role in achieving food and nutritional security, yet despite this, their production and cultivation have seen a rapid drop. Small millets are generally taken for coarse cereals including foxtail, finger, proso, barnyard, kodo, little millets, teff, fonio, job's tear, guinea, and browntop millet (Muthamilarasan and Prasad 2021). The main obstacles include inadequate policy backing compared to foods like wheat and rice, underdeveloped markets, and limited technology adoption, particularly in developing nations. Investments in manufacturing and processing facilities are insufficient. Concerns about the low production and consumption of small millets also include ignorance about the inadequate nutritional value of current dietary patterns and resistance to change them.

In rain-fed, semi-arid locations all throughout the world, little millets were a common traditional crop; however, the advent of cash crops limited their production to specific places. Millets can tolerate water-limiting situations because of their improved water and nitrogen usage efficiency, which are agroecological features. For instance, while wheat and maize need 450 and 500 g of water, respectively, to yield 1 g of dry biomass, foxtail millet only needs 250 g. Micro- and macronutrients, total protein, fiber, and resistant starch are all abundant in small millets as well. In contrast, tiny millet and barnyard millet have high iron concentrations (between 10 and 18 mg per 100 g), while finger millet is high in calcium (around 364 mg per 100 g) and potassium (about 320 mg per 100 g). Additionally, the majority of small millets are gluten-free, which makes it easier to prepare foods with a low glycemic index (Rao et al. 2011).

Although efficient procedures and equipment are available for main cereals to assure processing and storage with minimal wastage, such equipment for harvesting and/or threshing is not always available for small millets, which represent a bottleneck in the large-scale production of seed grains. All millets have different grain morphologies and structures; hence, it is necessary to create and employ crop-specific technology. Because millet grain tends to get rancid due to its higher lipid content, long-term storage of millet grains necessitates safety precautions such maintaining temperature above ideal to prevent sprouting or rotting. These measures must be taken to prevent widespread loss of valuable produce.

6.2 Millet Processing Technologies

The millet processing involves conversion of raw millets after harvest into a usable product which can be either directly consumed or can be subjected to further secondary or tertiary processing. The millet processing technologies can be classified into major two headings.

1. Mechanical processing technologies
2. Bioprocessing technologies

Before going through the details of small millet processing, there is a strong need to understand the structure of millet grains. Sorghum and millets have comparable anatomical and basic kernel structures. It is possible to distinguish between the pericarp (outer covering), endosperm (starchy section), and germ as the primary anatomical components (oily part). The pericarp of foxtail, common, and finger millets is like a bag that is only weakly connected to the endosperm at one location. In these utricle-type kernels, the pericarp easily separates, exposing the inner endosperm beneath the seed coat or testa. Sorghum and pearl millet have caryopsis-type kernels, which take a little more force to break the pericarp since it is entirely connected to the endosperm. These three main kernel constituents are distributed differently relative to one another. Pericarp, endosperm, and germ distribution in pearl millet are 8.4%, 75.0%, and 16.5%, respectively (Abdelrahman et al. 1984). In the sorghum kernel, the endosperm to germ ratio is 8.4:1, whereas it is 4.5:1 in pearl millet. Because the germ is so small in common and finger millets, the endosperm to germ ratio of 11:1 to 12:1 is substantially higher than it is in sorghum. From 5.6% to 14.8% of millet is protein.

Millet processing is similar to other cereal grains processing, where we transform the coarse cereal grains into usable and more palatable form or convert it into some useful, value-added products. We are well aware that millets are rich in fiber and micronutrients. Now the focus has changed to secondary metabolites including bioactive phytochemicals. Germ, a starch-containing endosperm, and the pericarp, a protective layer, are the three main components of millets that are partially separated and/or modified during processing. The separation of the offal (part not typically used for human consumption) from the edible component is typically the first step in processing cereal or coarse grain. The pericarp and occasionally the germ make up the offal. Removal of offal is also known as decortication or dehulling. The primary reasons why millet foods are less popular among people who eat rice and wheat are the outer tough seed coat and related characteristic flavor (Malleshi et al. 1986), cultural attachments, and lack of processed millet goods equivalent to rice or wheat (Hadimani and Malleshi 1993). The millet processing machineries are scarce. Minor millets have more limitations in processing compared to major millets because of their small size and irregular grain shapes. Most of the commercial industries are also involved in the processing of rice and wheat, which are grown and consumed on a very large scale. There are still few machineries which are used for primary processing of millets. Decortication is an important unit operation in

Fig. 6.1 Traditional small millet processing manually in Jawadhu Hills. (Photo: Ms Maria, ODI, UK)

millet, which enhances biological availability of nutrients, reduces antinutrients, and enhances protein availability (Pushpamma et al. 1990). Decortication of millets are generally done by rice dehullers. Some unit operations like parboiling prior to decortication facilitates bran removal. Other operations like dehulling, soaking, germination, roasting, drying, polishing, and milling are discussed in detail in subsequent sections (Fig. 6.1).

6.3 Conventional Processing of Small Millets

One of the significant traditional food groups that has recently been removed from most people's diets is little millets. Small millets have been grown for more than 3000 years, making them an essential component of Indian history and culture. They play a significant role in the food system and culture of the scheduled tribes and scheduled castes, as well as other marginalized and impoverished populations living in deprived, rain-fed regions of India. Small millets, which are C4 crops, have a high tolerance for warmer temperatures and the capacity to bounce back quickly from drought and heat stress (Davis et al. 2018). They are also a safe source of food because they are grown with almost any agricultural chemicals (Kam et al. 2016).

They are a part of various cropping systems that also include several uncultivated greens and many nutrient-dense crops including horsegram, niger, cowpea, etc. They also provide wholesome fodder. Reviving the cultivation and consumption of small millet is one of the viable answers in the context of dwindling biodiversity, climate change, severe irrigation water scarcity, and pervasive hunger.

Despite all these advantages of millets, its utilization is not at par due to processing limitations. Also, the socio-economic factors affect its cultivation and utilization. The major interventions to be incorporated to enhance utilization of millets can be primary processing of small millets, small-scale mechanical processing, early adopters' experiences with mechanized processing, and implications of these achievements for boosting local and regional food systems and diversifying diets. To make small millets palatable, their husks must be removed. This is an essential step in the production of grain-rice and in the subsequent processing of grains for human use. Women have traditionally overseen manually processing small millets with husk in all communities that are in the small millet production regions of India. Several tasks are involved in manual processing, but the main ones are the following:

1. Drying the grains to the ideal moisture level
2. Cleaning the grains to remove small and large stones and other unwanted materials using a winnowing pan
3. Removing the husk using a wooden or stone grinder followed by drying and hand pounding in mortar or just through hand pounding in mortar
4. Separating the unwanted fractions from millet rice and grits

Up till clean small millet rice is obtained, the second and third processes will be repeated twice or three times. Small millets must be manually processed, which is a specialized job that requires a lot of labor. Processing 5–8 kg of wheat requires 4 h of labor from women. Most young women are not prepared for that level of work, and there has not been enough knowledge and skill transfer across generations related to manual processing. The cultivation and consumption of small millets with husk have drastically decreased as a result of this, along with the ease with which rice and wheat (considered as superior foods) are available in the Public Distribution System. Even in places where little millets were still produced, they have changed from being a food crop to a commercial commodity.

Variation in raw materials in the case of small millets and their shorter shelf life are major challenges in their processing. Various tiny millet crops have different grains with regard to shape, grain surface characteristics, hardness, husk-grain bonding, and anticipated rice recovery (Karthikeyan 2016). Additionally, there are variations in the same small millet crop due to differences in cultivars, farming methods, and microclimate in different production regions and years. Kodo millets have hardness of around 25.5 N with the force required to split the husk is around 18.5 N. The terminal velocity of grain is measured as 3.75 m/s, which is important in their separation using aspiration. The average recovery of whole-milled grain is around 60–64%. In the case of Barnyard millet, the hardness, force required to

dehusk, terminal velocity, and average recovery are 23.8 N, 17.3 N, 3.08 m/s, and 60–65%, respectively. Foxtail millet and little millet have better recovery in range of 70–75%, but they have lower terminal velocity (Muniappan et al. 2018).

Some of the mechanical processing equipment available for millets includes aspirator cum grader of capacity varying from 50 to 500 kg. The destoners are available for 50–500 kg capacity. Aspirator cum grader cum destoners are also available with some industries. AVM Engineering (AVM), VICTOR AGRO SALES (Victor), Perfura Technologies Private Ltd. (Perfura), KMS Industries (KMS), and Vishra Agro Sales are a few companies that manufacture these devices. In Karnataka, Bhavani Industries, Vishwa Agro Tech, and Bio-Tech are other companies that do the same. Some of the huller designs manufactured included single-chamber centrifugal impact huller developed by Victor and AWM and improved by Dhan foundation and TNAU; double-chamber centrifugal impact huller developed by Dhan foundation and TNAU; CIAE model abrasive huller by CIAE, Bhopal, offered by Perfura; portable impact huller, 200–400 kg/h capacity; and table top impact huller developed by Dhan foundation. These equipments were found to perform well for small millets; still there are several modifications which are required to harness best possible mechanical processing of small millets (Fig. 6.2).

A new improvised design of millet processing was developed by McGill. For usage in homes, a hand-operated rubber roller dehuller has been created. The prototype was tested on various millets, including foxtail and kodo millets, as part of Mr. Subhash Palaniswamy's master's thesis. After two passes, the efficiency of this dehuller for small and foxtail millets is over 90%. In India, the hand-operated dehuller is prepared for transfer and expansion. Dr. Samson Sotocinal of SAS Technologies designed and constructed a large-scale rubber roller dehuller at McGill with a theoretical processing capacity of 175 kg of small millet per hour. When it was first tested with small millet, it had a one-pass efficiency of about 90% (Fig. 6.3).

The development of a double-chamber centrifugal dehuller resulted in its basic components being a feed hopper, two cast iron centrifugal chambers, impellers with curved vanes, a blower, and separate exits for collecting kernel and husk. A 5-hp motor with an appropriate power transfer system powers the device. Additionally, a grain elevator is offered to make it simple to feed grains into the machine. When the machine is running, the hopper's contents are thrown against the cast iron chamber at a high speed after entering the impeller through the feed housing, where they gain momentum. Impact causes the husk to split, and the kernel is then freed from the husk (Durairaj et al. 2019).

The 2–3-month shelf life of dehulled tiny millets is a significant barrier to the commercialization of small millet products. The presence of more fat than other cereals and the degradation of its triglycerides through lipolysis and subsequent oxidation of de-esterified unsaturated fatty acids are blamed for the poor keeping quality of small millet grains. The hydrolysis of the triglyceride, which causes the off-odor and taste in the flour and its products, is caused by the lipase enzyme, which is concentrated in the pericarp, aleurone layer, and germ. Several institutes are conducting storage trials incorporating three different settings, namely, vacuum

Fig. 6.2 Victor single-chambered CF dehuller (left) and AVM single-chamber CF dehuller with grader (right)

packing, modified atmosphere packaging, and hermetic storage, to extend the shelf life.

6.4 Bioprocessing Processing in Millets

Due of polyphenols' positive impact on diabetes prevention, interest in them has increased. Millets are a group of ancient plants that are both food and animal feed and are high in polyphenols. They are cultivated all throughout the world and have been developed to produce under hot, dry circumstances. Millets include polyphenols that have antidiabetic effects. Millet, however, is typically consumed following heating, germination, fermentation, and other processing techniques, which could change the polyphenol content and hence impair their capacity to fight diabetes. Millets are typically processed using a variety of techniques at home. These techniques include milling, soaking, cooking, roasting, germination and fermentation. The number of polyphenols in the finished product can be altered

Fig. 6.3 Enterprise level rubber roller type dehuller developed by McGill

by methods. There are other additional processing techniques as well, including as soaking, grinding, shelling, high-pressure, ultrasound, and microwaving. These processes can alter the polyphenol content of millets and the physicochemical features and eating quality qualities of millets. Cereal processing methods include soaking and dehulling. Typically, millets are dehulled before processing. Millets can be processed, whereas soaking can be employed to lower the amount of several anti-nutritional components. In germinated foxtail, high-pressure soaking can raise the TPC and lower the levels of tannins and other antinutrients. In addition to soaking and dehulling, milling is frequently utilized to minimize particle size for further processing by maximizing endosperm, bran, and germ separation.

Additionally, the use of ultrasonography, enzyme therapy, and their combination have an impact on the amount of certain polyphenols in millets. Ultrasonication (UA) and UA following enzyme treatment with xylanase (XUA), both of which increased the TFC extraction rates, were 1.4- and 1.3-fold higher than tannin extraction rates were 1.1- and 1.2-fold higher. Additionally, XUA resulted in a greater extraction of phenolic compounds in finger tissues compared to UA. In finger millet treated with UA and XUA samples, there were 3-(3′′-malonyl) glucoside

cyaniding, 6-C-pentosyl-8-C-pentosyl luteolin, and trimers of catechins. Moreover, the caffeic acid derivatives, which may have been released by xylanase, were exclusively found in finger millet that had undergone XUA processing, such as caffeoyl and dicaffeoyl shikimic acids. Although polyphenols are initially found in a stable. Exogenous enzyme therapy can lower millets' polyphenol content in their natural grain form. Additionally, microwave therapy caused various effects on the polyphenol content of various millets (Wang et al. 2022).

Numerous research has exclusively examined the changes in the past in the antioxidant capacity and polyphenol levels before and after processing. There has not been many comparison research on how polyphenols change both before and after processing impact diabetics. During processing, polyphenols are reduced potentially interfere with any positive effects on diabetes. Moreover, even though catechins and ferulic acid, two polyphenols, are well-known for their effects on inflammation, blood sugar, and antioxidants, among others. Additionally, millets' traces of polyphenols and their derivatives must be investigated to determine their possible health advantages for people.

6.5 Conclusions

Small millets processing units are being encouraged at the village level in production regions because the presence of local processing infrastructure is anticipated to lessen labor-intensive processing, which is anticipated to increase small millets' uptake. Various preliminary processing of millets has been developed and improved. In bioprocessing of millets, several technologies are still in pipeline. Several methods of extraction, encapsulation, and bioavailability have been researched in recent past to improve the utilization of millets, as the millets are hub of many nutrients. There is a wide scope of processing in millets to achieve its maximum utility and to realize it as wonder food using modification in traditional processing and improved processing technologies.

References

Abdelrahman A, Hoseney RC, Varriano-Marston E (1984) The proportions and chemical compositions of hand-dissected anatomical parts of pearl millet. J Cereal Sci 2(2):127–133

Davis KF, Chiarelli DD, Rulli MC, Chhatre A, Richter B, Singh D, DeFries R (2018) Alternative cereals can improve water use and nutrient supply in India. Sci Adv 4(7):eaao1108

Durairaj M, Gurumurthy G, Nachimuthu V, Muniappan K, Balasubramanian S (2019) Dehulled small millets: the promising nutricereals for improving the nutrition of children. Matern Child Nutr 15:e12791

Hadimani NA, Malleshi NG (1993) Studies on milling, physico-chemical properties, nutrient composition and dietary fibre content of millets. J Food Sci Technol (India) 30(1):17–20. https://dhan.org/smallmillets2/sm-processing-equipments.html

Kam J, Puranik S, Yadav R, Manwaring HR, Pierre S, Srivastava RK, Yadav RS (2016) Dietary interventions for type 2 diabetes: how millet comes to help. Front Plant Sci 7:1454

Karthikeyan M (2016) Small millets in mainstream diets: promoting decentralised processing infrastructure. IDRC

Malleshi NG, Desikachar HSR, Tharanathan RN (1986) Physico-chemical properties of native and malted finger millet, pearl millet and foxtail millet starches. Starch-Stärke 38(6):202–205

Muniappan K, Raghavan V, Nachimuthu V, Raveendran M, Panaiyuran S, Vediyappan V, Nayak BK (2018) CIFSRF final technical report: Scaling up small millet post-harvest and nutritious food products project (CIFSRF Phase 2). IDRC

Muthamilarasan M, Prasad M (2021) Small millets for enduring food security amidst pandemics. Trends Plant Sci 26(1):33–40

Pushpamma P, Ejeta G, Mertz ET, Rooney LW, Schaffert R, Yohe J (1990) Importance of sorghum as food in Asia. In: Proceedings of international conference an sorghum nutritional quality, vol 26, pp 229–241

Rao BR, Nagasampige MH, Ravikiran M (2011) Evaluation of nutraceutical properties of selected small millets. J Pharm Bioallied Sci 3(2):277

Wang H, Fu Y, Zhao Q, Hou D, Diao X, Xue Y, Shen Q (2022) Effect of different processing methods on the millet polyphenols and its anti-diabetic potential. Front Nutr 9:780499

Nutritional Aspects, Phytochemical Composition and Potential Health Benefits of Small Millets

7

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Abstract

Small millets comprise of a group of cereals widely cultivated and consumed across the arid and semi-arid parts of the world. These cereals are highly nutritious and show excellent adaptability to various biotic and abiotic stress conditions. Nevertheless, these grains have remained largely underutilized owing to their coarse nature. With climate change and malnutrition becoming major concerns across the globe, the small millets are receiving greater attention recently. Furthermore, the increased incidence of chronic lifestyle disorders has led to an upsurge in consumer preference towards foods including the small millets with potential health-promoting attributes. The small millet grains are nutritionally rich and are good sources of protein and dietary fibre. Further, these grains are rich sources of micronutrients including minerals, viz. calcium, iron, zinc and B vitamins including thiamine, niacin, riboflavin, etc. The small millet grains are also good source of phytochemicals especially phenolic compounds with potential human health beneficial effects. In this chapter, the nutritive value, phytochemical composition and potential health promoting effects of small millets including finger, foxtail, little, proso, kodo and barnyard are presented. We have also described the impact of different processing techniques on the grain nutritional characteristics.

Keywords

Nutri-cereals · Micronutrients · Antidiabetic · Processing

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7.1 Introduction

Millets encompass a group of small-seeded cereals belonging to the grass family, *Poaceae*. They are widely cultivated across the arid and semiarid parts of the world, where they assume significance as staple crops, providing food and nutritional security to the resource-constrained people. Millets include the major millets as well as the small or minor millets; Sorghum (*Sorghum bicolor*) and pearl millet (*Pennisetum glauccum*) are the major millets. The small millets, named so owing to their small size, include finger millet (*Eleusine coracana* (L.) Gaertn.), foxtail millet (*Setaria italica* (L.) P. Beauv.), proso millet (*Panicum miliaceum* (L.)), barnyard millet (*Echinochloa frumentacea* (Indian barnyard millet); *Echinochloa esculenta* (A.) Braun; Japanese barnyard millet), kodo millet (*Paspalum scrobiculatum* (L.)) and little millet (*Panicum sumatrense* Roth. ex. Roem. & Schult.).

Millets including the small millets are hardy crops and exhibit agronomical superiority in terms of their shorter growing seasons, wider adaptability to different environments, ability to grow in marginal lands and requirement of lesser inputs and minimum incidences of biotic stress (Bandyopadhyay et al. 2017; Vetriventhan et al. 2020; Malleshi et al. 2020). Small millet grains are regarded as 'nutri-cereals', as they are highly nutritious and possess excellent nutraceutical value. They house a plethora of micronutrients and bio active phytochemicals with human health-promoting attributes. Despite their nutritional and agronomical superiority, these grains have been largely ignored for long years mainly because of the lack of awareness (Majid and Priyadarshini 2020). However, recently, as there is an increased consumer preference for healthy foods, these grains are regaining their significance globally. Increasing research on its health benefits shows that small millets can be exploited as potential therapeutic foods in the management of chronic lifestyle disorders including diabetes mellitus, hypertension, obesity, etc. (Chaudhary Kanchan 2013; Ren et al. 2016; Anis and Sreerama 2020). These grains are now regarded as promising crops for the future owing to their climate-smart nature and sustainable food and nutritional security and adding diversity to food basket. This chapter is focussed on the nutritional aspects, composition of phytochemicals and the potential human health benefits of the underutilized small millets.

7.2 Nutritive Value of Small Millets

The small millet grains differ from one another in different characteristics including grain type, size, shape, colour, 1000 kernel weight, etc. (Fig. 7.1; Table 7.1). Most small millets possess husk which has to be removed to produce edible form of the grain. An exception is finger millet; as are utricles, the pericarp layers can be easily removed by rubbing.

The nutritional composition of small millets may vary depending on the grain, genotype and environment among other factors (Vetriventhan et al. 2020). The balanced nutritional composition of small millets in comparison with other

Fig. 7.1 Different types of small millets

commonly consumed cereals including rice, wheat and maize is presented in Table 7.2. The small millet grains contain about 60–78% carbohydrates, 6–12.3% protein, 1.9–4.3% fat and 1.3–2.7% ash. The grains are rich in total dietary fibre and also micronutrients including minerals and B vitamins. They also contain phytochemicals such as phenolic compounds with potential bioactivities.

7.2.1 Carbohydrates

As with most other cereals, carbohydrates are the major nutritional component of small millets with starch as the main carbohydrate. The total starch of small millet grains varies with a content of 55–65% (finger millet), 56–73% (foxtail millet), 42–51% (little), 58–77% (proso millet), 47–60% (kodo millet) and 48–60% (barnyard millet) (w/w) db (reviewed in Kaimal et al. 2021). Millet starch is composed of approximately 20–30% amylose and 70–80% amylopectin. Nevertheless, small millets including foxtail, finger and proso also have genotypes with waxy (high amylopectin)-type grains like that of sorghum (Serna-Saldivar and Espinosa-Ramírez 2019; Taylor 2017).

Regarding the starch granule morphology, finger, proso, kodo and little millets contain two types of starch granules, viz. large polygonal type and small polygonal and/or round type. Finger millet also contains compound type granules, which is

Table 7.1 Small millets: general description

Common name	Scientific name	Grain type	Grain colour	Grain shape; size	1000 kernel weight (g)
Finger millet	*Eleusine coracana*	Utricle	Light to dark brown	Round or globose 1.2–1.8 mm (d)	2.3
Foxtail millet	*Setaria italica*	Caryopsis	Pale to yellow orange	Ovoid 1–2 mm (d)	2.0
Little millet	*Panicum sumatrense*	Caryopsis	Grey, pale to straw	Elliptical to oval 1.8–1.9 mm (l)	1.9
Proso millet	*Panicum miliaceum*	Utricle	White to yellow orange	Oval 3 mm (l) and 2 mm (d)	6.1
Kodo millet	*Paspalum scrobiculatum*	Caryopsis	Dark brown to blackish	Elliptical to oval 2.5 mm (l)	6.7
Barnyard millet	*Echinochloa frumentacea* (Indian) *Echinochloa esculenta* (Japanese)	Caryopsis	Pale	Tiny elliptical to oval 2 mm (l)	4.2

Information from Taylor (2017); Serna-Saldivar and Espinosa-Ramírez (2019)
d diameter, *l* long

Table 7.2 Nutritional composition of small millets in comparison with rice, wheat and maize

Grain	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)	Total dietary fibre (TDF)	Energy
Finger millet	7.16	1.92	2.04	66.8	11.2	320
Foxtail millet	12.3	4.30	2.6	60.1	10.7	331
Little millet	10.13	3.89	1.34	65.5	7.7	346
Proso millet	11.5	3.5	2.7	64.5	9.6	341
Kodo millet	8.92	2.55	1.72	66.2	6.4	331
Barnyard millet	6.2	2.20	1.3	65.5	12.6[a]	307
Rice, raw, milled	7.9	0.52	0.56	78.24	2.81	356
Wheat, whole	10.59	1.47	1.42	64.72	11.2	321
Maize, dry	8.8	3.7	1.17	64.7	12.2	334

Source: Longvah et al. (2017); Indian food composition tables; Malleshi et al. (2020)
[a] Roopashree et al. (2014)

made up of closely packed small granules (McDonough et al. 1986; Kumari and Thayumanavan 1998). Depending on the rate of enzymatic hydrolysis upon ingestion, starch can be categorized in to three types, viz. rapidly digestible (RDS), slow digestible (SDS) and resistant starch (RS). Though there is a variation in the time taken for hydrolysis (as the name suggests), both RDS and SDS are absorbed in the small intestine, while RS remains unabsorbed and gets fermented in the large intestine (Kaimal et al. 2021). RS imparts several health benefits including potential antidiabetic effect. As they are resistant to digestion, RS is now a days included along with the dietary fibre component of food. A study of nine land races from Taiwan reported RS content, which amounted up to 35.2–51.2% (% of total starch) which was reported (Yin et al. 2019). Sharma and Gujral (2020) reported the RS content of small millet whole grain flours in %/100 g starch: finger (30.17), foxtail (25.25), barnyard (28.17), kodo (30.87), little (29.93) and proso (21.99).

Dietary fibre is defined as 'the edible parts of plant or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine' (AACC 2000; https://www.cerealsgrains.org/resources/definitions/Pages/DietaryFiber.asp). Consumption of foods rich in dietary fibre is known to impart health benefits, viz. improved gut health, reduced gastrointestinal transition time and slow release of glucose in to the blood stream among others (Kaur et al. 2014). Hence, dietary fibre is regarded as a potential functional food component. Small millets are among the rich sources of dietary fibre. The total dietary fibre content in small millets range from about 6% in kodo millet to 12.6% in barnyard millet. As with other cereals, the dietary fibre is mainly distributed in the pericarp as well as endosperm cell walls. The total dietary fibre includes the soluble and insoluble dietary fibre; in general, insoluble dietary fibre is the major fraction in small millets contributing about from 58% to 95% of the TDF (Serna-Saldivar and Espinosa-Ramírez 2019). The insoluble and soluble dietary fibre content (%) of the small millets are finger (9.5;1.7), foxtail (8.7;1.8), little (5.5;2.3), proso (9.3;1.9), kodo (4.3;2.1) and barnyard (8.8;1.1), respectively (Malleshi et al. 2020; Longvah et al. 2017).

The soluble sugars, viz. glucose, fructose and sucrose, form the minor carbohydrate component of the small millet grains with a content of approximately 1% (Taylor 2017).

7.2.2 Protein

Small millets are gluten-free and hence serve as important source of dietary protein for celiac patients. The highest protein content among the small millets is reported in foxtail millet (12.3%) and proso millet (11.5%). Grain proteins are mainly concentrated in the protein bodies of endosperm. Prolamin is the major protein fraction in the small millets, viz. finger and foxtail millet, while, glutelins contribute the major protein fractions in barnyard, proso, kodo and little millet. In finger millet, along with prolamins, glutelins also form a major fraction (Taylor 2017; Serna-Saldivar and Espinosa-Ramírez 2019; Sachdev et al. 2020). The essential amino acid

Table 7.3 Essential amino acids present in millets (g/100 g protein)

Cereals	HIS	ILE	LEU	LYS	MET	PHE	THR	TRP	VAL
Finger millet	2.37	3.70	8.86	2.83	2.74	5.70	3.84	0.91	5.65
Foxtail millet	2.14	4.55	11.96	1.42	2.69	6.27	3.89	1.32	5.49
Little millet	2.35	4.14	8.08	2.42	2.21	6.14	4.24	1.35	5.31
Proso millet	2.1	4.1	12.2	1.5	2.2	5.5	3.0	0.8	5.4
Kodo millet	2.23	4.44	10.84	1.91	2.73	9.56	3.85	1.32	6.78
Barnyard millet	2.42	6.04	12.46	2.13	3.08	6.86	4.72	1.31	6.59

Source: Longvah et al. (2017), Indian food composition table; Sachdev et al. (2020)

composition of small millets is presented in Table 7.3. Small millets have a balanced essential amino acid composition; however, like in most other cereals, lysine is the limiting essential amino acid. Apart from the essential amino acid profile, the protein quality is characterized in terms digestible indispensable amino acid score (DIAAS). The DIAAS for proso and foxtail millet are only about 7 and 10, respectively, as compared to the pseudocereal common buckwheat with DIAAS of 68, implicating the latter is a better-quality protein source (Han et al. 2019; Sachdev et al. 2020).

7.2.3 Fat

Fat is relatively a minor constituent of small millet grains. It is mainly concentrated in the germ layer like in other cereals. Among the small millets, foxtail millet has relatively higher fat content (4.3%) probably due to its larger germ layer. Finger millet has a lower fat content of about 1.9% and has been implicated in the better storage stability of the grains (Malleshi et al. 2020). With respect to fatty acid composition, small millets contain a good amount of unsaturated fatty acids. Further, the essential fatty acids including linoleic and linolenic acids have been detected in small millets. The monounsaturated oleic acid is an important component of fats in small millets (Taylor 2017).

7.2.4 Micronutrients

Minerals and vitamins constitute the important micronutrients. Small millets are good sources of mineral nutrients; the important minerals present in small millets as compared to wheat, rice and maize are presented in Table 7.4. The minerals are mainly located in the outer layers, viz. bran of the grain. The calcium content of finger millet is 364 mg/100 g, which is much higher as compared other small millets and cereals. Barnyard millet (5 mg/100 g) and also finger millet (4.6 mg/100 g) are good sources of iron, which can meet about 90% of the daily recommended iron. Foxtail millet contains good amount of iron and zinc. Also, all the small millets are good source of potassium and magnesium among other the minerals.

Table 7.4 Micronutrient composition of small millets (mg/100 g)

	B vitamins			Minerals			
Grain type	Thiamine (mg)	Riboflavin (mg)	Niacin (mg)	Calcium (mg)	Magnesium (mg)	Iron (mg)	Zinc (mg)
Finger millet	0.37	0.17	1.3	364	146	4.6	2.5
Foxtail millet	0.59	0.11	3.2	31	81	2.8	2.4
Little millet	0.26	0.50	1.3	16	91	1.3	1.8
Proso millet	0.20	0.18	2.3	15	153	0.8	1.4
Kodo millet	0.29	0.20	1.5	15	122	2.3	1.6
Barnyard millet	0.33	0.10	4.2	20	82	5.0	3.3
Wheat (whole)	0.46	0.15	2.7	39.3	125	3.97	2.8
Rice (raw, milled)	0.05	0.05	1.7	7.5	19.3	0.65	1.21
Maize (dry)	0.33	0.09	2.69	8.9	145	2.5	2.3

Source: Longvah et al. (2017), Indian food composition tables

Regarding vitamins, all the small millets like other cereals, are good sources of B vitamins including thiamine, riboflavin, niacin and folic acid (Table 7.4). The B vitamins are concentrated mainly on the bran layer of the grains (Taylor 2017). The small millets contain vitamin E (tocopherols) with contents like most other cereals with contents in finger, little and kodo millets 0.16, 0.55 and 0.07 mg α-tocopherol equivalents/100 g, respectively (Longvah et al. 2017). Carotenoids, the terpenoid pigments with various physiological roles, have been reported in small millets. In addition to their role in phytohormone synthesis, protection from UV radiation and antioxidant activity, certain carotenoids, viz. α- and β-carotene, exhibit pro-vitamin A activity. The total carotenoid content of small millets including finger, little, foxtail and proso millets varied from 199, 78, 173 and 366 μg/100 g, respectively (Asharani et al. 2010). The major carotenoids identified in proso millet includes lutein and zeaxanthin which exhibits very little pro-vitamin activity (Zhang et al. 2014). Similarly, all-*trans* lutein and all-*trans* zeaxanthin are among the prominent carotenoids reported in foxtail millet (Shen et al. 2015). β-carotene is reportedly among the minor carotenoids in small millets (Shen et al. 2015; Taylor 2017).

7.2.5 Antinutrients: Tannins and Phytic Acid

Like most other cereal grains, small millets also contain some phytochemicals with anti-nutritional properties. Phytic acid (myo-inositol 1,2,3,4,5,6-hexakis dihydrogen

phosphate) is among the major anti-nutrients identified in small millets. Phytate serves as the major storage form of phosphorous in cereals. It forms complex with mineral ions especially divalent cations, viz. Ca^{2+}, Mg^{2+}, Zn^{2+}, etc., and interferes with their bioavailability. Protease inhibitors including the trypsin and chymotrypsin inhibitors have been reported in small millets, viz. finger and barnyard millets (Pattabiraman 1986; Udupa and Pattabiraman 1985). Also, among the small millets, some cultivars of finger millet reportedly have condensed tannins. Protease inhibitors and condensed tannins are known to interfere with the digestibility of small millet grains.

7.3 Phytochemical Composition of Small Millets

In addition to the balanced macronutrient content, millets also have a rich content of phytochemicals with potential bioactivities. Phenolic compounds are among the dominant phytochemicals in millets as in most cereals. Also, millets, both major and minor millets, consist of substantially higher amounts of phenolic phytochemicals than in other staple cereals (Taylor and Duodu 2015). The composition of phenolic compounds and their abundance depend on the variety of small millet and also the environmental factors among others.

Phenolic compounds are secondary metabolites that serve several physiological functions in plants including defence against biotic stress, viz. pathogens. Phenolic compounds are distributed along the outer layers of the grain including the bran layer. Small millet phenolics include the soluble and insoluble phenolics: the soluble forms are those found in free forms or those which are esterified or etherified to soluble carbohydrates; insoluble form includes the phenolics that are found esterified to the cell wall polysaccharides. The general structure of phenolic compounds consists of a phenol benzene ring with hydroxyl OH group as the basic components which is further substituted. Like in the major millets, viz. sorghum and pearl millet, phenolic acids and flavonoids are the major phenolic compounds present in small millets also. Further, tannins (discussed along with the antinutrients), the polyphenolic compounds consisting of flavan-3-ol units including catechin and/epicatechin and epigallocatechin, are found in some varieties of finger millet (Dykes and Rooney 2006).

The total phenolic content (TPC) of finger millet genotypes has been reported in several studies (Viswanath et al. 2009; Chandrasekara and Shahidi 2010; Kumari et al. 2016) with each of these studies quantifying the phenolic content in a different fraction, viz. soluble, bound, hull, whole grain and or dehulled finger millet grain. The TPC of soluble phenolic fraction as reported by Chandrasekara and Shahidi (2010) in finger millet were up to 31.39 μmol ferulic acid equivalents/g (FAE/g) of defatted meal and for the bound fraction; it was up to 3.83 μmol FAE/g of defatted meal. Thus, in comparison with the bound fraction, soluble fraction had been found to have higher phenolic contents (Chandrasekara and Shahidi 2010). Regarding the total flavonoid content (TFC), the soluble fraction of finger millet exhibited about 7 μmol catechin equivalents/g (CE/g) of defatted meal, while in the bound fraction, it

was 1.05 μmol CE/g of defatted meal (Chandrasekara and Shahidi 2010). Also, the condensed tannin content (CTC) was reported to be 17.65 ± 3.95 mg CE/100 g dry weight (DW) (Ofosu et al. 2020).

For foxtail millet, earlier studies revealed a similar TPC in the soluble and bound fractions with a content of 10.79 ± 0.82 and 11.59 ± 0.23 μmol FAE/g of defatted meal, respectively (Chandrasekara and Shahidi 2010). A study on the TPC of whole, dehulled, hull, pearled and bran fractions showed that the hull and bran fractions possessed higher content of phenolics (Pradeep and Sreerama 2018). The TFC content in foxtail millet was reported as 1.26 ± 0.03 CE/g of defatted meal in the soluble fractions which is higher as compared to the bound fractions (0.47 ± 0.09 μmol CE/g of defatted meal).

A high phenolic content of up to 368 mg catechol equivalents/100 g was reported for the dry flour of kodo millet (Hegde and Chandra 2005). Further, TPC determination by Chandrasekara and Shahidi (2010) revealed that the TPC of the whole kodo millet was 32.39 and 81.64 μmol FAE/g defatted meal, respectively, in the soluble and the bound fractions. Further, the TFC of kodo millet ranged up to 33.71 and 4.53 μmol CE/g defatted meal of the soluble and bound fractions. Also, the study showed that whole grain kodo millet displayed the highest phenolic content among the studied grains, finger, foxtail, little and proso millet among others.

Regarding proso millet, the TPC of bound extracts was lower as compared to the soluble fraction with a content of 7.19 and 2.21 μmol FAE/g defatted meal in the soluble and bound phenolic fractions, respectively (Chandrasekara and Shahidi 2010). More recently, evaluation of TPC in 14 proso millet cultivars from Northern China showed that soluble fraction exhibited TPC of 592–1510 mg FAE/kg DW, while in the insoluble fraction, 1146–2436 mg FAE/kg DW were found in the insoluble phenolic fractions (Yuan et al. 2021). The TFC of 1.18 and 0.44 μmol CE/g of defatted meal in the soluble and bound fractions of whole proso millet has been reported (Chandrasekara and Shahidi 2010). Only few studies are available regarding the phenolic content and composition in barnyard and little millet grains. Ofosu et al. (2020) reported the TPC of 129.5 ± 4.95 mg FAE/100 g DW, TFC of 101.3 ± 10.4 mg CE/100 g DW and CTC of 59.54 ± 4.63 mg CE/100 g DW in barnyard millet. Little millet whole grain was found to exhibit a TPC of 12.67 and 9.64 μmol FAE/g of defatted meal in the soluble and bound extracts, respectively. Further, Pradeep and Sreerama (2018) reported a TPC of 9.6–20.9 and 3.0–4.5 μmol FAE/g in the soluble and bound phenolic fractions, respectively, in little millet cultivars. Both the reports show that the phenolic content is higher in the soluble fractions. The major phenolic acids and flavonoids detected in the small millets (both in bound and soluble fractions) are presented in Tables 7.5 and 7.6, respectively.

Table 7.5 Phenolic acids identified in small millets

Small millets	Phenolic acids		References
	Hydroxy benzoic acid	Hydroxy cinnamic acid	
Finger millet	*p*-Hydroxybenzoic, protocatechuic, syringic, gentisic, gallic and vanillic acid	Sinapic, ferulic, *p*-coumaric, caffeic, chlorogenic acid and *trans*-cinnamic	Viswanath et al. (2009), Chandrasekara and Shahidi (2011), Hithamani and Srinivasan (2014)
Foxtail millet	Gallic, protocatechuic, *p*-hydroxy benzoic, gentisic, vanillic and syringic acid	Caffeic, *trans*-cinnamic, *p*-coumaric, chlorogenic acid, sinapic and *trans*-ferulic acid	Chandrasekara and Shahidi (2011), Zhang and Liu (2015), Kumari et al. (2016), Pradeep and Sreerama (2017)
Little millet	Gallic acid, dihydroxy benzoic acid and vanillic acid	Caffeic acid, chlorogenic acid, ferulic acid, sinapic acid and *p*-coumaric acid	Pradeep and Sreerama (2018)
Proso millet	Vanillic acid, *p*-hydroxy benzoic acid, syringic acid	Caffeic acid, ferulic acid, *p*-coumaric acid, chlorogenic acid; Chlorogenic acid and ferulic acid dehydrodimers	Zhang et al. (2014), Mattila et al. (2005), Chandrasekara and Shahidi (2010, 2011)
Kodo millet	Protocatechuic, *p*-hydroxy benzoic, gallic, syringic, vanillic	Caffeic, *p*-coumaric acid, chlorogenic, sinapic, *trans*-ferulic, cinnamic acid	Chandrasekara and Shahidi (2011)
Barnyard millet	–	–	–

Table 7.6 Flavonoids identified in small millets

Small millet	Flavonoids	References
Finger millet	Quercetin, epicatechin and apigenin, catechin derivatives, daidzein, gallocatechin and epigallocatechin	Xiang et al. (2019a), Chandrasekara and Shahidi (2011)
Foxtail millet	Catechin, myricetin, luteolin, daidzein, quercetin, apigenin, naringenin and kaempherol	Chandrasekara and Shahidi (2011), Pradeep and Sreerama (2018)
Little millet	Apigenin, kaempferol and luteolin	Chandrasekara and Shahidi (2011), Pradeep and Sreerama (2018)
Proso millet	Rutin and kaempherol	Seo et al. (2011)
Kodo millet	Vitexin, isovitexin, apigenin, luteolin and quercetin	Chandrasekara and Shahidi (2011)
Barnyard millet	Luteolin, tricin, catechin, kaempferol, apigenin, isorhamnetin and 3,7-dimethylquercetin	Watanabe et al. (1999), Ofosu et al. (2020)

7.4 Potential Human Health Benefits of Small Millets

Small millets, owing to their balanced macronutrient content and abundant micronutrients and phytochemicals, exhibit potential human health beneficial effects. Recent research has demonstrated the potential of small millets and/or their bioactive components in imparting beneficial effects in ameliorating chronic diseases through in vivo and in vitro studies.

The potential health benefits of small millets and/or their bioactive principles are described here under:

7.4.1 Mitigation of Micronutrient Deficiencies

The rich content of micronutrients present in small millets helps to tackle micronutrient deficiencies that are prevalent across all age groups. Regular consumption of small millets could supplement the micronutrients including Ca, Mg, K, Fe, Zn, etc. which are vital for human health and wellbeing. Finger millet, which is an abundant source of calcium, could alleviate the complications associated with calcium deficiency. Studies on calcium retention in children in the age group of 9–12 suggested that there was significant calcium retention which could in turn help in bone accretion during growth upon consumption of finger millet-based diets (Joseph et al. 1958; Kurien and Doraiswamy 1967). Further, a recent study by Sahaya et al. (2021) reported that finger millet supplement along with physical activity could improve the calcium levels and bone mineral density in premenopausal women.

The role of millets in improving haemoglobin levels and reducing iron deficiency anaemia have been reported (Anitha et al. 2021). Supplementation of health mix based on small millets, viz. kodo, little, foxtail and finger millet and wheat as well as pulses among other ingredients, could improve haemoglobin level in primary school children. Also, Moharana et al. (2020) reported the significant increase in haemoglobin levels upon consumption of finger millet-based meal, ladoo, in a study conducted in adolescent female subjects.

7.4.2 Antioxidant Activity

Oxidative stress and the concomitant generation of free radicals are among the factors implicated in the development and progression of several lifestyle disorders, viz. cardiovascular diseases, diabetes mellitus, etc. Dietary antioxidants play an important role in improving the redox status and thus preventing these ailments. Hegde and Chandra (2005) evaluated the DPPH radical scavenging activity of methanolic extracts of millets including kodo, finger, little, foxtail and barnyard among others by ESR spectroscopy and showed 28–70% radical scavenging activity. Among the studied small millets, kodo millet was found to show the maximum activity. Furthermore, a lower radical scavenging activity in the white varieties of

small millets as compared to their coloured counter parts was demonstrated in the study, implicating the role of seed coat phenolics in imparting antioxidant activity. High-antioxidant activity in terms of reducing power, trolox equivalent antioxidant capacity, β-carotene linoleate system and ferrous ion chelation have been reported in the soluble and bound phenolic extracts of kodo, finger, foxtail, proso and little millet whole grains (Chandrasekara and Shahidi 2010). Also, the ferrous ion chelating activity and singlet oxygen quenching ability of the phenolic extracts of the small millet phenolic extracts have been previously demonstrated (Chandrasekara and Shahidi 2011). The effect of variety and cultivation location on antioxidant activity of Sri Lankan proso, finger and foxtail millet grains as studied in vitro through DPPH radical scavenging, ferrous ion chelating ability, reducing power and trolox equivalent antioxidant capacity and β-carotene linoleate system have been reported (Kumari et al. 2016). The study showed that finger millet exhibited higher phenolic content and antioxidant activity as compared to the proso and foxtail millet grains. Recent in vitro studies reporting the antioxidant activities in small millet-bioactive components have been presented in Table 7.7.

7.4.3 Antihypertensive Activity

Recent studies report the antihypertensive role of small millets. One such study evaluated the ability to reduce blood pressure in spontaneously hypertensive rats by feeding 200 mg/kg o foxtail millet (raw, extruded and fermented) protein hydrolysates for 4 weeks. As compared to control, the rats fed with the hydrolysate exhibited lower blood pressure with the raw and extruded hydrolysates being more effective. Also, the serum angiotensin converting enzyme and angiotensin II levels were low in the treated rats, demonstrating the potential role of the hydrolysates in ameliorating hypertension (Chen et al. 2017).

7.4.4 Hypolipidemic Activity

Small millets including foxtail, barnyard, etc. exhibit lipid-lowering effect. Nishizawa et al. (2009) reported decrease in serum triglycerides and increase in HDL cholesterol upon feeding Japanese barnyard millet protein to diabetic mice. Lowering of serum triglycerides upon feeding foxtail and proso millet on hyperlipidaemic rats was reported (Lee et al. 2010). In another study, aqueous extract of foxtail millet grains was found to show beneficial effects in terms of lowering triglycerides, total cholesterol and LDL cholesterol in diabetic, treated rats as compared to the control (diabetic, untreated) (Sireesha et al. 2011).

Table 7.7 Studies establishing antioxidant activity of small millet grains and/or their bioactives

Small millet	Source	Antioxidant property	Reference
Finger millet	Methanolic extract	Quenching of DPPH and hydroxyl radicals	Sripriya et al. (1996)
	Crude phenolic extract	DPPH radical scavenging	Chethan et al. (2008)
	Seed coat phenolic extract	Inhibition of free radicals generated through β-carotene-linoleate system	Viswanath et al. (2009)
	Phenolic extract	DPPH, ABTS scavenging and ORAC	Xiang et al. (2019a)
	Peptide from finger millet protein hydrolysate	ABTS, DPPH, hydroxyl radical scavenging and metal chelating	Agrawal et al. (2019)
	Ethanolic extract	DPPH and ABTS radical scavenging	Ofosu et al. (2020)
	Methanolic and ethanolic extract	ABTS, DPPH scavenging	Jayawardana et al. (2022)
Foxtail millet	Whole flour and bran rich methanolic and ethanolic fraction	DPPH radical scavenging and reducing power	Suma and Urooj (2012)
	Insoluble fibre from white and yellow foxtail millet	DPPH and ABTS radical scavenging activity	Bangoura et al. (2013)
	Alkaline extracted polysaccharide	DPPH and hydroxyl radical scavenging activity	Zhu et al. (2015)
	Phenolic extract	DPPH scavenging, reducing power, hydrogen peroxide scavenging, ferrous ion chelating	Pradeep and Sreerama (2018)
	Phenolic extract	DPPH, $ABTS^{+}$ scavenging and ORAC assays	Xiang et al. (2019b)
	Ethanolic extract	DPPH and ABTS radical scavenging	Ofosu et al. (2020)
	Ethanol extract and alkali extract	DPPH, FRAP scavenging	Kuruburu et al. (2022)
Little millet	Phenolic extract	DPPH scavenging, reducing power, hydrogen peroxide scavenging, ferrous ion chelating ability	Pradeep and Sreerama (2018)
Proso millet	Phenolic extract	Peroxy radical scavenging effect	Zhang et al. (2014)
	Methanolic extract	DPPH, ABTS and FRAP assay	Shen et al. (2018)
	Phenolic extract	DPPH, ABTS and FRAP assay	Yuan et al. (2021)
Kodo	Phenolic and γ-amino butyric acid extracts of kodo millet flour	Total antioxidant capacity, DPPH, FRAP, metal chelating ability and hydrogen peroxide scavenging activity	Sharma et al. (2021)
		Peroxy radical scavenging activity	

(continued)

Table 7.7 (continued)

Small millet	Source	Antioxidant property	Reference
Barnyard millet	Ethanol extract of Japanese barnyard millet		Watanabe (1999)
	Ethanolic extract	DPPH and ABTS radical scavenging	Ofosu et al. (2020)

DPPH 2,2′-Diphenyl-1-picrylhydrazyl radical, *ORAC* oxygen radical absorbance capacity, *ABTS* 2,2′-Azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, *FRAP* ferric-reducing antioxidant power/ferric-reducing ability of plasma assay

7.4.5 Anti-inflammatory Activity

Small millets exhibit an anti-inflammatory effect, in turn reducing the risk of acute and chronic diseases associated with inflammation. Lee et al. (2010) reported significantly low levels of C-reactive protein in hyperlipidaemic rats fed with foxtail millet, thus demonstrating the beneficial effect of foxtail millet on the inflammation. The anti-inflammatory effect of peptide fraction derived by Alcalase treatment from foxtail millet prolamin has been studied using LPS-stimulated RAW264.7 murine macrophages. The results demonstrated that the peptide fraction, MPP (MW $<$ 1 kDa), could significantly reduce inflammation by inhibiting nitric oxide and pro-inflammatory cytokines, viz. TNF-α, interleukin-6 and interleukins, IL-1β and IL-6 (Ji et al. 2020). More recently, the in vitro anti-inflammatory effect of methanolic and ethanolic extract of finger millet in terms of arachidonate 5-lipoxygenase, xanthine oxidase, hyaluronidase and oxidative burst inhibitory activities have been evaluated. The study reported potential anti-inflammatory role of finger millet with the methanolic extracts showing higher arachidonate 5-lipoxygenase, xanthine oxidase inhibitory activity (Jayawardana et al. 2022).

7.4.6 Anticancer Activity

Most of the in vitro studies involving anticancer activity of small millets are based on the inhibition of cancer cell proliferation by small millet bioactive components including the phenolics and peptides. Phenolic extracts from whole millet grains including those of foxtail, finger, proso, kodo and little among others were evaluated for its antiproliferative activity in adenocarcinoma cell lines showed that all the millet extracts could inhibit cell proliferation, with kodo and proso millet extracts showing 100% inhibition. Furthermore, the extracts could also demonstrate in vitro inhibition of DNA scission induced by hydroxyl and peroxyl radicals and lipid peroxidation in liposomes showing their potential in preventing the initiation and progression of cancer (Chandrasekara and Shahidi 2011). Zhang et al. (2014) evaluated the in vitro antiproliferative activity of free and bound phenolic extracts of proso millet in MDA human breast cancer as well as HepG2 human liver cancer cells. A dose-dependent antiproliferative activity on both the cell lines with free

extracts showing higher activity has been reported. The study showed that the antiproliferative activity observed was not owing to cytotoxicity. In another study, Shen et al. (2018) also demonstrated the antiproliferative activity of proso millet on MDA-MB-231-human breast cell have been reported. Also, the anti-colon cancer effect and the mode of action of a 35 kDa peroxidase protein extracted from foxtail millet bran have been demonstrated in nude mice (Shan et al. 2014, 2015, 2020). More recently, Kuruburu et al. (2022) analysed the antiproliferative potential of ethanol and alkali extracts of foxtail millet seeds against breast cancer cells through sulforhodamine-B assay and reported that both extracts could arrest cancer cell proliferation.

7.4.7 Antidiabetic Activity

Perhaps the most researched health beneficial effect of millets including the small millets is their role in the management of diabetes and its associated complications. Both in vivo and in vitro studies demonstrate the antidiabetic activities of small millets which are reportedly contributed by its high dietary fibre, resistant starch, phenolic compounds and bio-active peptides.

Choi et al. (2005) demonstrated the beneficial effect of proso millet protein on diabetes through improved insulin sensitivity in type 2 diabetic mice. Improved glycaemic response upon feeding proso millet protein in obese type 2 diabetic mice has been demonstrated (Park et al. 2008). Beneficial effect on plasma glucose level and improved insulin sensitivity upon feeding Japanese barnyard millet protein to diabetic mice has been reported (Nishizawa et al. 2009). Sireesha et al. (2011) demonstrated significant decrease in fasting blood glucose with a significant improvement in glycaemic control as evidenced by lower levels of HbA1c by feeding foxtail millet seed aqueous extract in diabetic treated rats. Bangoura et al. (2013) reported the in vitro hypoglycaemic effect of insoluble fibres from white and yellow foxtail millet grains and found that the hypoglycaemic effect comparable to commercial soy fibre.

More recently, Ren et al. (2021) investigated the mechanism underlying hypoglycaemic effect of foxtail millet and identified the role of gut microbiota. Supplementation of foxtail millet in diabetic rats (high-fat diet as well as streptozotin induced) exhibited hypoglycaemic effect in terms of reduced fasting glucose, glycated serum protein, etc. Further, through 16S rRNA and liver RNA sequencing, it was demonstrated that the improved glucose metabolism was at least in part through the increased abundance of lactobacillus, activation of the PI3K/AKT signalling pathway and inhibition of the NF-κB signalling pathway. The effect of Japanese barnyard millet bran on diabetes has been recently investigated (Ito et al. 2022). They demonstrated that rats fed with bran diet showed reduced increase in blood glucose post feeding as compared to control rats. Bran diet could also lower polyuria associated with diabetes as well as the HbA1C in diabetic rats. Furthermore, diabetic rats fed with bran diet had a higher expression of the antioxidant enzyme, haeme oxidase 1 in the liver.

The inhibition of carbohydrate-digesting enzymes including α-amylase and α-glucosidase is considered as a key antidiabetic property as it could help in regulating the post prandial blood glucose levels. There are several studies demonstrating the in vitro α-amylase and α-glucosidase inhibitory effect of small millets especially the phenolic extract. Shobana et al. (2009) demonstrated the α-amylase and α-glucosidase inhibitory activity of phenolic extracts of finger millet seed coat. Also, α-amylase and α-glucosidase inhibitory activity ethanolic extracts of foxtail and proso have been reported (Ju-Sung et al. 2011). More recently the phenolic extracts of finger, foxtail and barnyard millet (Ofosu et al. 2020) and Sri Lankan finger millet varieties (Jayawardana et al. 2022) were reported to exhibit α-amylase and α-glucosidase inhibitory activity.

The role of small millets in reducing the risk of associated complications of diabetes mellitus has been studied. Hegde et al. (2002) demonstrated the beneficial effect of methanolic extract of finger and kodo millet by imparting protection against glycation as well as collagen crosslinking in vitro. Furthermore, the ability of small millet phenolics in inhibiting nonenzymatic glycation has been reported (Ofosu et al. 2020). Also, Chethan et al. (2008) demonstrated the effect of aldose-reductase inhibitory activity of finger millet phenolic extracts, thus helps in the management of diabetes-induced cataract.

7.4.8 Antimicrobial Activity

Several studies reported the antimicrobial activity of small millet phenolics as well as peptides. Viswanath et al. (2009) found that the seed coat phenolic extracts from finger millet could inhibit the microorganisms, viz. *Bacillus cereus* and *Aspergillus niger*. Further, foxtail millet peptide with antifungal activity has been reported by Xu et al. (2011). In another study, Banerjee et al. (2012) demonstrated the antibacterial activity of finger millet crude phenolic extracts through agar diffusion assay. Recently, Sharma et al. (2016) showed that kodo millet polyphenols could inhibit bacterial test indicators, viz. *Staphylococcus aureus*, *Leuconostoc mesenteroides*, *Bacillus cereus* and *Enterococcus faecalis*. Bisht et al. (2016) reported antibacterial activity of peptide from finger millet against *Pseudomonas aeruginosa* and *Salmonella enterica*.

7.4.9 Prebiotic Effect

Prebiotics include non-digestible food components that enhance the growth and activity of the gut associated beneficial bacteria, thereby imparting health benefits to the host (reviewed in Abdi and Joye 2021). The potential prebiotic role of dietary fibre from millets including foxtail millet have been found in a study by Farooq et al. (2013). The study demonstrated that millet dietary fibre upon fermentation by probiotic bacteria, viz. *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Bifidobacterium longum* and *Bifidobacterium bifidum*, produced short-chain fatty

acids including acetate, propionate and butyrate which are known to promote gut health.

7.5 Effect of Grain Processing on Nutritional Characteristics of Small Millets

Millets including small millets are subjected to different types of processing, viz. decortication, malting and fermentation, and different types of thermal treatments that convert them in to edible form. These treatments induce changes in nutritional, phytochemical and organoleptic characteristics of the grains among others. Dehulling or decortication is a primary processing employed in small millets to remove the coarse pericarp. As this process involves the removal of outer layers, the nutritional components distributed on this part of the grain may get affected. As the phenolic compounds are mainly concentrated in the bran layer of the grain, decortication is known to reduce the phenolic content substantially; Shobana and Malleshi (2007) reported the reduction of polyphenolic compounds including tannins in finger millet upon decortication. Dehulling of foxtail millet reduced the protein and ash content by 4.14% and 37%, respectively. Also, dehulling reduced phytic acid content of the grains by 5.20% (Pawar and Machewad 2006). While studying the effect of polishing on milling characteristics as well as proximate principles in barnyard millet, Lohani et al. (2012) observed that the contents of protein, fat, ash and the fibre contents were reduced with increased moisture, milling time and degree of polishing. The study observed that 3-min polishing of barnyard millet in rice polisher could help in minimum nutritional losses.

Malting in foxtail millet significantly enhanced carbohydrates and reduced the crude fat and crude fibre content as compared to the raw grains (Choudhury 2011). The enhanced total carbohydrates may be attributed to the partial degradation of amylopectin and increased amylose content during germination (Gokavi and Malleshi 2000; Choudhury 2011). Enhanced carbohydrates and energy values were observed in popped foxtail millet sample with significant reductions in the crude fat and crude fibre contents (Choudhury 2011). Pradeep and Sreerama (2015) observed an improved phenolic content and thus enhanced antioxidant activity in small millet grains including barnyard, foxtail and proso by germination. Improved protein quality upon lactic acid fermentation and malting has been reported in finger and foxtail millets among other millets (Taylor et al. 2017). Further, malting for 4 days enhanced riboflavin content by 2.5-fold in finger millet (Malleshi and Klopfenstein 1998).

Antony et al. (1996) determined the effect of fermentation on foxtail millet nutrients and observed the improved protein extractability and retained beneficial fatty acid profile as in raw flour. Also, they observed reduced total starch and corresponding increase in total and reduced sugar. Also, fermentation in finger millet could enhance the mineral extractability by 20–30% which could be attributed to the reduction in antinutrients including phytate and tannin upon fermentation (Antony and Chandra 1999). Recently Sharma et al. (2016) studied the effect of germination

on phenolics, dietary fibre and mineral content in barnyard millet grain. They found that germination could increase TPC, TFC, dietary fibre and minerals, while phytic acid content was reduced. Pradeep and Guha (2011) reported that germination, steaming and roasting of little millet could improve TPC, TFC and tannin content. Dehulling, soaking and cooking of foxtail millet resulted in 6.41% and 50.60% reduction in protein and ash content, respectively. The phytic acid content was also reduced by 49.89% in the dehulled, soaked and cooked grains (Pawar and Machewad 2006).

The processing methods have a substantial impact on the in vitro starch and protein digestibility (IVPD) of millets (reviewed in Annor et al. 2017). Dehulling as well as a combination of treatments including dehulling, soaking and cooking resulted in enhanced IVPD in foxtail millet (Pawar and Machewad 2006). In finger millet, a combination of enzymatic treatment with cellulase and hemicellulase and directed fermentation has enhanced IVPD along with acceleration of fermentation process (Antony and Chandra 1999). Further, Choudhury et al. (2011) studied the starch digestibility and IVPD in two varieties of popped foxtail millet grains (yellow and purple). The results showed that popping significantly enhanced starch digestibility and IVPD of both varieties of foxtail millet. The improved susceptibility of starch to enzymatic digestion, owing to its release from the protein matrix during popping, might have enhanced the starch digestibility, and the expanded endosperm due to the localized rupture of cell wall during popping might have contributed to enhanced protein digestibility. Also, the same study observed that malting significantly improved the starch digestibility of both varieties. Various changes during the germination and/malting process, viz. rupturing of starch granules, activation of amylases, decrease in antinutrients including the amylase inhibitors and the partial digestion of the starch, might have contributed to the improved starch digestibility. The protein digestibility was also improved during malting with the purple variety, showing significant enhancement, while only slight improvement was observed in the yellow variety. In finger millet, improvement in RDS was observed upon puffing (Roopa and Premavalli 2008).

7.6 Conclusion and Future Perspectives

Small millets including the finger, foxtail, little, proso, kodo and barnyard are gluten-free grains with excellent nutritional value and hence are regarded as ‘nutri-cereals’. These grains house plenty of phytochemicals with potential bioactivities. Owing to their good micronutrient, dietary fibre and phenolic contents, these grains impart human health benefits including the potential to alleviate micronutrient deficiency and management of chronic lifestyle disorders among others. In addition to its nutritional superiority, these grains are climate resilient, which makes them a promising crop for the future.

With increased consumer preference for foods with therapeutic benefits, small millets are regaining their significance from the coarse cereal status. One of the major hurdles in the widespread adoption of these grains as staple cereals is the difficulty in

their processing, owing its miniscule nature. Developing improved processing technologies, especially for the small millets would help to improve the availability of these cereals and hence its utilization. Further, the nutritional value of small millets is not fully exploited; especially the phytochemical profile of the small millets including those of barnyard, kodo and little millet among others needs wide spread research attention. Also, the available data on health benefits of the small millets are still limited. More studies on several aspects including the mechanism of action of the bioactive components of the grains in imparting the health benefits are needed. Also, studies involving controlled human trials aimed at elucidating the role of small millets in imparting health benefits would enable promoting these grains as sources of functional food ingredients. There is a tremendous opportunity in placing these nutri-cereals as a sustainable source of nutrition thereby ensuring global food and nutritional security.

References

AACC (American Association of Cereal Chemists) (2001) The definition of dietary fiber: report of the dietary fibre definition committee to the board of directors of the American association of cereal Chemists. Cereal Foods World 46:112–129

Abdi R, Joye IJ (2021) Prebiotic potential of cereal components. Foods 10(10):2338. https://doi.org/10.3390/foods10102338

Agrawal H, Joshi R, Gupta M (2019) Purification, identification, and characterization of two novel antioxidant peptides from finger millet (*Eleusine coracana*) protein hydrolysate. Food Res Int 120:697–707. https://doi.org/10.1016/j.foodres.2018.11.028

Anis MA, Sreerama YN (2020) Inhibition of protein glycoxidation and advanced glycation end-product formation by barnyard millet (*Echinochloa frumentacea*) phenolics. Food Chem 315:Article 126265. https://doi.org/10.1016/j.foodchem.2020.126265

Anitha S, Kane-Potaka J, Botha R, Givens DI, Sulaiman NLB, Upadhyay S, Vetriventhan M, Tsusaka TW, Parasannanavar DJ, Longvah T, Rajendran A, Subramaniam K, Bhandari RK (2021) Millets can have a major impact on improving iron status, hemoglobin level, and in reducing iron deficiency anemia—a systematic review and meta-analysis. Front Nutr 8:725529. https://doi.org/10.3389/fnut.2021.725529

Annor GA, Tyl C, Marcone M, Ragaee S (2017) Why do millets have slower starch and protein digestibility than other cereals? Trends Food Sci Technol 66:73. https://doi.org/10.1016/j.tifs.2017.05.012

Antony U, Chandra T (1999) Enzymatic treatment and use of starters for the nutrient enhancement in fermented flour of red and white varieties of finger millet (*Eleusine coracana*). J Agric Food Chem 47(5):2016–2019

Antony U, Sripriya G, Chandra T (1996) The effect of fermentation on the primary nutrients in foxtail millet (*Setaria italica*). Food Chem 56(4):381–384

Asharani VT, Jayadeep A, Malleshi NG (2010) Natural antioxidants in edible flours of selected small millets. Int J Food Prop 13(1):41–50. https://doi.org/10.1080/10942910802163105

Bandyopadhyay T, Muthamilarasan M, Prasad M (2017) Millets for next generation climate-smart agriculture. Front Plant Sci 8:1266

Banerjee S, Sanjay KR, Chethan S, Malleshi NG (2012) Finger millet (*Eleusine coracana*) polyphenols: investigation of their antioxidant capacity and antimicrobial activity. Afr J Food Sci 6:362–374

Bangoura ML, Nsor-Atindana J, Ming ZH (2013) Solvent optimization extraction of antioxidants from foxtail millet species' insoluble fibers and their free radical scavenging properties. Food Chem 141:736–744

Bisht A, Thapliyal M, Singh A (2016) Screening and isolation of antibacterial proteins/peptides from seeds of millets. Int J Curr Pharm Res 8(3):96–99. https://doi.org/10.22159/ijcpr.2016v8i4.15271

Chandrasekara A, Shahidi F (2010) Content of insoluble bound phenolics in millets and their contribution to antioxidant capacity. J Agric Food Chem 58(11):6706–6714. https://doi.org/10.1021/jf100868b

Chandrasekara A, Shahidi F (2011) Determination of antioxidant activity in free and hydrolyzed fractions of millet grains and characterization of their phenolic profiles by HPLC-DAD-ESIMS. J Func Foods 3:144–158

Chaudhary Kanchan YN (2013) Evaluation of hypoglycemic properties of kodo millet based food products in healthy subjects. IOSR J Pharm 3(2):14–20. https://doi.org/10.9790/3013-32201420

Chen J, Duan W, Ren X, Wang C, Pan Z, Diao X, Shen Q (2017) Effect of foxtail millet protein hydrolysates on lowering blood pressure in spontaneously hypertensive rats. Eur J Nutr 56(6):2129–2138. https://doi.org/10.1007/s00394-016-1252-7

Chethan S, Dharmesh SM, Malleshi NG (2008) Inhibition of aldose reductase from cataracted eye lenses by finger millet (Eleusine coracana) polyphenols. Bioorg Med Chem 16:10085–10090

Choi YY, Osada K, Ito Y, Nagasawa T, Choi MR, Nishizawa N (2005) Effects of dietary protein of Korean foxtail millet on plasma adiponectin, HDL-cholesterol, and insulin levels in genetically type 2 diabetic mice. Biosci Biotechnol Biochem 69(1):31–37. https://doi.org/10.1271/bbb.69.31

Choudhury M, Das P, Baroova B (2011) Nutritional evaluation of popped and malted indigenous millet of Assam. J Food Sci Technol 48(6):706–711

Dykes L, Rooney LW (2006) Sorghum and millet phenols and antioxidants. J Cereal Sci 44:236–251

Farooq U, Mohsin M, Liu X, Zhang H (2013) Enhancement of short chain fatty acid production from millet fibres by pure cultures of probiotic fermentation. Trop J Pharm Res 12(2):189

Gokavi SS, Malleshi NG (2000) Malting characteristics of a few Indian wheat and chickpea varieties. J Food Sci Technol 37:586–591

Golda SR, Swaminathan A, Vijayaraghavan R (2021) Effectiveness of physical activity and finger millet-based food supplement on biochemical parameters and bone mineral density among premenopausal women. Evid Based Complement Alternat Med 2021:4757991. https://doi.org/10.1155/2021/4757991

Han F, Han F, Wang Y, Fan L, Song G, Chen X, Jiang P, Miao H, Han Y (2019) Digestible indispensable amino acid scores of nine cooked cereal grains. Br J Nutr 121(1):30–41. https://doi.org/10.1017/S0007114518003033

Hegde PS, Chandra TS (2005) ESR spectroscopic study reveals higher free radical quenching potential in kodo millet (*Paspalum scrobiculatum*) compared to other millets. Food Chem 92:177–182

Hegde P, Chandrakasan G, Chandra T (2002) Inhibition of collagen glycation and crosslinking in vitro by methanolic extracts of finger millet (*Eleusine coracana*) and kodo millet (*Paspalum scrobiculatum*). J Nutr Biochem 13(9):517. https://doi.org/10.1016/s0955-2863(02)00171-7

Hithamani G, Srinivasan K (2014) Effect of domestic processing on the polyphenol content and bioaccessibility in finger millet (*Eleusine coracana*) and pearl millet (*Pennisetum glaucum*). Food Chem 164:55–62. https://doi.org/10.1016/j.fooFzhangdchem.2014.04.107

Ito Y, Suzuki A, Nasukawa H, Miyaki K, Yano A, Nagasawa T (2022) Ameliorative effects of Japanese barnyard millet (*Echinochloa esculenta* H. Scholz) bran supplementation in streptozotocin-induced diabetic rats. Food Sci Technol Res 28(5):431–439. Released on J-STAGE September 20, 2022, Advance online publication June 10, 2022, Online ISSN 1881-3984, Print ISSN 1344-6606. https://doi.org/10.3136/fstr.FSTR-D-22-00079

Jayawardana SAS, Samarasekera JKRR, Hettiarachchi GHCM, Gooneratne MJ (2022) Antidiabetic properties of finger millet (*Eleusine coracana* (L.) Gaertn.) varieties cultivated in Sri Lanka. J Herb Med 32:100534. https://doi.org/10.1016/j.hermed.2022.100534.jcs.2014.10.009

Ji Z, Mao J, Chen S, Mao J (2020) Antioxidant and anti-inflammatory activity of peptides from foxtail millet (*Setaria italica*) prolamins in HaCaT cells and RAW 264.7 murine macrophages. Food Biosci 36:e100636

Joseph K, Kurien PP, Swaminathan M, Subramaniyan V (1958) The effect of partial or complete replacement of rice in poor vegetarian diets by ragi (Eleusine coracana) on the metabolism of nitrogen, calcium and phosphorus. Br J Nutr 12:213–218

Ju-Sung K, Tae Kyung H, Myong-Jo K (2011) The inhibitory effects of ethanol extracts from sorghum, foxtail millet and proso millet on alpha-glucosidase and alpha-amylase activities. Food Chem 124:1647–1651

Kaimal A, Mujumdar A, Thorat B (2021) Resistant starch from millets: recent developments and applications in food industries. Trends Food Sci Technol 111:563. https://doi.org/10.1016/j.tifs.2021.02.074

Kaur KD, Jha A, Sabikhi L, Singh AK (2014) Significance of coarse cereals in health and nutrition: a review. J Food Sci Technol 51:1429–1441

Kumari SK, Thayumanavan B (1998) Characterization of starches of proso, foxtail, barnyard, kodo, and little millets. Plant Food Hum Nutr 53:47–56

Kumari D, Madhujith T, Chandrasekara A (2016) Comparison of phenolic content and antioxidant activities of millet varieties grown in different locations in Sri Lanka. Food Sci Nutr 5(3):474–485. https://doi.org/10.1002/fsn3.415

Kurien PP, Doraiswamy TR (1967) Effect of replacing cereal in a poor diet with whole or refined ragi four on the nutritional status and metabolism of nitrogen, calcium and phosphorus in children (boys). J Nutr Dietet 4:102–109

Kuruburu MG, Bovilla VR, Leihang Z, Madhunapantula SV (2022) Phytochemical-rich fractions from foxtail millet (*Setaria italica* (L.) P. Beauv) seeds exhibited antioxidant activity and reduced the viability of breast cancer cells in vitro by inducing DNA fragmentation and promoting cell cycle arrest. Anti Cancer Agents Med Chem 22(13):2477–2493. https://doi.org/10.2174/1871520622666220215122141

Lee SH, Chung IM, Cha YS, Park Y (2010) Millet consumption decreased serum concentration of triglyceride and C-reactive protein but not oxidative status in hyperlipidemic rats. Nutr Res 30(4):290–296. https://doi.org/10.1016/j.nutres.2010.04.007

Lohani UC, Pandey JP, Shahi NC (2012) Effect of degree of polishing on milling characteristics and proximate compositions of barnyard millet (Echinochloa frumentacea). Food Bioprocess Technol 5:1113–1119

Longvah T, Ananthan R, Bhaskarachary K, Venkaiah K (2017) Indian food composition tables. National Institute of Nutrition, Indian Council of Medical Research, Hyderabad

Majid A, Priyadarshini CGP (2020) Millet derived bioactive peptides: a review on their functional properties and health benefits. Crit Rev Food Sci Nutr 60(19):3342–3351. https://doi.org/10.1080/10408398.2019.1686342

Malleshi NG, Klopfenstein CF (1998) Nutrient composition, amino acid and vitamin contents of malted sorghum, pearl millet, finger millet and their rootlets. Int J Food Sci Nutr 49:415–422

Malleshi NG, Tiwari A, Agarwal A, Sood S (2020) Nutritional quality and health benefits. In: Singh M, Sood S (eds) Millets and pseudo cereals (genetic resources and breeding advancements). Woodhead Publishing (Elsevier), pp 159–168. https://doi.org/10.1016/B978-0-12-820089-6.00009-4

Mattila P, Pihlava J, Hellstrom J (2005) Contents of phenolic acids, alkyl- and alkenylresorcinols, and avenanthramides in commercial grain products. J Agric Food Chem 53:8290–8295

McDonough CM, Rooney LW, Earp CF (1986) Structural characteristics of *Eleusine coracana* (finger millet) using scanning electron and fluorescence microscopy. Food Microstruct 5:247–256

Moharana A, Khosla P, Nayak D, Tripathy P (2020) Effect of finger millet [ragi] ladoo consumption on the level of hemoglobin. Eur J Mol Clin Med 7:1018–1022

Nishizawa N, Togawa T, Park KO, Sato D, Miyakoshi Y, Inagaki K, Ohmori N, Ito Y, Nagasawa T (2009) Dietary Japanese millet protein ameliorates plasma levels of adiponectin, glucose, and lipids in type 2 diabetic mice. Biosci Biotechnol Biochem 73(2):351–360. https://doi.org/10.1271/bbb.80589

Ofosu FK, Elahi F, Mwine Daliri EB, Chelliah R, Ham HJ, Kim JH et al (2020) Phenolic profile, antioxidant, and antidiabetic potential exerted by millet grain varieties. Antioxidants (Basel) 9:254. https://doi.org/10.3390/antiox9030254

Park KO, Ito Y, Nagasawa T, Choi MR, Nishizawa N (2008) Effects of dietary Korean proso-millet protein on plasma adiponectin, HDL cholesterol, insulin levels, and gene expression in obese type 2 diabetic mice. Biosci Biotechnol Biochem 72(11):2918–2925. https://doi.org/10.1271/bbb.80395

Pattabiraman TN (1986) Trypsin/chymotrypsin inhibitors from millets. In: Friedman M (ed) Nutritional and toxicological significance of enzyme inhibitors in foods, Advances in experimental medicine and biology, vol 199. Springer, Boston, MA. https://doi.org/10.1007/978-1-4757-0022-0_25

Pawar VD, Machewad GM (2006) Processing of foxtail millet for improved nutrient availability. J Food Process Preserv 30:269–279

Pradeep SR, Guha M (2011) Effect of processing methods on the nutraceutical and antioxidant properties of little millet (*Panicum sumatrense*) extracts. Food Chem 126(4):1643–1647. https://doi.org/10.1016/j.foodchem.2010.12.04

Pradeep PM, Sreerama YN (2015) Impact of processing on the phenolic profiles of small millets: evaluation of their antioxidant and enzyme inhibitory properties associated with hyperglycemia. Food Chem 169:455–463. https://doi.org/10.1016/j.foodchem.2014.08.010

Pradeep PM, Sreerama YN (2017) Soluble and bound phenolics of two different millet genera and their milled fractions: comparative evaluation of antioxidant properties and inhibitory effects on starch hydrolysing enzyme activities. J Funct Foods 35:682–693

Pradeep PM, Sreerama YN (2018) Phenolic antioxidants of foxtail and little millet cultivars and their inhibitory effects on α-amylase and α-glucosidase activities. Food Chem 247:46–55

Ren X, Chen J, Molla MM, Wang C, Diao X, Shen Q (2016) In vitro starch digestibility and in vivo glycemic response of foxtail millet and its products. Food Funct 7(1):372–379. https://doi.org/10.1039/c5fo01074h

Ren X, Wang L, Chen Z, Hou D, Xue Y, Diao X, Shen Q (2021) Foxtail millet improves blood glucose metabolism in diabetic rats through PI3K/AKT and NF-κB Signaling pathways mediated by gut microbiota. Nutrients 13(6):1837. https://doi.org/10.3390/nu13061837

Roopa S, Premavalli K (2008) Effect of processing on starch fractions in different varieties of finger millet. Food Chem 106(3):875–882

Roopashree U, Chimmad B, Naik R, Bharati P, Itagi S (2014) Glycemic index and significance of barnyard millet (Echinochloa frumentacae) in type II diabetics. J Food Sci Technol 51:392–395

Sachdev N, Goomer S, Singh LR (2020) Foxtail millet: a potential crop to meet future demand scenario for alternative sustainable protein. J Sci Food Agric 101(3):831–842. https://doi.org/10.1002/jsfa.10716

Seo M-C, Ko J, Song S-B, Lee J-S, Kang J-R et al (2011) Antioxidant compounds and activities of foxtail millet, proso millet and sorghum with different pulverizing methods. J Korean Soc Food Sci Nutr 4:790–797

Serna-Saldivar SO, Espinosa-Ramírez J (2019) Grain structure and grain chemical composition. In: Sorghum and millets. AACC International Press, pp 85–129. https://doi.org/10.1016/B978-0-12-811527-5.00005-8

Shan S, Li Z, Newton IP, Zhao C, Li Z, Guo M (2014) A novel protein extracted from foxtail millet bran displays anti-carcinogenic effects in human colon cancer cells. Toxicol Lett 227(2):129–138. https://doi.org/10.1016/j.toxlet.2014.03.008

Shan S, Shi J, Li Z, Gao H, Shi T, Li Z, Li Z (2015) Targeted anti-colon cancer activities of a millet bran-derived peroxidase were mediated by elevated ROS generation. Food Funct 6(7): 2331–2338. https://doi.org/10.1039/c5fo00260e

Shan S, Wu C, Shi J, Zhang X, Niu J, Li H, Li Z (2020) Inhibitory effects of peroxidase from foxtail millet bran on colitis-associated colorectal carcinogenesis by the blockage of glycerophospholipid metabolism. J Agric Food Chem 68(31):8295–8307

Sharma B, Gujral HS (2020) Influence of nutritional and antinutritional components on dough rheology and *in vitro* protein & starch digestibility of minor millets. Food Chem 299:125115. https://doi.org/10.1016/j.foodchem.2019.125115

Sharma S, Sharma N, Handa S, Pathania S (2016) Evaluation of health potential of nutritionally enriched kodo millet (*Eleusine coracana*) grown in Himachal Pradesh, India. Food Chem 214: 162–168. https://doi.org/10.1016/j.foodchem.2016.07.086

Sharma S, Jan R, Riar CS, Bansal V (2021) Analyzing the effect of germination on the pasting, rheological, morphological and in-vitro antioxidant characteristics of kodo millet flour and extracts. Food Chem 361:130073. https://doi.org/10.1016/j.foodchem.2021.130073

Shen R, Yang S, Zhao G, Shen Q, Diao X (2015) Identification of carotenoids in foxtail millet (*Setaria italica*) and the effects of cooking methods on carotenoid content. J Cereal Sci 61:86–93

Shen R, Ma Y, Jiang L, Dong J, Zhu Y, Ren G (2018) Chemical composition, antioxidant, and antiproliferative activities of nine Chinese proso millet varieties. Food Agric Immunol 29(1): 625–637. https://doi.org/10.1080/09540105.2018.1428283

Shobana S, Malleshi N (2007) Preparation and functional properties of decorticated finger millet (Eleusine coracana). J Food Eng 79:529–538. https://doi.org/10.1016/j.jfoodeng.2006.01.076

Shobana S, Sreerama YN, Malleshi N (2009) Composition and enzyme inhibitory properties of finger millet (Eleusine coracana L.) seed coat phenolics: mode of inhibition of α-glucosidase and pancreatic amylase. Food Chem 115:1268–1273. https://doi.org/10.1016/j.foodchem.2009.01.042

Sireesha Y, Kasetti RB, Nabi SA, Swapna S, Apparao C (2011) Antihyperglycemic and hypolipidemic activities of *Setaria italica* seeds in STZ diabetic rats. Pathophysiology 18(2): 159–164. https://doi.org/10.1016/j.pathophys.2010.08.003

Sripriya G, Chandrasekharan K, Murty VS, Chandra TS (1996) ESR spectroscopic studies on free radical quenching action of finger millet (*Eleusine coracana*). Food Chem 57(4):537–540

Suma PF, Urooj A (2012) Antioxidant activity of extracts from foxtail millet (*Setaria italica*). J Food Sci Technol 49(4):500–504. https://doi.org/10.1007/s13197-011-0300-9

Taylor J (2017) Millets: their unique nutritional and health-promoting attributes. In: Gluten-free ancient grains, Woodhead Publishing Series in food science, technology and nutrition. Woodhead Publishing, pp 55–103. https://doi.org/10.1016/B978-0-08-100866-9.00004-2

Taylor JRN, Duodu KG (2015) Effects of processing sorghum and millets on their phenolic phytochemicals and the implications of this to the health-enhancing properties of sorghum and millet food and beverage products. J Sci Food Agric 95:225–237

Udupa SL, Pattabiraman TN (1985) Isolation and characterization of a trypsin/chymotrypsin inhibitor from the millet *Echinochloa frumentacea*. J Agric Food Chem 33:642–646

Vetriventhan M, Azevedo VCR, Upadhyaya HD et al (2020) Genetic and genomic resources, and breeding for accelerating improvement of small millets: current status and future interventions. Nucleus 63:217–239. https://doi.org/10.1007/s13237-020-00322-3

Viswanath V, Urooj A, Malleshi NG (2009) Evaluation of antioxidant and antimicrobial properties of finger millet polyphenols (*Eleusine coracana*). Food Chem 114:340–346

Watanabe M (1999) Antioxidative phenolic compounds from Japanese barnyard millet (*Echinochloa utilis*) grains. J Agric Food Chem 47:4500–4505. https://doi.org/10.1021/jf990498s

Xiang J, Apea-Bah FB, Ndolo VU, Katundu MC, Beta T (2019a) Profile of phenolic compounds and antioxidant activity of finger millet varieties. Food Chem 275:361–368

Xiang J, Zhang M, Apea Bah FB, Beta T (2019b) Hydroxycinnamic acid amide (HCAA) derivatives, flavonoid C-glycosides, phenolic acids and antioxidant properties of foxtail millet. Food Chem 295:214–223. https://doi.org/10.1016/j.foodchem.2019.05.058

Xu W, Wei L, Qu W, Liang Z, Wang J, Peng X, Zhang Y, Huang K (2011) A novel antifungal peptide from foxtail millet seeds. J Sci Food Agric 91(9):1630–1637. https://doi.org/10.1002/jsfa.4359

Yin SY, Kuo SM, Chen YR, Tsai YC, Wu YP, Lin YR (2019) Genetic variation of physicochemical properties and digestibility of foxtail millet (*Setaria italica*) landraces of Taiwan. Molecules 24(23):4323. https://doi.org/10.3390/molecules24234323

Yuan Y, Xiang J, Zheng B, Sun J, Luo D, Li P, Fan J (2021) Diversity of phenolics including hydroxycinnamic acid amide derivatives, phenolic acids contribute to antioxidant properties of proso millet. LWT 18:112611. https://doi.org/10.1016/j.lwt.2021.112611

Zhang LZ, Liu RH (2015) Phenolic and carotenoid profiles and antiproliferative activity of foxtail millet. Food Chem 174:495–501. https://doi.org/10.1016/j.foodchem.2014.09.089

Zhang L, Liu R, Niu W (2014) Phytochemical and antiproliferative activity of proso millet. PLoS One 9(8):e104058

Zhu A, Tang L, Fu Q, Xu M, Chen J (2015) Optimization of alkali extraction of polysaccharides from foxtail millet and its antioxidant activities *in vitro*. J Food Biochem 39:708–717

Physiological Traits Associated with Genetic Improvement of Small Millets

8

Shailesh Kumar, Trisha Sinha, and Sweta Mishra

Abstract

Global warming has already destroyed the natural harmony in soil and water ecosystems in many regions all over the globe. Extremities in weather events such as temperature, moisture, and others have produced harmful effects for agriculture. Increasing rate in production of major crops has reduced a lot from the previous era. Conventional practices like intensive agriculture and shifting cultivation have resulted in soil erosion and degradation. Alternative ways such as introducing less popular crops for cultivation like small millets could bring a revolution in the world of agriculture again. Small millets have a great future in them as they are blessings for the humanity. They are rich source of many important nutrients and proteins, which are a good option for malnourished population. Other beneficial roles of these crops include lowering blood pressure, reducing diabetes, cure to heart diseases, prevention from celiac diseases, helping in reducing obesity, and improving skin elasticity. The lesser intake of resources like water and nutrients makes them easier in the competition. Another crucial role played by small millets is to provide antioxidants to the consumers to combat various stress factors. People are still ignorant to exhaust the maximum possible potential of small millets resulting in their lesser yield. The path in between

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optimum yield and maximum yield in small millets is left uncrossed due to some complexities owing to their smaller size and crop physiology for breeding program. As per some previous studies, multiple numbers of physiological traits such as plant height, leaf area, growth parameters, photosynthesis, soluble protein content, grain nutrients, yield parameters, resistance to abiotic and biotic stresses, etc. can be used as improved traits for genetic improvement of several small millets. Thus, improved yield in small millets will be obtained by utilizing the full potential relying on their breeding through improved physiological traits.

Keywords

Small millets · Global warming · Food security · Nutrients · Physiological approaches

8.1 Introduction

As per the reports, the global mean temperature is predicted to rise by 0.5–4 °C in the twenty-first century (Zandalinas et al. 2021). To save the environmental harmony from outer interference is literally not possible; thus, alternative ways of tolerance have to be implemented in order to save the environment from current climatic conditions under change. Yielding more from unit area using same inputs is the new target that the agricultural scientists have set to reach the food demand of the globe. This big target in front of us seems more challenging as sustainability comes to cross the road. The revolution in the last 1960s has served as both a boon and curse. Agriculture has grown doubly or triply since then as the scientists dared introducing high-yielding varieties of crops and chemical fertilizers that collectively resulted for the higher crop production. No one could visualize the upcoming threats in the world of Indian agriculture post the revolutionary phase. Soils have degraded a lot due to repeated and inefficient use of chemical fertilizers. Near future generation is going to witness another revolution in agriculture again with all its changed patterns, production techniques, and cultivable crops in the wish list. The dominating crops in India such as rice, wheat, sugarcane, and other important crops have lost the glory due to their connection with soil quality degradation. The urge for changes in overall agriculture has also replaced some dominant crops with less popular crops in the bucket list (Goron and Raizada 2015). Small millets are some of them in the new wish list. Small millets, as we know, are a group of crops with small seeds consumable for both humans and animals. Examples include finger millet, proso millet, barnyard millet, fonio, little millet, foxtail millet, kodo millet, job's tears, browntop millet, guinea millet, etc. (Gupta et al. 2010; Divya et al. 2018; Das et al. 2019; Vetriventhan et al. 2020). The world of agriculture has now accepted small millets as the most promising crops of future sustenance. The reasons could be their lesser dependence on inputs (Goron and Raizada 2015; Das et al. 2019), lesser requirement of water (Khound and Santra 2020), and a good number of potential traits such as stress tolerance (Dube et al. 2018), production of various antioxidants (Chandrasekara and Shahidi 2011b), and containing higher amounts of nutrients

(Hegde et al. 2005; Lata et al. 2013). These all, in together, made people think to go for small millets under the availability of limited resources in hand. The future of small millets will also depend on how efficiently they have been cultivated obtaining all possible outcomes from each potential plant parts of the small millets. Reports have confirmed of their ample contents of nutrients in leaves and seeds such as zinc, iron, phosphorus, magnesium, calcium, manganese, vitamins, and others (Leder 2004). Future will tell us whether these emerging crops could take a permanent place in Indian agriculture or will be fed away under the dominance of rice-wheat-sugarcane cropping system. A lot of its fate is in our hand as we could ensure best cultivation managements to protect their potential traits toward obtaining a bright and hunger-free India, moreover globe. Science with its blessing hands has to offer a lot. Genetic improvement relying on development of improved physiological traits could be one of the best solutions to overcome the shortcomings of small millets.

8.2 Prospective of Small Millets in the Globe and in India

Millets are small-seeded grasses belonging to the family Poaceae (Macron 1994; Ceasar and Ignacimuthu 2009; Vetriventhan et al. 2020). Like other group members of this family, millets are also cultivated as cereal crops for their grains as consumable part for humans and feed for livestock (Lata 2015). Asia and Africa dominate the world in terms of producing millets of various kinds (Vetriventhan et al. 2020; Meena et al. 2021). They are excellent fodder as they contain most of the necessary ingredients for livestock. Their easy availability due to short duration nature (Senthil et al. 2018) also provides farmers an excellent opportunity for serving cattle and humans. Bekkering and Tian (2019) reported that about two billion people are under the direct effects of hidden hunger due to lack of micronutrients in the regular diet. Hyponutrition or undernourishment in most of the cases is due to lack of micronutrients such as iron, zinc, and selenium (Combs 2001; Kennedy et al. 2003). Small millets suitably fit as the perfect diet because they provide high energy, high dietary fiber, proteins, many essential minerals, vitamins, antioxidants, and have a low glycemic index (Kam et al. 2016; Singh and Chauhan 2019). Not only that, small millets are also consumed as fermented beverages (Vetriventhan et al. 2020), making increased availability of nutrients, proteins and minerals, and easier digestibility and reduce antinutritional factors (Nkhata et al. 2018). Another best thing of small millets includes their wide range of adaptability in the environment due to their diverse properties (Ceasar and Ignacimuthu 2009; Stanly and Shanmugam 2013; Anbukkani et al. 2017). According to Reddy et al. (2011), small millets are even potent enough for pest-resistance, making them empowered with the longer storability. Small and marginal farmers of India cannot always generate income security by failing to run with the ever-increasing costs of cultivation due to high-input costs (Lokesh et al. 2022). Economically also, small millets are complete blessings for the farmers. According to Adekunle et al. (2018), the average price of small millets in India was 70 Rs/kg compared to that of rice 40 Rs/kg, whereas in West Africa the price of a kilo of fonio was twice that of rice (Vodouhe et al. 2007).

8.2.1 Small Millets: Classification, Origin, and Distribution

India practices cultivation of only six crops as small millets, i.e., finger millet, foxtail millet, proso millet, little millet, Indian barnyard millet, and kodo millet (Divya et al. 2018). The details of their origin, botanical classification, and distribution have been described in Table 8.1.

8.2.2 Current Status

Borlaug (2002) predicted that the global food demand would be 2.5–4.5 t/ha by the year 2025. Simultaneously, the global population is projected to reach 9 billion by 2050 (Beddington 2010); feeding which an increase of 60–70% in food production from the current level will be required (Vetriventhan et al. 2020). But achieving this huge target could not be possible relying only on major cereals, pulses, and oilseeds that are largely in practice of cultivation. In this era of climate change, limited water and soil availability, the path toward yield progress will be full of hurdles (Tester and Langridge 2010; Lesk et al. 2016; Gupta et al. 2017). The supporting hands of less popular crops like small millets are not be neglected to feed this sea of population. The importance of small millets was understood long back and India started

Table 8.1 Botanical name, origin, and distribution of some important small millets

Sl. no.	Crop	Botanical name	Origin	Distribution	References
1.	Finger millet	*Eleusine coracana* (L.) Gaertn.	Uganda, Ethiopia	India, Nepal, China, Myanmar, Sri Lanka, Uganda, Kenya, Sudan, Zimbabwe, etc.	de Wet et al. (1984), Dwivedi et al. (2012)
2.	Kodo millet	*Paspalum scrobiculatum*	India	India, western Africa	Christopher et al. (1987), Bhatt et al. (2003)
3.	Indian barnyard millet	*Echinochloa frumentacea*	India	India, Nepal, Pakistan, and central Africa	Vetriventhan et al. (2020)
4.	Proso millet	*Panicum miliaceum* (L.)	China and Europe	India, China, Japan, Afghanistan, Russia, Iran, Iraq, Syria, Turkey, Mongolia, Romania, USA	Hunt et al. (2011)
5.	Foxtail millet	*Setaria italica*	China, Europe and Afghanistan-Lebanon	China, Europe, India, Nepal, Japan, Korea, Afghanistan-Lebanon	Dwivedi et al. (2012), Diao (2017)
6.	Little millet	*Panicum sumatrense*	India	India, Sri Lanka, Nepal, Pakistan, Myanmar, and eastern Indonesia	de Wet et al. (1983), Bhatt et al. (2003)

improvement program for small millets during the beginning of the twentieth century (Seetharam 1998). The establishment of All India Coordinated Small Millets Improvement Project in 1986 with its headquarter at Bangalore, India, witnessed more improvement in small millets production and breeding. The exact production of different small millets globally and in India is not well-documented due to their classification as "millets" as a whole (Vetriventhan et al. 2020). The reports of FAOSTAT (2018) say that the area under millets cultivation have reduced from 433.98 lakh hectares in 1961–1963 to 322.38 lakh hectares in 2016–2018 in average. The same reports also said that the average production of millets increased from 249.70 lakh tonnes in 1961–1963 to 290.31 lakh tonnes in 2016–2018. Millets in India could be a game changer in the rainfed region of the country contributing to 60% of the total area (Anbukkani et al. 2017). FAO (2018) reported India as the leading producer of millets in the world followed by Niger and China. State-wise, Rajasthan in India leads in the production of millets followed by Maharashtra and Gujarat (Adekunle et al. 2018). Still, it is not satisfactory as these crops are not at their fullest. A lot of barriers are prominent in exhausting the maximum possible yields from these crops. The gaps are to be filled by genetic improvements with development of improved physiological traits. Physiology opens a lot of closed doors by widening the opportunity of development of improved traits with genetics hand-in-hand. As India has the highest number of undernourished people in the world, quite efforts have to be applied to cross the long road between the starting point, i.e., food production, and the end point, i.e., food for everyone. Millets can play a vital role here providing both food and nutritional security at a time. A good amount of research work is going on in India and abroad to find out the full potential of small millets by the use of genetics. The present chapter will be discussing those scopes of improvement in breeding of small millets by physiological aspects.

8.2.3 Scope of Small Millets

People have changed a lot with time in terms of food habits leading to multiple numbers of health complexities and deadly diseases. Longer dependence on sugar-based diets and rich meals has cost a number of lives. Currently, people are becoming more health conscious for which they have already replaced some dominating foods prepared from rice and wheat in the daily diets. The long awaiting of small millets to enter in the daily diets could end here. Small millets are less sugar containing, and thus they could gain the trust of diabetes patients all over the world. Not only has that, their rich contents of fibers also assured good health for the obese people. People are gaining more faith on light food than the heavy diets made up of major cereals. Though small millets are light and less sugar containing, they are no less than a complete food because of their higher sources of several essential minerals and nutritional factors. Otherwise, these crops also contain various phenolic compounds and antioxidants that could function as anticarcinogenic (Chandrasekara and Shahidi 2011a). Amadou et al. (2013) reported that benefits from small millets are not limited to these; but they can also prevent heart diseases and help in

increasing energy levels and improvement of the muscular system. Another big advantage of these crops is that they can complete their life cycle within a short span of 60–90 days (Hunt et al. 2014). Therefore, small millets can fit in a multicropping system both under irrigated and dry farming condition empowered with this trait (Stanly and Shanmugam 2013; Vetriventhan et al. 2020). Therefore, considering the nutritional profile and other valuable properties, it can be said that small millets are one of the promising crops of current times and future.

8.3 Nutritional Composition and Food Value of Different Small Millets

The small millets are ample sources of important nutrients and essential components making a complete daily diet. This feature makes small millets "smart-food crops" and "nutri-cereals" (Vetriventhan et al. 2020). But the availability and amount of nutrients varies with the type and variety of small millets. According to Anbukkani et al. (2017), finger millet ranks in nutrition value per 100 g in terms of calcium, while in terms of phosphorus, it is only next to maize, wheat, and foxtail millet. Protein content per 100 g is highest in proso millet (Anbukkani et al. 2017; Hassan et al. 2021) followed by foxtail millet, wheat, and pearl millet (Anbukkani et al. 2017). On the other hand, barnyard millet stands first for containing both fats and crude fiber in highest amount among all cereals. A detailed discussion is being made about the nutritional composition of different small millets in Table 8.2.

Food value of small millets is decided by the presence of various compulsory materials such as starch, lipids, proteins, and amino acids in variable amounts. In some instances, small millets are of higher food values even than main course cereals

Table 8.2 Major nutritional properties of small millets and their significant roles

Sl. no.	Nutrients	Significant roles	References
1.	Magnesium	Reduced risk of heart attack; anticarcinogenic	Chandra et al. (2018), Hassan et al. (2021)
2.	Phosphorus	Growth and energy generation	Chandra et al. (2018)
3.	Calcium	Essential component of bones	Upadhyaya et al. (2011), Bachar et al. (2013), Goron and Raizada (2015)
		Improved production of breast milk	Kimeera and Sucharitha (2019)
4.	Zinc	Improved growth	Upadhyaya et al. (2011), Bandyopadhyay et al. (2017)
5.	Iron	Improved hemoglobin in blood	Arora (1977), Upadhyaya et al. (2011), Deshpande et al. (2015)
6.	Potassium	Improved drought tolerance	Hassan et al. (2021)
7.	Sodium	Not specified	Amadou et al. (2013)

Table 8.3 Food value of small millets with their valuable roles

Sl. no.	Crop	Nutrients	Significant roles	References
1.	Finger millet	Polyphenols, antioxidants	Anticarcinogenic	Viswanath et al. (2009), Devi et al. (2014)
		Essential amino acids, i.e., valine, lysine, threonine, tryptophan, and methionine	Superior food value to other cereals and oilseeds	Ravindran (1991), Mbithi-Mwikya et al. (2000), Bhatt et al. (2011)
		Unsaturated fatty acids	Improved health of heart	Kunyanga et al. (2013)
2.	Kodo millet	High dietary fibers, carbohydrates	Easy digestibility and excellent food sources for diabetic patients	Geervani and Eggum (1989), Deshpande et al. (2015)
3.	Proso millet	Proteins, amino acids, and fibers	Easy digestibility, antidiabetic, anticarcinogenic	Zhang et al. (2014), Vetriventhan and Upadhyaya (2018)
		Vitamin B_1 and B_3	Energy production, muscle contraction	Kalinova and Moudry (2006)
4.	Foxtail millet	Proteins, fats, crude fibers, carbohydrates	Energy production, improved gut health, antidiabetic	McIntosh et al. (2003), Upadhyaya et al. (2011), Ch et al. (2020)
5.	Little millet	Fats, carbohydrates, fiber, proteins, phenolic acids	Improved digestibility and health	Pradeep and Guha (2011)
6.	Indian barnyard millet	Crude fibers, carbohydrates, protein	Improved nutrition and health benefits	Anbukkani et al. (2017)
7.	Japanese barnyard millet	Good lipids and proteins	Healthy diets, improved heart conditions	Kim et al. (2011)
8.	Browntop millet	Crude fibers, proteins, fat	Lowered glycemic index	Ch et al. (2020)

like rice and wheat for their diverse benefits (Hegde et al. 2005; Hassan et al. 2021). A brief idea of the food value of small millets is given in Table 8.3.

8.4 Gaps in Cultivation of Small Millets in India

In spite of carrying this much of beneficial roles, production and productivity of small millets are not satisfactory. According to Sood et al. (2020), millet farming is mainly concentrated in developing nations where average productivity is still below the world average. This might be due to fear of uncertain market return from millets

among farmers across the world. Meanwhile, in developed nations and some developing nations such as India and China, farmers having relatively better socioeconomic conditions, well-developed marketing system, and easier accessibility to inputs including improved varieties can work harder in order to get the full potential of millets of various kinds (Meena et al. 2021). Besides these small seed size of millets causes difficulties for mechanical planting and harvest and ultimately for their commercialization. Hybridization in small millets is comparatively more difficult due to their smaller size of florets (Gupta et al. 2012). Bio Yerima and Achigan-Dako (2021) emphasized this as the reality behind the lack of improved varieties of millets leading to lower productivity in comparison to the major cereal crops.

Climate, the most important factor, has various direct and indirect impacts on cultivation of various millets. Dominance of climate has sent every other factor back to it. Climatic factors such as rainfall patterns and distribution, soil type and fertility, and ambient environment play a big role in determining the outcome from cultivation of millets (Sood et al. 2019). Other biggest constraints effect in the form of various abiotic and biotic stress factors. Das (2013) reported that small millets have to face various deadly diseases such as smut, blast, and rust among others. Lall and Yadav (1982) reported that higher weed intensification during the early growth phases of small millets is a natural barrier for the crops in terms of increased completion for nutrients, light, and water, resulting in ultimate loss of crop productivity. Among abiotic factors, high-temperature stress, moisture deficiency, and salinity have the most deleterious impacts on small millets. As grown in semiarid and arid regions, the exposure to terminal moisture and high-temperature stress for small millets is a common thing, due to which these crops give their highest possible yields. Deadly effects of salinity are expressed by lowering germination, growth, and overall physiological processes in small millets. Thus, interest in cultivation of small millets was lost in the middle resulting in lowered area of cultivation and production per unit area. The period of long negligence for these crops has come to an end looking into their significant roles for environment and human health as well. However, the full understanding of the physiology of the crops along with their genetics is required in order to exhaust the maximum achievable output from small millets. The combination of physiology and genetics can bring real revolution by removing all physiological barriers and developing varieties with improved physiological traits.

8.5 Disadvantages of Small Millets

The abundant blessings of small millets in the form of various nutrients cannot reach to humans and other animals in their fullest. Along with the nutrients, small millets, unfortunately, contain a number of phytochemicals that exert negative impacts on the nutritive value of the millets by reducing the digestibility of nutrients thus interfere with their regular activities (Sarita and Singh 2016). These phytochemicals, also known as anti-nutrients, mainly include phytates, polyphenols, oxalic acids, tannins, digestive enzyme inhibitors, and amylase and protease inhibitors leading to

Table 8.4 Antinutrients present in small millets and their impacts on health upon consumption

Sl. No.	Antinutrients	Impacts	References
1.	Tannins	Reduced feed intake, impaired nutrient digestibility, and nitrogen retention	Mohammedain et al. (1986), Kumar et al. (2016)
2.	Phytates	Reduced digestibility of phosphorus	Kumar et al. (2016), Boncompagni et al. (2018)
3.	Tannins and phenols	Reduced utilization of nutrients	Pelig-Ba (2009), Singh and Raghuvanshi (2012)
4.	Protease inhibitors	Reduced activities of useful enzymes, i.e., trypsin and chymotrypsin	Ravindran (1991), Kumar et al. (2016)
5.	Non-starch polysaccharides, oxalates	Reduction in digestibility of nutrients	Abate and Gomez (1984), Malathi and Devegowda (2001)
6.	Amylase inhibitors	Reduced carbohydrate utilization	Kumar et al. (2016)

altered metabolic activities (Singh and Sarita 2016; Vinoth and Ravindhran 2017; Hassan et al. 2021). Table 8.4 discusses about some of the antinutrients of small millets with their associated health impacts.

8.6 Genetic Improvement of Small Millets Through Physiological Approaches

Since the beginning of improvement in small millets back in twentieth century, India has witnessed a quite good progress with development and release of new varieties for various small millets. Genetics figures out all the physiological, morphological, and molecular drawbacks in a crop and then studies thoroughly where and how it can work to overcome all the drawbacks in order to get them superior to previous ones with insertion of preferable traits. Current era is characterized by extremities in weather parameters for which crop production has come uncertain. Implementation of climate-resilient approaches can assure better resistance to all climatic barriers in front of crop production. Shifting to less popular cereals, i.e., small millets, is also part of climate-resilient approaches that could stand well under climate-changing conditions. This will also need the backup of improved physiological traits such as leaf size, leaf numbers, drought resistance, high temperature tolerance, nutrient use efficiency, antioxidant properties, disease resistance, etc. Improvement of small millets targets breeding of those with the improved physiological traits. This section will be discussing some of the possibilities and successes in genetic improvement in small millets based on physiological traits.

8.6.1 Improved Physiological Traits

The complete knowledge of genetic composition and their full exposure for trait improvement need omics studies which offer a lot of opportunities. Omics studies compose various branches under biotechnology, i.e., genomics, proteomics, transcriptomics, phenomics, etc. (Fritsche-Neto and Borém 2014). A lot of parameters have been studied with the use of omics tools. In the case of small millets, stover quality matters a lot; thus, breeding for improved stover quality will broaden the future prospects and improving feed value (Schiere et al. 2004). Other traits include resistance to abiotic and biotic stresses, improved protein content, improved crude fiber content, promoted activities of antioxidants conferring anticarcinogenic effects, fortified nutrients, and also improved yield as well. Padulosi et al. (2009) reported that minor millets such as finger, kodo, foxtail, little, proso, and barnyard have the ability to grow successfully in diverse soils, varying rainfall regimes, diverse photoperiods and in marginal, due to their genetic adaptation. A brief discussion has been made about some important physiological traits that can be improved with using genetic tools (Table 8.5).

8.6.1.1 Leaf Area

Leaf area for any crop is an important physiological trait; it is in direct relationship with photosynthetic capacity for the crop. So, larger leaf area denotes more photosynthesis. This trait not only secures good photosynthesis but also denotes the capacity of a plant to face the challenges of various stressful conditions by maintaining dry matter accumulation. According to Ludlow and Muchow (1990), reduction in leaf area is also associated with checked growth of leaf and hastened leaf senescence. A number of studies confirmed lower water uptake and water use efficiency; hampered growth and dry matter accumulation; and lower leaf numbers and area under drought, salinity, and high temperature for small millets, which might

Table 8.5 Breeding of some small millets for introduction of improved traits

Sl. no.	Millet type	Tools used	Traits	References
1.	Finger millet	Genome-wide association studies (GWAS)	Improved contents of nutrients like zinc, iron, calcium, magnesium, and potassium	Puranik et al. (2020)
		Molecular markers	Tryptophan accumulation and association	Babu et al. (2014a, b)
2.	Fonio millet	Comparative genomics	Seed size, lodging, and shattering	Wang et al. (2021)
3.	Foxtail millet	Genome-wide association studies (GWAS)	Nutritional traits	Jaiswal et al. (2019)
4.	Proso millet	Molecular markers	Development of glutinous verities	Araki et al. (2012)

be due to obstructed cell enlargement and cell division (Ludlow and Muchow 1990). Thus, improved varieties with higher leaf area will certainly show positive connection in terms of tolerance to abiotic stresses such as moisture scarcity, high temperature, especially at reproductive phase, and salinity, thus securing improved dry matter accumulation and photosynthetic rate for small millets.

8.6.1.2 Net Assimilation Rate and Leaf Area Duration

Net assimilation rate (NAR) and leaf area duration (LAD) both represent the health status of a plant. A healthy plant generally has higher NAR and LAD, while a stressed plant tends to show lower values of NAR and LAD with increasing magnitude of stress. Senthil et al. (2018) also found decreasing values NAR and LAD under terminal drought condition for small millets like finger millet, little millet, and barnyard millet. They also reported that the reduced NAR and LAD were associated with the rapid decrease in leaf number and leaf area under terminal drought condition. According to Chetti and Sirohi (1995), LAD is a useful growth parameter that indicates the photosynthetic efficiency due to its direct association with dry matter accumulation. Divya et al. (2018) reported that variations in LAD in five small millets, viz., finger miller, kodo millet, little millet, barnyard millet, and foxtail millet, were not only due to differences in species but also dependent on the crop growth stage. Thus, development of varieties of small millets with improved NAR and LAD will be of great benefit for climate-changing conditions.

8.6.1.3 Photosynthesis

Photosynthetic rate in any plant is decided by the factors such as chlorophyll content (Yoshida 1972), structure and number of leaves, and also the mechanism involved. The differences between the C_3 and C_4 mechanisms of photosynthesis have classified plants accordingly. The efficiency of C_4 crops for photosynthesis is greater than the C_3 crops due to more utilization of CO_2 by double chloroplast layers. Another advantage of the C_4 crops is their ability to suppress the photorespiration that is one of the major constraints of reduced crop production in C_3 crops. All the millets being the C_4 crops also possess the ability to yield more through activation of the carboxylation surpassing the oxygenation by the RuBiSCO enzyme that shows more affinity to atmospheric CO_2 (Aubry et al. 2011). Millets have expressed immediate water and nitrogen use efficiency of nearly 1.5- to fourfold higher than C_3 photosynthesis, as reported by Sage and Zhu (2011). Also, millets show reducing trend in photosynthetic rate in moderate to excessive adverse conditions. However, Channappagoudar et al. (2008) reported that high-yielding genotypes of barnyard millet expressed higher photosynthetic rates. Thus, improved verities of various small millets with enhanced photosynthetic activity can be released with the help of genetics.

8.6.1.4 Total Carbohydrates

Millets of various kinds possess carbohydrate in different forms such as starch, dietary fibers, and soluble sugars which help in prevention of many life-style diseases (Hassan et al. 2021). Soluble sugars not only decide the outcome of

photosynthesis but also possess the properties of resistance to several abiotic stresses such as drought, salinity, and high temperature under which plant becomes scarce of inputs like water to produce enough food by photosynthesis. During stress, soluble sugar moves to the region of the plant requiring food sources to survive. Wankhede et al. (1979) reported that finger millet and foxtail millet contained starch, pentosans, cellulose, and lignin with the maximum content of starch varying from 59.5% to 61.2%. Thus, considering the tidy contents of carbohydrates, small millets can be grown with the target of developing improved varieties.

8.6.1.5 Soluble Protein Content

Soluble proteins are important for plants. The reverse connection of proteolytic activities and soluble proteins content make the complex physiology of plant understood in an easier way. The decreasing trend of soluble proteins content under stress conditions has been reported from a lot of earlier studies. Senthil et al. (2018) also reported reduction in soluble protein content at reproductive stage upon exposure to drought condition for three small millets, i.e.. finger millet, little millet, and barnyard millet. On the other hand, foxtail millet has an envious content of protein (7.17–15.73%) that is the target of many scientists to exploit and use for development of more improved verities employing breeding program (Senthil et al. 2018). But foxtail millet also shows reducing trend in soluble protein content under drought, as reported by Dai et al. (2012). Soluble proteins in sufficient amounts increase the food value for any grain as proteins have direct role in growth and development of living bodies. Thus, genetic improvement can be done based on soluble protein content for several small millets.

8.6.1.6 Grain Nutrients

Grains of all the small millets provide ample amount of various essential nutrients (Mallikarjun et al. 2013; Cedric et al. 2017; Ch et al. 2020). These nutri-cereals are the future of agriculture, sustainability, and climate-resilience. The desire of improved varieties of the small millets with enhanced nutrient contents has been increasing for which genetics can help a lot.

8.6.1.7 Yield and Yield Attributing Traits

Like other crop, yield in small millets is associated with the combined effects of a number of factors. Those include plant height (Divya et al. 2018), dry matter accumulation (Chidambaram and Palanisamy 1996), number of tillers per plant (Divya et al. 2018), 1000-grain weight (Naidu et al. 2021), harvest index (Channappagoudar et al. 2007), etc. In little millet, 1000-grain weight and photosynthetic rate individually built strong and positive correlation with yield (Channappagoudar et al. 2007). Grain yield is affected due to direct impacts on yield attributing parameters such as 1000-grain weight, leaf area, panicle dry weight per plant, etc. Zhang et al. (2022) reported positive correlation of grain yield with physiological traits like leaf area, 1000-grain weight, panicle dry weight per plant, and harvest index for both well-watered and water stress conditions in foxtail millet. However, chlorophyll content was found to be negatively correlated under water

stress condition for the same study in contrast to the well-watered condition in which it was in positive correlation with grain yield. In another study on little millet, Naidu et al. (2021) reported significant and positive correlation between grain yield and traits such as plant height, panicle length, SPAD chlorophyll meter reading, and fodder yield per plant individually. So, a number of promising traits can be improved with the target of improved yield attainment. But most of the neglected crops including small millets carry some have undesirable properties such as that small seed size, lodging, and seed shattering that lead to limited yield and poor quality of grains (Hinterthuer 2017). With the recent advances in genetics and breeding tools like genomics, it is possible to overcome these drawbacks.

8.6.2 Stress Tolerance

8.6.2.1 Abiotic Stress Tolerance

Abiotic stresses are one of the biggest challenges plant has to face to survive and stand well. Abiotic stress in the form of moisture scarcity, flood, salinity, and high and low temperatures has been reported to damage crops on a serious note. Weather events in extremities are occurring in higher frequency due to changing patterns of climate. These, together, are adding fuel to the problem of stress inducing effects. There are genes that could unlock the potential of various crops fostering agricultural productivity, even under stressful condition. For instance, a number of genes, responsible to induce tolerance to abiotic and biotic stresses for plants have been introduced into selected genotypes in order to obtain the specified trait (Ribaut and Hoisington 1998).

Millets have been reported with tolerance to abiotic stresses of variable intensities depending on the species and variety. Liu et al. (2015) reported salinity tolerance in proso millet with the highest level of tolerance by the most tolerant varieties. Drought tolerance has been identified in proso millet (Seghatoleslami et al. 2008), finger millet (Bhatt et al. 2011), little millet (Ajithkumar and Panneerselvam 2014), and foxtail millet (Sudhakar et al. 2015). Some of the targeted traits regarding abiotic stress tolerance for different small millets have been discussed in Table 8.6.

Some specific genes have been identified to confer tolerance to multiple abiotic stresses for the small millets. Veeranagamallaiah et al. (2009) reported the display of salinity tolerance for foxtail millet by improved activity of aldose reductase. Tolerance to salinity, cold stress, and moisture scarcity by foxtail millet was possible through the promoted gene *C2H2 type of zinc finger transcription factors* (*TFs*), as reported by Muthamilarasan et al. (2014). Similarly, improved drought tolerance has been observed in finger millet by the gene *Dehydrin7*, found from tobacco (Singh et al. 2015). Thus, there is a huge scope for genetic improvement of various small millets by the means of abiotic stress tolerance.

8.6.2.2 Biotic Stress Tolerance

Biotic stress accounts for huge crop loss in terms of growth and yield. Small millets also attract some diseases affecting their overall growth, survival, and yield.

Table 8.6 Specific abiotic stress tolerance traits targeted for different small millets

Sl. no.	Type of small millet	Targeted traits	References
1.	Proso millet	Drought tolerance and improved WUE	Goron and Raizada (2015)
2.	Japanese barnyard millet	Herbicide resistance	Yang et al. (2013)
3.	Foxtail millet	Salinity and drought tolerance	Wang et al. (2011)
		Herbicide resistance	Diao (2017)
		Resistance to high temperature and excess moisture	Wang et al. (2012)
4.	Little millet	Glyphosate herbicide resistance	Goron and Raizada (2015)
5.	Finger millet	Drought resistance	Parvathi et al. (2013)

However, some small millets contain promising traits that can surpass biotic stress. As for example, Indian barnyard millet contains some antifeedants at concentrations ever greater than those in rice. According to Kim et al. (2008), these antifeedants display resistance to the feeding activity of brown planthopper (BPH) in Indian barnyard millet. But all the small millets have the susceptibility toward some specific diseases to overcome which a number of associated resistant genes have been identified. A brief discussion of the breeding program of various small millets against biotic stress has been made.

Finger millet (*Eleusine coracana*) is prone to blast disease caused by *Pyricularia grisea* (Ceasar and Ignacimuthu 2009). This confers huge loss in yield of finger millet in response to which identification of stable sources of resistance to blast disease of finger millet and their deployment in breeding research has been highly desired in the country high-yielding blast-resistant cultivars in finger millet in the country (Byre Gowda et al. 1999). Genetic engineering has worked in the development of blast-resistant varieties in finger millet. This involves the transfer of gene resistant to the fungus responsible for the disease from the source organism to the desired organism (Ceasar and Ignacimuthu 2008). Latha et al. (2005) developed a transgene from prawn antifungal protein PIN-resisting blast in finger millet. Foxtail millet (*Setaria italica*) is also severely affected by the diseases like blast, smut, and rust (Vetriventhan et al. 2016). Sharma et al. (2014) reported germplasm sources for blast resistance in foxtail millet. Kodo millet (*Paspalum scrobiculatum*) gets affected by a number of diseases among which sheath blight and smut seem to be more dangerous. Genetics focuses for development of improved kodo millet varieties resistant to sheath blight, shoot fly, and head smut (Upadhyaya et al. 2015; Vetriventhan et al. 2020). Fonio millet (*Digitaria exilis* (L.) Stapf) shows susceptibility toward a number of diseases like bacterial blight, rust, fungal diseases, etc. It has been suggested that the use of marker assisted backcrossing in pyramiding genes for improving resistance in fonio to diseases will be successful (Hsu et al. 2020; Bio Yerima and Achigan-Dako 2021).

8.7 Future of Agriculture Through Small Millets

Current agriculture is focused on sustainability. Climate resilience is the new addition in the list. Drought, salinity, and high temperature stresses are becoming stronger with time due to impacts of climate change. Thus, sustainability with the implementation of climate-resilient approaches is the only solution to the complicated problem of climate change, soil erosion, limited water availability, and increasing population pressure. Small millets have long back been identified as excellent climate-resilient crops (Padulosi et al. 2009). Li and Brutnell (2011) reported that small millets surpass the prevalence of stress conditions and their consequences by implementing several traits such as developing shorter stature, smaller leaf area, thickening cell walls, and forming dense root system. Occurrences of diseases to plant are also becoming intense due to alternations in the natural environment and growth habit for various pathogens like fungi, viruses, and bacteria. Small millets also attract some pathogens which damage the crops. But a number of genes have been developed conferring tolerance to pathogens. Small millets can certainly be the crops of future for their richer sources of nutrients, antioxidants, and other beneficial properties.

8.8 Conclusions

It is true that a number of factors decide the overall potential of a crop. The piercing factors like population pressure and sustainability have made it harder to achieve the target of higher productivity, exhausting the fullest possibilities from major crops like rice, wheat, oilseeds, and pulses. Adopting millet cultivation has been felt necessary toward the attainment of sustainability. As it is known that no big target is fulfilled without struggles, so is the target of reaching food security to people from every single part of the world which also has to pass the tough state of affairs like limited natural resources, climate changing patterns, and uncertainty of incomes from agriculture. The shortcomings in small millets are also to be overcome also in order to utilize their full potential. The agricultural community is in continuous search of new innovations in terms of latest technologies, adoption of alternative cultivation pattern, and release of new varieties. The target of achieving improved traits like resistance to abiotic and biotic stresses, improved nutritional factors, improved morphophysiological traits, and yield will be met through breeding of small millets for which an exhaustive study is needed to get complete knowledge of physiology of different small millets. Therefore, physiological breeding approaches are the deciding factor in genetic improvement of the small millets which are yet to open many closed doors of the future sustenance.

References

Abate AN, Gomez M (1984) Substitution of finger millet (*Eleusine coracana*) and bulrush millet (*Pennisetum typhoides*) for maize in broiler feeds. AFST 10:291–299

Adekunle A, Lyew D, Orsat V et al (2018) Helping agribusinesses—small millets value chain to grow in India. Agriculture 8:44

Ajithkumar IP, Panneerselvam R (2014) ROS scavenging system, osmotic maintenance, pigment and growth status of Panicum sumatrense roth. Under drought stress. Cell Biochem Biophys 68: 587–595. https://doi.org/10.1007/s12013-013-9746-x

Amadou I, Gounga ME, Guo-Wei L (2013) Millets: nutritional composition, some health benefits and processing—a review. Emir J Food Agric 25(7):501–508. https://doi.org/10.9755/ejfa.v25i7.12045

Anbukkani P, Balaji SJ, Nithyashree ML (2017) Production and consumption of minor millets in India—a structural break analysis. Ann Agric Res 38(4):1–8

Araki M, Numaoka A, Kawase M et al (2012) Origin of waxy common millet, Panicum miliaceum L. in Japan. Genet Res Crop Evol 59:1303–1308. https://doi.org/10.1007/s10722-011-9755-9

Arora RK (1977) Job's-tears (*Coix lacryma-jobi*): a minor food and fodder crop of northeastern India. Econ Bot 31:358–366

Aubry S, Brown NJ, Hibberd JM (2011) The role of proteins in C_3 plants prior to their recruitment into the C_4 pathway. J Exp Bot 62:3049–3059. https://doi.org/10.1093/jxb/err012

Babu BK, Agrawal PK, Pandey D et al (2014a) Association mapping of agro-morphological characters among the global collection of finger millet genotypes using genomic SSR markers. Mol Biol Rep 41:5287–5297. https://doi.org/10.1007/s11033-014-3400-6

Babu BK, Agrawal PK, Pandey D et al (2014b) Comparative genomics and association mapping approaches for opaque2 modifier genes in finger millet accessions using genic, genomic and candidate gene-based simple sequence repeat markers. Mol Breed 34:1261–1279. https://doi.org/10.1007/s11032-014-0115-2

Bachar K, Mansour E, Ben KA et al (2013) Fiber content and mineral composition of the finger millet of the oasis of Gabes Tunisia. J Agric Sci. https://doi.org/10.5539/jas.v5n2p219

Bandyopadhyay T, Muthamilarasan M, Prasad M (2017) Millets for next generation climate-smart agriculture. Front Plant Sci 8:1266

Beddington J (2010) Food security: contributions from science to a new and greener revolution. Philos Trans R Soc B Biol Sci 365:61–71. https://doi.org/10.1098/rstb.2009.0201

Bekkering CS, Tian L (2019) Thinking outside of the cereal box: breeding underutilized (pseudo) cereals for improved human nutrition. Front Genet 10:1289

Bhatt A, Singh V, Shrotria PK et al (2003) Coarse grains of Uttaranchal: ensuring sustainable food and nutritional security. Indian Farmers Digest 7:34–38

Bhatt D, Negi M, Sharma P et al (2011) Responses to drought induced oxidative stress in five finger millet varieties differing in their geographical distribution. Physiol Mol Biol Plants 17:347–353. https://doi.org/10.1007/s12298-011-0084-4

Bio Yerima ARI, Achigan-Dako EG (2021) A review of the orphan small grain cereals improvement with a comprehensive plan for genomics-assisted breeding of fonio millet in West Africa. Plant Breed 140:561–574

Boncompagni E, Orozco-Arroyo G, Cominelli E et al (2018) Antinutritional factors in pearl millet grains: phytate and goitrogens content variability and molecular characterization of genes involved in their pathways. PLoS One 13:e0198394. https://doi.org/10.1371/journal.pone.0198394

Borlaug NE (2002) Feeding a world of 10 billion people: the miracle ahead. In Vitro Cell Dev Biol Plant 8:221–228

Byre Gowda M, Shankare Gowda BT, Seetharam A (1999) Selection for combining grain yield with high protein and blast resistance in finger millet (*Eleusine coracana* Gaertn.). Indian J Genet 59(3):345–349

Ceasar SA, Ignacimuthu S (2008) Efficient somatic embryogenesis and plant regeneration from shoot apex explants of different Indian genotypes of finger millet (*Eleusine coracana* (L.) Gaertn.). In Vitro Cell Dev Biol Plant 44:427–435
Ceasar SA, Ignacimuthu S (2009) Genetic engineering of millets: current status and future prospects. Biotechnol Lett 31:779–788
Cedric H, Janet B, Alpoim D et al (2017) Proso millet and its potential for cultivation in the Pacific Northwest, U.S. A review. Front Plant Sci 7(1):1–17
Ch H, Patro TSSK, Anuradha N (2020) Estimation of nutritive composition of seven small millets. J Pharmacogn Phytochem 9(3):1871–1874
Chandra A, Singh AK, Mahto B (2018) Processing and value addition of finger millet to achieve nutritional and financial security—case study. Int J Curr Microbiol Appl Sci 7:2901–2910
Chandrasekara A, Shahidi F (2011a) Antiproliferative potential and DNA scission inhibitory activity of phenolics from whole millet grains. J Funct Foods 3:159–170
Chandrasekara A, Shahidi F (2011b) Determination of antioxidant activity in free and hydrolyzed fractions of millet grains and characterization of their phenolic profiles by HPLC-DAD-ESI-MS. J Funct Foods 3:144–158. https://doi.org/10.1016/j.jff.2011.03.007
Channappagoudar BB, Hiremath SM, Biradar NR et al (2007) Morpho-physiological traits influencing the grain yield potential in little millet. Karnataka J Agric Sci 20(3):473–476
Channappagoudar BB, Hiremath SM, Biradar NR et al (2008) Influence of morpho-physiological and biochemical traits on the productivity of barnyard millet. Karnataka J Agric Sci 20(3): 477–480
Chetti MB, Sirohi GS (1995) Effect of water stress on leaf characteristics and its recovery in mung bean (*Vigna radiata* L. Wilczek) cultivars. J Maharashtra Agric Univ 20:85–87
Chidambaram S, Palanisamy S (1996) Dry matter production and harvest index in little millet (*Panicum sumatrense* Roth Ex. Roem and Schult). Madras Agric J 83:15–17
Christopher J, Raj PS, Pillai KG (1987) Cytological studies of three species of *Paspalum* Linn. from South India. Cytologia 52:487–491
Combs GF (2001) Selenium in global food systems. Br J Nutr 85(5):517–547
Dai HP, Shan CJ, Wei AZ et al (2012) Leaf senescence and photosynthesis in foxtail millet (*Setaria italica* (L.) P. Beauv) varieties exposed to drought conditions. Aust J Crop Sci 6(2):232–237
Das IK (2013) Disease management in grain, forage and sweet sorghum. In: Chapke RR, Bhagwat VR, Patil JV (eds) Sorghum cultivation for value-added diversified products and sweet sorghum perspectives. Directorate of Sorghum Research, Hyderabad, pp 99–104
Das S, Khound R, Santra M et al (2019) Beyond bird feed: proso millet for human health and environment. Agriculture 9:64. https://doi.org/10.3390/agriculture9030064
de Wet JMJ, Rao KEP, Brink DE (1983) Systematics and domestication of *Panicum sumatrense* (Graminae). J d'agriculture Tradit Bot appliquée 30:159–168
de Wet JMJ, Rao KEP, Brink DE et al (1984) Systematics and evolution of *Eleusine coracana* (Gramineae). Am J Bot 71:550–557
Deshpande SS, Mohapatra D, Tripathi MK et al (2015) Kodo millet—nutritional value and utilization in Indian foods. J Grain Process Storage 2(2):16–23
Devi PB, Vijayabharathi R, Sathyabama S et al (2014) Health benefits of finger millet (*Eleusine coracana* L.) polyphenols and dietary fiber: a review. J Food Sci Technol 51:1021–1040. https://doi.org/10.1007/s13197-011-0584-9
Diao X (2017) Production and genetic improvement of minor cereals in China. Crop J 5:103–114
Divya K, Senthil A, Sritharan N et al (2018) Morpho-physiological traits influencing the grain yield potential in small millets. Madras Agric J 105(7–9):476–479. https://doi.org/10.29321/MAJ.2018.000188
Dube T, Mlilo C, Moyo P et al (2018) Will adaptation carry the future? Questioning the long-term capacity of smallholder farmers' adaptation strategies against climate change in Gwanda District. Zimbabwe J Hum Ecol 61(1–3):20–30
Dwivedi S, Upadhyaya H, Senthilvel S et al (2012) Millets: genetic and genomic resources. In: Janick J (ed) Plant breeding reviews. Wiley, Hoboken, NJ, pp 247–374

FAO (2018). http://www.fao.org/wiews-archive/germplasm_query.htm?i_l=EN
FAOSTAT (2018) Production-yield quantities of millets in world + (total) 1962–2018. https://www.fao.org/faostat/en/#data/QC/visualize
Fritsche-Neto R, Borém A (2014) Omics: opening up the "black box" of the phenotype. In: Borém A, Fritsche-Neto R (eds) Omics in plant breeding. Wiley, New York, pp 1–11
Geervani P, Eggum BO (1989) Nutrient composition and protein quality of minor millets. Plant Foods Hum Nutr 39:201–208. https://doi.org/10.1007/BF01091900
Goron TL, Raizada MN (2015) Genetic diversity and genomic resources available for the small millet crops to accelerate a new green revolution. Front Plant Sci 6:Art 157. https://doi.org/10.3389/fpls.2015.00157
Gupta A, Mahajan V, Gupta HS (2010) Genetic resources and varietal improvement of small millets for Indian Himalaya. In: Tewari LM, Pangtey YPS, Tewari G (eds) Biodiversity potentials of the Himalaya. Gyanodaya Prakashan, Nainital, India, pp 305–316
Gupta S, Kumari K, Sahu PP et al (2012) Sequence based novel genomic microsatellite markers for robust genotyping purposes in foxtail millet [*Setaria italica* (L.) P. Beauv]. Plant Cell Rep 31:323–337. https://doi.org/10.1007/s00299-011-1168-x
Gupta SM, Arora S, Mirza N et al (2017) Finger millet: a "certain" crop for an "uncertain" future and a solution to food insecurity and hidden hunger under stressful environments. Front Plant Sci 8:643. https://doi.org/10.3389/fpls.2017.00643
Hassan ZM, Sebola NA, Mabelebele M (2021) The nutritional use of millet grain for food and feed: a review. Agric Food Secur 10:16. https://doi.org/10.1186/s40066-020-00282-6
Hegde PS, Rajasekaran NS, Chandra TS (2005) Effects of the antioxidant properties of millet species on oxidative stress and glycemic status in alloxan-induced rats. Nutr Res 25:1109–1120. https://doi.org/10.1016/j.nutres.2005.09.020
Hinterthuer A (2017) Can ancient grains find their way in modern agriculture? CSA News 62:4–9. https://doi.org/10.2134/csa2017.62.0412
Hsu Y-C, Chiu C-H, Yap R et al (2020) Pyramiding bacterial blight resistance genes in Tainung82 for broad-spectrum resistance using marker-assisted selection. Int J Mol Sci 21:1281. https://doi.org/10.3390/ijms21041281
Hunt HV, Campana MG, Lawes MC et al (2011) Genetic diversity and phylogeography of broomcorn millet (*Panicum miliaceum* L.) across Eurasia. Mol Ecol 20:4756–4771
Hunt HV, Badakshi F, Romanova O et al (2014) Reticulate evolution in *Panicum* (Poaceae): the origin of tetraploid broomcorn millet, *P. miliaceum*. J Exp Bot 65:3165–3175
Jaiswal V, Bandyopadhyay T, Gahlaut V et al (2019) Genome-wide association study (GWAS) delineates genomic loci for ten nutritional elements in foxtail millet (*Setaria italica* L.). J Cereal Sci 85:48–55. https://doi.org/10.1016/j.jcs.2018.11.006
Kalinova J, Moudry J (2006) Content and quality of protein in proso millet (*Panicum miliaceum* L.) varieties. Plant Foods Hum Nutr 61:45–49
Kam J, Puranik S, Yadav R et al (2016) Dietary interventions for type 2 diabetes: how millet comes to help. Front Plant Sci 7:1–14
Kennedy G, Nantel G, Shetty P (2003) The scourge of "hidden hunger": global dimensions of micronutrient deficiencies. Food Nutr Agric 32:8–16
Khound R, Santra DK (2020) Omics for proso millet genetic improvement. Nucleus 63:241. https://doi.org/10.1007/s13237-020-00339-8
Kim C-S, Alamgir KM, Matsumoto S et al (2008) Antifeedants of Indian barnyard millet, *Echinochloa frumentacea* Link, against brown planthopper, *Nilaparvata lugens* (Stal). Z Naturforsch 63:755–760
Kim JY, Jang KC, Park BR et al (2011) Physicochemical and antioxidative properties of selected barnyard millet (*Echinochloa utilis*) species in Korea. Food Sci Biotechnol 20:461–469. https://doi.org/10.1007/s10068-011-0064-z
Kimeera A, Sucharitha KV (2019) Millets-review on nutritional profiles and health benefits. Int J Recent Sci Res 10(7):33943–33948

Kumar SI, Babu CG, Reddy VC et al (2016) Anti-nutritional factors in finger millet. J Nutr Food Sci 6:3. https://doi.org/10.4172/2155-9600.1000491

Kunyanga CN, Imungi JK, Velingiri V (2013) Nutritional evaluation of indigenous foods with potential food-based solution to alleviate hunger and malnutrition in Kenya. J Appl Biosci 67: 5277–5288

Lall M, Yadav LNS (1982) Critical time of weed removal in finger millet. Indian J Weed Sci 14:85–88

Lata C (2015) Advances in omics for enhancing abiotic stress tolerance in millets. Proc Indian Natl Sci Acad 81:397–417

Lata C, Gupta S, Prasad M (2013) Foxtail millet: a model crop for genetic and genomic studies in bioenergy grasses. Crit Rev Biotechnol 33:328–343. https://doi.org/10.3109/07388551.2012.716809

Latha AM, Rao KV, Reddy VD (2005) Production of transgenic plants resistant to leaf blast disease in finger millet (*Eleusine coracana* (L.) Gaertn.). Plant Sci 169:657–667. https://doi.org/10.1016/j.plantsci.2005.05.009

Leder I (2004) Sorghum and millets. In: Füleky G (ed) Cultivated plants, primarily as food sources, in Encyclopedia of Life Support Systems (EOLSS), Developed under the auspices of the UNESCO. EOLSS Publishers, Oxford, UK

Lesk C, Rowhani P, Ramankutty N (2016) Influence of extreme weather disasters on global crop production. Nature 529:84–87. https://doi.org/10.1038/nature16467

Li P, Brutnell TP (2011) *Setaria viridis* and *Setaria italica*, model genetic systems for the panicoid grasses. J Exp Bot 62:3031–3037. https://doi.org/10.1093/jxb/err096

Liu M, Qiao Z, Zhang S et al (2015) Response of broomcorn millet (*Panicum miliaceum* L.) genotypes from semiarid regions of China to salt stress. Crop J 3:57–66. https://doi.org/10.1016/j.cj.2014.08.006

Lokesh K, Dudhagara CR, Mahera AB et al (2022) Pharma Innov J SP-11(4):75–84

Ludlow MM, Muchow RC (1990) A critical evaluation of traits for improving crop yields in water limited environments. Adv Agron 43:107–153

Macron AE (1994) Wheat streak mosaic virus resistance in foxtail millet *Setaria italica* L. Beauv. and factors related to resistance. M.Sc. thesis, University of Nebraska, Lincoln. p 78

Malathi V, Devegowda G (2001) *In vitro* evaluation of non-starch polysaccharide digestibility of feed ingredients by enzymes. Poult Sci 80:302–305

Mallikarjun Y, Hemalatha S, Meghana DR et al (2013) Evaluation of Little Millet (*Panicum sumatrense*) land races for cooking and nutritional composition. Curr Res Biol Pharm Sci 2(1):7–11

Mbithi-Mwikya S, Ooghe W, Van Camp J et al (2000) Amino acid profiles after sprouting, autoclaving, and lactic acid fermentation of finger millet (*Eleusine coracana*) and kidney beans (*Phaseolus vulgaris* L.). J Agric Food Chem 48:3081–3085. https://doi.org/10.1021/jf0002140

McIntosh GM, Noakes M, Royle PJ et al (2003) Whole-grain rye and wheat foods and markers of bowel health in overweight middle-aged men. Am J Clin Nutr 77:967–974

Meena RP, Joshi D, Bisht JK et al (2021) Global scenario of millets cultivation. In: Kumar A et al (eds) Millets and millet technology. Springer. https://doi.org/10.1007/978-981-16-0676-2_2

Mohammedain GM, Babiker SA, Mohammed T (1986) Effect of feeding millet, maize and sorghum grains on performance, carcass yield and chemical composition of broiler meat. Trop Agric 63:173–176

Muthamilarasan M, Venkata SB, Pandey G et al (2014) Development of 5123 intron-length polymorphic markers for large-scale genotyping applications in foxtail millet. DNA Res 21:41–52. https://doi.org/10.1093/dnares/dst039

Naidu BN, Kumar MH, Sekhar MR (2021) Character association analysis for morphological and physiological characters in little millet (*Panicum sumatrense* Roth. ex Roem and Schult). Pharma Innov J 10(8):605–607

Nkhata SG, Ayua E, Kamau EH et al (2018) Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. Food Sci Nutr 6:2446–2458

Padulosi S, Mal B, Ravi SB et al (2009) Food security and climate change: role of plant genetic resources of minor millets. Indian J Plant Genet Resour 22(1):1–16

Parvathi MS, Nataraja KN, Yashoda BK et al (2013) Expression analysis of stress responsive pathway genes linked to drought hardiness in an adapted crop, finger millet (*Eleusine coracana*). J Plant Biochem Biotechnol 22:193–201. https://doi.org/10.1007/s13562-012-0135-0

Pelig-Ba KB (2009) Assessment of phytic acid levels in some local cereal grains in two districts in the upper east region of Ghana. Pak J Nutr 8:1540–1547. https://doi.org/10.3923/pjn.2009.1540.1547

Pradeep SR, Guha M (2011) Effect of processing methods on the nutraceutical and antioxidant properties of little millet (*Panicum sumatrense*) extracts. Food Chem 126:1643–1647. https://doi.org/10.1016/j.foodchem.2010.12.047

Puranik S, Sahu PP, Beynon S et al (2020) Genome-wide association mapping and comparative genomics identifies genomic regions governing grain nutritional traits in finger millet (*Eleusine coracana* L. Gaertn.). Plants People Planet 2:649–662

Ravindran G (1991) Studies on millets: proximate composition, mineral composition and phytate and oxalate contents. Food Chem 39:99–107. https://doi.org/10.1016/0308-8146(91)90088-6

Reddy INBL, Reddy DS, Narasu ML et al (2011) Characterization of disease resistance gene homologues isolated from finger millet (*Eleusine coracana* L. Gaertn). Mol Breed 27:315–328. https://doi.org/10.1007/s11032-010-9433-1

Ribaut J-M, Hoisington D (1998) Marker-assisted selection: new tools and strategies. Trends Plant Sci 3:236–239. https://doi.org/10.1016/S1360-1385(98)01240-0

Sage RF, Zhu X-G (2011) Exploiting the engine of C_4 photosynthesis. J Exp Bot 62:2989–3000. https://doi.org/10.1093/jxb/err179

Sarita ES, Singh E (2016) Potential of millets: nutrients composition and health benefts. J Sci Innov Res 5(2):46–50

Schiere JB, Joshi AL, Seetharam A et al (2004) Grain and straw for whole plant value: implications for crop management and genetic improvement strategies. Exp Agric 40:277–294

Seetharam A (1998) Small millets—achievements during 1947–1997. Indian J Agric Sci 68 (Suppl):47–54

Seghatoleslami MJ, Kafi M, Majidi E (2008) Effect of drought stress at different growth stages on yield and water use efficiency of five proso millet (*Panicum miliaceum* L.) genotypes. Pak J Bot 40:1427–1432

Senthil A, Ashok S, Sritharan N et al (2018) Physiological efficiency of small millets under drought condition. Madras Agric J 105(7–9):363–367. https://doi.org/10.29321/MAJ.2018.000161

Sharma R, Girish AG, Upadhyaya HD et al (2014) Identification of blast resistance in a core collection of foxtail millet germplasm. Plant Dis 98:519–524

Singh S, Chauhan ES (2019) Role of underutilized millets and their nutraceuticals importance in the new era—a review. Int J Sci Res Rev 8(2):2844–2857

Singh P, Raghuvanshi RS (2012) Finger millet for food and nutritional security. Afr J Food Sci 6(4): 77–84

Singh E, Sarita SE (2016) Potential functional implications of finger millet (*Eleusine coracana*) in nutritional benefits, processing, health and diseases: a review. Int J Home Sci 2:151–155

Singh RK, Singh VK, Raghavendrarao S et al (2015) Expression of finger millet EcDehydrin7 in transgenic tobacco confers tolerance to drought stress. Appl Biochem Biotechnol 177:207–216. https://doi.org/10.1007/s12010-015-1738-4

Sood S, Joshi DC, Chandra AK et al (2019) Phenomics and genomics of finger millet: current status and future prospects. Planta 250:731–751

Sood S, Joshi DC, Pattanayak A (2020) Breeding advancements in barnyard millet. In: Gosal SS, Wani HS (eds) Accelerated plant breeding, cereal crops. Springer Nature, Switzerland, pp 391–410

Stanly JM, Shanmugam A (2013) A study on millets based cultivation and consumption in India. Int J Market Finan Serv Manag Res 2(4):49–58

Sudhakar C, Veeranagamallaiah G, Nareshkumar A et al (2015) Polyamine metabolism influences antioxidant defense mechanism in foxtail millet (*Setaria italica* L.) cultivars with different salinity tolerance. Plant Cell Rep 34:141–156. https://doi.org/10.1007/s00299-014-1695-3

Tester M, Langridge P (2010) Breeding technologies to increase crop production in a changing world. Science 327:818–822

Upadhyaya HD, Ravishankar CR, Narasimhudu Y et al (2011) Identification of trait-specific germplasm and developing a mini core collection for efficient use of foxtail millet genetic resources in crop improvement. Field Crop Res 124:459–466

Upadhyaya HD, Vetriventhan M, Dwivedi SL et al (2015) Proso, barnyard, little and kodo millets. In: Singh M, Upadhyaya HD (eds) Genetic and genomic resource of grain cereal improvement. Academic, Oxford, pp 321–343

Veeranagamallaiah G, Ranganayakulu GS, Thippeswamy M et al (2009) Aldose reductase expression contributes in sorbitol accumulation and 4-hydroxynon-2-enal detoxification in two foxtail millet (*Setaria italica* L.) cultivars with different salt stress tolerance. Plant Growth Regul 59: 137–143. https://doi.org/10.1007/s10725-009-9396-6

Vetriventhan M, Upadhyaya HD (2018) Diversity and trait-specific sources for productivity and nutritional traits in the global proso millet (*Panicum miliaceum* L.) germplasm collection. Crop J 6:451–463

Vetriventhan M, Upadhyaya HD, Dwived SL et al (2016) 7-Finger and foxtail millets. In: Singh M, Upadhyaya HD (eds) Genetic and genomic resources for grain cereals improvement. Academic, San Diego, pp 291–319. https://doi.org/10.1016/B978-0-12-802000-5.00007-1

Vetriventhan M, Azevedo VCR, Upadhyaya HD (2020) Genetic and genomic resources, and breeding for accelerating improvement of small millets: current status and future interventions. Nucleus 63:217–239

Vinoth A, Ravindhran R (2017) Biofortifcation in millets: a sustainable approach for nutritional security. Front Plant Sci 8:29

Viswanath V, Urooj A, Malleshi NG (2009) Evaluation of antioxidant and antimicrobial properties of finger millet polyphenols (*Eleusine coracana*). Food Chem 114(1):340–346. https://doi.org/10.1016/j.foodchem.2008.09.053

Vodouhe R, Dako GA, Dansi A (2007) Fonio: a treasure for West Africa. In: Plant genetic resources and food security in west and central Africa, Regional conference, 26–30 April 2004, vol 472. Bioversity International, p 219

Wang M, Pan Y, Li C et al (2011) Culturing of immature inflorescences and *Agrobacterium*-mediated transformation of foxtail millet (*Setaria italica*). Afr J Biotechnol 10:16466–16479

Wang C, Jia G, Zhi H et al (2012) Genetic diversity and population structure of Chinese foxtail millet [*Setaria italica* (L.) Beauv.] landraces. G3 2:769–777. https://doi.org/10.1534/g3.112.002907

Wang X, Chen S, Ma X (2021) Genome sequence and genetic diversity analysis of an underdomesticated orphan crop, white fonio (*Digitaria exilis*). Gigascience 10:Giab013

Wankhede DB, Shehnaj A, Rao MR (1979) Carbohydrate composition of finger millet (*Eleusine coracana*) and foxtail millet (*Setaria italica*). Plant Foods Hum Nutr 28:293–303

Yang X, Yu X-Y, Li Y-F (2013) De novo assembly and characterization of the Barnyard grass (*Echinochloa crus-galli*) transcriptome using next-generation pyrosequencing. PLoS One 8: e69168. https://doi.org/10.1371/journal.pone.0069168

Yoshida S (1972) Physiological aspects of grain yield. Annu Rev Plant Physiol 23:437–464

Zandalinas SI, Fritschi FB, Mittler R (2021) Global warming, climate change, and environmental pollution: recipe for a multifactorial stress combination disaster. Trends Plant Sci 26:588–599. https://doi.org/10.1016/j.tplants.2021.02.011

Zhang L, Liu R, Niu W (2014) Phytochemical and antiproliferative activity of proso millet. PLoS ONE 9:e104058. https://doi.org/10.1371/journal.pone.0104058

Zhang W, Wang B, Liu B et al (2022) Trait selection for yield improvement in foxtail millet (*Setaria italica* Beauv.) under climate change in the North China plain. Agronomy 12:1500. https://doi.org/10.3390/agronomy12071500

Reproductive Biology, Genetics, Evolution, and Diversity in Finger Millet (*Eleusine coracana* (L.) Gaertn.)

9

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Abstract

Finger millet (*Eleusine coracana* (L.) Gaertn.) is a nutrient-dense climate-resilient major grain crop in Asian and African subcontinents. Being future smart food crop, its production will help in improving the livelihood of people by improving nutritional status. Thus, it is essential to understand its floral biology, genetics, origin, evolution, and genetic diversity to achieve the goal of nutritional security in the developing countries. The origin of finger millet traced back to 5000 years, and Africa and India were recognized as the primary and secondary centers of diversity. Finger millet is a highly self-pollinated allotetraploid crop with as low as 1% cross-pollination aided by wind; thus, artificial hybridization techniques can permit its further improvement immensely. Identification of modern emasculation techniques like chemically hybridizing agent (ethrel), hot water emasculation, cold water, and plastic bag method transformed from traditional to modern production system due to complex architecture and tiny nature of florets of finger millet. Study of genetic diversity for identification of resistance against various biotic and abiotic stresses as well as high calcium, zinc, and iron-containing species helps in classification and improving the overall production aspect of finger millet. Thus, it is vital to preserve the germplasm for the conservation of genetic diversity and understanding the crop in-depth. This chapter discusses the details about finger millet origin, evolution, floral biology, and cytogenetics, and genetic diversity also highlights the germplasm for overall improvement of finger millet.

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Keywords

Finger millet · Genetics · Floral biology · Origin · Evolution and diversity

In the field of applied science like crop improvement, where genetic variation is a prerequisite for any improvement in a crop understanding, evolutionary biology will only enhance the opportunities in plant breeding and improve the efficiency of strategies for crop improvement. However, our understanding of crop evolution from a place of origin through domestication, adaptation, and selection remains limited which slows the improvement process. Crop evolution involves domestication of wild plants and its subsequent adaptation to a new environment outside their ancestor's origin during their spread all over the globe.

9.1 Introduction

Finger millet (*Eleusine coracana* (L.) Gaertn.) is a minor millet having great nutritional, cultural, and historical importance, particularly in the Asian and African regions. It is commonly known as *ragi* in India. It is documented to have its origin in Asia and Africa's arid and semiarid tropics, where it is grown for food, fodder, and medicinal purposes (Phillips 1974). It has been reportedly used as a traditional treatment for a number of illnesses, including liver disease, measles, pleurisy, pneumonia, and small pox, in Africa and India. It has high nutritional value directly comparable to wheat and rice, hence finds its importance as nutrient-rich grain super food in today's health-conscious world. The name finger millet is derived from its shape of panicles, which takes the form of several fingers. After sorghum, pearl millet, and foxtail millet, finger millet is the fourth most significant millet in the world (Sood et al. 2019). Apart from this, finger millet is a resilient major grain crop with the prospect of upscaling the nutritional status and livelihood of people around the word especially of the developing countries. India is the largest producer with an area, production, and productivity of 1.14 million hectares, 1.52 million tons, and 1661 kg per ha, respectively (PIB report 2021). This crop can be grown in a wide range of climatic conditions. It can be grown in a hot weather (long duration cultivars) as well as in a cold season also (early maturing varieties). This crop is also tolerant to various biotic and abiotic stresses like disease, fungi, insects, mildew, and viruses. It can be grown in soil condition like alkali, salt, slope, drought, and laterite with pH variation 5.0–8.2. Finger millet or *ragi* is an allotetraploid crop ($2n = 4x = 36$). The genus *Eleusine* includes nine diploid and can be either annual or perennial herbs. The cultivated species also have several races and subraces, making finger millet a highly variable and diverse crop for agronomically important traits such as growth habit, good vigor, large panicle size, numbers of finger, and branching and high-density of grain filling.

Therefore, studying the germplasm diversity of the finger millet will be rewarding for the selection of superior genotypes and will make way for a successful breeding program for the crop improvement. Understanding the biology of the plant and identifying the existing variability and diversity in the species is crucial for planning a successful breeding program. This chapter reviews the information and resources generated for finger millet, including the plant botany, floral reproductive biology, species characterization, wild relatives, and achievements through breeding.

9.2 Taxonomic Classification of Finger Millet

Rank	Scientific names
Kingdom	*Plantae*
Subkingdom	*Tracheobionta*
Superdivision	*Spermatophyta*
Division	*Magnoliophyta*
Class	*Liliopsida*
Subclass	*Commelinidae*
Order	*Cyperales*
Family	*Poaceae*
Genus	*Eleusine* Gaertn
Species	*Eleusine coracana* (L.) Gaertn.
Synonyms	*Ragi*, *madwa*, *nachani*, *mundua*, *mandika*, *Marawah*, *African millet*, *finger millet*, *goosegrass*

9.3 Origin of Finger Millet

Finger millet or *ragi* (*Eleusine coracana* (L.) Gaertn.), a domesticated cereal of African origin, was later introduced to Asia. Finger millet is an allotetraploid ($2n = 4x = 36$), which is majorly believed to have originated in the tropics (arid and semiarid) and subtropics (Fakrudin et al. 2004) of India, Myanmar, Sri Lanka, Nepal, China, and Japan in Asia, while in Africa, it is cultivated in Uganda, Kenya, Tanzania, Ethiopia, Eritrea, Rwanda, Zaire, and Somalia (Upadhyaya et al. 2010). The *africana* ssp. are found along the highlands of East Africa and the grasslands of Southern Africa. Some reports suggest that it is also found in India and the UK. Kennedy-O'Byrne (1957) was the first to recognize *africana* as a distinct species. Earlier De Candolle (1886), De Wildeman (1940), Greenway (1945), Cobley (1956), and Vishnu-Mittre (1968) believed that origin of *ragi* was India; however, during later years Portères (1951, 1970, 1958) and Mehra (1963a, b) suggested that it was of African origin. Vavilov (1951) reported that finger millet may have originated independently in India and Africa. However, later Fuller (2002, 2006) did an in-depth review on the origin of genus *Eleusine* and confirmed it to be of African origin. On the basis of claims, it was suggested the Indian origin of finger

millet was widely based on misidentified material of other species. Himalayas (India, Nepal, and southern China) appears to be a secondary center of adaptation since upland races of Asia are widely distributed here. According to Phillips (1972), the genus *Eleusine* comprises nine annual and perennial species of which eight are African and the remaining one is a New World species (*E. tristachya* Lam.) native to Argentina and Uruguay (Lovisolo and Galati 2007). East Africa is considered the center of diversity of the genus *Eleusine* and eight other species (*E. africana*, *E. coracana*, *E. kigeziensis*, *E. indica*, *E. floccifolia*, *E. intermedia*, *E. multiflora*, and *E. jaegeri*) (Mehra 1963a, b; Phillips 1972). Species under genus *Eleusine* are represented in Table 9.1.

It is now however accepted that evolution in cultivated finger millet species races occurred in Africa before its introduction into India. The exotic germplasm mainly from Africa was introduced to India during the 1970s. Race *coracana* is extensively grown across India and Africa and gave rise to all four other races of cultivated species through selection under domestication. Race *vulgaris*, *elongata*, *plana*, and *compacta* may have evolved separately in India and Africa under similar environmental conditions from race coracana.

In the cultivated species, comparatively less racial evolution took place in India (De Wet et al. 1984). Due to the high diversity of *E. coracana* in Africa and India and morphological similarity to both *E. indica* and *E. africana*, the place of origin of *E. coracana* has remained controversial.

9.4 Evolution of Finger Millets

Eleusine is an annual allotetraploid crop with chromosome and ploidy $2n = 4x = 36$, (AABB) that includes two distinct subspecies named *Eleusine coracana* ssp. *coracana* (L.) Gaertn. and *Eleusine coracana* ssp. *africana* (Hilu 1994; Hilu and de Wet 1976). The cultivated species is *coracana* and *africana* is a wild species. The cultivated *E. coracana* subsp. *coracana* is an allotetraploid ($2n = 4x = 36$, AABB) and is morphologically similar to two weedy species, *E. coracana* subsp. *africana* ($2n = 36$) and *E. indica* ($2n = 18$). These two species are widely sympatric in Africa, with *E. indica* extending to Asia. *E. indica* has been proved as the maternal diploid genome donor (AA) of *E. coracana* subsp. *coracana* as well as *E. coracana* subsp. *Africana* through various studies based on cytology, isozymes, RAPD, chloroplast DNA, and genomic in situ hybridization (GISH) which revealed that *E. indica* has contributed one of the genome (AA) to the genome (AABB) of cultivated *E. coracana* (Bisht and Mukai 2001a, b; Chennaveeraiah and Hiremath 1974; Hilu 1988, 1995; Hilu and de Wet 1976; Werth et al. 1994). The evolution of *Eleusine coracana* is shown in Fig. 9.1. In GISH experiments, Bisht and Mukai (2000) suggested *E. floccifolia* as the B genome donor to the polyploid species *E. coracana*. However, Neves et al. (2005) rejected this claim of *E. floccifolia* being the B genome donor to the species *E. coracana* which is a polyploid, based on nuclear internal transcribed spacers (ITS) and plasmid trnT-trnF sequences. According to Liu et al. (2014), paternal parent contributing B genome of *E. coracana* seems to have become

Table 9.1 Species under genus *Eleusine*. (Source: Mirza and Marla 2019)

Species	Growth Habit	Ploidy	Characteristics					References
			Leaf	Spikelet/ inflorescence	Florets	Rhizome	Others	
Eleusine floccifolia	Perennial	Diploid ($2n = 2x = 18$)	Thin whitish hairs found on leaf surface				Can be grown at high elevated areas	Phillips (1972, 1974, 1995), Sisay and Baars (2002)
Eleusine intermedia	Perennial	Diploid ($2n = 2x = 18$)	Leaf are small and soft with straight hairs on margins (pilose)	Spikelets are laxly arranged to resemble inflorescence	Three-nerved lemma with distinct one-nerved keel or ridge on the floret	Stout		Grassland Index (2009), Phillips (1972)
Eleusine jaegeri	Perennial	Diploid ($2n = 2x = 20$)	Leaves are pale green in color. Leaf margin is rough. Leaf have whitish overlapping leaf-sheath	Culms are aggressively branched abundantly to form thick and coarse tussocks or tuft to resemble the inflorescence			Grown commonly in grasslands of east African mountainous regions and unpalatable to livestock; used for basket making	Grassland Index (2009), Phillips (1972, 1974, 1995)
Eleusine kigeziensis	Perennial	Diploid ($2n = 2x = 38$)	Leaves are soft covered with long soft hairs. Hairs may be or may not be present	Inflorescence is generally long and open	Florets have lemma with a central (three-nerved keel) and two inconspicuous lateral nerves	Short slender ascending		Phillips (1972, 1974, 1995)

(continued)

Table 9.1 (continued)

Species	Growth Habit	Ploidy	Characteristics					References
			Leaf	Spikelet/ inflorescence	Florets	Rhizome	Others	
Eleusine multiflora	Annual	Diploid ($2n = 2x = 16$)		A dense cluster of 2–8 oblong to ovate, 1.5–3-cm-long spikes alternately arranged on a short axis at the apex of the culm				Hilu and Johnson (1997), Neves (2011) and Werth et al. (1994)
Eleusine tristachya	Annual	Diploid ($2n = 2x = 18$)		Digitate inflorescence with densely grouped flowers at the top of the axis	Short, oblong spikes with spikelets neatly placed perpendicular to the spike axis		Important as a forage grass. Only species native to South America	Clayton et al. (2009), Ellis et al. (2004), Hansen (1980), Hilu (1980, 2003), Elorza et al. (2001), Lovisolo and Galati (2007), Phillips (1972, 1974, 1995)
Eleusine indica	Annual	Diploid ($2n = 2x = 18$)		Short shattering spikelets with small seeds covered inside	Short glumes and thin lemma (three-nerved lemmas with a three-nerved keel)		Known as goosegrass; as a major weed worldwide	Phillips (1972), Neves (2011)

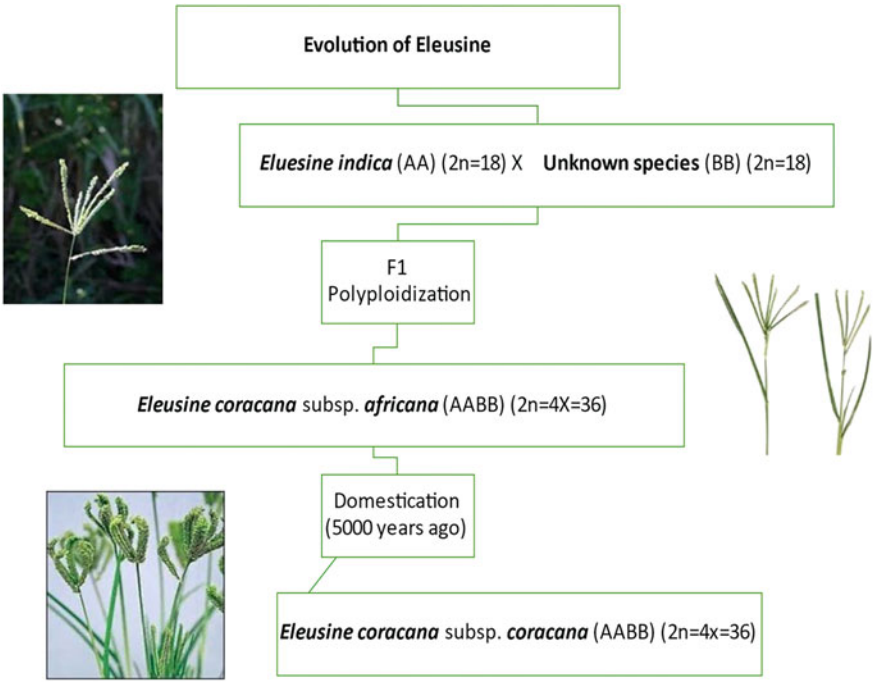

Fig. 9.1 Evolution of *Eleusine coracana*

extinct. Later, after considering the morphological similarities and studies of chromosome numbers and genome size, it was considered as a subspecies of *E. indica* (Lye 1999; Phillips 1972, 1974). When attempts to cross the tetraploid *E. africana* ($2n = 4x = 36$, AABB) and the diploid *E. indica* ($2n = 2x = 18$, AA) were made, it resulted in sterile plants (Chennaveeraiah 1973; Hiremath and Salimath 1992). On the other hand, *E. africana* was observed to hybridize ssp. *coracana* naturally and hence classified as a subspecies of *E. coracana*. *E. africana* ($2n = 36$), also tetraploid, resembles *E. coracana* in morphological characteristics. *E. coracana* possibly originated from *E. africana* through selection and further mutation which resulted in larger grains since both *E. coracana* and *E. africana* are related genetically and the hybridization occurs naturally (Hilu and De Wet 1976).

9.5 Diversity in Finger Millet

Finger millet has a rich diversity, and large collections are conserved in long-term storage in various gene banks across the globe. *Eleusine coracana* is highly variable in their centers of origin in Africa and the Indian subcontinent. The gene pool of finger millet is represented in Fig. 9.2. The species *E. coracana* comprises subspecies *africana* and *coracana*. The subspecies *africana* is a wild type and consists of

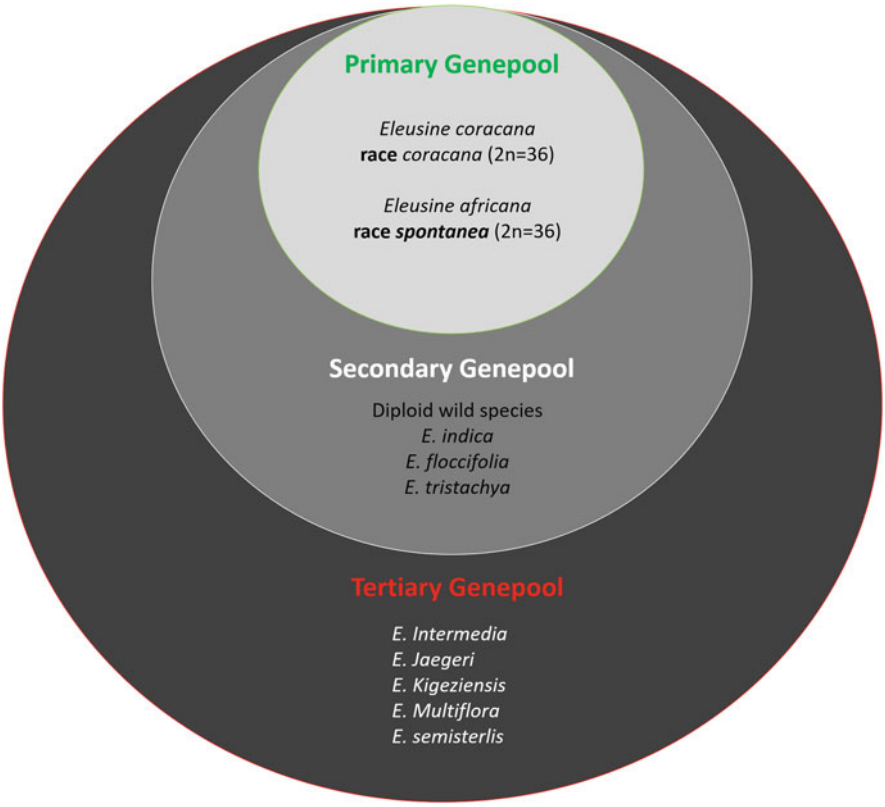

Fig. 9.2 Gene-pool of finger millet

africana and *spontanea* races. The subspecies *coracana* is a cultivated type and is further classified into four races, namely, *elongata*, *plana*, *compacta*, and *vulgaris* based on the type of inflorescence (Prasada Rao et al. 1993).

The race *Elongata* has three subraces, *laxa*, *reclusa*, and *sparsa*; *Plana* also consists of three subraces, i.e., *seriata*, *confundere*, and *grandigluma*, while the race *Compacta* has no subrace, while the race *Vulgaris* has four subraces, *liliaceae*, *stellata*, *incurvate*, and *digitata* (Prasada Rao et al. 1993). The classification of the subspecies and races of *E. coracana* is represented in Tables 9.2 and 9.3 and Fig. 9.3.

9.6 Botanical Description

Finger millet has an annual or perennial erect, decumbent, and prostrate growth habit. The plant is robust, free tillering, and annual tufted grass tall up to 170 cm. The culms are compressed and green colored, and their nodes are glabrous with hollow or solid internodes. The lower side internodes are short, the longest being the terminal node carrying the inflorescence. From the top nodes, culms frequently branch to

Table 9.2 Different subspecies of *E. coracana*

Subspecies	No. of races	Origin	Growth habit	Characteristics					Remarks	References
				Leaf	Spike/ inflorescence	Spikelets	Florets	Grain		
Africana	Two races (*Africana* and *spontanea*)	Highlands of East Africa and the grasslands of Southern Africa	Annual plant that grows in dense tufts	Long	Thick and long spikes, nearly 135-cm-tall flowering culms and a long, open spike make up the inflorescence branch	Spikelets are organized in rows of two on each side of the rachis	Ascending culms that are geniculate and start branching at the lower nodes, Glumes are shorter than spikelet and are lanceolate-oblong, usually less than 5 mm and narrow-winged along the keel, the ligule has a clear ciliate fringe	Granular surface grain with barely visible ridges	Subspecies *Africana* have similar morphology to *E. indica*	Bharathi (2011), Chen and Phillips (2006), Chennaveeraiah (1973), Phillips (1972, 1995)
Coracana	Four cultivated races (*elongata*, *plana*, *compacta*, *vulgaris*)		Annual		Inflorescence branches are slender to robust. Inflorescences are often digitated or sub-digitate, with one or more racemes	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	Culms usually branches from the top nodes to produce secondary inflorescences	No true caryopsis instead it have an utricle. The grains are globose with black, brown, red or even white color. Grains are exposed between the florets in the non-shattering spikelets when ripe		McDonough et al. (1986), De Wet et al. (1984), Hilu and de Wet (1976), Upadhyaya et al. (2007), Phillips (1972, 1974, 1995), Dida and Devos (2006)

Table 9.3 Different races of different subspecies of *E. coracana*

Sub species	Type		Races	Found in	Description
E. coracana spp. *africana*	Wild type	1	Africana	Burundi, Uganda, Malawi, Tanzania and Ghana	Plants can grow up to 165 cm tall and can be erect, prostrate, or decumbent. This race has the long open inflorescence and panicle bearing 4–14 fingers which extend 14 cm in length. Generally the grains are brown to dark brown color
		2	Spontanea	Kenya, India, and also in the UK	Plants are upright and can either be green or colored. *Spontanea* is capable of standing at a maximum height of 120 cm. The fingers are often incurved and measure 6–9 cm. The inflorescence typically has 6–8 fingers, however sometimes 10 fingers also reported. The color of grains is light to dark brown
E. coracana spp. *coracana*	Cultivated type	1	Elongata	India, Nepal, Zimbabwe, Nigeria, Uganda, and South Africa	Digitate inflorescence with long, slender, and spreading spikelets. The grains color ranges from light brown to reddish and dark brown. It comprises three subraces, laxa, reclusa, and sparsa. Both erect and decumbent plant kinds can be found in laxa. Reclusa's fingers are open or curled at the top and are rather short (12 cm). Typically, sparsa inflorescences are pendulous (drooping). It has multiple branches and lengthy fingers up to 14.8 cm in length (7–13)
		2	Plana	India, Zimbabwe, Kenya, Nigeria, Uganda, and Ethiopia	Plants are either erect or decumbent. Generally the plants are green in color and pigmented also. This race is distinguished by large spikelets (6–17 cm long) arranged in two or

(continued)

Table 9.3 (continued)

Sub species	Type		Races	Found in	Description
					more rows along the rachis, which give the inflorescence branch a flat, ribbon-like appearance. Plana also comprises three subraces: seriata, confundere, and grandigluma. Although short and open spikes are also known, seriata spikes are typically curved at the top and have 6–15 fingers. The grains range in color from light to dark brown. The spikes on confundere are curled at the top and often have 6–7 fingers. However, reports from Uganda indicate that there might be up to 23 panicle branches per plant. The grains are mostly reddish brown in color. The grandigluma subrace is characterized by large, pointed glumes and extremely long fingers up to 17 cm. The spikes are top-curved and have 5–10 fingers. The grains range in color from mild to reddish brown
		3	Compacta	Asia	The growth characteristics of the plants are both erect and decumbent, and they are both green and colored. They can contain 5–14 spikelets (often 6–8), and since the tips are typically in-curved, the inflorescence matures looking rather compressed or semi-compact. (Guarino 2012). It has four subraces. Both the stellata and the incurvata subraces have in-curved fingers, with the incurvata subrace having more of an appearance of a fist. Both subraces grains

(continued)

Table 9.3 (continued)

Sub species	Type		Races	Found in	Description
					are predominantly brown or reddish brown in color. In subrace liliaceae, the fingers are reflexed or short open, but in subrace digitata, the fingers are top-curved. The grains of the subrace digitata are ragi brown, ranging in color from reddish brown to dark brown
		4	Vulgaris	India, Kenya and Uganda	In Asia and Africa, they are known as cockscomb finger millet. The decumbent or erect plants are pigmented and green. The fingers (4–11) of the inflorescence axis are in-curved at the top to resemble a giant fist, and the inflorescence axis is separated at the base upward. The grains can be reddish, dark brown, or light brown

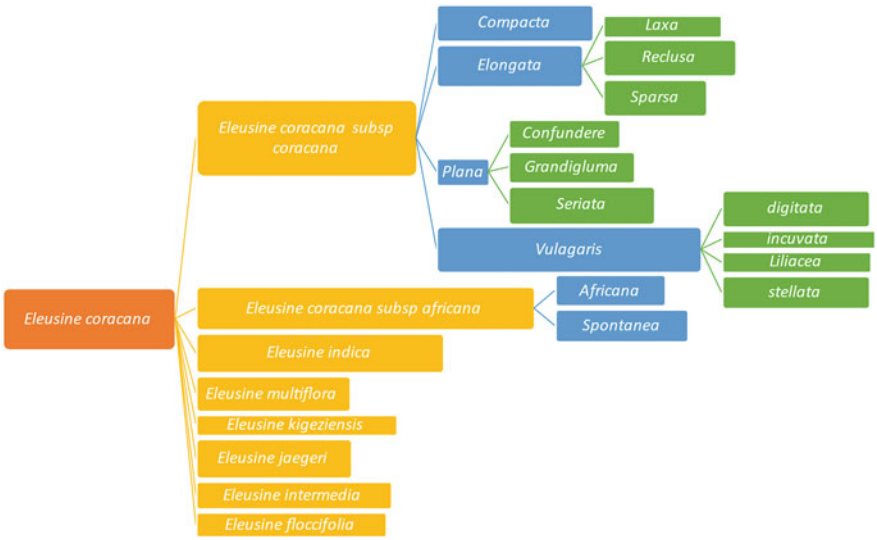

Fig. 9.3 Races and subraces of the species *Eleusine coracana*

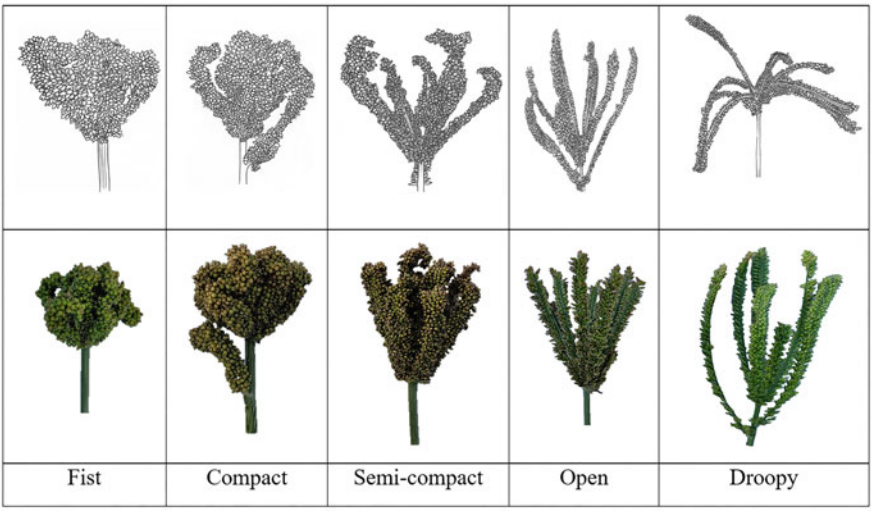

Fig. 9.4 Variations in head shapes of finger millet. (Photo taken by Aachal Futane)

form secondary inflorescences. The leaf sheaths have noticeable keeling and flattening. The leaf blades are linear and tapered, narrow, flat, or folded with prominent midrib, fimbriate ligule of 1–2 mm, a fringe of hairs, and ciliated margins. There are four types of panicle shapes, namely, (1) fist, (2) compact, (3) semi-compact, (4) open, and (5) droopy as shown in Fig. 9.4.

The plant's inflorescence is a bunch of 5–26 fingers, composed of dense spikelets where the grain, or seed, is produced. The inflorescence comprises of spikes in a terminal umbel form and open digitate terminal whorl-bearing spikes. Spikelets are compressed laterally, and two curved rows are present in the outer side of the spike. Each spikelet has flowers/blooms and approximately 1000–3000 flowers per earhead (Fig. 9.5b, c). The opening of the florets and grain filling starts from bottom to top within the spikelets. The spikelet-bearing grains are typically covered by a thin, visible pericarp of brown color between the lemma and palea. Variations in head shapes and grain shapes of finger millet aids to differentiate closely related species (De Wet et al. 1984). The grains have globular shape. The endosperm is starchy and testa strongly adheres to the aleurone layer. The five-layered structure of testa of finger millet is kind of unique among the other cereal grains. The top surface has a thin autofluorescent layer. The inner layers contain tannins, while the outer layer contains phenolic compounds that autofluoresce. The finger millet plant possesses an active adventitious and very strong fibrous root system, making it difficult to take out of the soil. In a recent study by Mirza et al. (2014), various components and stages of developing inflorescence were described.

Fig. 9.5 Panicle at flowering stage. (**A**) Anthers are visible at florets tips, (**B**) flowering in semi-compact panicle, and (**C**) flowering in open type panicle

9.6.1 Floral Morphology and Breeding Behavior

Finger millet is a highly self-pollinated crop with as low as 1% cross-pollination. Its floral structure has been described by many researchers in the past. Coleman (1920) and Ayyangar (1932) described the flower as a bird foot due to its appearance and arrangement of the fingers bearing 2–10 whorls but only 5–6 spikes. At the end of a stem, the lowest spike arises called thumb (first claw of bird) which is parted by 2–5-cm distance from other spikes (fingers) represented in Fig. 9.6. Emrey (2022) identified the following floral structures of finger millet as each finger millet plant

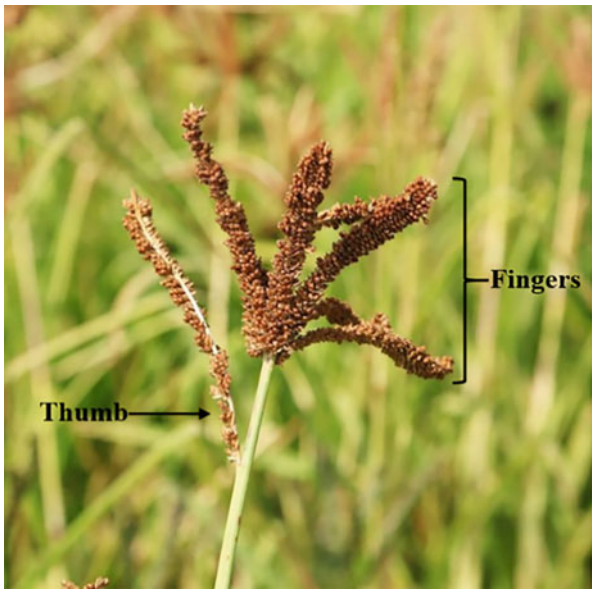

Fig. 9.6 Inflorescence with fingers and thumb. (Source: https://www.genebanks.org/resources/crops/finger-millet/)

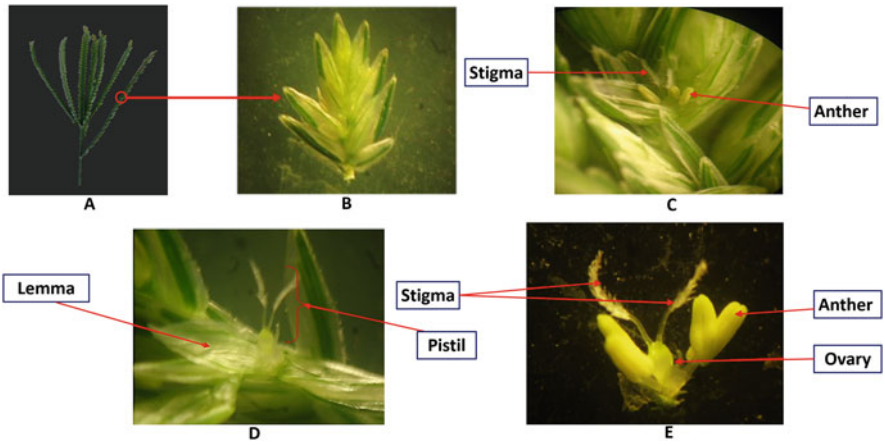

Fig. 9.7 Floral biology. (**A**) Inflorescence (fingers), (**B**) floret or spikelet, (**C**) floret within lemma and palea, (**D**) Pistil (female part) with ovary and stigma, (**E**) Anthers and pistil without lemma and palea. (Source: Emrey 2022)

has several fingers (inflorescence), each finger has in turn several spikelets, each spikelet further contains florets, and each floret has three anthers around the stigma as you observed under a microscope as illustrated in Fig. 9.7a, b. The inflorescence consists of straight or little curved fingers or spikes with a whorl having 3–11 digitate (Gupta et al. 2011a, b). The inflorescence is supported by a peduncle from which spikes extend in the shape of a whorl that resembles fingers.

In each finger, there are about 70 spikelets, each spikelet having 2–13 complete flowers. Florets are hermaphrodite (bisexual) except terminal florets which are sterile in nature. Florets consist of the lemma, palea, stamens, pistil, anthers, and ovary making it the perfect flower, and an earhead contains about 1000–3000 flowers (Patroti and Gowda 2015).

The fully matured flower consists of three stamens, short or big anthers, and a longer or shorter filament—illustration of female and male parts of flower Fig. 9.7d, e. Stigma is white or brown colored with a branched structure; styles are free to their bases with the glabrous ovary.

The florets are in the axil of the lemma, and the lemma is boat-shaped with small appendages. Lemma and pelea are awnless having three and two nerves, respectively. The floret is covered by two large unproductive or barren glumes, enclosed between a pair of palea (Fig. 9.7c; Emrey (2022)). Palea is short with two lodicules and the androecium almost surrounds the stigma, ensuring self-pollination having three long or short filamentous stamens with non-penicillate anther and the gynoecium is bi-carpellary, unilocular with superior ovary having two styles with plumose stigma (Ganapathy 2017).

9.6.1.1 Anthesis and Pollination

In finger millet the mechanism of anthesis and pollination were thoroughly examined by various researchers in the past decades like Ayyangar (1932), Ayyangar and Wariar (1934), Chavan et al. (1955), Chavan and Shendge (1957), and Emrey (2022). According to Ayyangar and his team, the inflorescence takes around 10 days to emerge fully. The anthesis (first opening of the flower) occurs between 1.00 a.m. and 5.00 a.m. As soon as lemma and palea start to separate, the stigma and anthers appear almost synchronously. The anthers dehiscence longitudinally and occurs before the florets' opening (Emrey (2022)). Soon after the dehiscence of anthers, the flower is observed to be closed with no traces of stigma (Gupta et al. 2011a, b). The inflorescence appears yellow after anthers emerge and the florets grow more distinct and clearer. The flowering in the finger millet coincides in all the florets. One floret in a spikelet opens each day. A spikelet opens from top to bottom in an acropetal manner, whereas a floret within a spikelet opens in a besipetal manner from the bottom toward the top. The florets are compact before anthesis, and the androecium and gynoecium are tiny, densely clustered, and pale in color (Mirza and Marla 2019). Generally, the flowering gets completed in 3–4 days, but due to variation in temperature and humidity, the flowering may vary from 5 to 8 days (Patroti and Gowda 2015). After the flowering initiation, maximum flower opening is seen on the third day, while the florets at the terminal end remain sterile.

At the time of dehiscence inside the flower, the oblong anthers and sticky stigma attained the same height to ensure self-pollination. The anthers burst to pollinate their stigmas. The pollen viability remains only for 15–20 min, while the stigma is receptive for up to 5 h (Dodake and Dhonukshe 1998). Therefore, this mechanism and pollen and stigma characteristics do not permit cross-pollination beyond 1% in finger millet. Anthers become yellowish and inflorescence due to pollens, and the ovary became swollen after the anthesis as other parts of androecium and gynoecium

gradually increase in size. After flower opening, feathery stigma and anthers get clearly visible at the floret's tips (Fig. 9.5a).

9.6.1.2 Emasculation and Pollination Techniques

In millets, generally the flowers are tiny and complex; hence, the emasculation is very critical and tedious. The skillful act of the removal of stamens or anthers or the killing of pollen grains without affecting the female reproductive organ is known as emasculation. The floral architecture of finger millet makes it very difficult to emasculate and hybridize. In sorghum, the immature anthers from the florets, which are most likely to open the following morning, are forcibly removed from flowers using forceps or needles making hand emasculation feasible. However, due to the tiny size and complexity of the florets, these techniques are challenging with finger millet and other minor millets, so the contact method of hybridization is generally followed, whereas the success rate of more than 50% can be achieved by hand emasculation method (Shailaja et al. 2010). The technique of altering the temperature and humidity to open the flowers is also used where the fingers are gently sprinkled with cold water and after smoothly bagging them airtight with the help of polythene bags. As the glumes start to open, the exposed anthers are carefully removed without harming any female parts of the flowers, i.e., stigma, hence completing the emasculation (Ganapathy 2017). The pistil is very sensitive to dry environment; hence, it needs to be sprinkled with cold water to avoid drying in the emasculated fingers. Pollination is the key to make the hybridization successful. The desired male parent is selected, and the pollens are dusted on the emasculated fingers for pollination. For this the emasculated female and pollinating male parent panicles are tied together to achieve the hybridization by intertwining the fingers of the male panicle inside the female panicle and covering them with appropriate size butter paper bags to avoid any external pollen contamination. For identification of the true F_1 hybrids, the male-pollinating parent is chosen wisely with pigmentation on the nodes for easy identification of hybrids (Gupta 2006).

Hand Emasculation

Day 1: For proper hand emasculation, the new panicles come out from the leaf sheath are selected (Fig. 9.8a). The hand emasculation should be performed in the evening hours**,** generally within 3–5 days of the emergence of panicles. Panicles that were likely to open the next day should be selected. The selected panicles must be health and immature. The tips of the selected panicle required to be cut off using sterilized scissors (Fig. 9.8b). Then the panicles must be sprinkled with cold water and firmly covered with a polythene bag (Fig. 9.8c; Step 1).

Day 2: Emasculation is carried out in the morning hours between 3 a.m. and 6 a.m. because at this time the pistil slowly opens and the glumes contains premature anthers.

Using sterile fine-tipped forceps, alternate spikelets should be removed from each finger. (Fig. 9.8c; Step 2). The sharp sterile needles and magnifying glass should be

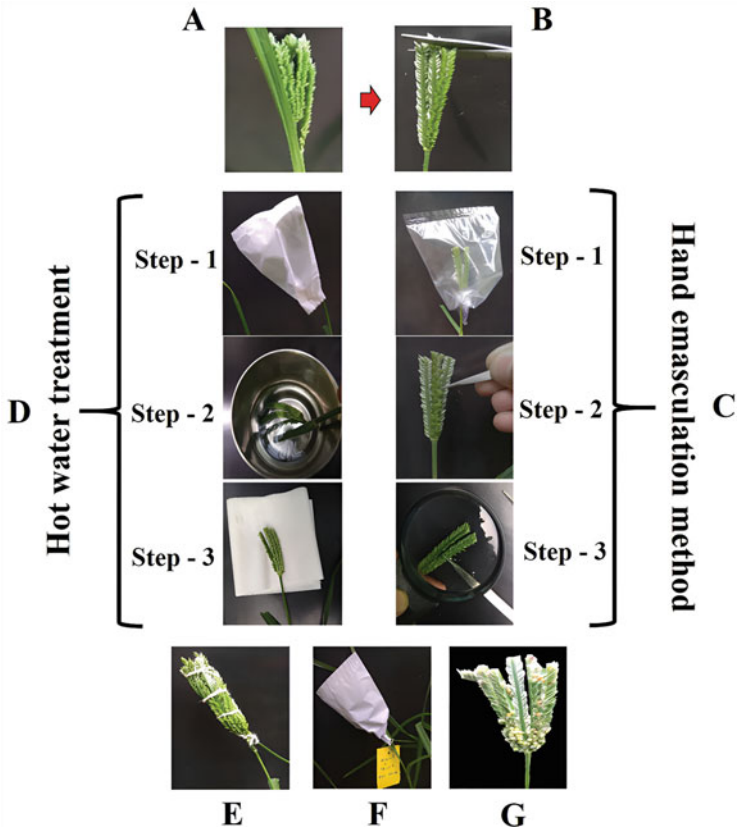

Fig. 9.8 Crossing technique for finger millet illustrated employing hand emasculation and hot-water emasculation methods. (**A**) Female parent: panicle initiation from leaf sheath. (**B**) Female parent: the tips of fingers are removed/cut using scissors by maintaining only 2–3 fingers per panicle. (**C**) Hand-emasculation method: Step 1: Spraying the cold water on spikelets of female parent and covering it air-tightly with polythene bag. Step 2: Using fine-tipped forceps to remove alternative spikelets of the female parent. Step 3: Anthers are carefully removed with the help of a magnifying glass lens and fine needle from the female parent. (**D**) Hot-water treatment: Step 1: Panicles of female parents are covered with butter paper bags. Step 2: The hot water of 52°C is taken into flask and panicles are immersed for 2–5 min. Step 3: Removal of water content from the inflorescence in female parent using tissue paper. (**E**) Male inflorescence and emasculated female parent inflorescence are tied together of with a thread. (**F**) Butter paper bags are placed over the inflorescences of both flowers to prevent the cross-pollination by foreign pollen grains and to ward off insect attack. (**G**) Seed setting after pollination at 35–40 days after crossing. (The photographs taken from Krishna et al. 2020)

used to open the glumes of carefully selected flowers/florets, and anthers are removed without injuring the stigma (Fig. 9.8c; Step 3).

As the pistil get dry quickly, spray the cold water to maintain the moisture. Now the panicles are ready for crossing.

Hot-Water Treatment

Emasculation is a crucial step for finger millet as it is a bisexual plant. Hot-water-based emasculation is used to make female parents sterile. In hot-water emasculation, the appropriate stage panicles of female parent should be selected and immersed in hot water for 2–5 min. The water temperature should be 48–52°C depending on the location and climatic conditions of the area to make emasculation effective. Inducing male sterility in finger millet by treating the inflorescence (3–4 days after emergence) with hot water at 52°C for 5 min has been successful (Gupta et al. 2011a, b).

The steps in this process are described below in detail:

Day 1: Panicles expected to flower in 2–3 days are likely selected, and the tips are excised with sterile scissors (Fig. 9.8a) (premature stage of anthers), and the panicles are properly covered in butter paper bag (Fig. 9.8d; Step1). This process should be done evening (Fig. 9.8b).

Day 2: In the morning hours of the next day between 3 a.m. and 6 a.m., hot-water emasculation is performed. For emasculation, most of fingers are removed leaving only 3–4 fingers. Take the hot water with temperature 48–52°C in the thermoflask-like container to maintain the constant temperature. Then immerse the whole panicle into the hot-water container for 2–5 min (Fig. 9.8d; Step 2). Then remove the panicles, and completely dry them using tissue paper (Fig. 9.8d; Step 3). This will impart the flower opening and pollination can be carried out.

Use of Chemical-Hybridizing Agents

Chemical-hybridizing agents are used to make plant sterile by killing or inactivating the stamens of the flowers. These are also called androcides or male gametocides, generally used in self-pollinating plants.

There are many different CHAs present, but only a few are effective for finger millet emasculation. Ethrel and GA3 are found to be suitable to some extent for emasculation in finger millet. Application of ethrel at proper concentration at the proper stage of the crop is essential to achieve effective emasculation. Studies have been conducted to understand the effect of ethrel for emasculation at different concentrations ranging from 1000 to 3000 ppm. Ethrel application at 2000 ppm concentration at the booting stage was most effective for emasculation (Kunguni 2016). Ethrel cause male sterility of between 15% and 38% on finger millet when applied at concentrations of between 1500 and 2000 ppm at DS 45 (Oduori 2008). The application of GA3 at the concentration of 1600 ppm at fifth leaf stage has been found reliable to create male sterility (Ganapathy 2017).

Though this emasculation technique is not highly successful, more studies on its concentration and time of application can make it reliable.

Use of Genetic Male Sterility

Emasculation using genetic male sterility involves the use of nucleus (GMS) or cytoplasmic male sterility (CMS) genes for making plants (female) sterile. Genetic male sterility aids in an effective hybridization program by making the pollens infertile by nuclear genes. Cytoplasmic male sterility genes halt pollen production due to a mitochondrial gene defect (House 1985). Techniques such as environment sensitive genetic male sterility (EGMS), photoperiod genetic male sterility (PGMS), and thermosensitive genetic male sterility (TGMS) are effective but not found yet in finger millet as present in rice.

9.6.2 Crossing

The crossing, i.e., pollination, can be executed distantly after emasculation. The un-emasculated male inflorescence which is healthy and mature enough can be directly used for pollination. Both the inflorescence, male (un-emasculated) and female (emasculated) are tied together (Fig. 9.8e) and immediately covered with a fresh butter paper bag (Fig. 9.8f) to complete the crossing process.

9.7 Genetics and Cytogenetics of Finger Millet

Both *Eleusine coracana* subsp. *coracana* and *E. coracana* subsp. *africana* possess tetraploid genomes with a somatic chromosomal count of $2n = 4x = 36$ chromosomes, denoted by the genomic notation AABB (Chennaveeraiah and Hiremath 1974; Hiremath and Salimath 1992). The A genome is derived from the diploid *E. indica*, while the origin of the B genome remains unknown (Hilu 1988; Bisht and Mukai 2001a). Nevertheless, research utilizing genomic in situ hybridization has revealed that the perennial *E. floccifolia* serves as the source of the B genome for the aforementioned tetraploids (Bisht and Mukai 2001a, b). Cultivated finger millet can cross-breed with *E. coracana* subsp. *africana* and *E. kigeziensis* ($2n = 4x = 38$), an allotetraploid endemic to southwestern Uganda and Rwanda (Phillips 1974). Subspecies africana is native to the African continent.

Compared to other main grains, finger millet's genome sequencing has been delayed. In contrast to rice, whose first draft genome was published in 2005 (International Rice Genome Sequencing 2005) and whose gene annotation was finished in 2013 (Sakai et al. 2013), the first draft genome of finger millet was published in 2017 (Hittalmani et al. 2017). Fewer genomic research studies are being conducted due to delayed genome sequencing and insufficient financial allocation. In the upcoming years, the draft genome will be a crucial tool for improving the genetics of finger millet. Currently, only two draft finger millet sequences are accessible in the NCBI database.

In comparison to rice, maize, and barley, finger millet has a very small amount of expressed sequence tags (ESTs). Compared to hundreds of other main crops, finger millet has just 139 gene sequences in the NCBI database. Several genome

Table 9.4 Genomic and associated data for finger millet in NCBI database

Database	Number	Database	Number
Genome	1	Protein	482050
Gene	132	Protein structure	3
dbSNP	0	Bio-assays	18
Identical protein Groups	55351	Pathways	0

assemblies are available for different cereals; however, finger millet (ASM218045v1) only has one. Similar to this, only three proteins from finger millet have had their structures determined (Strobl et al. 1995; Gourinath et al. 2000). Finger millet has not been improved using single nucleotide polymorphism (SNP) markers. There are just a few stress situations with high-grain Ca concentration and a small number of transcriptomes for finger millet. However, most studies have not yet validated differentially expressed genes and characterized them. To address issues in ragi, further work is required to collect the transcriptome of genotypes under biotic and abiotic challenges as drought, salinity, and blast. Finger millet's publicly available entire genome sequence would be a significant step toward finding and using other genome-dependent resources. For instance, the finger millet genome may be blasted with RNAseq readings to identify important genes and their roles in processes (Table 9.4).

9.7.1 Genome Sequences

Using Illumina and SOLiD (sequencing by oligonucleotide ligation and detection) sequencing technology, the whole genome of the finger millet genotype ML-365 (a blast-resistant, drought-tolerant cultivar with high cooking quality) was sequenced (Hittalmani et al. 2017). Nearly 45 Gb of paired end data and 21 Gb of mate-pair data were produced. In the assembled genome, there were 525,759 scaffolds (more than 200 bp), with an average scaffold length of 2275 bp and a N50 length of 23.73 kb (Hittalmani et al. 2017). In this work, plants of genotype ML-365 that were well-watered (WW) (53,300 unigenes) and mild moisture stressed (LMS) (100,046 unigenes) had their transcriptomes effectively sequenced and assembled. Using protein sequences from Viridiplantae, the UniProt database was used to annotate around 64% of the unigenes. According to gene expression analyses, there were 12,893 unique unigenes in the LMS condition, 2267 unique unigenes in the WW condition, and 111,096 common unigenes in both the LMS and WW situations. Protein-protein homology modelling was used to identify transcription factors (TFs), which revealed similarity with 56 of the recognized TF families. In comparison to rice and other plants of the Poaceae family, foxtail millet was shown to have greater colinearity of genes. According to this study's findings (Hittalmani et al. 2017), the wild cousin *E. coracana* subspecies *africana* and the farmed finger millet *E. coracana* subspecies *coracana* have identical genome sizes. This suggests that

E. coracana subspecies *africana* was the source of domesticated finger millet (Dida et al. 2008).

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9.7.2 QTLs for Agronomic Traits

In nature, yield traits essential for agronomy are controlled by multiple genes with minor cumulative effects or epistasis. Simple sequence repeats (SSR) markers were used to study agronomically important features in finger millet, including grain production, disease resistance, drought tolerance, and nutritional quality parameters. Identification of QTLs associated with nutritional attributes based on association mapping would benefit bio-fortification programs aimed at treating nutritional deficiencies. Using 23 anchored SSR markers, association mapping for calcium concentration in 113 genotypes identified 9 QTLs (Kumar et al. 2015). Four QTLs (UGEP81, UGEP24, FMBLEST32, and RM262) for blast resistance were discovered by Babu et al. (2014a, b) utilizing association mapping in 190 genotypes of finger millet and 104 SSR markers. Using 120 SSR markers, the tryptophan content quantitative trait loci (QTLs) OM5 and FM8 and the protein content QTLs FMO2EST1 were found, and it was found that these QTLs were connected to opaque2 modifiers (Opm) (Babu et al. 2014b). The two necessary amino acids for humans are lysine and tryptophan. Cereals lack these amino acids since they only contain 1.5–2% lysine and 0.25–0.5% tryptophan, but humans need 5% lysine and 1.1% tryptophan for adequate nutrition. Compared to cereals, finger millet has comparatively high tryptophan content. Further enhancing the quality of finger millet germplasm will benefit from the discovery of the QTLs connected to the Opm gene, which is responsible for the tryptophan content. Ramakrishnan et al. (2017) used association mapping under P-adequate and P-deficient settings to identify four QTLs (qLRDW.1, qLRDW.2, qHSDW.1, and qHRL.1) related to root dry weight, shoot dry weight, and root length. P shortage has a significant negative impact on shoot and root development throughout the seedling stage. It

would be necessary to confirm the discovered QTLs before using them for marker-assisted selection.

9.7.3 Functional Characterization of Important Genes

The identification of a gene's function is achieved through gene annotation research, which involves the use of related and model crop species. To verify gene activity, gene expression investigations are conducted. It is crucial to characterize key attributes of genes to breed enhanced varieties. In the case of finger millet, genomic research is uncovering candidate genes related to signaling and nutrition transport (Sood et al. 2016). When compared to other grains, finger millet has tenfold greater calcium content. Finding key genes and their signaling can aid in understanding the process and, if required, allow for the use of these genes in the bio-fortification of cereal crops.

9.7.3.1 Genes in Calcium Metabolism

A key nutrient needed for the growth and development of both plants and animals, including humans, is calcium. It comes from the earth and makes up the intermediate lamellae between two neighboring cells. The finger millet seed's aleurone layer has the highest concentration of calcium, followed by the seed coat and embryo (Nath et al. 2013). The expression of transporter genes involved in Ca signaling has been found to be inversely correlated with calcium levels (Carter et al. 2004). The transfer of nutrients between maternal and filial tissues occurs through apoplast since nutrients are not transmitted via the transpiration stream for developing embryos (Patrick and Offler 2001). So calcium levels of mature embryo or seed coat, which are thought to be controlled by Ca^{2+} transporter genes, might fluctuate in apoplast of the maternal tissue. It may be possible to transmit this feature to other millets grains by characterizing the genes involved in Ca buildup. CaM-stimulated type IIB Ca^{2+} ATPase, Ca^{2+}/H^{+} antiporter (CAX1), two CaM-dependent protein kinases (CaMK1 and CaMK2), and two pore channel 1 (TPC1) transcript levels have been analyzed in two contrasting genotypes of finger millet (GP-1, low Ca, and GP-45, high Ca) to determine their levels of expression (Mirza et al. 2014). Eighty-two Ca sensor genes were found in the transcriptome of growing spikes of the genotypes GP-1 and GP-45 (Singh et al. in 2014). In the spike samples of GP-45 and GP-1, respectively, it was discovered that the expression levels of 24 genes and 11 genes were increased. Seven genes in GP-45 that are highly expressed code for CaML, seven for CIPK, five for CBL, four for CDPK, and two for CRK. In the same genotypes, the gene CIPk24 was also studied (Chinchole et al. 2017). When compared to genotype GP1, the expression of this gene was found to be greater in the shoot, root, leaf, and growing spike tissues of GP-45. Through in silico analysis employing model plant genomes, nine single nucleotide polymorphisms (SNPs), one extra beta sheet domain in protein, and changes in protein localization in vacuoles between model crop plants were discovered. EcCIPK24 (GP-1) was shown to have a stronger affinity for the proteins EcCBL4 and EcCBL10 than EcCIPK24 (GP-45). It has been suggested that

elevating calcium levels in seeds can be significantly aided by EcCIPK24's activation of the EcCAX1b protein (Chinchole et al. 2017).

The effort of finding the genes involved for Ca metabolism in finger millet will go much more quickly now that the genome is available. Finger millet's Ca^{2+} transport pathways might be studied using reverse genetics for Ca metabolism. As the whole genome sequence for ragi is now available for site-directed mutagenesis to prevent off-target effects, gene editing techniques like CRISPR/Cas9 may be used to create mutations in putative genes thought to be involved for Ca transport and its buildup in grain (Ceasar et al. 2016). For comparable investigations, the CRISPR/Ca9 method has been effectively used in several crops.

9.7.3.2 Genes in Nitrogen Metabolism

Studies have been done to identify the main genes responsible for N transport in finger millet. Finger millet genotypes PRM-1, PRM-701, and PRM-801 were studied for the expression of PBF Dof (prolamin-binding factor DNA binding with one finger only), a transcription factor involved in the regulation of seed protein storage. These tissues included stem, root, and flag leaf at the vegetative stage as well as the development of spikes (Gupta et al. 2011a, b). The gene was more highly expressed in developing spikes than in other tissues across genotypes, according to the pattern of its expression. It was discovered that these genotypes' increased seed protein content was linked to enhanced PBF Dof expression during the early development stages (Gupta et al. 2011a, b). Other important genes, such as EcHNRT2 (*Eleusine coracana* high-affinity nitrate transporter), EcLNRT1 (*Eleusine coracana* low-affinity nitrate transporter), EcNADH-NR (*Eleusine coracana* nitrate reductase), EcGS (*Eleusine coracana* glutamine synthetase), EcFd-GOGAT (*Eleusine coracana* glutamine The findings revealed that, within 30 min of exposure to N deficit, all examined genes, with the exception of EcHNRT2, were expressed in the leaves of GE-3885. In the roots of GE-1437 and GE-3885, the gene EcNADH-NR was seen to be overexpressed and normally expressed, respectively, when plants were subjected to greater nitrate concentrations, but not in. According to their findings (Gupta et al. 2013), GE-3885 (high protein genotype) perceives nitrogen earlier than low-protein genotype. Dof1 and Dof2 exhibit antagonistic behavior in the regulation of genes involved in C and N metabolism, according to an investigation of EcDof1 and EcDof2 expression patterns in the same genotypes (GE3885 and GE1437). According to Gupta et al. 2014, roots from GE-3885 were found to have higher EcDof1/EcDof2 ratios than those from GE-1437, indicating more gene activation linked to N absorption and assimilation that resulted in higher grain protein accumulation.

9.7.3.3 Genes in Abiotic Stress Tolerance

Due to its excellent adaptation to tropical semiarid climate, finger millet is widely renowned for being drought-resistant. In an effort to enhance crops, efforts have been made to pinpoint the genes that are important for drought tolerance. Drought is the most significant abiotic stressor impacting agricultural ecosystem production. The finger millet gene EcDehydrin7 that responds to drought was identified (Singh

et al. 2015) and over-expressed in tobacco. EcDehydrin7-transfected tobacco plants demonstrated drought tolerance. In genotype GPU-28 under drought stress, it was discovered that additional genes sensitive to drought (metallothionein, farnesylated protein ATFP6, protein phosphatase 2A, and farnesyl pyrophosphate synthase) were overexpressed (Parvathi et al. 2013). Further research on these genes' expression patterns will aid in the discovery of distinctive signals involved in drought tolerance in finger millet. These genes are thought to have a significant role in drought tolerance. By searching a cDNA library of finger millet for orthologous sequences, a regulatory gene TBP associated factor6 (EcTAF6) responsible for drought response in finger millet was discovered. Ragi genotype GPU-28's expression levels in response to various stress exposures were examined. In vitro exposure to NaCl (salinity stress), PEG (osomotic stress), and methyl viologen (oxidative stress) was observed to increase the expression of the gene EcTAF6 relative to control conditions (Parvathi and Nataraja 2017). Additionally, genes have been discovered and verified in finger millet utilizing a drought responsive transcriptome by cDNA subtraction (Ramegowda et al. 2017). Ectopic expression of EcGBF3 in *A. thaliana* was used to identify the gene as a prospective candidate. In Atgbf3 mutant lines, high EcGBF3 expression in *A. thaliana* offered resistance to the aforementioned conditions (Ramegowda et al. 2017). Due to the difficulties in producing mutant lines in finger millet for functional genomics investigations, only *A. thaliana*, a model plant, was used for the expression analyses.

RNAseq was used to analyze the transcriptomes of the salt-tolerant variety Trichy 1 and the salinity-susceptible variety Co-12 under salinity conditions (Rahman et al. 2014). Rice's capacity to withstand salt and drought was enhanced by the overexpression of the EcNAC67 TF gene (Rahman et al. 2016). In the finger millet genotype GPU-28, transcription factors from the bZIP family (EcbZIP60) and basic helix-loop-helix (bHLH) family (EcbHLH57) were found to be in charge of the abiotic stress response in response to drought, osmotic, salt, and methyl viologen (MV) stresses (Babitha et al. 2015a, b). The CBL interacting protein kinase31 (EcCIPK31-like) gene for drought tolerance in finger millet was discovered and characterized (Nagarjuna et al. 2016). According to Ramakrishna et al. 2018, a new bZIP TF gene called EcbZIP17 that is exclusive to the endoplasmic reticulum of finger millet was identified and overexpressed in tobacco. Heat stress and salinity stress tolerance were displayed by the plants overexpressing EcbZIP17.

There are several areas of work with tremendous possibility in finger millet as it is a gold mine for nutrition enhancing and stress resistance genes. Identification and use of such genes would not only benefit crop improvement of finger-millet but also would serve as gene resource for other crops. Genome sequencing has been done; however, more sequencing efforts are needed for sequencing other varieties. This would help in targeting variation present in the crop for breeding at haplotype and gene level. The technologies like CRISPR/Cas require genome sequences for its proper use. More is needed to be done with respect to mutant and genetic studies. Millets such as ragi as a nutrient rich crop has to be given more attention as they have can ameliorate nutritional imbalance in human diet and address hidden hunger of the world.

9.8 Plant Genetic Resources

ICRISAT in Patancheru, India, manages a total of 5957 accessions in their global-in-trust collections. Among these accessions, 4585 are of non-Indian or exotic origin, including 105 wild species, 5665 landraces, 137 improved cultivars, and 50 breeding/research materials. Seven hundred sixty-six finger millet accessions, including 17 wild relatives (*E. floccifolia*, *E. indica*, *E. jaegeri*, *E. multiflora*, and *E. tristachya*), are kept at the USDA's Agricultural Research Station in Gryphon, Georgia. These collections are from 11 countries: Ethiopia, India, Kenya, Nepal, Pakistan, South Africa, Tanzania, Uganda, Zaire, Zambia, and Zimbabwe. Other South Asian countries like Nepal (877), Sri Lanka (393), and Bhutan (84) also hold significant collections. African regions such as Kenya (1902), Zimbabwe (1158), Uganda (1155), Zambia (497), Tanzania (293), Malawi (145), Eritrea (120), Burundi (113), Ethiopia (71), Nigeria (20), and South Africa (17) have reported their collections. Additionally, China (300), the Russian Federation (110), and Vietnam (52) are known to conserve finger millet collections. The database of finger millet accessions is maintained by Genesys portal online which shows classify the accession according to different parameters (Figs. 9.9, 9.10, 9.11, and 9.12).

Diversity in different traits among accession procures from different centers of diversity has been extensively studied and reported. In their study Bedis et al. (2006) reported a large variability among the germplasm studied for flowering, maturity, ear length, finger number, fodder, and grain yield, while Upadhyaya et al. (2007) observed extensive variability for plant pigmentation, growth characters, flowering, plant height, and length of inflorescence and width and grain color. Chemeda and Gemechu (2010), however, reported that the geographical origin had little to no

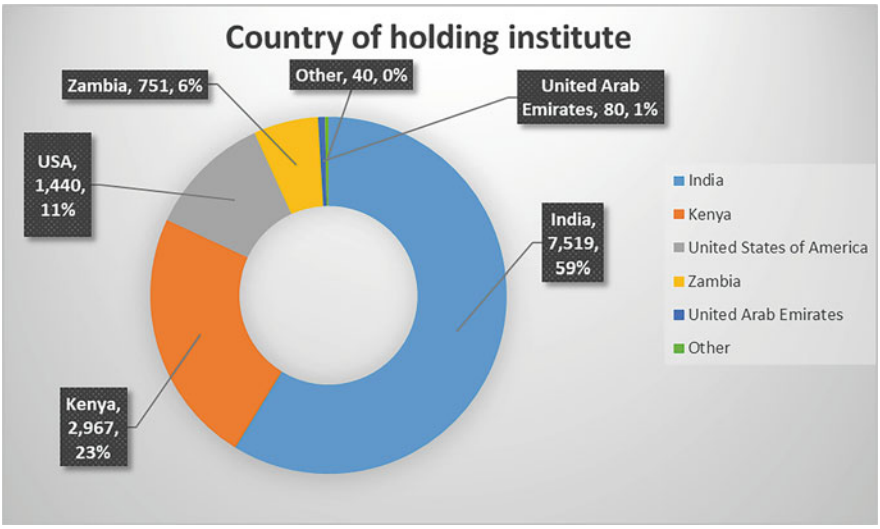

Fig. 9.9 Pie chart of countries that maintain accessions of finger millet

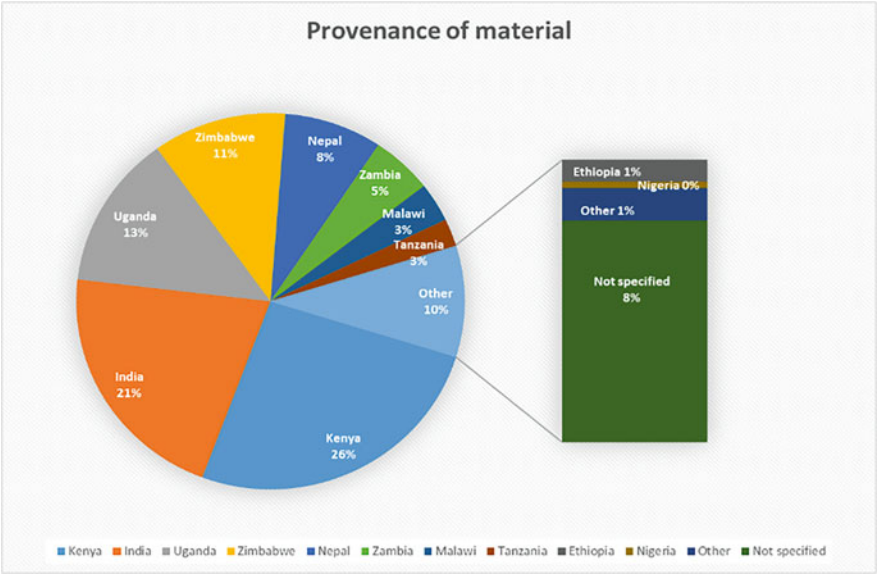

Fig. 9.10 Countries where accessions of finger millet are maintained

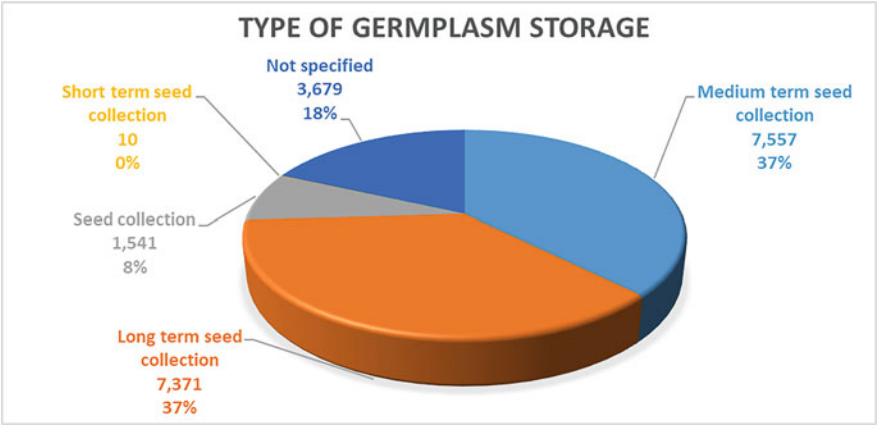

Fig. 9.11 Types of germplasm collection used for storing accessions of finger millet in the world

impact on Ethiopia's pattern of diversity in native and exotic collections. In addition they suggested that biomass, ear weight, and grain weight contributed more toward the observed diversity. Based on their research of 196 germplasm, Mnyenyembe and Gupta (1998) showed substantial variability for flowering, plant height, finger

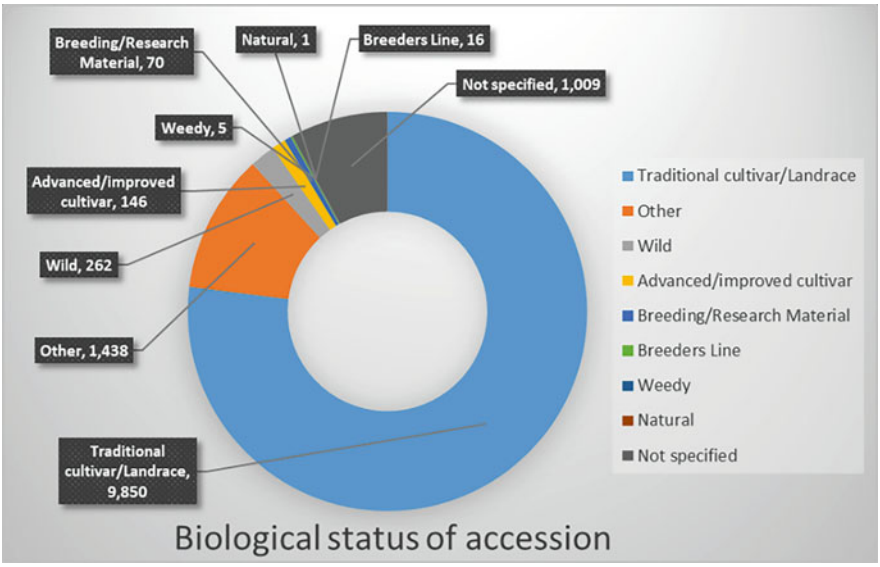

Fig. 9.12 The biological status of accessions of finger millet in world

length, finger breadth, number of fingers, number of productive tillers, panicle production, grain yield, and finger blast resistance. Reddy et al. (2009) characterized 5949 germplasm accessions for a wide range of qualitative and quantitative traits, with about 2000 of these accessions coming from Eastern Africa. They found significant variation for the days to flowering, which ranged from 50 to 120 days, with the majority of early flowering accessions coming from Burundi and late accessions coming from Ethiopia. Among the eastern African collection, green plant types were dominant over pigmented plants with a dominance of erect growth habit. The majority of lodging-resistant accessions were from Uganda. The east African germplasm was dominated by race *vulgaris*. Large ears, higher grain density, thick, robust stems, and broad, dark green leaves were all characteristics of the African germplasm (Seetharam 1982).

Upadhyaya et al. (2007) reported that in 909 finger millet germplasm accessions introduced from the gene bank at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Bulawayo, Zimbabwe in 2001, more than 50% green plant accessions were from Kenya, Nepal, Zambia, and Zimbabwe. Among the 909 accessions, 65.3% were green plant type accessions, and 34.7% were pigmented type accessions. Decumbent and erect types of growth habits were dominant with 92.8% erect type. In the same study, plant height of the collection varied from 50 to 120 cm in the collections from Zimbabwe to 85–130 cm (Ethiopia, Malawi, Uganda, and Zaire), suggesting that the dwarf plant had high frequency in

Table 9.5 Sources for micronutrients (Ca, Zn, and Fe)

S. no.	Micronutrient	Rich in genotypes	References
1	Calcium	CFMV 1 (Indravati), CFMV 2	Yadava et al. (2020)
2	Zinc	CFMV 1 (Indravati), CFMV 2	Yadava et al. (2020)
3	Iron	VR 929 (Vegavathi), CFMV 1 (Indravati), CFMV 2, KMR-216, BR-36, PR-10-21, GPU-28	Yadava et al. (2020), Babitha et al. (2015a)

(up to 75 cm) accessions from Zimbabwe than in the entire collection from Southern and Eastern Africa while days to 50% flowering varied from 62 to 96 days in the accessions from Kenya to 73–81 days in accessions from Tanzania and Zaire, indicating that early flowering accessions occurred in Kenya, and late flowering accessions were found in Tanzania and Zaire. They also reported an extensive range of grain colors (dark brown, light brown, ragi brown, reddish brown, and white) were observed in the finger millet germplasm collection introduced from Southern and Eastern Africa. The majority of the accessions were light brown, reddish brown, dark brown, *ragi* brown, and white. The inflorescence length varied from 40 to 215 mm in the collections from Kenya, Zimbabwe, and unknown origin to 60–180 mm in accessions from Nepal, Ethiopia, and Tanzania, indicating that the narrowest inflorescence width accessions were found in Kenya, Zimbabwe, and unknown origin. In contrast, the widest width accessions were found in Nepal, Ethiopia, and Tanzania (Table 9.5).

9.9 Achievements

See Table 9.6.

Table 9.6 Resistant sources for biotic and abiotic stresses

S. no.	Biotic stresses	Resistant sources	References	Abiotic stresses	Resistant sources	Source
1	Blast	GE 1559 (IE 990), GE 569 (IE 339), GE 1330 (P228), GE 4440, GE 4449, GE 669 (IE 1012), GE 1356 (P282), GE 1026 (HR 23-8-9), GE 5192 (IE 3655), GE 132 (IE 329), GE 145 (IE 293) GPU 28, GPU 45, VL 315, GPU 48, OEB 259, VL 340, PRM 9809, HR 374, GE 496, GE 3090, GPU 26, GPU 66, GE 1787, GE 1382, GE 1402, L5, VR 762, VR 847, VL 149, Gautami, KM 65, Suraj (VR 520), Saptagiri (PR 2614), PR 230 (Maruthi), PRM 1, KMR 301, KOPN 235, KMR 340, A 404, BM 9-1, Chilika (OEB 10), Bharathi (VR 762), Sri Chaitanya (VR 847), VL 352, OEB 532, VL 376, BM 2, VL Mandua—348, OEB 526, PPR 2700 (Vakula), GN-5, GNN-6, KRI 013-11, WN 259, DHFMV78-3-1, TNEC 1256, PPR 1040, BR 45, KRI 013-18, GPU 88, BR	Ganapathy (2017), Hiremath and Gowda (2018), Manjappa et al. (2018) Ayyangar (1932), Patro and Madhuri (2014), Netam et al. (2019)	Drought	GE 208, GE 496, GE 596, GE 1855, GE 4434, GE 4730, GE 4976, PR 1044, A 404, BM 2, GN 2, GE 436, MR 6, RAU 8, GN 3, Suraj (VR 520), Saptagiri (PR 2614), PPR 2700 (Vakula), VR 708	Ganapathy (2017), Manjappa et al. (2018)

		90, TNEC 1234, DHFMV10-2-1, GK 1, VL 376, GPU 92, GPU 67, KMR 316, KMR 228 and GPU 93, GPU 99, GPU 98 and GSMC-1, PES 400, PES 176, VL 146				
2	Blight	PR 230 (Maruthi), KMR 340	Ayyangar (1932)	High temp. (>3°C)	GE 4, GE 99, GE 128, GE 145, GE 909, GE 1013, GE1028, GE 1274, GE 1815, GE 2370, GE 2911, GE 3303, GE 3265, GE3266, GE 3885, GPU 26, L-5, PES 110, Chilika, KMR 301, Poorna	Ganapathy (2017)
3	Brown spot	BM 9-1	Ayyangar (1932)	Cold tolerance	Indaf 7	Manjappa et al. (2018)
4	Stem borer	Chilika (OEB 10), KMR 340	Ayyangar (1932)	Salinity	TRY 1, Trichy 1, VR-1076, GPU 28, GPU 67, ML 365, Udurumallige, PYR1 and GPU 48	Ayyangar (1932), Anonymous (2021), Sujatha and PushpaLatha (2019), Kumari and Sharavanan (2022)
5	Aphids	KMR 340	Ayyangar (1932)	Non-lodging Non-shattering	GPU 67, VL Mandua—348 Indira ragi 1, OEB 532 Indira ragi 1, OEB 532	Ayyangar (1932) Ayyangar (1932)

References

Anonymous (2021) PIB report 2021. First advance estimates of production of major kharif crops. https://pib.gov.in/PressReleasePage.aspx?PRID=1756743

Ayyangar GNR (1932) The inheritance of characters in ragi, Eleusine coracana Gaertn. Madras Agric J 20:1–9

Ayyangar GR, Wariar UA (1934) Anthesis and pollination in ragi, Eleusine coracana Gaertn., the finger millet. Indian J Agric Sci 4:386–393

Babitha KC, Ramu SV, Nataraja KN, Sheshshayee MS, Udayakumar M (2015a) EcbZIP60, a basic leucine zipper transcription factor from Eleusine coracana L. improves abiotic stress tolerance in tobacco by activating unfolded protein response pathway. Mol Breed 35(9):1–17

Babitha KC, Vemanna RS, Nataraja KN, Udayakumar M (2015b) Overexpression of EcbHLH57 transcription factor from Eleusine coracana L. in tobacco confers tolerance to salt, oxidative and drought stress. PLoS One 10(9):e0137098

Babu BK, Dinesh P, Agrawal PK, Sood S, Chandrashekara C, Bhatt JC, Kumar A (2014a) Comparative genomics and association mapping approaches for blast resistant genes in finger millet using SSRs. PLoS One 9(6):e99182

Babu BK, Agrawal PK, Pandey D, Kumar A (2014b) Comparative genomics and association mapping approaches for opaque2 modifier genes in finger millet accessions using genic, genomic and candidate gene-based simple sequence repeat markers. Mol Breed 34(3): 1261–1279

Bedis MR, Ganvir BN, Path PP (2006) Genetic variability in finger millet. J Maharashtra Agric Univ 31(3):369–370

Bharathi A (2011) Phenotypic and genotypic diversity of global finger millet (Eleusine coracana (L.) Gaertn.). Composite collection. Doctoral dissertation, Tamil Nadu Agricultural University

Bisht MS, Mukai Y (2000) Mapping of rDNA on the chromosomes of Eleusine species by fluorescence in situ hybridization. Genes Genet Syst 75(6):343–348

Bisht MS, Mukai Y (2001a) Genomic in situ hybridization identifies genome donor of finger millet (Eleusine coracana). Theor Appl Genet 102(6):825–832

Bisht MS, Mukai Y (2001b) Identification of genome donors to the wild species of finger millet, Eleusine africana by genomic in situ hybridization. Breed Sci 51(4):263–269

Carter C, Pan S, Zouhar J, Avila EL, Girke T, Raikhel NV (2004) The vegetative vacuole proteome of Arabidopsis thaliana reveals predicted and unexpected proteins. Plant Cell 16:3285–3303. https://doi.org/10.1105/tpc.104.027078

Ceasar SA, Rajan V, Prykhozhij SV, Berman JN, Ignacimuthu S (2016) Insert, remove or replace: a highly advanced genome editing system using CRISPR/Cas9. Biochim Biophys Acta Mol Cell Res 1863(9):2333–2344

Chavan VM, Shendge PY (1957) Plant breeding in South Asia with reference to millets. Indian J Genet Plant Breed 17:156–175

Chavan VM, Gopalkrishna N, Khadilkar BT (1955) Blooming and anthesis in nagli (Eleusine coracana (Linn.) Gaertn). Poona Agric Coll Mag 46:175–179

Chemeda D, Gemechu K (2010) Morpho-agronomic classification of some native and exotic finger millet (Eleusine coracana L.) germplasm accessions in Ethiopia. East Afr J Sci 4(1):20–26

Chen S, Phillips SM (2006) Eleusine. In: Flora of China, Poaceae, vol 22. Science Press/PRC and Missouri Botanical Garden Press, Beijing

Chennaveeraiah MS (1973) Genome relationships of Eleusine tristachya and E. floccifolia. J Cytol Genet 8:1–5

Chennaveeraiah MS, Hiremath SC (1974) Genome analysis of Eleusine coracana (L.) Gaertn. Euphytica 23(3):489–495

Chinchole M, Pathak RK, Singh UM, Kumar A (2017) Molecular characterization of EcCIPK24 gene of finger millet (Eleusine coracana) for investigating its regulatory role in calcium transport. 3 Biotech 7(4):1–10

Clayton WD, Harman KT, Williamson H (2009) GrassBase—the online world grass flora (version 29 Jan 2008)
Cobley LS (1956) An introduction to the botany of tropical crops. Longmans
Coleman LC (1920) The cultivation of ragi in Mysore. Printed at the Government Press
De Candolle A (1886) Origin of cultivated plants (Hafner, New York, 1967). English translation of the second edition, pp 316–321
De Wet JMJ, Rao KP, Brink DE, Mengesha MH (1984) Systematics and evolution of Eleusine coracana (Gramineae). Am J Bot 71(4):550–557
De Wildeman E (1940) De l'origine de certains éléments de la flore du Congo belge et des transformations de cette flore sous l'action de facteurs physiques et biologiques. G. van Campenhout
Dida MM, Devos KM (2006) Finger millet. In: Cereals and millets. Springer, Berlin, pp 333–343
Dida MM, Wanyera N, Harrison Dunn ML, Bennetzen JL, Devos KM (2008) Population structure and diversity in finger millet (Eleusine coracana) germplasm. Trop Plant Biol 1(2):131–141
Dodake SS, Dhonukshe BL (1998) Variability in floral structure and floral biology of finger millet (Eleusine coracana (L.) Gaertn.). Indian J Genet Plant Breed 58(01):107–112
Ellis MH, Rebetzke GJ, Kelman WM, Moore CS, Hyles JE (2004) Detection of Wheat streak mosaic virus in four pasture grass species in Australia. Plant Pathol 53(2):239
Elorza MS, Dana E, Sobrino E (2001) Aproximación al listado de plantas alóctonas invasoras reales y potenciales en España. Lazaroa 22:121–131
Emrey TM (2022) Investigation of finger millet floral structure and hand emasculation. J Plant Physiol Pathol 10(6):2
Fakrudin B, Shashidhar HE, Kulkarni RS, Hittalmani S (2004) Genetic diversity assessment of finger millet, Eleusine coracana (Gaertn), germplasm through RAPD analysis. PGR Newslett 138:50–54
Fuller DQ (2002) Fifty years of archaeobotanical studies in India: laying a solid foundation. Indian Archaeol Retrospect 3:247–363
Fuller DQ (2006) Agricultural origins and frontiers in South Asia: a working synthesis. J World Prehist 20(1):1–86
Ganapathy KN (2017) Improvement in finger millet: status and future prospects. In: Millets and sorghum: biology and genetic improvement. Wiley, pp 87–111
Gourinath S, Alam N, Srinivasan A, Betzel C, Singh TP (2000) Structure of the bifunctional inhibitor of trypsin and α-amylase from ragi seeds at 2.2 Å resolution. Acta Crystallogr D Biol Crystallogr 56(3):287–293
Grassland Index (2009) Grassland species profiles. FAO, Rome. http://www.fao.org/ag/AGP/AGPC/doc/GBASE/
Greenway PJ (1945) Origins of some East African food plants: Part V. East Afr Agric J 11(1):56–63
Guarino L (2012) Global strategy for the *ex-situ* conservation of finger millet and its wild relatives. Global Crop Diversity Trust, ICRISAT, Patancheru
Gupta A (2006) Improvement of millets and pseudo-cereals for rainfed agriculture in hill region. In: Sustainable production from agricultural watersheds in North West Himalaya. Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, Uttaranchal, pp 163–174
Gupta A, Sood S, Agrawal PK, Bhatt JC (2011a) Floral biology and pollination system in small millets. Eur J Plant Sci Biotechnol 6:81–86
Gupta N, Kumar Gupta A, Singh NK, Kumar A (2011b) Differential expression of PBF Dof transcription factor in different tissues of three finger millet genotypes differing in seed protein content and color. Plant Mol Biol Report 29(1):69–76
Gupta AK, Gaur VS, Gupta S, Kumar A (2013) Nitrate signals determine the sensing of nitrogen through differential expression of genes involved in nitrogen uptake and assimilation in finger millet. Funct Integr Genomics 13:179–190. https://doi.org/10.1007/s10142-013-0311-x
Gupta S, Gupta SM, Gupta AK, Gaur VS, Kumar A (2014) Fluctuation of Dof1/Dof2 expression ratio under the influence of varying nitrogen and light conditions: involvement in differential

regulation of nitrogen metabolism in two genotypes of finger millet (Eleusine coracana L.). Gene 546(2):327–335
Hansen A (1980) Eleusine. In: Flora Europaea, vol 5. Cambridge University Press, Cambridge, pp 258–259
Hilu KW (1980) Eleusine tristachya (Lam.) Lam. (Poaceae). Madrono 27:177–178
Hilu KW (1988) Identification of the "A" genome of finger millet using chloroplast DNA. Genetics 118(1):163–167
Hilu K (1994) Validation of the combination Eleusine coracana subspecies africana (Kennedy-O'Byrne) Hilu et Dewet. Phytologia 76(5):410–411
Hilu KW (1995) Evolution of finger millet: evidence from random amplified polymorphic DNA. Genome 38(2):232–238
Hilu KW (2003) Eleusine. In: Flora of North America north of Mexico, vol 25. Oxford University Press
Hilu KW, De Wet JMJ (1976) Domestication of Eleusine coracana. Econ Bot 30(3):199–208
Hilu KW, Johnson JL (1997) Systematics of Eleusine Gaertn. (Poaceae: Chloridoideae): chloroplast DNA and total evidence. Ann Missouri Bot Garden 84:841–847
Hiremath C, Gowda J (2018) SSR based genetic diversity in blast resistant and susceptible accessions of finger millet (Eleusine coracana. L). Electron J Plant Breed 9(2):400–408
Hiremath SC, Salimath SS (1992) The 'A' genome donor of Eleusine coracana (L.) Gaertn. (Gramineae). Theor Appl Genet 84(5):747–754
Hittalmani S, Mahesh HB, Shirke MD, Biradar H, Uday G, Aruna YR, Lohithaswa HC, Mohanrao A (2017) Genome and transcriptome sequence of finger millet (Eleusine coracana (L.) Gaertn.) provides insights into drought tolerance and nutraceutical properties. BMC Genomics 18(1): 1–16
House LR (1985) A guide to sorghum breeding. International Crops Research Institute for the Semi-Arid Tropics
Kennedy-O'Byrne J (1957) Notes on African grasses: XXIX. A new species of Eleusine from tropical and South Africa. Kew Bull 12:65–72
Krishna TA, Maharajan T, Roch GV, Ramakrishnan M, Ceasar SA, Ignacimuthu S (2020) Hybridization and hybrid detection through molecular markers in finger millet [Eleusine coracana (L.) Gaertn.]. J Crop Improv 34(3):335–355
Kumar A, Yadav S, Panwar P, Gaur VS, Sood S (2015) Identification of anchored simple sequence repeat markers associated with calcium content in finger millet (Eleusine coracana). Proc Natl Acad Sci India B Biol Sci 85(1):311–317
Kumari AN, Sharavanan PT (2022) Early identification of salt-tolerant genotypes in finger millet (Eleusine coracana L.) at germination stage by observing the morphological characters. Indian J Agric Res 1:7
Kunguni JS (2016) Investigation of ethrel gametocide in finger millet (Eleusine coracana, L. Gaertn) hybrids through genetic analysis. Doctoral dissertation
Liu Q, Jiang B, Wen J, Peterson PM (2014) Low-copy nuclear gene and McGISH resolves polyploid history of Eleusine coracana and morphological character evolution in Eleusine. Turk J Bot 38(1):1–12
Lovisolo MR, Galati BG (2007) Ultrastructure and development of the megagametophyte in Eleusine tristachya (Lam.) Lam.(Poaceae). Flora Morphol Distrib Funct Ecol Plants 202(4): 293–301
Lye KA (1999) Nomenclature of finger millet (Poaceae). Lidia 4:149–151
Manjappa SD, Rangaiah S, Gowda MVC (2018) Assessment of molecular diversity in an elite set of finger millet (Eleusine coracana (L.) Gaertn.) genotypes using SSR markers. Electron J Plant Breed 9(2):564–576
McDonough CM, Rooney LW, Earp CF (1986) Structural characteristics of Eleusine coracana (finger millet) using scanning electron and fluorescence microscopy. Food Struct 5(2):9
Mehra KL (1963a) Consideration of the African origin of Eleusine coracana (L.) Gaertn. Curr Sci 32:300–301

Mehra KL (1963b) Differentiation of cultivated and wild Eleusine species. Phyton 20:189–198
Mirza N, Marla SS (2019) Finger millet (Eleusine coracana L. Gartn.) breeding. In: Advances in plant breeding strategies: cereals. Springer, pp 83–132
Mirza N, Taj G, Arora S, Kumar A (2014) Transcriptional expression analysis of genes involved in regulation of calcium translocation and storage in finger millet (Eleusine coracana L. Gartn.). Gene 550(2):171–179
Mnyenyembe PH, Gupta SC (1998) Variability for grain yield and related traits in finger millet germplasm accessions from Malawi. Afr Crop Sci J 6(3):317–322
Nagarjuna KN, Parvathi MS, Sajeevan RS, Pruthvi V, Mamrutha HM, Nataraja KN (2016) Full-length cloning and characterization of abiotic stress responsive CIPK31-like gene from finger millet, a drought-tolerant crop. Curr Sci 111:890–894
Nath M, Roy P, Shukla A, Kumar A (2013) Spatial distribution and accumulation of calcium in different tissues, developing spikes and seeds of finger millet genotypes. J Plant Nutr 36(4): 539–550
Netam PS, Thakur AK, Kumar P, Netam RS (2019) Screening of finger millet (Eleusine coracana) varieties for resistant to blast (Magnaporthe grisea) disease in Bastar District, Chhattisgarh, India. Int J Curr Microbiol App Sci 8(12):2664–2668
Neves SS (2011) Eleusine. In: Wild crop relatives: genomic and breeding resources. Springer, pp 113–133
Neves SS, Swire-Clark G, Hilu KW, Baird WV (2005) Phylogeny of Eleusine (Poaceae: Chloridoideae) based on nuclear ITS and plastid trnT–trnF sequences. Mol Phylogenet Evol 35(2):395–419
Oduori CO (2008) Breeding investigations of finger millet characteristics including blast disease and striga resistance in Western Kenya. Doctoral dissertation
Parvathi MS, Nataraja KN (2017) Discovery of stress responsive TATA-box binding protein associated Factor6 (TAF6) from finger millet (Eleusine coracana (L.) Gaertn). J Plant Biol 60(4):335–342
Parvathi MS, Nataraja KN, Yashoda BK, Ramegowda HV, Mamrutha HM, Rama N (2013) Expression analysis of stress responsive pathway genes linked to drought hardiness in an adapted crop, finger millet (Eleusine coracana). J Plant Biochem Biotechnol 22(2):193–201
Patrick JW, Offler CE (2001) Compartmentation of transport and transfer events in developing seeds. J Exp Bot 52(356):551–564
Patro TSSK, Madhuri J (2014) Identification of resistant varieties of finger millet for leaf, neck and finger blast. Int J Food Agric Vet Sci 4(2):7–11
Patroti P, Gowda J (2015) Floral biology studies in three different varieties of finger millet (Eleusine coracana (l.) Gaertn.). J Res PJTSAU 46
Phillips SM (1972) A survey of the genus Eleusine Gaertn. (Gramineae) in Africa. Kew Bull 27: 251–270
Phillips SM (1974) Eleusine. In: Polhill RM (ed) Flora of tropical East Africa. Crown Agents for Overseas Governments and Administrations, pp 260–267
Phillips S (1995) Poaceae (Gramineae), vol 7. Addis Ababa University, Addis Ababa
Portères R (1951) Eleusine coracana Gaertn. Céréale des humanités pauvres des pays tropicaux. Bulletin de l'Institut Français de l'Afrique Noire 13(1951):1–78
Porteres R (1958) Le millet Eleusine de l'Inde et de l'Afrique Oriental (E. coracana). J Agric Trop Bot Appl 5:463–486
Portères R (1970) Primary cradles of agriculture in the African content. African Prehistory
Prasada Rao KE, De Wet JMJ, Gopal Reddy V, Mengesha MH (1993) Diversity in the small millets collection at ICRISAT. In: Riley KW, Gupta SC, Seetharam A, Mushonga JN (eds) Advances in small millets. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, India
Rahman H, Jagadeeshselvam N, Valarmathi R, Sachin B, Sasikala R, Senthil N, Sudhakar D, Robin S, Muthurajan R (2014) Transcriptome analysis of salinity responsiveness in contrasting genotypes of finger millet (Eleusine coracana L.) through RNA-sequencing. Plant Mol Biol 85(4):485–503

Rahman H, Ramanathan V, Nallathambi J, Duraialagaraja S, Muthurajan R (2016) Over-expression of a NAC 67 transcription factor from finger millet (Eleusine coracana L.) confers tolerance against salinity and drought stress in rice. BMC Biotechnol 16(1):7–20

Ramakrishna C, Singh S, Raghavendrarao S, Padaria JC, Mohanty S, Sharma TR, Solanke AU (2018) The membrane tethered transcription factor EcbZIP17 from finger millet promotes plant growth and enhances tolerance to abiotic stresses. Sci Rep 8(1):1–14

Ramakrishnan M, Ceasar SA, Vinod KK, Duraipandiyan V, Ajeesh Krishna TP, Upadhyaya HD, Al-Dhabi NA, Ignacimuthu S (2017) Identification of putative QTLs for seedling stage phosphorus starvation response in finger millet (Eleusine coracana L. Gaertn.) by association mapping and cross species synteny analysis. PLoS One 12(8):e0183261

Ramegowda V, Gill US, Sivalingam PN, Gupta A, Gupta C, Govind G, Nataraja KN, Pereira A, Udayakumar M, Mysore KS, Senthil-Kumar M (2017) GBF3 transcription factor imparts drought tolerance in Arabidopsis thaliana. Sci Rep 7(1):1–13

Reddy VG, Upadhyaya HD, Gowda CLL, Singh S (2009) Characterization of eastern African finger millet germplasm for qualitative and quantitative characters at ICRISAT. J SAT Agric Res 7:9

Sakai H, Lee SS, Tanaka T, Numa H, Kim J, Kawahara Y, Wakimoto H, Yang CC, Iwamoto M, Abe T, Yamada Y (2013) Rice Annotation Project Database (RAP-DB): an integrative and interactive database for rice genomics. Plant Cell Physiol 54(2):e6

Seetharam A (1982) Finger millet improvement [cultivation of improved varieties, India]. Indian Farming

Shailaja HB, Thirumeni S, Paramasivam K, Ramanadane T (2010) Combining ability analysis in finger millet (Eleusine coracana (L.) Gaertn.) under salinity. Electron J Plant Breed 1(2): 129–139

Singh UM, Chandra M, Shankhdhar SC, Kumar A (2014) Transcriptome wide identification and validation of calcium sensor gene family in the developing spikes of finger millet genotypes for elucidating its role in grain calcium accumulation. PLoS One 9:e103963

Singh RK, Singh VK, Raghavendrarao S, Phanindra MLV, Raman KV, Solanke AU et al (2015) Expression of finger millet EcDehydrin7 in transgenic tobacco confers tolerance to drought stress. Appl Biochem Biotechnol 177:207–216. https://doi.org/10.1007/s1201

Sisay A, Baars RMT (2002) Grass composition and rangeland condition of the major grazing areas in the mid Rift Valley, Ethiopia. Afr J Range Forage Sci 19(3):161–166

Sood S, Kumar A, Babu BK, Gaur VS, Pandey D, Kant L, Pattnayak A (2016) Gene discovery and advances in finger millet [Eleusine coracana (L.) Gaertn.] genomics—an important nutri-cereal of future. Front Plant Sci 7:1634

Sood S, Joshi DC, Chandra AK, Kumar A (2019) Phenomics and genomics of finger millet: current status and future prospects. Planta 250(3):731–751

Strobl S, Muehlhahn P, Bernstein R, Wiltscheck R, Maskos K, Wunderlich M, Huber R, Glockshuber R, Holak TA (1995) Determination of the three-dimensional structure of the bifunctional alpha-amylase/trypsin inhibitor from ragi seeds by NMR spectroscopy. Biochemistry 34(26):8281–8293

Sujatha B, PushpaLatha AHD (2019) Evaluation of sodium chloride stress tolerance in finger millet (Eleusine coracana L.) cultivars by observing morphological characters. Int J Adv Res 7:149–153. (ISSN 2320-5407)

Upadhyaya HD, Gowda CLL, Reddy VG (2007) Morphological diversity in finger millet germplasm introduced from r Africa. J SAT Agric Res 3(1):1–3

Upadhyaya HD, Sarma NDRK, Ravishankar CR, Albrecht T, Narasimhudu Y, Singh SK, Varshney SK, Reddy VG, Singh S, Dwivedi SL, Wanyera N (2010) Developing a mini-core collection in finger millet using multilocation data. Crop Sci 50(5):1924–1931

Vavilov NI (1951) The origin, variation, immunity and breeding of cultivated plants, vol 72(6). LWW, p 482

Vishnu-Mittre M (1968) Protohistoric records of agriculture in India. Trans Bose Res Inst 31:87–106

Werth CR, Hilu KW, Langner CA (1994) Isozymes of Eleusine (Gramineae) and the origin of finger millet. Am J Bot 81(9):1186–1197

Yadava DK, Choudhury PR, Hossain F, Kumar D, Sharma TR, Mohapatra T (2020) Biofortified varieties: sustainable way to alleviate malnutrition. Indian Council of Agricultural Research, New Delhi, p 19

Websites

UKEssays (2018) Salinity responsiveness in finger millet analysis. https://www.ukessays.com/essays/sciences/salinity-responsiveness-finger-millet-5814.php?vref=1

https://www.millets.res.in/technologies/finger_millets_varieties.pdf

https://www.apnikheti.com/en/pn/agriculture/crops/fodder/finger-millet

https://wcd.nic.in/sites/default/files/ICAR_AKS_Biofortification_19-09-2017_0.pdf

Breeding Finger Millet (*Eleusine coracana* L. Gaertn) for Improvement of Quality Characters and Yield

10

Botta Thandava Ganesh, Kyada Amitkumar Dilipbhai, Shridhar Ragi, and Ashvinkumar Katral

Abstract

Finger millet (*Eleusine coracana* L. Gaertn) also known as "ragi" belongs to family "*Poaceae*" (*Gramineae*) is an important millet after Jowar and Bajra. Among millets, finger millet is a sustainable future crop which offers a source of food, fodder, forage, energy (fuel), and nutritional security to the world. Finger millet can be eaten as cakes, porridges, pudding, and biscuits. It is better for its suitability to dry land, hilly and tribal agriculture of Asian and African countries. It grows under less irrigated conditions due to its C_4 nature and low water requirement (350 mm). It will be grown under various agro-climatic zones and agroecological zones in relative to cereal crops. It can be stored safe for nearly 50 years without pest infestation and can be used during famine. Finger millet has a rich nutritional profile like carbohydrates (65–75%), crude fibers (15–20%), proteins (5–8%), and also a better stock of minerals (2.5–3.5%) like calcium (344 mg/100 g), phosphorus (283 mg/100 g), iron (3.9 mg/100 g), and zinc (2.79 mg/100 g). Men and women are suggested to eat finger millet as it is hypoglycemic due to more amylose to amylopectin ratio, hypocholesterolemic due to high dietary fiber, thereby management of obesity, diabetes, and anemia. Genetic variability in finger millet genotypes is a prior requirement in breeding of crop breeding to develop stable genotypes across the various seasons and locations for their yield and its contributing traits and quality characters. Identifying candidate genes for quality characters like calcium, protein, antioxidants, and "gluten free" finger millet using marker assisted selection (MAS) and genomic selection which increases the genetic gain per unit time. Removal of anti-nutritional factors like phytic acid, phenols, and tannins and thereby increases bioavailability of Fe, Zn, Ca, Mg, and Mn. Variability in

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germplasm of finger millet is to be harnessed for breeding of varieties with high yield and quality.

Keywords

Finger millet · Quality · Yield · Variability · Stability

10.1 Introduction

Finger millet (*Eleusine coracana* (L.) Gaertn) is an important food source in dryland agriculture. The "finger millet" got its name because of finger like branching pattern of the earhead. As traditionally, from time immemorial, "Ragi" got its name from "ragika" which means goddess of cereals. Finger millet is generally grown in tropical and subtropical regions in different countries such as India and its surrounding nations like Nepal, Sri Lanka, China, and Japan in Asia whereas Ethiopia, Kenya, Rwanda, Zairein Africa. India is rather a few steps ahead in finger millet production and productivity in Asia with an area of one million hectares and a production of 1.76 million metric tons with a productivity of 1747 kg/ha (EPWRF India Time Series, 2019–2020). It is known for its suitability to dry land, hill and tribal agriculture of Asian and African countries. Moreover, as it is a C_4 plant and have a low water requirement (350 mm), it can be grown under low soil moisture conditions and also suitable for various contingent cropping systems. Finger millet crop can be grown all around the year, depending on irrigation conditions. It will be grown under various agro-climatic zones and agroecological zones when compared to other cereal crops (Ashoka and Halikatti 1997). Straw of finger millet can be profoundly used as an animal feed hence it is used as both human food and animal feed as a forage crop. Climate change like heat waves leading to terminal moisture stress, untimely rains during flowering and harvesting stages thereby, reduction in production as in case of cereals like wheat in recent times affecting global food supply and biotic and abiotic factors limiting the yield and also with less quality. So, giving emphasis on finger millet which is resistant to all biotic and abiotic factors results in sustained stability in yield with high quality. However, identifying the candidate genes for the traits governing quality like calcium and protein is utmost important. Hence, the genotypes should be assessed in order to evolve superior and stable varieties across the various environments/seasons with high yield (Thandava Ganesh et al. 2021) and quality parameters by keeping in mind the "gluten free," removal of anti-nutritional factors like phytic acid, phenols, and tannins, and availability of Fe, Zn, Ca, Mg, and Mn. The crop breeding strategies for the yield and quality improvement are the dire need for finger millet for food and nutritional security across the world by reducing food shortage and malnutrition.

10.2 Taxonomy

The chromosome number of *Eleusine coracana* is $2n = 4x = 36$ (AABB, tetraploid). Ragi is called as utricle because seed coat and pericarp are not fused completely. It is a self-pollinated crop and hand emasculation is difficult because of small size of the flowers. A contact method of crossing is practiced in ragi. The genus *Eleusine* contains 11 species out of which 6 are diploids and five are tetraploids. The species *Eleusine indica* is diploid ($2n = 2x = 18$), while *Eleusine coracana* and *Eleusine africana* are tetraploids ($2n = 4x = 36$). *Eleusine coracana* has originated from the *Eleusine africana* as a gene introgression mutant (Hilu and Wet 1976). Moreover, finger millet is classified into African ragi (*E. africana*) and Indian ragi (*E. coracana*) on the basis inflorescence morphology. Long fingers, bold grains with stiff straw are present in African types. Short fingers, small grains, and photo insensitive come under Indian types. Coming to the progenitors, *Eleusine indica* is wild species in Africa and India, while *Eleusine stricta* is wild in Africa alone. The Indian-African interspecific cross derivatives led to Indaf varieties.

10.3 Yield of Finger Millet

Reduction in yields in finger millet is due to biotic factors as variability is not utilized for developing varieties. The devastating disease in finger millet is blast (*Pyricularia grisea*), the disease occurs common in other cereal crops and grasses. A ragi root aphid (*Tetraneura nigriabdominalis*) infects from the roots and results in wilting of plants and drying in patches in ragi fields. Hence, genetic variability in finger millet genotypes is a prior requirement in crop improvement programs like pre breeding and transfer of resistant genes through back cross method for development of a better varieties to tackle biotic stresses and thereby increase the grain and fodder yield. The genetic variability studies help in achieving this goal by assessing the genetic variability in the genotypes and thereby evolving high yielding varieties based on fodder and grain yield and their influencing traits.

10.3.1 Variability Studies in Finger Millet

The grain yield generally quantitative in nature is not only influenced by factors of environment and but also depends on its associated traits. Therefore, the selection of high yielding RILs (Recombinant inbred lines)/ABLs (Advanced Breeding lines) should not be based on just a grain yield, rather its contributing traits are also important. When finger millet is grown in different seasons or environments, germplasm show variable range of fluctuations in their yield and its related traits. The stability of a crop to perform well over a range of environments or seasons is as important as its potential for yield per se. Lack of high and stable yielding varieties is one of major debacles for increasing production of finger millet in India and across the world.

Bezaweletaw et al. (2006) reported that high PCV and GCV were portrayed by plant height while the low values were recorded for 1000-grain weight in 64 land races. The characters, viz., finger width, finger length, and grain yield per plant showed a large part of the phenotypic difference accounted by the hereditary component individually. This demonstrated the existence of enormous variability that stayed and without any change by natural conditions among the germplasm, which was progressively valuable for hybridization or selection. Moreover, they found higher heritability for finger width.

Shet et al. (2010) tested three unique varieties of cultivable species of *Eleusine coracana* with *Eleusine africana* for genetic variability. They studied that the F_2 population of two crosses enrolled higher PCV and GCV for grain yield per plant.

Ulaganathan and Nirmalakumari (2011) recorded genetic variability values moderate for 1000-grain weight, fingers per earhead, finger length, and plant height, while low for days to maturity and finger width in 105 genotypes of finger millet. High heritability was notified for finger width, grain yield per plant, fingers per earhead, and days to maturity. It demonstrated marginal to high genotype × season interaction in germplasm.

Jayashree and Nagarajaiah (2013) evaluated 689 accessions of finger millet and recorded high heritability for finger length and plant height.

Karad and Patil (2013) tested 65 finger millet accessions. The results noted regarding phenotypic and genotypic coefficients of variation were high for grain yield per plant, finger length, number of productive tillers per plant, iron percent, number of earhead per plant, average fingers per earhead, fodder yield per plant, and 1000-grain weight.

Reddy et al. (2013) recorded a wider range of variability in 18 elite finger millet accessions for number of fingers per earhead, grain yield per plant, and plant height. However, PCV and GCV were low for finger width and moderate for finger length while higher for number of fingers per earhead.

Mahanthesha et al. (2017) analyzed 48 genotypes and observed higher GCV and PCV values for number of tillers per plant, grain yield per plant, and finger length.

Devaliya et al. (2017) assessed 68 finger millet genotypes for their genetic variability and its twelve contributing traits. “Grain yield per plant” showed high PCV and GCV. The characters viz., number of productive tillers per plant, main earhead length, and number offingers per earhead showed a medium amount of variation. Higher heritability along with high genetic advance was noted for grain yield per plant, main earhead length.

10.3.2 Stability in Yield Across Seasons

An identification and assessment of stable and high yielding genotypes under diverse seasonal conditions before releasing as a variety is the foremost step in plant breeding and this influences directly on the adoption of the cultivars by the farmers. Therefore, stability in production is a significant for the plant breeder to evolve varieties in finger millet with stable and high yield across the various seasons.

Kandpal et al. (1981) conducted experiments relating to changes in the enzyme activities regarding proline metabolism in ragi (*Eleusine coracana*) leaves in summer related to water stress. Free proline in ragi leaves increased from 6 to 85-fold as water stress created by treatment of polyethylene glycol was extended.

Badu-Apraku et al. (2012) analyzed 12 extra early maturing corn genotypes in 17 different places from 2006 to 2009 and they used GGE biplot and AMMI analysis for estimating the G × E interaction for grain yield.

Fentie et al. (2013) conducted the experiment in northwestern Ethiopia to know the genotype effect, season effect in finger millet germplasm for yield. Nine genotypes were laid down in RCBD having three replications at six sites in northwestern Ethiopia. The combined ANOVA revealed for genotypes, seasons, and their interactions is highly significant for grain yield.

Malambane et al. (2014) evaluated 35 finger millet genotypes in the dry season 2010/2011 and the rainy season 2011. Variation due to season resulted in a large proportion of variations on plant height (94.60%), yield per plot (97.40%) and days to flowering (78.00%). However, variations due to genotype were 38.50% for finger number, 41.80% for finger width, and 16.80% for days to flowering.

Venkateshbabu et al. (2015) identified ragi genotypes which are having high water use efficiency and yield for rainfed conditions based on water use efficiency traits viz., relative water content (RWC), SPAD chlorophyll meter readings (SCMR), specific leaf area (SLA), and yield parameters in ten genotypes viz., GP3, GP-24, GP-25, GP-104, GP-111, GP-27, GP-149, GP-153, GP-23, and GP-160.

Nishar et al. (2020) tested ragi variety "BirsaMadua-1" for its productivity and cost of cultivation in four villages of Ranchi in *kharif*, 2018. They found that there was an alteration of rainfall in the district and its period of *kharif* crops is declining. There was a raise in potential evapotranspiration (PET) and temperature. Even under the changing environment, Ragi showed stability and adaptability with a Benefit-Cost ratio of 2.28.

10.4 Quality Parameters

The mineral content of millets is mostly influenced by the genetic factors and environmental conditions prevailing in growing region. The results indicated the significant amount of variability present among the RILs studied for mineral nutrient. Finger millet is nutritious in terms of high phosphorous, calcium, zinc, and iron. The daily intake of finger millet is good for bone health and could reduce risk of fracture and keeps diseases such as osteoporosis away. Even though it is rich in nutraceutical property, anti-nutritional factor makes bioavailability of minerals lower to monogastric animals (Thompson 1993). Phytic acid even though anti-nutritional factor like polyphenols and tannins, found relatively high amounts but also acts like antioxidant activity which is an important factor in health, aging, and metabolic diseases. Finger millet is called as "Nutri-cereal" because of its plethora of nutritional advantages, and also contains rich amounts of micronutrients like Fe,

Zn, Ca, Mg, and Mn. As finger millet is rich in dietary fibers, it helps in reducing constipation and smooth functioning of digestive system. Because of high amylose content in grains, the starch is slowly converted to sucrose, thereby reducing the sugar levels in blood and is a boon to diabetic patients. Finger millet has a rich nutritional reservoir like carbohydrates (65–75%), crude fibers (15–20%), and proteins (5–8%). It is a better source of minerals (2.5–3.5%) like calcium (344 mg/100 g), phosphorus (283 mg/100 g), iron (3.9 mg/100 g), and zinc (2.79 mg/100 g). People are suggested to eat ragi and other millets instead of cereals like wheat and rice, etc., as millets are hypoglycemic due to more amylopectin, hypocholesterolemic due to high dietary fiber and food stays longer in digestive tract which results in slow release of glucose into blood and thereby management of obesity and diabetes and also helps in management of anemia (Chethan and Malleshi 2007).

10.4.1 The Procedure of Chemical Analysis of Grain Samples of Finger Millet

10.4.1.1 Collection and Preparation of Grain Samples

The grain samples collected from the experimental plots are transferred to muslin bag and transported to the experimental laboratory immediately. For long travel the samples should be transported under cool conditions. To remove the moisture and to inactivate the enzyme activities, the samples are dried in an oven at temperature of 65–70 °C for 48 h. The oven dried samples are powdered using a Willey mill machine and stored in polythene bags to prevent contamination. The powdered samples are subjected for analysis of secondary macro nutrients and micronutrients. The details of the methods for the estimation of nutrient content of grains are presented in Table 10.1.

10.4.1.2 Digestion of the Powdered Grain Samples

Digestion of the powdered grain samples should be done by following the di-acid digestion for the determination of Ca, Mg, Mn, Zn, Fe, and Cu. The di-acid digestion is carried out using a 9:4 mixture of HNO_3:$HClO_4$. Pre-digestion using 25 mL

Table 10.1 Methods to estimate grain nutrient content

Sl. No.	Parameters	Method
Secondary macronutrients		
1	Calcium (mg/100 g)	Titration method (Piper 1966)
2	Magnesium (mg/100 g)	
Micronutrients		
3	Zinc (mg/100 g)	Atomic absorption spectroscopy (AAS) Lindsay and Norvell (1978)
4	Iron (mg/100 g)	
5	Manganese (mg/100 g)	
6	Copper (mg/100 g)	

Table 10.2 Grouping of finger millet germplasm based on calcium and magnesium

Calcium (mg/100 g)		Magnesium (mg/100 g)	
Class	Selection criteria	Class	Selection criteria
Low	<306.00	Low	<175.00
Medium	306.00–364.00	Medium	175.00–186.00
High	>364.00	High	>186.00

Table 10.3 Grouping of finger millet germplasm based on iron, manganese, zinc, and copper contents

Class	Iron (mg/100 g)	Manganese (mg/100 g)	Zinc (mg/100 g)	Copper (mg/100 g)
Low	<4.24	<30.10	<3.42	<0.50
Medium	4.24–4.60	30.10–37.90	3.42–3.95	0.50–0.65
High	>4.60	>37.90	>3.95	>0.65

HNO_3/g sample is done to avoid explosion, if the sample is high in fats/oils. The procedure follows as below:

1. One gram of ground sample in 100 mL conical flask and add 10 mL of di acid 9:4 mixture of HNO_3:$HClO_4$ and mix them.
2. Red N 2 fumes ceases sometime after keeping in low heat hot plate in digestion chamber.
3. Volume is reduced to 3–5 mL and liquid becomes colorless.
4. After cooling down, make up volume to 100 mL double distilled water and filter it through Whatman No. 1 paper and aliquot for determination of Ca, Mg, Zn, Fe, Cu, and Mn.

After determination of results, grouping of finger millet genotypes has to be classified based on contents of calcium and magnesium (Table 10.2) and micronutrients (Table 10.3) as low, medium, and high classes.

10.4.2 Determination of Calcium and Magnesium Content in Grain Samples

Calcium and magnesium in the digested grain sample can be estimated by using of Patton Reeders and EBT indicators against standard Versenate solution, respectively for calcium and calcium plus magnesium whereas magnesium was determined by difference (Piper 1966).

$$\text{Ca (mg/100 g)} = \frac{A \times N \times 0.02 \times \text{Volume of sample (digested)} \times 1000}{\text{Aliquot taken} \times \text{Sample weight}} \times 100$$

$$\text{Mg (mg/100 g)} = \frac{[A \times B] \times N \times 0.012 \times \text{Volume of sample (digested)} \times 1000}{\text{Aliquot taken} \times \text{Sample weight}} \times 100$$

where, N is normality of EDTA, A and B are the titer values.

10.4.3 Determination of Micronutrients (Fe, Mn, Zn, and Cu) Content in the Grain Samples

Using the appropriate hallow cathode lamp, feed the samples to the atomic absorption spectrophotometer after the proper dilution of the di-acid extract.

$$\text{Micronutrient conc. (ppm)} = \frac{\text{Graph ppm} \times \text{Volume of digested sample}}{\text{Weight of sample}}$$

Note: 1000 ppm = 1 mg/g.

Babu et al. (1987) studied six hybrid varieties, viz., C 157, APK 2, VR 250-6, VZM 1, VZM 2, and Godavari of finger millet for their grain nutrient content. The concentrations of Ca and Cu were more in VZM 1 than the other hybrids. VR 250-6 showed the highest Fe content.

Ravindran (1991) analyzed grain samples of three varieties of finger millet for their mineral composition. The content of minerals was high in relative to other common cereal seeds and particularly the high level of calcium (24%). The results informed that finger millet as dietary food source has to be explored for nutrients.

Bachar et al. (2013) studied the grain nutrient content for 30 genotypes of finger millet. Experimental results given information that magnesium and calcium were 84.71–567.45 mg/100 g and 189.93–1272.36 mg/100 g, respectively, the most concentrated nutrients among the studied genotypes.

Solomon et al. (2014) studied the variation in nutritional status in six finger millet genotypes viz., KNE-479 and KNE 1034 from International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Gulu-E and FMV-1 from Kenya Agricultural Research Institute, and local landraces Ateso and Nyaikuro, were compared. The genotype "Gulu-E" has highest calcium content of 3199 mg/100 kg while KNE-479 had the lowest (2736 mg/kg) and mean calcium content of 2944 mg/kg was observed. For iron "KNE 1034" had the highest (147 mg/kg) and Gulu-E (33 mg/kg) the least and mean zinc content of the genotypes was 16.9 mg/kg.

Tahsin and Sanjay (2017) analyzed landraces for eight mineral elements Cu, Zn, Fe, Mn, Ca, and Mg by tri-acid digestion method. The Ca and Fe are present in high concentrations.

Badigannavar and Ganapathi (2018) experimentally studied the mineral nutrients among 49 indigenous finger millet germplasm lines at Bhabha Atomic Research Centre (BARC), Mumbai, India. They observed variability for micronutrients. Across the germplasm lines studied, calcium ranged from 135.0 to 312.0 mg/

100 g, iron ranged from 2.0 to 21.6 mg/100 g and magnesium ranged from 31.0 to 139.0 mg/100 g.

10.4.3.1 Anti-nutritional Content and Bioavailability of Micronutrients in Finger Millet

More amount of anti-nutrients in finger millet make the micronutrients less bio-available which can be removed by processing techniques such as germination, sprouting, soaking, roasting, dehulling, cooking, malting, and fermentation which are simple traditional food processing methods and therefore be used to increase bio availability of minerals. Germination could increase the anti-oxidant activity and reduce the anti-nutritional content of finger millet. There is a gradual decrease in the anti-nutritional contents of the flour as the days of germination increases. The phenolics, one of the secondary metabolic compounds, synthesized as a result of plant-environment interaction and acts as defense factors. Phenols are classified into two types as simple phenols and polyphenols. Polyphenols are once again classified into flavonoids and non-flavonoids. Tannins come under the non-flavonoid group. Phenolics have anti-oxidant property, anti-microbial activity, and high tendency to chelate positive metal ions like copper and iron. Phytic acid (IUPAC ID: (1*r*,2*R*,3*s*,4*S*,5*R*,6*S*)-cyclohexane-1,2,3,4,5,6-hexayl hexakis[dihydrogen (phosphate)]) and having formula of ($C_6H_{18}O_{24}P_6$) is a highly reactive chemical compound that readily binds mineral cations and in this complexed form is called as phytin. It is a sixfold dihydrogen phosphate ester of inositol, also called as inositol polyphosphate. IP_3 plays a role in signalling pathways like Ca^{2+} channel opening and rapid influx of calcium from the plant vacuole into the cytosol is limited by the very short life span (often less than 1 s) and constituent of cell membrane.

10.5 Challenges and Prospects

Unravelling the potential of finger millet as a climate resilient crop for achieving global food and nutritional security is an immediate task before us. The pioneering work on finger millet resulted in identification of Indaf (like Indaf 7 and Indaf 9) series in Asia. Stalwarts such as Dr. Lesli Coleman and Dr. Ragi Lakshmanaiah have made noteworthy contributions in the field of finger millet breeding particularly in hybridization of Indian and African finger millet. Dr. C. H. Lakshmanaiah initiated hybridization to generate genetic variability by crossing Indian varieties with African eco-types during 1964 that resulted in identification of 16 Indo-African varieties, known as Indaf varieties. It is essential to conserve the genetic resources, trait discovery and pre-breeding, conventional improvement, genomics and crop improvement, molecular breeding, and new vistas has to be laid out. For nutritional and health benefits, processing, value addition of traditional foods is of utmost important. Market linkages and entrepreneurship development and international market and export results in supply across the globe. There is urgent need to formulate government policies and support systems at national level, millet involvement in public distribution system and social welfare schemes such as nutrition

schemes and increase the role of N.G.O.s and Food and Agricultural Organization (FAO) of the United Nations Organization at international level in millets promotion. Establishment of premier agricultural research institutes engaged in basic and strategic research on millets distinguished for their excellence in agricultural education, research, and outreach activities. International institutes like International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) which is located in Hyderabad, India, and it is an organization that involved in agricultural research for development (R&D) in the drylands of Asia and African countries. It is actively involved in identification of crop wild relatives and use of multi-omics approaches to understand the mechanisms of complex traits to develop superior finger millet varieties. Combined efforts of target participants like researchers, industrialists, professionals, nutritionists, entrepreneurs, policy makers, other stakeholders of finger millet, and students would create a new avenue for development of breeding of finger millet. The fundamental mission of finger millet breeding is to serve the farming community by providing technologies for the wellbeing and socio-economic enhancement of the farmers. It is the crop specific sites in the country for agro-climatic zones to cater to the regional requirements. Constantly striving toward excellence in agricultural research and extension regarding breeding, the research stations should make persistent efforts in research and development and to release numerous high yielding and quality varieties in finger millet. The governments should make the research stations to be the hub for finger millet research and has actively involved in imparting education and awareness related activities among farmers through field demonstrations and other extension activities. The international conferences would be a platform for researchers across the globe to exchange their experiences, marketing, and value-addition opportunities and policy issues for promotion. Recently conferences like "ICFM-2022" which was held in Bengaluru, India, would aptly act as a curtain raiser in the run up for international year of millets-2023. Finger millet improvement in past, present, and future has to be assessed. Finger millet genome sequencing and annotation of candidate genes for "yield and quality parameters" status and its utility, evolutionary aspects in finger millet, global finger millet genetic resources their conservation and utilization, genomic and transcriptomic insights into nutraceutical properties of finger millet. At global level, finger millet improvement in Africa (Eastern and Southern Africa), crop production systems in Africa, ICRISAT-India and ICRISAT-Africa initiatives in finger millet improvement has been taking place. Crop production perspectives for drylands, physiological adaptations for climate resilience, economics of finger millet production systems, and latest developments in finger millet processing are the main arena for development of finger millet with high yield and quality parameters. Commercialization of finger millet-based products and avenues for export promotion in finger millet, nutritional programs in government funded schemes, private firms and corporates in sponsoring programs in public and private partnership (PPP) mode are way forward for millets development at national and global scenario. Our vision should transform millets cultivation from subsistence farming to globally competitive enterprise through cost-effective and environment friendly production,

processing, and value addition technologies and supply chain networks and basic studies regarding finger millet.

References

Ashoka MB, Halikatti SI (1997) Performance of finger millet genotypes to sowing dates. J Adv Agric 10:9–11

Babu BV, Ramana T, Radhakrishnan TM (1987) Chemical composition and protein content in hybrid varieties of finger millet. Short communication. Indian J Agric Sci

Bachar K, Mansour E, Khaled AB, Abid M, Haddad M, Yahya LB, Ferchichi A (2013) Fiber content and mineral composition of the finger millet of the Oasis of Gabes Tunisia. J Agric Sci 5(2):219

Badigannavar A, Ganapathi TR (2018) Genetic variability for mineral nutrients in indigenous germplasm lines of finger millet (*Eleusine coracana* Gaertn.). J Cereal Sci 84:1–6

Badu-Apraku B, Oyekunle M, Obeng-Antwi K, Osuman AS, Ado SG, Coulibay N, Didjeira A (2012) Performance of extra-early maize cultivars based on GGE biplot and AMMI analysis. J Agric Sci 150(4):473–483

Bezaweletaw K, Sripichitt P, Wongyai W, Hongtrakul V (2006) Genetic variation, heritability and path-analysis in Ethiopian finger millet [*Eleusine coracana* (L.) Gaertn] landraces. Agric Nat Resources 40(2):322–334

Chethan S, Malleshi NG (2007) Finger millet polyphenols: characterization and their nutraceutical potential. Am J Food Technol 2(7):582–592

Devaliya SD, Singh M, Intawala CG (2017) Genetic divergence studies in finger millet [*Eleusine coracana* (L.) Gaertn.]. Int J Curr Microbiol Appl Sci 6(11):2017–2022

Fentie M, Assefa A, Belete K (2013) AMMI analysis of yield performance and stability of finger millet genotypes across different environments. World J Agric Sci 9(3):231–237

Hilu KW, Wet JD (1976) Racial evolution in *Eleusine coracana* ssp. coracana (finger millet). Am J Bot 63(10):1311–1318

Jayashree MK, Nagarajaiah C (2013) Genetic variability and character association studies in African and Indian finger millet (*Eleusine coracana* L. Gaertin) accessions. Environ Ecol 31(4A):1950–1953

Kandpal RP, Vaidyanathan CS, Kumar MU, Sastry KS, Rao NA (1981) Alterations in the activities of the enzymes of proline metabolism in Ragi (*Eleusine coracana*) leaves during water stress. J Biosci 3(4):361–370

Lindsay WL, Norvell W (1978) Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Sci Soc Am J 42(3):421–428

Mahanthesha M, Sujatha M, Ashok KM, Pandravada SR (2017) Studies on variability, heritability and genetic advance for quantitative characters in finger millet (*Eleusine coracana* (L.) Gaertn) germplasm. Int J Curr Microbiol Appl Sci 6(6):970–974

Malambane G, Jaisil P, Sanitchon J, Suriharn B, Jothityangkoon D (2014) Variations of genotypes, seasons and genotype by season interactions for yield, yield components and agronomic traits in finger millet (*Eleusine coracana* L Gaertn). Plant Knowl J 3(1):8–14

Nishar A, Anwesha D, Gufran A, Firoz A, Mahtab R (2020) Potential and economics of ragi (*Eleusine coracana* (L.) Gaertn) cultivation under changing weather conditions of Ranchi, Jharkhand. Int J Curr Microbiol Appl Sci 9(2):467–476

Piper CS (1966) Soil and plant analysis. Hans Publishers, Bombay

Ravindran GJFC (1991) Studies on millets: proximate composition, mineral composition, and phytate and oxalate contents. Food Chem 39(1):99–107

Reddy CV, Reddy PVRM, Munirathnam P, Gowda J (2013) Studies of genetic variability in yield and yield attributing traits of finger millet [*Eleusine coracana* (L.) Gaertn]. Indian J Agric Res 47(6):549–552

Shet RM, Jagadeesha N, Lokesh GY, Gireesh C, Jayarame G (2010) Genetic variability, association and path coefficient studies in two interspecific crosses of finger millet [*Eleusine coracana* (L.) Gaertn]. Int J Plant Sci (Muzaffarnagar) 5(1):24–29

Solomon IS, Oliver N, Richard O, J, A. (2014) Variation of nutritional and anti-nutritional contents in finger millet (*Eleusine coracana* L. Gaertn.) genotypes. J Agric Vet Sci 7(11):06–12

Karad SR, Patil JV (2013) Assessment of genetic diversity among finger millet (*Eleusine coracana* L.) genotypes. Int J Integr Sci Innov Technol Sec C 2(4):37–43

Tahsin K, Sanjay GA (2017) Screening of higher mineral containing finger millet landraces from Maharashtra. Int J Food Sci Nutr 2:21–25

Thandava Ganesh B, Ramanappa TM, Ramesh S, Mohan Rao A (2021) MSc (Agri) Thesis, University of Agricultural Sciences, Bangalore. https://krishikosh.egranth.ac.in/handle/1/5810191732

Thompson LU (1993) Potential health benefits and problems associated with antinutrients in foods. Food Res Int 26(2):131–149

Ulaganathan V, Nirmalakumari A (2011) Genetic variability for yield and yield related traits in finger millet [*Eleusine coracana* (L.) Gaertn] genotypes. Department of Millets, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, India

Venkateshbabu D (2015) Identification of highwater use efficiency and high yielding ragi genotypes for rainfed conditions. Ecoscan 3:121–124

Breeding Finger Millet for Abiotic Stress Tolerance: Strategies and Challenges

11

Vadakkemuriyil Divya Nair and Reeta Devi

Abstract

The term "millet" refers to a varied group of small-seeded annual C4 panicoid grasses, the biomass of which is used as feed and the seeds as food. A vital climate-resilient nutrimillet with a wealth of elite genes and alleles is finger millet. The most effective and long-term tactics for increasing abiotic stress resistance in millet crops could be found in advanced biotechnology applications, like "omics" approaches. Abiotic stress tolerance-boosting strategies for millet crops could be discovered in cutting-edge biotechnology applications like "omics" techniques. An enhanced breeding method called genomics-assisted breeding takes into account both phenotypic selection and genetic information at the same time when creating phenotypes also can contribute to the abiotic stress tolerant variety development of the specie. In addition, application of novel technologies viz. gene manipulation technologies, tissue culture techniques, genetic diversity assessments, and germplasm conservation techniques can contribute to the enhancement of abiotic stress tolerance capacity of finger millet.

In this chapter is discussing the various research approaches that have been focused to improve abiotic stress tolerance in finger millet. Also, this chapter provides the specifics of the phenomic and genomic methods used to improve finger millet.

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Keywords

Finger millet · Abiotic stress tolerance · Germ plasm conservation · Omics approaches

11.1 Introduction

Global food security is severely hampered by abiotic stressors, which not only reduces productivity but can also degrade the standard of the harvested product (Wang and Frei 2011). Millets are among the widely cultivated crop due to their strong capacity for adaptation to a variety of ecological circumstances, high resistance for abiotic stresses, and minimal input requirements (Bandyopadhyay et al. 2017a, b). Millets' resistance to abiotic stress is influenced by a variety of morphological, genetic, and biochemical characteristics, such as early flowering or flexibility in response to rainfall patterns, reduced leaf area, waxy coating on plant body, thickened cell walls, and very well developed root systems. Genetic peculiarities like highly expressed genes of abiotic stress, and physiological peculiarities like increased photosynthetic and metabolic rate. Finger millet is an allo-tetraploid crop that was domesticated in East Africa and is extremely self-pollinating.

Population growth puts increased pressure on agriculture to use the available area to raise more food. The development of crops that are adaptable to climate change faces new obstacles. In the future decades, the world's developing countries will have to deal with the need for more food and make measures to combat hunger. *E. coracana* is therefore thought of as a nutraceutical crop or nutracereal that could aid in eradicating world hunger and malnutrition, but finger millet productivity is adversely impacted by abiotic restrictions which adversely affect the agriculture yield and food quality. Therefore, it is vital to improve finger millet to get over abiotic constraints. The advent of molecular breeding has accelerated the pace of plant breeding as a result of the development of genetic markers and genomics.

Additionally, the selection process for plants has steadily shifted from morphology-based to genetic-based. It is revealing that in order to improve crops, genome-assisted breeding approaches are helpful.

It is mostly cultivating in the world's arid and semiarid regions in more than 25 countries (Fakrudin et al. 2004). Although it has great potential and represents the sixth most significant cereal crop, it is not used as much as other commonly consumed cereals. There are around 22,583 finger millet genotypes found in India alone out of the more than 34,160 genotypes that are available world-wide as India is the country that produces the most finger millet (Ramakrishnan et al. 2015). Africa produces the most finger millet annually, 2.5 million metric tonnes, next to India, which produces 12 lakhs in metric tonnes (Sakamma et al. 2018).

The model plant for dietary supplements, *E. coracana* (Fig. 11.1), possesses all the quantitative and qualitative qualities necessary. It is more nutritionally beneficial other any other well-known cereal crops (Latha et al. 2005; Chandrasekara and Shahidi 2010).

Fig. 11.1 View of finger millet in field

Eleusine coracana (L.) Gaertn., vernacular - ragi, nachani, or nagali, belong to gramineae family. It is a type of crop plant that is allotetraploid ($2n = 4X = 36$). The generic term "Eleusine" derives from the historic city of "Eleusis," which was dedicated to Demeter and the Greek goddess of agriculture, Eleusis. The specific epithet "coracana" is derived from the Sri Lankan Sinhalese word "kurukkan." The particular epithet "coracana" is derived from the word "kurukkan" in Sri Lankan Sinhalese. The particular epithet "coracana" is derived from the word "kurukkan" in Sri Lankan Sinhalese. The panicle's finger-like branching (Fig. 11.2) is what gives finger milletits common name (Mirza and Marla 2019).

Both *E. Indica* ($2n = 18$) and E. Africana ($2n = 36$) share physical similarities with finger millet, and they are both descended from the wild species *E. Indica* and *E. tristachya* or *E. floccifolia* (Hiremath and Salimath 1992; Babu et al. 2013a, b). Abiotic stress, which also affects the quantity and quality of crops, is a significant barrier to global food security. It significantly reduces finger millet yield and growth (Saha et al. 2017; Ceasar et al. 2018; Maharajan et al. 2019).

The main abiotic stressors (Fig. 11.3) to finger millet are reduced soil fertility (soil deficient in nutrients), a lack of soil moisture (drought), and high soil salinity (Krishnamurthy et al. 2014; Maharajan et al. 2019; Mukami et al. 2020; Talwar

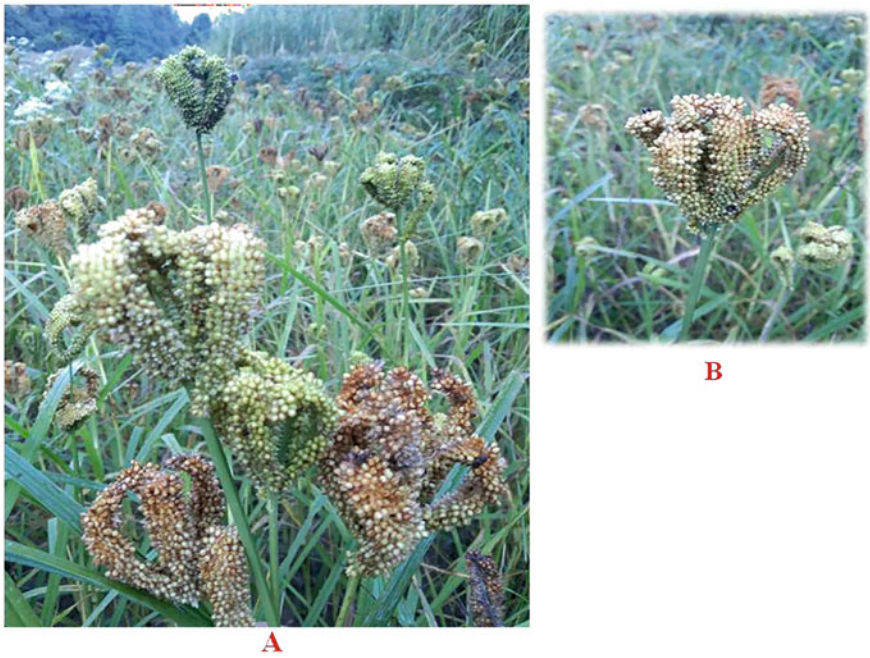

Fig. 11.2 (**a**) Inflorescence of finger millet in filed. (**b**) Enlarged view of an inflorescence

et al. 2020). In the regions where finger millet is grown, drought is the most important abiotic constraint (Ceasar et al. 2018). Drought is the most significant abiotic limitation in the areas where finger millet is farmed.

The plant finger millet possesses capacity to cop free radicals and withstand drought stress (Bhatt et al. 2011). Activation of numerous gene responses to drought stress enables the finger millet to mitigate the stress (Parvathi et al. 2013).

Soil salinity also hinders the growth of finger millet, but it does not affect the crop as much as drought and nutrient deficiency do (Krishnamurthy et al. 2014; Mukami et al. 2020).

Finger millet under salinity stress experiences a drop in grain weight, a postponement of blooming, and a decrease in water content, plant height, leaf expansion, and finger length and width (Anjaneyulu et al. 2014).

It is reported that salinity also adversely affects the plant development, shoot and root biomass and terminal leaf elongation of variety GPU-28, of finger millet (Hema et al. 2014; Parvathi and Nataraja 2017; Rahman et al. 2014a, b).

The production of finger millet is drastically reduced by all abiotic restrictions. Because of this, producing finger millet that is tolerant to abiotic stress is necessary to increase production. Germplasm characterization and gene finding are also crucial. Concerted efforts to utilize all the available advanced sophisticated technologies are now under trial (Fig. 11.4) to enhance the production of finger millet.

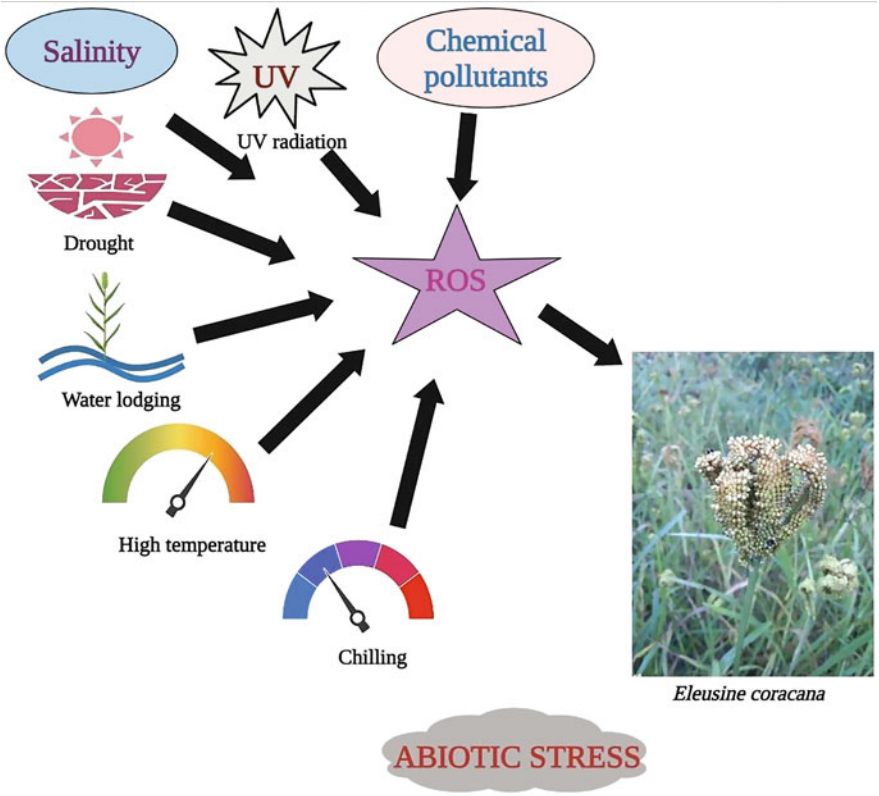

Fig. 11.3 Schematic representation of various abiotic stress which affects the finger millet production

11.2 History and Significance of Finger Millet

Next-generation breeding for abiotic stress tolerant varieties finger millet will depend on identification of adaption traits present in various populations and germplasm collections due to the increasingly unfavorable environmental circumstances.

Eleusine coracana subsp. Africana, which is indigenous to Africa, has a wider dispensation to damp regions of the world, including Asia and South America, is the source of the domesticated finger millet (*Eleusine coracana* subsp. coracana; $2n = 4x = 36$) (Dida et al. 2007, 2008; Gliblin and Fuller 2011; Fuller and Hildebrand 2013). Therefore, the persistence of landraces and their undomesticated varieties in various agro-ecosystems offers possibility for vast and distinctive phylogenetically adaptive diversity. It may present excellent prospects for the development or selection of cultivars with consistent yields that are able to adjust to both biotic (such as pests and diseases) and abiotic (such as drought) challenges.

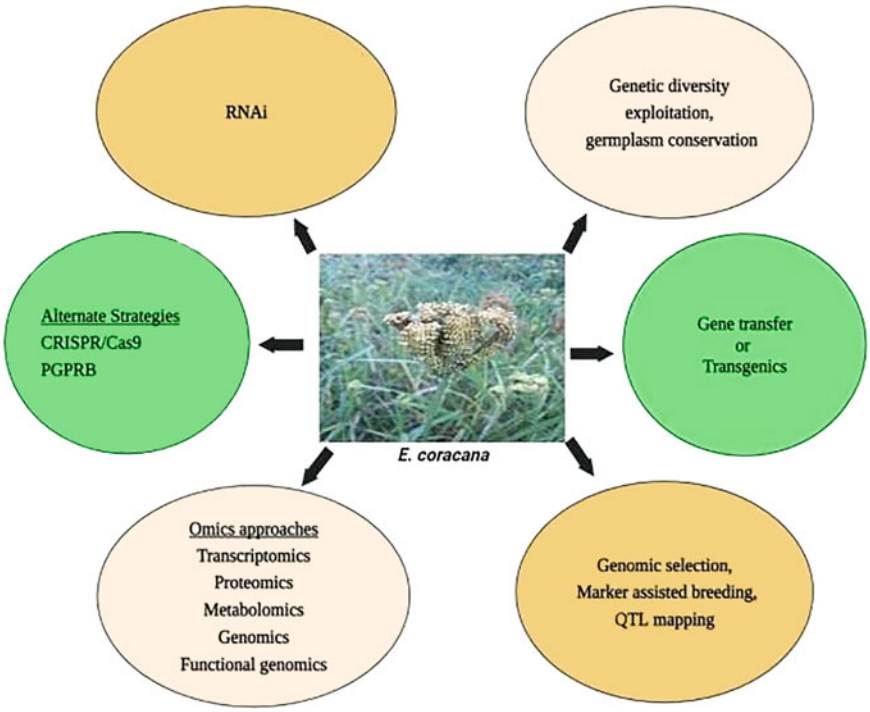

Fig. 11.4 Schematic representation of various research approaches that have been focused to improve abiotic stress tolerance in finger millet

New finger millet varieties that will be well suited to challenges brought on by the climate are already being developed as a result of national and international breeding projects. A variety of millet germplasm, including those of the crop's wild cousins, will certainly see an increase in demand as a result.

The primary location of origin, which is highly variable, is in Africa, and a secondary center is in India. Together, these two centers of variety account for all of the undomesticated varieties' natural dispersal (Fuller and Hildebrand 2013; Onziga 2015; Tesfaye and Mengistu 2017).

Since they make up the main gene pool that is freely cross-compatible, both the wild *E. Africana* ($2n = 4x = 36$; AABB genome) and the farmed finger millet. *E. coracana* ($2n = 4x = 36$; AABB genome) are significant from the perspective collection of germplasm and its' conservation. Wild relatives are found in two centers with sufficient variations, one of which is the highly variable original center of origin (Africa) and the other of which is a secondary center (India) (Fuller and Hildebrand 2013; Onziga 2015; Tesfaye and Mengistu 2017).

A similar allotetraploid, *Eleucine kigeziensis* ($2n = 4x = 38$; AADD genome), which is only found on the African continent and is highly endemic in eastern Africa, and the farmed finger millet are cross compatible (Dramadri 2015). The secondary

gene pool is made up of the interbreedable diploid wild species *E. intermedia* ($2n = 18$; AB genome), *E. tristachya* ($2n = 18$; AA genome), *E. floccifolia* ($2n = 18$; BB genome), and *E. indica* ($2n = 2x = 18$; AA genome). *E. jaegeri* ($2n = 2x = 20$; DD genome), *E. multiflora* ($2n = 2x = 16$; CC genome), and *E. verticillata* ($2n = 2x = 18$) are three diploid species that make up the tertiary gene pool. It is also well demonstrated that the diploid *E. indica* (wild goosegrass) is the source of the A genome in finger millet, but there is still a lack of information about the origins of the B genome (Devarumath et al. 2005; Liu et al. 2014; Hatakeyama et al. 2017; Zhang et al. 2019). Additionally, it is confirmed that that the diploid *E. indica* (wild goose grass) is the source of the finger millet's A genome, but there is a lack of information about the origins of the finger millet's B genome (Devarumath et al. 2005; Liu et al. 2014; Hatakeyama et al. 2017; Zhang et al. 2019). However, research on those species may uncover novel genes that can be incorporated into cultivable finger millet to create high-yielding and stress-resilient varieties, particularly against extreme drought, heat, salinity, low nutrient levels, and the deadly finger millet blast disease, all of which are made worse by climate change.

11.3 Effects of Climate Change and Associated Abiotic Stress on Finger Millet and Their Amelioration Strategies

Abiotic stress, which also affects the quantity and quality of crops, is a significant barrier to global food security. It significantly reduces finger millet yield and growth (Saha et al. 2017; Ceasar et al. 2018; Maharajan et al. 2019). The production of finger millet is adversely affect through all abiotic limitations. Because of this, producing finger millet that is tolerant to abiotic stress is necessary to increase production. Germplasm characterization and gene finding are also crucial. There has been extensive research on how climate change affects agricultural crops (Liu and Howell 2010; Liu et al. 2014; Lule et al. 2018; Manyasa et al. 2015). By virtue of alarming pace at which the climate is changing, food supplies around the world are directly impacted by a variety of abiotic pressures that are having an impact on food crops. Stress from drought, heat, flooding, and water logging are among the main effects of climate change on millet crops, as is lodging. After 4 weeks of seeding, full yield reduction was documented in different landraces of finger millet that had been exposed to drought (Maqsood and Ali 2007; Mgonja et al. 2011). A yield loss of over 60% was seen as a result of terminal dryness, which occurred from flowering till maturity (Miglani 2017). The majority of millets, especially finger millet, can live in extremely dry soil. For instance, it was noted that the biomass of millet and sorghum was equivalent in the Sahel region of West Africa, where the precipitation level is very little (Pandian et al. 2017). Drought has been linked to adverse impacts on crops' productivity as well as their nutritional quality, particularly the mineral and protein content of grains (Pandian et al. 2017; Panwar et al. 2010). Despite the fact that the majority of finger millet varieties are heat-tolerant, heat causes numerous physiological and biochemical changes. The most vulnerable biological functions to

heat stress are photosynthesis and respiration, which has a significant impact on crop output (Ramegowda et al. 2017).

Plants have a variety of defenses against the stress of water logging, which is brought on by hypoxia or anoxia. Plants participate in anaerobic respiration in response to hypoxia, finger millet has also been found to do this *(Eleusine coracana*).

Although anaerobic metabolism is less effective than aerobic metabolism, ATP generated during fermentation temporarily supports the cell. Because this method demands more sugar than aerobic metabolism, species that can withstand water logging such as rice and finger millet exhibit changes in their carbohydrate metabolism (Tumwesigye et al. 2019).

The greatest gene banks in the world, which are maintained by numerous domestic and international research organizations, have more than 34,675 accessions of finger millet. Dwivedi et al. (2012). Most of them also exhibit cross-compatibility with domesticated species, which increases the possibility of improving climate stress tolerance (Dwivedi et al. 2012; Saha et al. 2017; Gupta et al. 2017). Due to the high expenditures involved in analyzing every accession for traits important to agricultural development programs, these vast collections, which provide a promising genetic diversity, have a limited scope of application.

Researchers have begun preliminary screening efforts, nevertheless, in an effort to maximize the usefulness of the saved germplasm. Core collections comprising 622 accessions (Upadhyaya et al. 2005) and a miniature collection of 80 accessions (Upadhyaya et al. 2006; Babu et al. 2013a, b) have been produced on the basis of phenotypic characterization and multi-locational evaluation data acquired for quantitative aspects. There is now access to germplasm that is of short lifespan, high yielding, and resistant to blast disease as result of the evaluations' and identification of valuable variants. The evaluations identified beneficial variations, resulting in the development of early maturing, high yielding, and blast-resistant germplasm. Also a few accessions that can tolerate salinity and drought have been discovered; these can be employed in hybridization programs to boost finger millet tolerance to environmental factors. The one wild (*E. africana*) race and the four domesticated (*E. Coracana*) races (vulgaris, plana, elongate, and compacta) that belonged to the biotic stress accessions all exhibited a wide range of agronomic characteristics, such as duration for maturity, plant height, and panicle type. The same holds true for various accessions that include grains high in protein, calcium (Ca), iron (Fe), and zinc (Zn), which can be utilized to give finger millet and other crops a more nutrient-dense soil (Babu et al. 2013a, b). Along with the core and miniature-core assessments, progressive research initiatives to identify or develop genotypes of finger millet that are tolerant to adverse environmental circumstances commenced in the past few years at various research organizations.

In countries like India, Malawi, Zimbabwe, Kenya, Tanzania, and Ethiopia, only a small number of traditional breeding programs have so far resulted in varieties with higher yield and resilience to climate strain (Lenne et al. 2007; Mgonja et al. 2011; Sreenivasaprasad et al. 2004).

The gene banks' current repository of germ plasm that offers resilience to a variety of climatic challenges for climate-smart breeding may benefit from the addition of these enhanced genotypes and screened materials as more important sources of variation. A wider genetic base for finger millet improvement against diverse environmental conditions may also come from future usage of the mutant (both induced and natural) lines, but the mutant genetic resources accessible to finger millet are very less and require attention (Saha et al. 2017). There hasn't been much research done on the causes of finger millet's resistance to abiotic stresses such heat stress, water logging, salt, toxicity from aluminum, and nutritional deprivation. Additionally, the majority of reported screening trials were often founded on phenotypic data.

Therefore, it is anticipated that the recent developments in finger millet genomics studies would offer vivacious evidence for precise and expedited assessment of the existing finger millet genetic resources for resistance to diverse pressures. In the end, this will increase the effectiveness of research programs' breeding efforts and speed up the development and deployment of finger millet varieties for the stress tolerance.

11.4 Genomic Resource Availability of Finger Millet to Adapt with the Climate Change

Compared to other main cereals, finger millet has generally limited genetic resources, according to the database of NCBI (https://www.ncbi.nlm.nih.gov/), which hinders further development of this crop. There are only 1934 ESTs in finger millet that are linked to features that are resistant to disease, salt, and drought Ceasar et al. (2018).

In comparison to rice, maize, and barley, these are roughly 662.4, 1046.3, and 434.5 times less, respectively. Still, comparative genomics has been extensively investigated within the grass family, though, and can significantly support efforts to use markers to aid in selection for climatic stress resistance in finger millet (Yadav et al. 2014a, b; Ramakrishnan et al. 2016, 2017; Hittalmani et al. 2017; Pandian et al. 2018a, b, c). The NCBI Genbank database now contains a sizable quantity of sequence information for additional grasses that was produced by EST programs. SSR markers could be discovered using in silico analysis for the genomes of grass with the aid of bioinformatics tools. Studies have also shown that SSR markers with strong transferability are linked to climatic stress resistance and excellent agronomic traits, including finger millet grass blast tolerance, Ca content, and yield (Yadav et al. 2014a, b; Ramakrishnan et al. 2016). The substantial gene-level synteny shared by genomes of grass and the transferability of genetic resources from other thoroughly researched poaceae members to finger millet may aid finger millet's adaptation to a variety of challenging climatic situations or abiotic stress. Using the Roche 454 and Illumina Next Generation Sequencing (NGS) technologies, 10,327 SSRs and 23,285 non-homologous first SNPs were found in finger millet (Gimode et al. 2016).

Furthermore, a wealth of genetic resources with numerous chances for abiotic stress adaptation have been explained in the wake of the current development of

whole genome research for finger millet (Hittalmani et al. 2017). This vast collection of high-quality genomic data, which also includes 330 genes related to calcium transport and accumulation as well as SSRs - 114,083, R-gene −1766, drought responsive genes −2866, C4 –pathway genes—146, and 56 families of transcription factors (TFs), is available to the public at the NCBI Genbank database. It might be used as a guide for updating finger millet molecular research in the future.

11.5 Enhancing Abiotic Stress Through Genetic Modification and Transgenic Methods in Finger Millet

11.5.1 Genetic Modification to Improve Crops

Today's advancements in agriculture heavily rely on the development of tolerance to environmental cues through gene exchange between unrelated species. For abiotic stress tolerance, a remarkable number of transgenic crop plants have been grown in various nations (Shrawat and Lörz 2006; Ali et al. 2011). For the effective introduction of genetic material in finger millet using physical, chemical, and biological means, the right gene transfer technology is required. Numerous research publications are available regarding the gene transfer of finger millet to improve nutritional quality or tolerability to biotic or abiotic stresses (Gupta et al. 2001; Latha et al. 2005; Ignacimuthu and Ceasar 2012; Jagga-Chugh et al. 2012; Satish et al. 2017). Using genetic engineering techniques, finger millet can be improved to be more resilient to abiotic stress. The substantial gene-level synteny seen in grass genomes and the ability of transfer of genetic resources from other widely-analyzed poacean members to finger millet may facilitate finger millet's adaptation to a wide range of complex climatic circumstances. Roche 454 and Illumina Next Generation Sequencing (NGS) technology were used to find 10,327 SSRs and 23,285 non-homologous SNPs in finger millet (Gimode et al. 2016).

To increase finger millet's resistance to abiotic stress, a few initiatives have been conducted over the past 20 years. As opposed to other environmental stressors, the majority of efforts in the case of finger millet have been aimed to develop transgenics tolerant to salinity stress. For the first time, Mahalakshmi et al. (2006) describe the creation of genetically modified finger millet lines that can survive abiotic stress. In this study, they used the biolistic approach to transfer the serine-rich protein (PcSrp) gene from *Porteresia coarctata* under the Actin-1 promoter from rice for salinity stress resistance to the embryonic calli derived from shoot tips. In this experiment, tungsten particles were bombarded with sorbitol and mannitol for 4 h prior to and after the bombardment, with a target distance of 7 cm. Transformants were used in a medium that had been pre-treated with 250 mM NaCl for the experiment Babu et al. (2013b). A binary vector called pCAMBIA that contains the PDH45 gene was modified to provide salt tolerance using Agrobacterium-mediated gene transfer. Embryonic calli were used as explant and to ensure the transformation hptII primers were used in this study. Also PCR and RTPCR were used to verify the expression of transferred gene in the host.

Bayer et al. (2014) developed lineages of transgenic organisms that are resistant to the dinitroaniline family of herbicides using a biolistic and an Agrobacterium-mediated procedure. The biolistic gene gun method was employed to transfer mutant 1-tubulin genes from *Eleusine indica* and Hordeum vulgare using the plasmid pAHTUAml. In this study, it is reported that the phosphinothricin resistance (bar) gene served as a selection marker, the maize ubiquitin promoter (PUbi) gene as a selectable marker, and the nopaline synthase (NOS) gene as a terminator. The binary vector pBIRUB8 with the TUBm1 and HvTUB1 gene and CaMV35S promoter were used in agrobacterium mediated gene transfer. The transformants were picked based on their development phases in vitro on a trifluralin-containing substrate. The application of Agrobacterium virulence gene activators boosted the incidence of transformation in *E. coracana*.

In order to improve the ability of finger millet to survive salt stress, Anjaneyulu et al. (2014) reported using Agrobacterium-mediated gene transfer to introduce the vacuolar H+-pyrophosphatase (SbVPPase) gene from Sorghum into finger millet. The transgene expression was investigated in normal and transgenic plants grown in 100 mM and 200 mM NaCl under the observation of particular biochemical and physiological growth parameters and observed that the transgenic plants demonstrated resistance to salt compared to controls.

Jayasudha et al. (2014) had reported the introduction of the H+-pyrophosphatase (AVP1) from *Arabidopsis thaliana* and the Na+/H+ antiporter of *Pennisetum glaucum* (PgNHX1) through Agrobacterium-mediated transformation in to *E. cocracana* where the transgenic plants shown to be successful in resistance to osmotic stress viz. salinity and drought stress. Gupta et al. (2001) demonstrated the effectiveness of various validated promoters, CaMV35S, Act I (rice), UqI (maize), RbcS, and FtuidA using β-glucuronidase (GUS) and they also documented the biolistic method of gene transfer. In 2011, Ceasar and Ignacimuthu, has been reported a methodology to improve Agrobacterium-mediated gene transfer via shoot apex. The Agrobacterium strain LBA4404 carried the GUS reporter gene and the hygromycin phosphotransferase (hptII) selectable marker gene on the plasmid binary vector CAMBIA1301. The result of the study reported that under ideal circumstances, there were 3.8% steady transformation efficiency and 19% transient expression in genotype GPU 45. Using a seed-derived callus as an explant, Jagga-Chugh et al. (2012) reported an improved biolistic mediated transformation procedure where the best circumstances for biolistic-mediated genetic transformation in finger millet, per this study, were reported to be 1100 psi pressure to rupture disc, 3 cm for the microcarrier, and 12 cm for the microprojectile. It also demonstrated highly effective transformation when the callus was treated with 0.4M sorbitol. they reported to be used the CaMV35S promoter for effective and precise gene transfer. Gene transfer was verified using DIG-labeled hptII as a probe for Southern hybridization and PCR with hptII primers.

The genetic modification of *E. coracana* was reported by Satish et al. (2017) utilizing Agrobacterium strain EHA105 harboring binary vector pCAMBIA1301. In this study utilizing the shoot apical meristem as an explant, a better procedure for gene transfer was created. In this study, a number of variables, including the age of

the explants, the amount of antibiotic (hygromycin), the density of the culture, the length of the infection, the period of co-cultivation, and the amount of acetosyringone concentration, were optimized. Without going through the callus phase, this study gives finger millet the highest transformation efficiency (85.1%) in 45 days. Southern blotting or PCR were used to assure the transformation.

In order to alter *E. coracana*, Yemets et al. (2008) established a biolistic technique employing a naturally mutated a-tubulin gene from *E. indica* that confers resistance to the herbicide Trifluralin, TFL (2,6-dinitro-*N,N*-dipropyl-4-(trifluoromethyl) benzenamine). Two a-tubulin 1 alleles that confer either a high level of tolerance to antimicrotubule herbicides, such as dinitroanilines and phosphoroamidates, or an intermediate level of tolerance to them, have been identified in goose grass (*E. indica*) which are the result of a single unique point mutation (Hussey and Anthony).

A steady expression of the bacterial mannitol-1-phosphate dehydrogenase (mtlD) gene in finger millet was attempted by Hema et al. (2014). Comparing the genetically modified plants to their respective natural kinds, the transgenic plants demonstrated resistance to both drought and salinity stress. Transgenic lines displayed greater chlorophyll retention than the drought stress induced normal plants (Hema et al. 2014).

The majority of crop improvement initiatives will focus on genetic transformation to address complicated climate-induced stresses under unfavorable climatic circumstances, as conventional approaches may be hampered by the lack of genetic diversity among diverse germplasm. For *E. coracana*, the application of gene modification for abiotic stress tolerance breeding is not yet much advanced, mostly due to difficulties linked to the scarcity of sufficient genomic resources (Hittalmani et al. 2017). This has mostly been due to the lack of ideal transformation techniques for producing mutant lines in finger millet based on a flexible manner of gene transfer mediated by Agrobacterium (Ceasar and Ignacimuthu 2014).

In finger millet, reports only a few transformation studies are available on transfer of genes linked to tolerance to salinity, drought, and leaf blast disease. The majority of crop improvement initiatives will focus on genetic transformation to address complicated climate-induced stresses under unfavorable climatic circumstances, as conventional approaches may be hampered by the lack of genetic diversity among cross-compatible germplasm. In order to handle complex climate-induced stresses due to the unfavorable climatic conditions, genetic modifications/gene transfer will be a key component of the majority of crop improvement initiatives, as cross-compatible germplasm's low genetic diversity may make conventional approaches ineffective.

Similar to this, the majority of earlier transformation research utilized biolistic-mediated gene transfer and were only able to evaluate expression of marker gene using model plants (Latha et al. 2005; Ceasar and Ignacimuthu 2014). Agrobacterium-mediated transformation, however, has made significant advancements in other well-analyzed cereals, such as rice (Beyer et al. 2002; Zhang et al. 2016). Similar to this, the majority of earlier transformation research utilized biolistic-mediated gene transfer and were able to evaluate the expression of

marker genes using model plants (Latha et al. 2005; Ceasar and Ignacimuthu 2014). Finger millet is mostly cultivated by resource-poor farmers, it is necessary to expand transgenic effort to finger millet within less time span in the genomic age to assure enhanced food and economic security.

It is hoped that the availability of the full finger millet genome assembly, which will enhance the Agrobacterium-mediated transformation procedures, will be helpful in the development of abiotic stress resistant finger millet transgenic crops.

This is especially true when T-DNA is given plant-specific promoters and terminators for situations in which the target loci come from lower or higher groups of other than plants.

The most recent Complete Genome Sequence resources for finger millet will typically act as a foundation for effective, thorough discovery and replication of new foreign stress-resistant genes from variations based on SNPs in different transformation studies for improving finger millet tolerance to numerous environmental cues.

For thorough discovery and cloning of new stress-tolerant genes from variations based on SNPs, and effectively utilizing them in various transformation studies for improving finger millet resistant to numerous climatic cues, the most recent Complete Genome Sequence resources for finger millet will typically serve as a reference.

11.6 Gene Silencing

In order to identify the function of an investigated gene that results in amend phenotypes, gene silencing is a crucial reverse genetics method. One technique for silencing genes is insertional mutagenesis (T-DNA insertions or transposable elements insertion) used to silence a target mRNA but has drawbacks when dealing with multigene families, necessitating extensive research to identify the required genes. Additionally, homologous recombination has not yet been successful in angiosperms to replace specific genes. Lately, virus-induced gene silencing (VIGS) via dsRNA has evolved as a significant functional genomics method that is quick and straight forward to use. Through the use of viral vectors that contain a target area of the host gene, VIGS can reduce the expression of the target plant gene.

Generally, it does not result in knockout mutants or stable RNA interference (RNAi), which can be used in varieties that are difficult to transform (Scofield and Nelson 2009). Although there are a number of vectors that can support VIGS in agricultural plants, finger millet have not yet seen a lot of use for them.

11.7 Application of Various Omics Approaches to Intensify the Abiotic Stress Resilience in Finger Millet

Advanced biotechnology applications, particularly "omics" techniques, may be the most practical and long-lasting means of enhancing abiotic stress resilience in finger millet. By using perception into the gene, protein, or metabolite profile and their morphological impacts offered by omics techniques, we can better comprehend the

molecule level foundation of stress tolerance and response. The accessibility of genetic sequences and ensuing advancements in "omics" technologies hold great potential for enhancing millets' capacity to tolerate abiotic stress. With the advent of whole-genome sequencing (WGS) for *E. coracana*, we can now pinpoint genetic coalitions that control genotypic diversity for agronomically desirable features in the elegant fostering populations (Hittalmani et al. 2017; Hatakeyama et al. 2017). Genome-assisted techniques allow for the quick identification and selection of novel beneficial genes in the germplasm. Genome-assisted methods enable the rapid discovery and selection of novel advantageous genes in the germplasm. It offers the opportunity to detect potential genes implicated in the potential to tolerate environmental cues. One must have a thorough understanding of both the target and the crop species on which the study was done in order to successfully utilize biotechnological applications to overcome abiotic constraints. With the development of "omics" technologies and the functional identification of particular genes, it has become evident that the environmental habituation that is necessary for plant survival is subject to stringent regulation (López et al. 2008; Muthamilarasan et al. 2014; Kissoudis et al. 2014). Our comprehension of the mechanisms underpinning stress tolerance has significantly advanced not just in model crops like Arabidopsis but also in other crops like garden and woody plants, cereals, and millets (Bokhari et al. 2007; Alvarez et al. 2008; Caruso et al. 2009; Degenkolbe et al. 2009; Shulaev et al. 2008; Watkinson et al. 2008; Ginzberg et al. 2009; Dwivedi et al. 2012; Lata et al. 2013; Muthamilarasan et al. 2014). Even while sequence data is an essential and required molecular biology beginning point, it is inadequate on its own to provide answers to concerns regarding the function of stress responsive genes, regulatory networks, and the pertinent biochemical processes. Therefore, greater techniques, such as bioinformatics and systems biology approaches, as well as quantitative and qualitative assessments of genetic expression products at the transcriptome, proteome, and metabolomics levels, are crucial. Therefore, it is critical to incorporate more comprehensive methodologies, such as bioinformatics and systems biology approaches, as well as quantitative and qualitative assessments of gene expression products at the transcriptome, proteome, and metabolomic levels.

Doing so will further enable a more focused application of marker assisted selection (MAS) and transgenic technologies (Dita et al. 2006). As a result, marker-assisted selection (MAS) and transgenic technologies will be applied in a more targeted manner.

The term "omics" broadly refers to all biotechnological applications that require a molecular understanding of stress response, such as genetic engineering, functional genomics, and gene expression, as well as their overall phenotypic effects in response to environmental perturbations, which are frequently accompanied by significant changes in the plant transcriptome, proteome, and metabolome (Ahuja et al. 2010).

Abiotic stress perception, response, and tolerance in millet crops are being effectively understood recently with the effective application of "omic" techniques. Various millet crops have undergone systematic transcriptome and expression profiling research to find potential genetic factor and controlling networks engaged

in abiotic stress responses. As described in more detail in the following sections, significant progress has been made so far in the study of prominent millets such as *Setaria italica*, *Pennisetum glaucum*, and *E. coracana* under abiotic stress.

11.7.1 Phenomics

Phenomics is a rigorous method to understand phenotypes, despite being inconsistent, time-consuming, and ecologically sensitive. A systematic way to studying phenotypes, however it is inconsistent, time-consuming, and environmentally delicate. Phenomics is a crucial "omics" technology for enhanced genomics and is equally significant. Phenomics is the assessment of an organism's "phenomes," or more specifically, its physical and biochemical features, which are subject to change as a result of environmental pressures or genetic mutation. The genomics platform's wealth of data must be appropriately linked to the phenotype in order to be effectively utilized.

In fact, it would be incorrect to label any of the aforementioned methods successful until the morphological manifestation of plants demonstrating any level of genomic, transcriptomic, proteomic, or metabolic profile is fully defined or comprehended. Between genotype and phenotype, there is a substantial knowledge gap, or the connection between genotype and phenotype is difficult to discern. (Furbank and Tester 2011). High capacity physiology and phenotyping has, in fact, become a new point of stagnation in plant breeding and stress biology. In reality, a new point of stagnation in breeding of plants and stress biology is high throughput physiology and phenotyping (Yang et al. 2013). Therefore, in order to conduct thorough assessments of any abiotic stress on crop plants, it is required to develop certain fundamental criteria for phenomics characterization. Though phenotyping plans for peal millet and foxtail millets are already been reported (Vadez et al. 2012; Lata et al. 2011), there are very less number of studies regarding the same on finger millets.

A methodical approach to researching phenotypes is phenomics though it is inconsistent, time-consuming, and ecologically delicate. For phenomic studies, advanced technologies are available because it is vital for characterization of the germplasm.

Automation, imaging, and software that successfully combines and improves phenotyping through optimization have become more widely available, which has benefitted high-throughput phenotyping investigations (Sood 2019). A high-capacity phenotyping system provides constant data homogeneity. Functional factors like morphological, phonological, physiological, and nutritional characteristics are used to predict how plants will react to abiotic stress. Therefore, it is essential to evaluate and characterize germplasm in order to create new breeding programs (Ulaganathan and Nirmalakumari 2015; Sood 2019; Krishna et al. 2020). Morphological markers and features play a substantial part role in the characterization of germplasm (Dasanayaka 2016). There are numerous research (Suman et al. 2019; Ulaganathan and Nirmalakumari 2015; Dhanalakshmi et al. 2014) that

compile and present the agro-morphological features of different germplasm stocks of finger millets.

Only a small amount of study has been done so far on the nutritional and nutrient components of finger millet morphotyping. In a study Ramakrishnan et al. (2017) grew 128 genotypes of finger millet in a greenhouse under low P circumstances, and these genotypes were split into low-P-tolerant and low-P-susceptible genotypes. The genotypes which were shown to have low-P-tolerance are GPU45, IE5201, IE2871, IE7320, GPU66, HOSUR1, TCUM1, IE2034, SVK1, RAU8, VR708, and IE3391.

Yamunarani et al. (2016) published a study on 319 genotypes of finger millet to examine their seed Zn content, and GEC164 and GEC543 showed greater grain Zn content.

In a similar manner, Nirgude et al. (2014) looked at 106 genotypes to evaluate how much protein and calcium were present in the grains and discovered that protein and calcium contents of these genotypes varied widely.

Finger millet genotypes differ significantly in terms of the nutritional value of the grain and their tolerance to abiotic stresses, offering the possibility to improve quality and production through breeding methods.

Studies on physical and functional properties of finger millet from sub-Saharan Africa were reported by Ramashia et al. (2018). The grain cultivars' physical characteristics, including their color attributes, sample weight, bulk density, true density, porosity, surface area, sample volume, aspect ratio, sphericity, dimensional characteristics, and moisture content, were determined. In this study finger millet showed superiority in characters like flour characters, dimensional properties than pearl millet variety which was used as control. The study is significant for the development of machinery for finger millet grain processing by agricultural and food engineers, designers, scientists, and processors. The findings should be helpful in determining the calibre of the grains used to enhance finger millet flour.

Phenotypic and genotypic diversity of composite global collection of finger millet was reported by Bharathi (2011). In this study they had used 1000 accessions which included 622 core collections and organized a set of 300 extreme diverse accessions as standard.

Agro-morphological character analysis on global collection of 190 genotypes of finger millet was reported by Babu et al (2014a, b). Overall, the findings demonstrated that genotypes of Asian were shorter in height, shorter in the length of the flag leaf, less numerous in the basal tiller, of early flowering and early maturity, shorter in the length of the ear head, and shorter in the length of the longest finger. For the purpose of developing effective breeding strategies for finger millet, taking into account its importance as a fantastic model system and also as an excellent source of nutrients, good phenotyping techniques, both high throughput and manual, must be developed.

11.7.2 Transcriptomics

A cell, tissue, or organism's entire population of messenger RNAs (mRNAs) is also known as the transcriptome, and the study of this population is known as transcriptomics. Genome-wide expression profiling analyses are subsequently effective techniques for locating potential genes implicated in a multitude of biological mechanisms and networks that control stress. Due to the fact that plants regularly suffer from cellular dehydration whether exposed to drought, salt, temperature extremes, or ABA, more than half of the genes expressed in all three circumstances are common.

Genes responsive to are typically divided into two classes, early- and late-responsive, based on the duration of their response. Genes which become active within a few seconds or minute to stress exposure are known as early responsive genes and the set of genes which take time, hours, days, or even weeks for response to abiotic stress is considered as late responsive genes (Ramanjulu and Bartels 2002).

Theoretically, early sensitive genes that are directly involved in signal detection, amplification, and transduction, such as protein kinases and transcription factors (TFs), are involved in initial protection and gene expression control. Therefore, it is hypothesized that early sensitive genes, such as protein kinases and transcription factors (TFs), are involved in initial protection and gene expression control by being immediately involved in signal detection, amplification, and transmission. The late responsive genes, on the other hand, are thought to aid in stress adaption as they encode for proteins including heat shock proteins (HSPs), reactive oxygen species (ROS) scavengers, and late embryogenesis abundant (LEA) proteins, among others (Bray 1997; Ramanjulu and Bartels 2002). The discovery that 11% of stress-inducible genes are essentially potential transcription factors (TFs) provides more proof of the significance of gene regulation in stress adaption and tolerance (Seki et al. 2002). The transcriptome procedure enables thorough study and measurement of alterations brought about by abiotic stressors at the level of the entire organism. Rice has been one of the most well studied monocots for gene expression research; the findings from these studies might potentially be applied to millets for comparative genomics. Although there are significant differences in the physiological processes between C3 and C4 crops, the model of rice is anticipated to be considerably different from millets with reference to stress response, physiology, architecture, and metabolism. So it has become more and more important in the current situation to design a C4 grass model system. Through the use of high throughput techniques like macro- and microarray analysis, model C4 crop foxtail millet has been used to make great strides in the genetic and genomic study of millets (Lata and Prasad 2013; Lata et al. 2013). The identification of responsible genes implicated in diverse metabolic processes and stress controlling networks is thus made possible by genome-wide expression profiling investigations, which are extremely effective methods. The majority of the recent developments in genomic research of *E. coracana* could direct or assist for studies on diversity of function, profiling of genome-wide transcript, and authentication of responsible genes linked to climatic stresses; particularly, genetic factors tangled in various nutrient signalling pathways, such as genes

responsible for uptake of calcium, phosphorus, and genes responsible for N and C metabolism, and abiotic stresses (drought, salinity). The complete genome's identified calcium genes will assist research into *E. coracana* germplasm for assimilation, translocation, and storage of nutrients like calcium in various tissues as well as the embellishment of other cereals with this essential nutrient as finger millet shows maximum calcium composition compared to other cereals (Kumar et al. 2016a, b). Li et al. (2020) had conducted study to assess differentially expressed proteins and differentially expressed genes towards drought stress at various phases of life cycle of *E. coracana.* In this combined transcriptome and proteome study, 80,602 and 3009 differentially expressed genes (DEGs) and proteins were recognized.

The biochemical and molecular underpinnings of salt resilience in two dissimilar *E. coracana* genotypes, CO 12 and Trichy 1, were investigate by Rahman et al. (2014a, b). In this research, the *E. coracana* transcripts were mapped and annotated towards rice gene models, resulting in the discovery of genes that respond to salinity and genotype-specific responses. It was discovered that the tolerant Trichy had much higher levels of many functional categories of genes, including transporters, transcription factors, gene included in cell signalling, osmotic balance, and the manufacture of suitable solutes. In another transcriptome study on finger millet variety GPU-28, Parvathi et al. (2019) had published that they had obtained 90,830 transcripts from drought tolerant samples. Different drought –stress signalling cascades of genes like, serene threonine protein phosphatase 2A (PP2A), calcineurin B-like interacting protein kinase 31 (CIPK31), farnesyl pyrophosphate synthase (FPS), signal recognition particle receptor (SRPRα), etc. were identified as part of pathway analysis and functional annotation of drought stress induced samples. Transcriptome characterization revealed nine regulatory genes of EAAs metabolic pathway in sustaining the quality and accumulation of seed storage protein (SSPs) in finger millet (Gururani et al. 2020). The regulatory genes are aspartate kinase, homoserine dehydrogenase, theronine synthase, theronnine dehydratase, dihydrodipicolinate synthase, cystathionine γ synthase, acetolactate synthase, and lysine 2-oxoglutarato reductase/saccharopine dehydrogenase (LOR/SD).

11.7.3 Proteomics

In order to comprehend biochemistry or underlying controlling mechanisms, a substantial examination of proteins expressed at a particular stage or environment in a cell, tissue, or organism is referred to as proteomics. The term "proteome" pertains to the total set of proteins generated or altered by an organism. Any organism's proteome, which functions as a connection between its transcriptome and metabolome, provides information about its current condition. Proteomics technique becomes fairly essential in assessing plant stress responses since proteins tend to operate directly on biochemical processes whereas mRNA levels are not always connected with protein accumulation (Gygi et al. 1999). Mass spectrometry (MS)-based proteomic methods are typically used to identify post-translationally

changed proteins (Barkley and Wang 2008). Contrary to other biological systems, publications on the use of proteomics to millet crops are few though there is high advancements in this field (Reddy et al. 2012). In addition, in contrast to transcriptome analysis, proteome analysis of millet plants responding to different abiotic stresses is still highly underdeveloped (Canovas et al. 2004).

Using two-dimensional electrophoresis, a proteomic analysis of salt-tolerant cv. "Prasad" seedlings that were 7 days old identified 29 proteins that were differently expressed and were included in signal transduction, photosynthesis, metabolism, and stress response (Veeranagamallaiah et al. 2008). Although information about sequence is a crucial and necessary jumping –off for molecular biology, it is not sufficient on its own to answer questions on the function of abiotic stress responsive genes, regulatory networks, or the relevant biochemical pathways.

The Jasmonate ZIM domain (JAZ) protein family, the key regulator of plant JA signalling pathway while methyl jasmonate treatment in *E. coracana* were reported through proteomic analysis by Sen et al. (2016).

11.7.4 Metabolomics

The term "metabolome" specifically describes to the entire collection of lighter molecular weight substances that are available in a sample and that are substrates or by products of enzymatic activities and have a tendency to directly affect the phenotypic of a cell, tissue, or organism. Thus, the aim of “metabolomics” is to identify metabolite profile of a sample at a particular stage, period, or set of environmental factors; this yields a clear functional description of physiological state of an organism. A further point illustrating the significance of metabolomics in the age of functional genomics is that changes in the cell metabolome are not always connected neither to changes in the transcriptome nor proteome. Several conceptual metabolomics approaches, such as target analysis, metabolite profiling, and fingerprinting, are useful for numerous large-scale applications, such as phenotyping of transgenics, determining gene function, conducting considerable equivalence tests, and monitoring stress responses. As a result, it is believed that metabolomics can bridge the genotype–phenotype gap. Consequently, a thorough knowledge of both gene function and the molecular processes prompting intricate biological processes depends on a coordinated analysis of the transcriptome, proteome, and metabolome.

There are a few reports of major millet crop’s metabolic, and the majority of metabolomics research has been done on model plants and staple cereals. It is reported that the examination of the transcriptome and metabolome of seedlings of *E. coracana* exposed to various treatments to induce osmotic stress viz Si (10 ppm), PEG (15%), and PEG (15%) + Si (10 ppm) and distilled water (DW) as the control, suggests that Si can affect some molecular processes to reduce osmotic stress (Mundada et al. 2021).

11.7.5 Genomics

For research on functional genomics and genome-assisted breeding, genomic resources must be accessible. Crop development efforts require fundamental genomic resources such as genetic maps, molecular markers, and sequencing data (Bansal et al. 2009). Direct screening of germplasm grown in fields and greenhouses no longer requires as much time or effort as it formerly did because to genome-based research. Because of this, selection in traditional plant breeding has steadily changed from phenotype-based to genotype-based (Leng et al. 2017). Genomic research is also employed to develop novel molecular markers and find QTL through MAS in order to improve cultivars (Maharajan et al. 2018). *E. coracana* typically is quite limited genetic resources in contrast to other prominent cereal crops, which makes it challenging to further develop this crop. Consequently, the finger millet genome resource is significant for its development (Saha et al. 2017; Ceasar et al. 2016). Molecular markers, genetic maps, and sequencing data are fundamental resources needed for genetic research and molecular breeding techniques. Molecular markers must be developed and quantitative trait loci (QTLs) must be modified using Marker Assisted Selection (MAS) for genomics as a whole to produce superior crops.

By eliminating the labor and time consuming direct screening of germplasm grown in fields and greenhouses, these genomic resources simplify breeding techniques for agricultural development initiatives. Comparative mapping and synteny investigations in agricultural plants are further aided by genetic maps and molecular markers. For instance, one recent study revealed the synteny between the chromosomes of pearl millet, foxtail millet, and other poaceae members (Rajaram et al. 2013; Fig. 11.2), but millets have lagged behind other cereal crops like rice, wheat, and maize in the development of gene linkage maps and the identification of DNA markers.

11.7.6 Genome Sequence of Finger Millets

The most modern technology of genome sequencing, known as next-generation sequencing (NGS), is beneficial for figuring out how the genomes of different crops are organized. The whole genomes of the finger millet varieties ML-365 (Hittalmani et al. 2017) and PR 202 (Hatakeyama et al. 2017) were constructed applying Illumina and SOLiD sequencing technologies. The whole genome sequencing (WGS) of the *E. coracana* variety ML-365 revealed approximately 45 Gb paired-end, 21 Gb mate-pair data, 525,759 scaffolds (>200 bp) with a N50 length of 23.73 Kb and an average scaffold length of 2275 bp, as well as 1766 genes for disease resistance and 2866, 330 genes, respectively, for drought-responsiveness and accumulation and transportation of calcium (Hittalmani et al. 2017). The genome size of the *E. coracana* genotype ML-365 was 1,453 Mb, with an average DNA content (2C) of 3.01 pg. The finger millet genome size is 82% covered by WGS, and a total of 85,243 genes are reported (Hittalmani et al. 2017). The variety PR-202's genome is stated to be 1.5 Gb in size in this study, with the assembled

genome coming in at 1189 Mb, or 78.2% of the genome (Hatakeyama et al. 2017). There is now more evidence of direct correlation between the *E. coracana* WGS and the model millet crop (Ceasar et al. 2018). The *E. coracana* genome sequence has insufficient annotation. For improved comprehension of genome organization, future high-resolution studies for the advancement of *E. coracana* will also need the finger millet genome sequence. Furthermore, there was little effort put into sequencing the transcriptome of specific variety subjected to abiotic cues including salinity, moisture, and drought. In the approaching years, more transcriptome sequencing of a specific abiotic stress (different macro- and micronutrients viz. P, N, and Zn) will be pivotal for improving *E. corancana*. It will help increase *E. coracana* productivity in conditions of changing climate. It will help to increase finger millet productivity in climate-changing conditions.

11.8 Finding Molecular Markers for Finger Millets' Abiotic Stress Tolerance

Several markers for use in genetic improvement have been discovered in light of the genome characterization of finger millet utilizing PCR and non-PCR based techniques. In order to evaluate the genetic diversity of the germplasm of *E. coracana*, numerous studies have used microsatellite and EST-based microsatellite markers (Arya et al. 2013; Babu et al. 2014a). Ten RAPD and ten ISSR markers were adopted by Gupta et al. (2010) to validate the genetic relationships among three different kinds of finger millets: PRM-1 (Brown), PRM-701 (White), and PRM-801 (Golden). The genetic adaptability of different finger millet genotypes to environmental cues has been described in various research reports using RAPD-based molecular characterization (Kumari and Pande 2010; Das et al. 2009; Babu et al. 2007; Ramakrishna et al. 2018; Fakrudin et al. 2004).

Crop development is influenced by two factors: choosing an effective hybridization technique and using molecular markers to verify the genetic purity of hybrids. There have been reports of hybridization and hybrid identification in finger millet from numerous eminent research groups (Krishna et al. 2020).

11.8.1 Breeding with the Aid of Markers and Genomic Selection

The description of the genomic selection and genetic interactions among the finger millet accessions depends heavily on research on genetic variation and population structure. The optimal breeding stock for improving finger millet has been shown to be determined by genotyping when paired with genetic variation and population structure (Dida et al. 2008). Therefore, the application of MAS-based rapid breeding methods is made simpler by genome-wide assessments of the genetic diversity of finger millet germplasm. There are numerous molecular marker systems, including single primer amplification reaction (SPAR) (Pandian et al. 2018a), interspersed simple sequence repeat (ISSR), random amplified polymorphic DNA (RAPD)

(Kumari and Pande 2010), and simple sequence repeat. Finger millet accessions can be distinguished genetically using the polymorphism of several markers. The diversity of the plant's accessions possibly useful in future breeding techniques to create finger millet that withstand abiotic stress. Marker-assisted breeding could significantly increase the efficacy and precision of traditional plant breeding through the use of MAS. The effectiveness and accuracy of traditional plant breeding could be greatly increased with the application of MAS in marker-assisted breeding. It is a crucial tool for identifying and enhancing specific abilities. The MAS, which are extensively employed in crop breeding programs, are built on the DNA-based molecular markers. As a result, after QTL are identified and mapped, genetic enhancement of such qualities may be attainable with the aid of marker-assisted breeding. Therefore, marker-assisted breeding may be able to augment the genetic make-up of such traits if QTL are found and mapped. Only a small number of QTL underlying agro-morphological and nutritional features were discovered under varied abiotic conditions. Therefore, following the identification and mapping of QTL, marker-assisted breeding may be able to improve the genetic makeup of such traits. Only association mapping has been applied so far for traits in finger millet, that are important from an agronomic standpoint. Using 87 genomic SSR markers in 128 genotypes of finger millet, 13 QTL were recently discovered that were associated with six agronomic traits, including plant length (UGEP50), root length (UGEP9 and UGEP57), seed yield (UGEP9, UGEP19, and UGEP80), number of fingers (UGEP104 and UGEP75), number of tillers (UGEP98 and UGEP6), and productive tillers (UGEP98) (Ramakrishnan et al. 2016, 2017). Agromorphological factors UGEP98 (basal tiller number), finger millet (plant height and flag leaf blade width), UGEP77 and UGEP90 (days to 50% flowering), and 190 finger millet genotypes were examined using 46 genomic SSR markers in a different study. The findings revealed four QTLs that were associated with these agromorphological factors (Babu et al. 2014a).Using 113 genotypes of finger millet and 23 anchored SSR markers, Kumar et al. (2015b) reported a total of nine QTL, including M2, M6, M11, M16, M26, M27, M36, M45, and M65 linked with calcium content. Additionally, 238 genotypes were defined by 85 genic and non-genic SSR markers, and a QTL (UGEP69) associated with the calcium content of finger millet grains was found (Yadav et al. 2017). It could aid in reducing future food demand and advancing farming practises for finger millet.

One of the crucial strategies used to distinguish and enhance specific features is the use of molecular markers. The DNA-based markers serve as the basis for a broad variety of molecular marker approaches that are extensively employed in crop improvement programs (Babu et al. 2007). When compared to conventional breeding, plant breeding with the support of molecular markers aids in more accurate tracking of characteristics. The investigation of genetic variation and QTL in *E. coracana* employing molecular indicators has been done in a number of papers, some of which are given here. Due to its small flowers and rapidly self-pollinating character, breeding efforts with this crop have been quite restricted. Additionally, the genetic pool of this crop is still largely uncharacterized, and there have only been a very limited number of papers in this area based on morphological, nutritional, and

other quantitative features (Upadhyaya et al. 2006). A higher degree of genetic variation was found between various cultivated finger millet types by Dida et al. (2008) using RAPD and microsatellites.

The production of an RFLP, AFLP, and SSR marker-based genetic linkage map of the A and B genomes of finger millet was the initial important advancement in finger millet genomics (Dida et al. 2007). Rice and finger millet were compared using a comparative analysis, which showed significant levels of preserved colinearity between gene orders. The research also allowed for the deduction of the putative ancestral chromosomal arrangements in grass species. The absence of studies on abiotic stress tolerance in *E. coracana* highlights the need for targeted research in this area.

Although there have been many data on genomics research for biotic stress resistance in finger millet (Panwar et al. 2010, 2011a, b; Babu et al. 2014a; b; c), these investigations on this plant have not been carried out. Ethiopian finger millet genotypes were analyzed for population structure using ISSR markers (Brhane et al. 2017). In this study the molecular variance analysis showed that there was more genetic variation within the populations (58.54%) than between the populations (41.45%) both with and without grouping. 5.88%, 38.33%, and 55.79% of the total genetic variations were attributed to populations within groups, differences within populations, and genetic diversity among groups, respectively. Eighty (80) accessions collected from different regions of Ethiopia, Zimbabwe and India obtained from Ethiopian Institute of Biodiversity were used in this study.

Villiers et al. (2015) in their study did a very useful genetic characterization of finger millet using 82 SSR markers published for finger millet across ten diverse accessions. The 52 well-performing markers amplified 274 alleles, with a range of 2–14, on average, across 88 samples, and a total of 88 loci. Major allele frequency varied between 0.18 and 0.93, with a mean of 0.57. The agronomically significant characteristics of finger millet, such as yield of grain, tolerance to disease, resilience to osmotic stress/water scarcity, and nutraceutical quality, have been identified using microsatellite markers. In order to address nutritional inadequacies, bio-fortification programs can benefit from the identification of QTLs for nutritional features using association mapping (Kumar et al. 2015a). For instance, employing 23 anchored SSR markers, 113 varieties of finger millet yielded a total of 9 QTLs related to Ca content (Kumar et al. 2015b). Therefore, identification of the responsible genes that affect Ca assimilation and QTLs influencing these traits are crucial for their successful integration in breeding and gene transfer techniques. In a different study, 190 varieties of finger millet were examined utilizing 46 genomic SSR markers to determine 4 agromorphological parameters, including number of basal tiller, width of flag leaf lamina, and length of plant (Babu et al. 2014a). Using 104 SSR markers, they also discovered four QTLs (UGEP81, UGEP24, FINGER MILLETBLEST32, and RM262) in the identical varieties of finger millet (Babu et al. 2014c). Using 120 SSR markers, two QTLs (OM5 and FINGER MILLET8) for tryptophan content and one QTL (FINGER MILLETO2EST1) for protein content in the above said finger millet varieties were discovered that year. These QTLs were connected to the opaque2 modifiers (Opm) gene (Babu et al. 2014b). Compared to other grains, finger

millet contains a high level of tryptophan. In light of this, finding the QTLs connected to the Opm gene that control the tryptophan concentration may be an important goal for further enhancing the quality of *E. coracana* variety. Furthermore, 87 SSR markers in 128 varieties of finger millet were used to identify 7 QTLs that were related with seven agronomic variables, including the number of productive tillers, yield of seed, tolerance to leaf blast (Ramakrishnan et al. 2016). By using association mapping in finger millet under altered concentration of P (P deficient or P sufficient) circumstances, four QTLs (qLRDW.1, qLRDW.2, qHSDW.1, and qHRL.1) linked with root/shoot dry weight and root length were recently discovered (Ramakrishnan et al. 2017). P shortage has a significant negative impact on shoot and root growth during the seedling stage. Therefore, the ability to tolerate a P shortage at the seedling stage is a crucial characteristic that cultivars of finger millet must possess (Ramakrishnan et al. 2017). With the help of marker-assisted selection and the information provided by this work, low P-tolerant varieties of *E. coracana* can be bred. Then, selected germplasm lines can be employed as cultivars for marginal lands where P insufficiency is an issue or as a source of P starvation tolerance QTLs. For QTL research in *E. coracana*, only population association mapping has been employed to date. Since it will perform a significant place in determining the agronomically relevant features, it is imperative to build the finger millet linkage maps for the identification of QTL. Additionally, the absence of the WGS has prevented the high throughput QTL mappings from being tried in finger millet. An effective tool for locating QTLs based on WGS is the genome-wide association study (GWAS). In the upcoming years, the build out of GWAS in *E. coracana* will be aided by the availability of WGS as well as cheap and accurate genome sequencing technologies. The ability to more precisely identify QTLs and related SNPs for important finger millet properties, such as grain Ca content, would be greatly aided by this.

11.8.2 Locating QTL That Are Connected to Features of Climatic Stress Tolerance

Most of the complex features that provide resistance to climatic stressors including disease, drought, salinity, and nutrient deficiency are controlled by QTL (Ceasar et al. 2018; Ramakrishnan et al. 2016, 2017; Serba and Yadav 2016; Sharma et al. 2018; Yadav et al. 2011, 2014a, b). As a result, genetic enhancement of qualities can be accomplished by marker-assisted breeding after the QTL are found and documented. The accurate discovery of stress tolerance QTL for MAS is anticipated to benefit from the use of entire genome-assisted research. It will eventually make it easier to choose cultivars for regions with obvious nutritional deficiencies or lines of germplasm that could serve as gene donors for stress resistance. As a result, this will increase the productivity and adaptability of resource-limited farmers, particularly those with limited access to pricey fertilizers, while also lowering greenhouse gas emissions by using less fertilizer. Even though the foundational research that were highlighted provide information on the genetic loci connected to different climatic

conditions, isolating targeted genes based on so few QTL mapping tests is often challenging. The latest complete genome sequence's accessibility will be crucial for the quick confirmation, finding, and fine-mapping of QTL linked to complicated stress-resistant traits. The ensuing high-density maps based on genome-wide next-generation markers, like as SNPs and SSRs, may enable the exact finding of genes, gene cloning, and gene pyramiding for improving finger millet traits that affect yield or/and impart tolerance to various climatic conditions.

The two most popular techniques for locating QTLs that condition complex traits are linkage and association mapping; these techniques have been used to locate genes and QTL in a variety of plant species (Zhang et al. 2016; Lule et al. 2018; Sharma et al. 2018). Using these techniques, genes and QTL have been discovered in several plant species (Yang et al. 2011; Zhang et al. 2016; Lule et al. 2018; Sharma et al. 2018). The two most popular techniques for locating QTLs that condition complex traits are linkage and association mapping; these techniques have been used to locate genes and QTL in a variety of plant species (Yang et al. 2011; Zhang et al. 2016; Lule et al. 2018; Sharma et al. 2018). The traditional linkage study of QTL for variables related to climatic stressors is constrained since the creation of bi-parental mapping populations for small millets is challenging. In order to produce high-resolution maps, huge mapping populations are also necessary. It is possible to assume that the development of the complete genome sequence will spark interest in genotyping-by-sequencing (GBS) and genome-wide association studies (GWAS) in finger millet. These could develop into potent second-generation biotech tools for high-resolution QTL mapping and SNP finding in finger millet, as well as in other well-studied cereals, such maize (Cappa et al. 2013; Samayoa et al. 2015; Zhang et al. 2016). As a result, this might enable us to get past the constraints imposed by the conventional QTL-analysis method. Furthermore, the accessibility of genome-wide high-density markers that span the entire genome may present additional chances to boost genetic gains for complex characteristics via genomic selection (GS). The next-generation marker technology may hold out hope for the selection of improved finger millet genotypes based on precise breeding values given the ongoing drop in sequencing price and the accessibility of high-throughput sequencing systems. In light of the current climate change situation, these could serve as the foundation for breeding populations of low-cost climate change resilient finger millet varieties.

11.8.3 Functional Genomics

The identification and functional characterization of important genes with noteworthy properties have been recognized as crucial for the development of variations with better qualities. The creation of transgenic, abiotic stress-resistant finger millet plants may benefit from functional genomics. In finger millet, there are many abiotic stress abiotic stress-responsive genes have so far been identified and investigated. Transgenic tobacco overexpressed the finger millet drought-responsive gene EcDehydrin7 (Singh et al. 2015a). According to the study's findings, the protein EcDehydrin7

significantly affects a plant's capacity to endure the stress of drought. Seven genes are responsive to drought in the finger millet leaf tissues under varying degrees of drought stress, including Metallothionein (MT), RISBZ4, Farnesylated Protein (ATFP6), Transcriptional Regulator (TR), Protein Phosphatase 2A (PP2A), Early Light Inducible Protein (ELIP), and Farnesyl Pyrophosphate Synthase (FPS) (Parvathi et al. 2013). The genes MT, ATFP6, RISBZ4, TR, and PP2A expressed in finger millet leaves under a 60% water scarcity stress.

It is reported that salt- resistant variety, Trichy 1 showed upregulation of several functional groups of genes from the families of transporters, transcription factors, cell signalling, osmotic homeostasis, and compatible solute biosynthesis. However, flavonoids biosynthetic activity was downregulated (Rahman et al. 2014a, b). However, there is very little information available on the use of genomics in finger millet, despite the fact that it is thought to be resilient to abiotic stress (Gupta et al. 2017). It has been proposed that functional gene characterization encoding important features is crucial for creating varieties with enhanced attributes. In finger millet, only early efforts have been done for these investigations. The discovery and characterization of potential genes contributing in nutrient sensing and transport in *E. coracana* are anticipated to be significantly aided by recent advances in genomic research (Sood et al. 2016). Dissecting the crucial genetic factors and signals engaged in Ca content of grain would be crucial for nutrient enriching other cereals because finger millet has seeds that are ten times higher in calcium than those of other cereals.

The preliminary research identifying and analyzing the functioning of potential genes in *E. coracana* are as follows. Two *E. coracana* varieties with different Ca traits (GP-1, low Ca, and GP-45, high Ca) were examined for the activation intensity of key genes engaged in Ca transport, including the Ca2C/HC antiporter (CAX1), two pore channel 1 (TPC1), calmodulin (CaM)-stimulated type IIB Ca2C ATPase, and two CaM dependent protein kinases (CaMK1 and CaMK (Mirza et al. 2014). Eighty-two Ca sensor genes were also discovered by the same team in the transcriptome of growing spikes of the GP-1 and GP-45 genotypes (Singh et al. 2014). This led to 24 genes having enhanced expression in the pooled spike sample of genotype GP-45 while only 11 genes had increased expression in the pooled spike sample of genotype GP-1. Twenty-four genes, including 7 CaML, 2 CRK, 5 CBL, 7 CIPK, and 4 CDPK genes, were strongly expressed in the GP-45 growing spikes. To identify important genes involved in Ca2C transport, profiling of whole genome and transcriptome were also carried out in the growing inflorescence of *E. coracana* (Singh et al. 2015a, b). The CIPk24 gene in these two finger millet varieties was also described (Chinchole et al. 2017). In comparison to GP1, this gene was over responsive in the developing spike, shoot, leaf, and root tissues of GP-45. Genome of other model plants were used in silico analysis to find nine SNPs, and an additional beta sheet domain, and variations in vacuolar localization. EcCBL4 and EcCBL10 were discovered to have a greater affinity for EcCIPK24 (GP-1) than EcCIPK24 (GP-45). EcCIPK24 is anticipated to play a significant function in high seed Ca buildup via activating EcCAX1b protein (Chinchole et al. 2017). Recent methods like CRISPR/Cas9, which require WGS to prevent any off-target effects, may be useful for creating mutants with deficiencies in crucial genes for

transportation of Ca transport and filling of grain (Ceasar et al. 2016). Numerous plants have been effectively used in such research with CRISPR/Ca9.

11.8.4 N Metabolism-Related Genes

In a few research, important genes in finger millet that are involved in N transport were also analyzed. The expression of the prolamin-binding factor DNA, binding with one finger only (PBF Dof), a transcription factor involved in the control of seed protein storage, in the root, stem, and flag leaf tissues of three genotypes of finger millet (PRM-1, PRM-701, and PRM-801) with different seed protein content and color was examined (Gupta et al. 2011). Three finger millet genotypes (PRM-1, PRM-701, and PRM-801) with different seed protein content and color were studied for their root, stem, and flag leaf tissues' activation of the prolamin-binding factor DNA binding with one finger only (PBF Dof) TF involved in the regulation of seed protein storage (Gupta et al. 2011).

All three genotypes showed a considerably higher function of this gene in growing inflorescence than in other tissues. It's interesting to note that these genotypes' increased activation of PBF Dof during the beginning stages of growth is correlated with protein content of grain (Gupta et al. 2011).

Two genotypes with contrasting grain protein contents (GE-1437, low-protein, and GE-3885, high-protein) were examined high-affinity nitrate transporter (EcHNRT2) of *E. coracana*. Ec low-affinity nitrate transporter (EcLNRT1) of *E. coracana*, Ec nitrate reductase (EcNADH-NR), Ec glutamine synthetase (EcGS), Ec glutamine oxoglutarate aminotransferase (EcFd-GOGAT), and Ec DNA binding with one finger 1 (EcDof1) (Gupta et al. 2013). According to this research, GE-3885 may be a quicker nitrogen sensor than the low-protein variety (Gupta et al. 2013). The activation patterns of EcDof1 and EcDof2 in the identical varieties (GE3885 and GE1437) were also examined by the same researchers (Gupta et al. 2014). Dof1 and Dof2 are transcription factors (TFs) that play opposing functions in controlling genes participated in C and N metabolism. In the roots of GE-3885, a larger EcDof1/EcDof2 ratio demonstrated greater activation of genes related to N absorption and assimilation, which resulted in increased grain protein accumulation (Gupta et al. 2014). Greater activation of genes involved in N uptake and assimilation, which results in high grain protein accumulation, was shown by a higher EcDof1/EcDof2 ratio in the roots of GE-3885 (Gupta et al. 2014).

11.8.5 Genes Related to the Metabolism of Carbon (C)

Malic enzyme (ME), sucrose phosphate synthase (SPS), pyruvate kinase (PK), and pyruvate di were reported to be analyzed in the genotype GE-1437 and GE 3885 of finger millet. These genes included 14-3-3, chlorophyll a/b binding protein (Cab), Rubisco (RBCS), phosphoenol pyruvate carboxylase (PEPC), phosphoen (Kanwal et al. 2014).

The validation of genes involved in C metabolism in finger millet has only ever been documented in this one publication. A greater number of genes involved in C metabolism will soon be found and characterized thanks to the WGS of finger millet.

11.8.6 Phosphate Transport-Related Genes

E. coracana genotypes RagiKorchara, Khairna, and VHC 3611 were used to study the activation of four phosphate transporter 1 (EcPT1 to EcPT4) genes (Pudake et al. 2017). Under various inorganic phosphate (Pi) regimes and with arbuscular mycorrhizae fungal colonization, the expression of these genes was confirmed (AMF). EcPT1 transcript levels were discovered to be almost five times greater in roots and leaves under deficient Pi than in normal. Under phosphate stress, the EcPT3 gene was activated in both the leaves and the roots. AMF was discovered to promote EcPT4 gene expression in tissues of root (Pudake et al. 2017). Only 4 EcPT1 genes have been found in *E. coracana* so far, but it appears that each plant has sufficient of these genes (Baker et al. 2015). *S. italica* is very close to finger millet, which has been shown to have 12PT genes, and their expression patterns, P transport test in yeast, their function using RNA interference (RNAi) were studied (Ceasar et al. 2014). According to the sequencing of the fragmentary transcripts sequences, these four PT genes in finger millet were discovered. The genome-wide identification and functional characterization of all the PT genes in finger millet will be aided by the recently published WGS.

11.8.7 Genes That Contribute to Abiotic Stress Tolerance

Because of how well finger millet has adapted to the semi-arid tropical climate, it has been regarded as a drought-resistant crop. Aiming to use the main genes involved in drought resistance for future applications, efforts have been made to describe these genes. One of the most significant non-biological elements influencing plant growth and productivity is drought stress. By isolating and over expressing the *E. coracana* drought- tolernacet EcDehydrin7 in tobacco, Singh et al. (2015a) attempted to describe the gene Increased EcDehydrin7 expression in tobacco plants increased drought tolerance. Seven drought-responsive genes, including metallothionein, farnesylated protein ATFP6, protein phosphatase 2A, and farnesyl pyrophosphate synthase, were discovered to be overexpressed in genotype GPU-28 during drought stress (Parvathi et al. 2013). The further analysis of these genes will assist in identifying any distinctive signals connected to finger millet's drought resistance. It is believed that these genes are essential for drought tolerance. Finger millet genotype GPU-28 was used to study the expression of TBP Associated Factor6 (EcTAF6), a gene that controls drought response, after a cDNA library of the plant was identified by screening (Parvathi and Nataraja 2017). In finger millet, drought resistance genes have also been discovered and verified utilizing the water scarcity tolerant transcriptome (Ramegowda et al. 2017). Ectopic expression in *A. thaliana*

was used to characterize one of these putative genes, EcGBF3. EcGBF3 overexpression in *A. thaliana* increased the ability of Atgbf3 mutant lines to tolerate osmotic, salt, and dry stressors (Ramegowda et al. 2017). This work also shown how challenging it is to create mutant *E. coracana* lines for studies on functional genomics; as a result, it was verified utilizing the model plant A. thaliana. Through RNAseq, various salt stress response genes were found in the leaves of Co-12 (susceptible) and Trichy 1 (tolerant) finger millet verities subjected to salinity conditions (Rahman et al. 2014a, b). The research group of Rahman et al. (2014a, b) also claimed that rice with EcNAC67 TF over expression had increased salinity and drought resistance (Rahman et al. 2016). EcNAC1, a stress-responsive NAC gene, was discovered to be significantly upregulated under salt stress and was implicated in the tolerance to high pH, and other abiotic stimuli (Ramegowda et al. 2012). Under drought, osmotic, salt, and methyl viologen (MV) stressors, the GPU-28 genotype of *E. coracana* was shown to express two abiotic stress sensitive TFs, EcbZIP60, and EcbHLH57 from the basic helix-loop-helix (bHLH) family, respectively. The CBL interacting protein kinase31 (EcCIPK31-like) gene that confers drought resistance in *E. coracana* was identified and reported by Nagarjuna et al. (2016). When finger millet was under water scarcity stress, the gene EcTAF6 for TATA box binding protein-associated factors (TAFs) was discovered (Parvathi and Nataraja 2017). A new *E. coracana* endoplasmic reticulum-specific bZIP TF gene (EcbZIP17) was recently discovered and introduced and reported to be over functional in tobacco (Ramakrishna et al. 2018). Comparatively to plants of the wild type, tobacco plants over active EcbZIP17 expressed resistance to heat and salinity stressors. These are the preliminary research on candidate gene identification and validation that were reported in finger millet. Unfortunately, unlike in model plants like rice and *A. thaliana,* these genes have not yet been further explained in *E. coracana*. As one has to create accurate genomic targets for such studies, the lack of WGS may be to blame. The recently revealed WGS is anticipated to aid in reverse genetic techniques such CRISPR-mediated mutant creation, promoter reporter fusion functional characterization, localization investigations, heterologous expression in yeast mutants, etc. In conclusion, *E. coracana's* WGS is anticipated to aid in numerous high precise studies to clarify the role of genes contributing in nutritional signalling and response to environmental cues. It may also be used in breeding initiatives to create better *E. coracana*. Due to the lack of WGS, only a small number of genetic resources are currently available. Though *E. coracana* has traditionally been thought of as a crop having capacity to tolerate climate- change and a crop for developing countries, recent research has shown that this crop is also susceptible to fungal blast, drought, salinity, and low nutrition stresses. Before the introduction of WGS, there had only been a small number of researches on the characterization of *E. coracana's* significant genes. Recently, two separate finger millet genotypes' WGS results were published (Hittalmani et al. 2017; Hatakeyama et al. 2017). This will make it easier to create numerous high-resolution experiments similar to those carried out in other model plants like rice and *A. thaliana*, and WGS may alter the future of *E. coracana* studies. This will make it easier to create numerous high-resolution experiments similar to those carried out in other model

plants like rice and *A. thaliana*, and Whole Genome Sequencing may alter the future of *E. coracana* research. The new genetic resource is anticipated to advance research on *E. coracana* in a variety of areas, such as the identification of genes by reverse genetic studies using precise mutants created through genome editing techniques like CRISPR/Cas9, boosted up functional genomics studies, and promoter binding of important genes with reporters like GFP for localization. In general, the *E. coracana* studies for breeding and enhancement are anticipated to be augmented by the recently revealed WGS. In general, it is anticipated that the lately revealed WGS of *E. coracana* would support study into its breeding and development. This will aid in the understanding of the main genes and controlling networks participated in nutrient translocation and abiotic stress tolerance and can be used to enhance other millets and non-millet grains production and nutritional quality, preserving the food security of the expanding global population.

11.8.8 Functional Characterization of Important Genes Linked to Pressures Brought on by Climate Change

Currently, it is known that abiotic stress due to climate change, including as illness, drought, salinity, and aluminum toxicity, has a significant impact on plants' ability to absorb nutrients and assimilate them, as well as how they use carbon (Maqsood and Ali 2007; Goron and Raizada 2015; Bista et al. 2018).

Future dangers associated with agricultural output are anticipated to be further exacerbated by these factors. Therefore, significant study attention could support the needed adaptation measures by identifying the candidate genes linked to climatic challenges and boosting finger millet abiotic stress resilience.

Future hazards to agricultural productivity are predicted to be made worse by these effects. In order to increase finger millet abiotic stress resilience, much study effort should be paid to finding the candidate genes linked to climatic pressures. This research might then help develop the necessary adaption techniques.

Given that the C4 crop finger millet contains more calcium than some other cereals (Kumar et al. 2016a, b), it is likely to have developed unique mechanisms that enable the plant to thrive in low nitrogen environments and adapt to a variety of environmental stresses, including drought, salinity, aluminum toxicity, and diseases (Goron and Raizada 2015; Gupta et al. 2012; Bandyopadhyay et al. 2017a).

It is hypothesized that potent promoters, transcription factors, and regulatory proteins control the genes responsible for these intricate systems (Gaur et al. 2018; Pudake et al. 2017; Rahman et al. 2016; Ramakrishna et al. 2018; Ramegowda et al. 2012; Sharma et al. 2017; Singh et al. 2014, 2015a, b). Contrary to other well-studied cereals like rice, a bottleneck has existed in our ability to fully grasp these underlying mechanisms, in part due to the limited genetic data that is now available. However, preliminary research on the profile of expression several genes linked to stress resilient has been described using model crops and comparative genomics. These findings could be used for breeding for abiotic stress tolerant varieties. In the post-genomic era, in-depth investigation using functional genomics methods such as

gene silencing, insertional mutagenesis, targeted induced local lesion in genome (TILLING), and over expression has been crucial in advancing our understanding of the complex regulatory networks involved in stress response, adaptation, and tolerance in plants. Numerous omics technologies are also generating enormous amounts of data that can be utilized to pinpoint key candidate genes for MAS or transgenic millets crop enhancement efforts. Reverse genetics methodologies will be essential to solve the difficulty of functionally validating thousands of identified genes or proteins despite the advent of proteomics and genome sequencing initiatives. A strong method for establishing a gene's function is to overexpress it in a homologous or homologous system while being regulated by a constitutive or stress-inducible promoter. Suppressing a gene's expression or knocking it out is a crucial way for assessing a gene's function.

Currently, it is known that stress caused by climate change, including as diseases, drought, salt, and aluminum toxicity, has a significant impact on plants' ability to absorb nutrients, assimilate them, and utilize carbon (Maqsood and Ali 2007; Goron and Raizada 2015; Bista et al. 2018).

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11.8.9 Specifics of Markers for Enhanced Agronomic Features and Climatic Stress Tolerance for Varieties Tolerant to Environmental Cues

Most plants, including finger millet, have nutrient-uptake proteins in their cell membranes that aid in the movement of minerals from the soil to their cells (Pudake et al. 2017; Sharma et al. 2017; Singh et al. 2014, 2015a, b). For example, the high-affinity AMT1 family of transporters is engaged in the intake of ammonium, whereas the NRT1 and NRT2 families of transporters are involved in the uptake of nitrate,

with low-affinity NO3 transporters at high N levels and high-affinity NO3 transporters at low N levels, respectively (Dechorgnat et al. 2018). Similar to how calcium (Ca) is absorbed through roots with the support of PHT1-type transport proteins (Pudake et al. 2017). In P-stressed plants, the majority of the roots-expressed genes for this PHT1 family are upregulated (Smith et al. 2003). Rahman et al. had published three ATP-binding cassette (ABC) transporters (located at loci Os11g39020, Os02g32690, and Os01g24010) as well as potassium-type KUP (potassium uptake permease) transporters (2014). Numerous transcription factors associated in nutrition signalling and abiotic stress response are also activated in the current released entire finger millet genome sequence (Hittalmani et al. 2017). As a result, the extensive collection of TFs contained in the recently made available complete genome sequence may offer new potential tools for modifying finger millet's susceptibility to stress. These results could improve research on crucial routes for the buildup of certain protective osmolytes, like proline, linked to physiological responses to osmotic stress brought on by salt or drought (Aleksza et al. 2017; Khatoon and Singh 2016). In adverse conditions, proline, which is regulated by the transcription factors of the MYB type PHR1 and PHR1-LIKE1 (PHL1), acts as an osmoprotectant by stabilizing cellular structures and enzymes, scavenging reactive oxygen species (ROS), and maintaining redox equilibrium (Sudan et al. 2015; Aleksza et al. 2017).

Model crops like A. thaliana, rice, and tobacco were used as the foundation for the majority of prior expression-profiling research for *E. coracana*. The availability of whole genome sequence data may serve as a launching point for research on model plants. It is envisaged that a comparative genomic analysis of finger millet and cognately related crops or other understudied cereals will present creative solutions for the quick application of genetic information.

With the introduction of full genome raw data, second-generation genomics biotechnologies based on reverse genetic mutagenesis, such as TILLING (Kashtwari et al. 2019), EcoTILLING (Bajaj et al. 2016), and CRISPR/Cas9, could aid gene-analysis investigations (Miglani 2017).

These non-transgenic reverse genetics approaches are projected to be more adaptable to various plant species, regardless of ploidy level or genome size (Barkley and Wang 2008).

11.8.10 Omics Research Using Bioinformatics and Systems Biology for Abiotic Stress Tolerance in Finger Millet

Understanding complex regulatory networks as well as the fundamental ideas underlying abiotic stress responses and tolerance in plants requires an understanding of genome-scale responses at various developmental stages and against a variety of environmental stressors. Usually, expression databases are accessible for model plants like *Arabidopsis* and rice. When these datasets are mined, a variety of clustering algorithms are typically used to search for collections or clusters of coexpressed genes that are predicted to be co-regulated by a similar set of

transcription factors in response to the same internal or external stimuli, and as a result, tend to form a transcriptional sub-network (Moreno- Risueno et al. 2010).

This idea can be utilized to determine the controlling hierarchy of expression of gene in various genera and species. Bioinformatics tools play a significant role in the further processing of more intricate and extensive gene expression data, which calls for extra advanced methodologies. Genome-scale data from numerous time-course experiments conducted in a variety of environmental conditions can be processed with ease using advanced bioinformatics tools and pipelines to detect and deduce significant patterns associated with particular cis-elements and regulatory genes, such as TFs, as well as other stress/stimuli-specific genes. This will aid in the reconstruction of regulatory and metabolic networks involving these genes and their regulatory interactions (Ma et al. 2007; Moreno- Risueno et al. 2010). Proteomics employs studies based on mass spectrometry (MS) to profile proteins, whereas metabolomics uses studies based on nuclear magnetic resonance (NMR) profile metabolites in specific cells, tissues, organelles, or entire plants. To identify different signalling and metabolic networks for a specific internal or environmental stimulus, the findings from these two investigations can be correlated with transcriptome profiles. In particular, metabolome investigations have the potential to shed light on biochemical networks, but unlike transcriptional or proteomic networks, undiscovered metabolites cannot be integrated into these networks by simply connecting analyses. In these circumstances, combining quantitative trait loci analysis with qualitative metabolite profiling may be useful for developing from scratch models of biochemical networks incorporating intricate plant responses. Since there has not been much work using systems biology approaches for understanding regulatory networks in millet crops, it is imperative that researchers worldwide make serious efforts in this direction. These orphan crops are otherwise important in terms of nutrition and economics, and it is important to understand the biological networks that are operating in them. These orphan crops are otherwise important in terms of nutrition and economics, and it is important to understand the biological networks that are operating in them. The foxtail millet genome sequence's accessibility and current genomics projects in pearl millet and finger millet are to be given credit for the significant growth of the application of omics for abiotic stress studies in millets. Using the current and planned high-throughput sequencing equipment, researchers can use a wide range of sequencing applications, such as the discovery of molecular markers, short RNAs, and SNPs. The issues raised by big genomes, especially those found in millets, can be solved by combining NGS methods with genome-wide expression profiling research. Proteomics and metabolomics research in millet crops is still behind those of genomics and transcriptomics.

The study of complex biological systems, such as those of millets, will be revolutionized by advances in high-throughput proteomics and metabolomics methods, such as flow injection - time-of-flight mass spectrometry, etc.

11.8.11 Pheno-Physiological Evaluation for Abiotic Stress Tolerance in Finger Millet; Diversity in Oxidative Stress Markers and Reactive Oxygen Species in Finger Millet

In a study reported by Bartwal et al. (2016) on the various genotypes of high abiotic stress tolerance on drought stress viz. PR 202, PES 400, PRM 6107, VL283, VL 328, and VL 149), it was showed that PRM 6107 and PR202 showed highest resilience and it also revealed that it is because of the accumulation of excessive reactive oxygen species (ROS) through the enzymatic antioxidants. Enhanced ascorbate peroxidise content, chlorophyll content, and the relative water content were shown by these varieties in this study. The results of this study suggest that the finger millet variants PRM6107 and PR202 have increased antioxidant potential and are more drought resistant. In order to create better varieties of economically significant crops, these stress-tolerant cultivars can be researched for mining of allele genes responsive to drought stress.

Mukami et al. (2020) had reported a study on six different varieties of finger millet seedling under draught stress. In this study they used mannitol to induce draught stress and the varieties under evaluation were GBK043137, GBKK043128, GBK043124, GBK043122, GBK043094, and GBK043050. The findings demonstrated that elevated levels of drought stress significantly reduced finger millet variety germination and early seedling growth and the root development were accelerated. Exposure to drought stress led to a considerable loss in relative water content and chlorophyll content, although the biochemical parameters assay revealed a less pronounced decline in RWC and an increased concentration of MDA content and proline content. In this study the varieties GBK043137 and GBK043094 show maximum tolerance to drought stress. This research reveals the variations in physiological and biochemical responses of *E. coracana* to drought, which is essential for breeding and choosing finger millet types that are resilient to drought.

Parvathi et al. (2013) had reported their research to identify candidate genes linked with the attribute in the drought-tolerant agricultural plant *Eleusine coracana* (L.) Gaertn, since cellular tolerance is one of the key traits in drought acclimation. In order to examine the stress responsiveness of a few chosen genes implicated in various stress response pathways, a unique gravimetric technique was used to replicate field level drought stress. E-northern analysis was used to study gene expression first, and various genotypes were later detected in leaf tissues. Particularly, the resistant IE 4709 and susceptible INDAF 7 were found to have the highest levels of genetic resistance to drought stress. These strains also contain metallothionein, farnesylated protein ATFP6, protein phosphatase 2A, RISBZ4, and farnesyl pyrophosphate synthase, all of which are likely important for the development of *E. coracana* hardiness. When viewed as a whole, the findings imply that numerous cellular tolerance pathways function in concert in drought-tolerant plants. This study observed at many gene types whose expression is connected to various cellular tolerance mechanisms related to drought stress. Such investigations are essential to identify prospective drought genes because stress responsive genes from an adapted organism like *E. coracana* are suitable and

preferable for targeted modulation of metabolic pathways for stress resistance. Cloning and characterizing putative stress genes from adaptable plants may provide new opportunities for enhancing related crops with focused biotechnological methods. Stacking several genes connected to various pathways would aid in regulating a polygenic characteristic like drought tolerance because multiple pathways and genes regulate cellular tolerance.

The many nutritional and physiological benefits of finger millet, particularly its capacity to prevent chronic diseases, have been discussed by Kannan (2010). Although there are finger millet types that are available in yellow, white, tan, red, brown, or violet, red versions are the most widely grown worldwide.

Ediga et al. (2013) have conducted the comparative analysis of two finger millet cultivars differing their sensitivity to salt stress. In this study the authors were compared the variation in response to salinity stress through damage of oxidation due to ROS in both enzymatic and non-enzymatic defense mechanism. Of the two varieties of finger millet that they are compared, PBR 2700 seems to be more resistant to NaCl stress than Sapthagiri.

Exploration of finger millet-growing regions of the Central Himalayan Region, Uttarakhand State, India, was carried out and reported by Trivedi et al. (2018). It is reported that 314 accessions were gathered from the 225 to 2250 masl altitude range of study area and were grown in common place for physiological diversity study. In this study two local accessions, Almora Local and Pithoragarh local, and two released varieties, i.e., VL-146 and VL-149, were used to as control to compare with the accessions under evaluation.

11.8.12 Alternative Methods for Millets to Improve Yield and Multi-stress Tolerance

The publically available genome sequences of foxtail millet (Bennetzen et al. 2012; Cannarozzi et al. 2014; Van Buren et al. 2020)

tef (Cannarozzi et al. 2014; Van Buren et al. 2020), pearl millet , finger millet [21], and proso millet (broomcorn) (Zou et al. 2019) are fantastic tools for the genetic advancement of these crops. Plants have a variety of defenses against the stress of waterlogging, which is brought on by hypoxia (low oxygen levels) or anoxia (Matsuura et al. 2016) (complete absence of oxygen). It has been noted that finger millet (*Eleusine coracana*) also engages in anaerobic respiration as a response to low oxygen levels (Hossain and Uddin 2011). Crop growth and development, and stress tolerance, have recently been improved using strategies like the application of plant growth promoting rhizobacteria (PGPRs). Additionally, with the development of next-generation sequencing technology, stress-tolerant crop varieties are being developed more and more frequently through genome editing using the clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) system. In addition to being high in calcium, methionine, tryptophan, fiber, and sulfur-containing amino acids, finger millet is also abundant in other important phytochemicals such as polyphenols (Nithiyanantham et al. 2019; Bandyopadhyay et al. 2017a, b). Minerals,

crude fibers, protein, and carbohydrates make up 2%, 4%, 9%, and 81% of the ingredients in finger millet. Comparing finger millet to wheat and rice, the mineral and fiber content of finger millet is higher. Furthermore, finger millet contains higher valine, threonine, and lysine levels compared to other millet species (Nithiyanantham et al. 2019; Bandyopadhyay et al. 2017a, b; Singh et al. 2018). Millets are excellent resources to use in the food industry because they contain important nutrients and phytochemicals with positive health effects (Nithiyanantham et al. 2019; Bandyopadhyay et al. 2017a, b; Nakarani et al. 2021; Disseka et al. 2018; Singh et al. 2018).

It is necessary to create stress-tolerant, high-yielding millets in order to combat the impacts of pressures brought on by climate change and to increase millets' output. Millets' ability to withstand stress and produce more can be increased in a number of ways. For instance, PGPR treatment has been utilized to increase yield and tolerance to stress in wheat (Nawaz et al. 2020; Islam et al. 2014; Barnawal et al. 2017) and rice (Sen et al. 2016; Ji et al. 2020; Joshi et al. 2020) but testing on millets has not yet been done. Another strategy for improving traits is through genome editing, which has recently attracted a lot of attention due to its potential for precise allele alteration. In order to change the genome or genes of plants to boost productivity and stress tolerance, the CRISPR/Cas9 system has emerged as a potential technique.

11.8.13 Utilization of Rhizobacteria That Promote Plant Growth (PGPRs)

Numerous crops have demonstrated the positive impact of PGPRs in increasing resistance to abiotic stress and productivity (Egamberdieva et al. 2017; Gamalero et al. 2010) Economically significant crops like rice, soybeans, lettuce, tomatoes, maize, and wheat have all benefited from the usage of PGPRs to reduce abiotic stressors and increase production. Additionally, it was shown that PGPRs induced the production of other growth regulators such salicylic acid and jasmonic acid as well as plant defense mechanisms (Castillo et al. 2015; Jogaiah et al. 2018). Additionally, it was found to facilitate plants' uptake of nutrients from the soil in testy situations.

11.8.14 Synthesis of IAA

Inoculating IAA-synthesizing bacteria in different species of plants led to better root growth, the development of root hairs, and lateral roots (Dimkpa et al. 2009) which enhance intake of nutrients and water (Mantelin and Touraine 2004) and supported plants' ability to withstand water/salinity stress (Egamberdieva and Kucharova 2009). IAA-synthesizing bacteria were injected into a variety of specie of plants, which resulted in improved root development, the growth of root hairs, and lateral roots (Dimkpa et al. 2009), which enhanced their capacity to absorb nutrients and

water (Mantelin and Touraine 2004) and supported their capacity to endure osmotic stress (Egamberdieva and Kucharova 2009). Azospirillum that synthesizes IAA boosted plants' ability to withstand drought by a factor of many (Dimkpa et al. 2009). The ability of bacteria to produce hormones and their ability in triggering endogenous hormones to stimulate resistance is greatly improved (Cassán et al. 2001). Azospirillum brasilense produces nitric oxide (NO), which is involved in IAA signalling and helps tomato plants (Solanum lycopersicum) grow adventitious roots ((Molina-Favero et al. 2008). Nitric oxide (NO), which is involved in IAA signalling and promotes the adventitious root growth of tomato plants (*Solanum lycopersicum*), is produced by *Azospirillum brasilense* (Molina-Favero et al. 2008).

11.9 Effects of PGPR on Root Morphology Under Drought

Plant cell physiological condition is mostly maintained by cell membranes. Rhizobacteria affect activities that happen within the cell membrane. A lack of water was found to improve phosphatidylcholine, change the composition of the phospholipids in the roots, and decrease phosphatidylethanolamine (Sueldo et al. 1996). Decreased phosphatidylethanolamine unsaturation and higher phosphatidylcholine were found, although Azospirillum treatment of wheat seedlings avoided these changes (Pereyra et al. 2006). The key mechanism for improved resistance to osmotic deficit is changes providing flexibility in the cell membrane of roots caused by modifications mediated by bacteria (Dimkpa et al. 2009).

Because PGPRs stimulate the antioxidant defense system and boost plant resilience to drought, they improve the rigidity of cell membranes in plants (Gusain et al. 2015). Activity of Rhizobacteria that synthesize ACC deaminase endogenous ethylene preserves homoeostasis under stress, which inhibits shoot and root growth. Aminocyclopropane-1-carboxylic acid (ACC) is a precursor for the production of ethylene (Polko and Kieber 2019), which the bacterial enzyme ACC deaminase reacts with to provide the plant with energy and nitrogen. Additionally, the absence of ACC permits the bacteria to lessen their ethylene toxicity, which fosters development and lessens stress (Glick 2005). It has been demonstrated that the ACC deaminase-producing Achromobacter piechaudii strain ARV8 increases the weights of tomato and pepper (*Capsicum annuum*) seedlings and reduces ethylene synthesis during saline stress (Mayak et al. 2004). In comparison to bacteria colonized from locations where water is readily available, PGPRs from water-deficient areas due to tandem dry spells are more stress-adapting and plant growth-promoting (Mayak et al. 2004). PGPRs from areas with a lack of water as a result of consecutive dry spells are more stress-adaptive and plant growth-promoting than bacteria inoculated from areas with easy access to water. When tomato seedlings were treated with *A. piechaudii* ARV8 from an arid location, growth was stimulated as opposed to seedlings treated with *P. putida* GR12-2 from a grass rhizosphere, where water was plentiful (Lifshitz et al. 1986).

11.9.1 Role of Volatile Compounds in Drought Tolerance

Dryness Tolerance and Volatile Compounds

In their interactions with plant roots, soil microorganisms either produce nonvolatile substances (siderophores and phytohormones) or organic volatile and inorganic volatile compounds in the form of gases that permeate through the pores in the soil particles (Kai et al. 2009). By enhancing plant biomass and enhancing plant defenses against plant diseases through inducing systemic resistance, these substances serve crucial roles in the food chain of microorganisms and in fostering plant growth (Ryu et al. 2004; Kai et al. 2009; Farag et al. 2013; Santoro et al. 2011). Volatiles must operate in plants when they are subjected to various stressors at once (Loreto and Schnitzler 2010; Holopainen and Gershenzon 2010). These chemicals, which are delivered under stressful circumstances, participate in signalling to produce systemic and priming effects in the same plant and neighboring plants (Loreto and Schnitzler 2010; Holopainen and Gershenzon 2010). Because of a significant decrease in volatile emissions and an improvement in photosynthesis, wheat seedlings enriched with the AZP2 strain of Bacillus thuringiensis had an enhanced biomass and a five-fold higher survival rate during water scarce circumstances (Timmusk et al. 2014). These results demonstrate that bacterial inoculation enhances plant stress resistance (Timmusk et al. 2014). These molecules represent important contributors to the assessment of drought and its mitigation using quick, non-intrusive procedures (Timmusk et al. 2014). *P. chlororaphis* O6 growth in roots decreased water loss via regulating stomata pores through the creation of the volatile metabolite 2R, 3R-butanediol; however, bacteria missing this metabolite production did not demonstrate any drought tolerance. During stressful times, this volatile compound also helps Arabidopsis develop resistance (Cho et al. 2008).

PGPRs have a significant potential to increase millets' productivity under abiotic stress circumstances due to their use in enhancing several crop plants' responses to stress, as described above, in different environments.

11.10 Use of CRISPR/Cas9 to Enhance Millets' Abiotic Stress Resistance

The last ten years have seen the development of site-specific nuclease (SSN)-based genome editing, which enables accurate and efficient gene alteration in plant and animal systems. When they interact with their target DNA, SSNs cause double-strand breaks (DSBs). These breaks are patched up using methods like homology-directed recombination (HDR) or nonhomologous end joining (NHEJ), which result in changes including substitutions and insertion/deletions in the target regions (Jinek et al. 2012). Consequently, the genome-modifying method is developing into a potent tool for functional genomics and crop breeding.

Compared to transgenic plants, plants with edited genomes have the advantage of containing modified DNA for a certain quality (Malzahn et al. 2017), and new kinds

created using this technology can be employed right away with fewer consumer safety concerns.

Plants that have undergone genome editing and carry novel alleles can also be used in breeding processes since they are subject to less strict regulatory requirements than plants that have undergone genetic modification (Waltz 2018).

More than 20 plant species have had their genomes modified using the CRISPR/Cas9 technology (Ricroch et al. 2017) to increase a variety of properties, including ability to withstand environmental cues and yield (Jaganathan et al. 2018; Shi et al. 2017). It is essential to choose a target gene to achieve the advancement of the quality of interest. Regulatory and structural genes are the two major classes of genes that can be used to modify traits. In contrast to regulatory genes, which function indirectly by regulating the expression of many more genes that might perhaps have a role in different biological processes, structural genes directly influence traits, such as abiotic stress tolerance, through the proteins they encode.

Furthermore, *cis*-regulatory sequences have a significant impact on how abiotic stress tolerance is modulated. (Shi et al. 2017), Cotton (Chen et al. 2017), maize (Char et al. 2017; Svitashev et al. 2016), rice [187, 188], and wheat [188, 189] are some examples of plant species where the CRISPR/Cas9 system has been used successfully to reduce abiotic stress. The CRISPR approach is only applied to a very limited number of plant species, and it has primarily been employed to improve features related to biotic pressures and its usage to increase agricultural productivity and resilience to abiotic challenges is still limited. Recently, CRISPR/Cas9 was employed to enhancing the target's resistance to heat by targeting the SlAGAMOUS-LIKE 6 (SIAGL6) gene, which enhanced tomato fruiting capacity under heat stress (Klap et al. 2017). By controlling the ARGOS gene, CRISPR/Cas9 was also used to increase drought tolerance in maize without lowering crop yields (Shi et al. 2017; Martignago et al. 2020). The simultaneous expression of numerous structural and regulatory genes in crop plants offers the CRISPR/Cas9 technology great promise for the development of multi-stress-tolerant crops. For several crops, such as cotton (Gao et al. 2017), maize (Char et al. 2017), wheat (Wang et al. 2018), and rice (Miao et al. 2013), multiple CRISPR/Cas9 gene editing procedures have been used.

Loss-of-function mutations can result from single-nucleotide alterations in a gene's critical domain. Single nucleotide changes in a gene's crucial region can cause loss-of-function mutations. This method is considered to have the potential to displace more conventional approaches to plant breeding, which mainly relied on populations with ample genetic diversity to introduce appealing characteristics to specific crop cultivars (Sharma et al. 2017; Bhat and Srinivasan 2002). A desirable characteristic that can be recognized by gRNA sequencing can be produced using the base-editing method of CRISPR/Cas9 in a specific population (Eid et al. 2018). As a result, neglected crops like finger millet could profit from the enormous potential of CRISPR/Cas9 genome editing technology for enhancing productivity and enhancing resilience to environmental stress. Thus, the enormous possibilities for using CRISPR/Cas9 genome editing technologies for enhancing productivity and enhancing resistance to environmental stress should assist underutilized crops like millet.

The enhancement of millets crops through the gene transfer techniques like use of CRISPR/Cas9 and other gene-transformation technologies is being investigated by the researchers since the inclusion of millet crops in food security initiatives is drawing expanding curiosity. For instance, Mamidi et al. (2020) reported the genome assembly of *Setaria viridis* in order to identify significant loci for features like loss of shattering and leaf angle, which are regarded as significant yield indicators for several grass crops.

Through CRISPR/Cas9 technology, it is verified that the Less Shattering1 (SvLes1) gene to regulate seed breaking. In earlier research, SiPHT1;2, SiPHT1;3, and SiPHT1;4 phosphate transporters (SiPHT1;2, SiPHT1;3, and SiPHT1;4) were downregulated in foxtail millet (*Setaria italica*) using transformation mediated by Agrobacterium Ceasar et al. 2017). For the nonredundant roles, they reported large increases in the quantity and density of roots and hairy roots in addition to significant decreases in total and inorganic phosphate in root and shoot tissues. The first millet crop to be sequenced was foxtail millet (Cannarozzi et al. 2014). The pearl millet genome sequence has also been made public (Varshney et al. 2017). According to a different study, transgenic finger millet (*Eleusine coracana* (L.) Gaertn.) plants were successfully grown using Agrobacterium (Ceasar and Ignacimuthu 2011). Four varieties of finger millet have been successfully revived using Agrobacterium transformation techniques (Satish et al. 2017). This approach for Agrobacterium-mediated transformation has been established for finger millet. Three significant millet crops—proso millet, small millet, and kodo millet—were the subject of genome-wide population analyses by Johnson et al. (2019).They discovered several SNIPs, including 1882 for proso millet, 3461 for tiny millet, and 2245 for kodo millet. These discoveries may aid in genome editing for improved crop resistance to stress. EMS-induced mutagenesis was recently used to produce a mutant of foxtail millet (Peng and Zhang 2020). In the light receptor gene phytochrome C (PHYC), which has a quick cycling time and is crucial for photoperiodic blooming, a point mutation known as "Xiaomi" was created (Yang et al. 2020).

Recently, we compiled the key genetic responses to abiotic stress found in related monocots including rice, wheat, and maize that are homologous to tef and suggested using CRISPR/Cas9 editing to increase tef's ability to withstand stress and produce better crops (Numan et al. 2021).

11.11 Conclusions

When compared to other grains, millet crops offer significant nutritional advantages. Although almost all millet crops have natural defenses against environmental cues, these problems still threaten millet production as the consequences of climate change grow. In order to reduce the effects of abiotic stressors and increase crop yield, methods like Plant Growth Promoting Rhizobacteria (PGPRs) and CRISPR/Cas9 are being utilized in various crops. The employment of Plant Growth Promoting Rhizobacteria (PGPR) and CRISPR/Cas9 will not only allow the plants to thrive well in challenging settings, but will also greatly increase their productivity,

according to our analysis of the literature on the topic. There have been some potential genes proposed as CRISPR/Cas9 system targets for millet crop improvement in terms of crop growth and production. Research in the fields of genomes, transcriptomics, metabolomics, proteomics, and other areas will be used to supplement the alternative strategy we propose. If we are to increase global food security in the face of climate change, which is negatively affecting the yield of staple crops, millets need increased attention from geneticists, biotechnologists, breeders. Millets have not been improved as much as major food crops.

Bibliography

Ahuja I, de Vos RCH, Bones AM, Hall RD (2010) Plant molecular stress responses face climate change. Trends Plant Sci 15:1360–1385

Aleksza D, Horvath GB, Sandor G, Szabados L (2017) Proline accumulation is regulated by transcription factors associated with phosphate starvation. Plant Physiol 1:555–567

Ali Q, Ahsan M, Tahir MHN, Elahi M, Farooq J, Waseem M (2011) Gene expression and functional genomic approach for abiotic stress tolerance in different crop species. IJAVMS 5: 21–248

Alvarez S, Marsh EL, Schroeder SG, Schachtman DP (2008) Metabolomic and proteomic changes in the xylem sap of maize under drought. Plant Cell Environ 31:325–340

Anjaneyulu E, Reddy PS, Sunita MS, Kavi Kishor PB, Meriga B (2014) Salt tolerance and activity of antioxidative enzymes of transgenic finger millet overexpressing a vacuolar H+-pyrophosphatase gene (SbVPPase) from Sorghum bicolor. J Plant Physiol 171(10):789–798

Arya L, Verma M, Gupta VK, Seetharam A (2013) Use of genomic and genic SSR markers for assessing genetic diversity and population structure in Indian and African finger millet (*Eleusine coracana* (L.) Gaertn.) germplasm. Plant System Evol 299:1395–1401. https://doi.org/10.1007/s00606-013-0822-x

Babu BK, Senthil N, Gomez SM, Biji KR, Rajendraprasad NS, Kumar S, Babu RC (2007) Assessment of genetic diversity among finger millet (*Eleusine coracana* (L.) Gaertn.) accessions using molecular markers. Genet Resources Crop Evol 54:399–404

Babu BK, Singh UM, Yadav S, Kumar A (2013a) Molecular marker technology for finger millet crop improvement: an under-utilized, food and nutritional security crop. Biotech Today Int J Biol Sci 2:57

Babu TK, Thakur RP, Upadhyaya HD, Reddy PN, Sharam R, Girish AG, Sarma NDRK (2013b) Resistance to blast (Magnaporthe grisea) in a mini-core collection of finger millet germplasm. Eur J Plant Pathol 135:299–311

Babu BK, Agrawal P, Pandey D, Jaiswal J, Kumar A (2014a) Association mapping of agro-morphological characters among the global collection of finger millet genotypes using genomic SSR markers. Mol Biol Rep. 41:5287–5297. https://doi.org/10.1007/s11033-014-3400-6

Babu BK, Agarwal PK, Pandey D, Kumar A (2014b) Comparative genomics and association mapping approaches for opaque2 modifier genes in finger millet accessions using genic, genomic and candidate gene-based simple sequence repeat markers. Mol Breeding 34:1261–1279

Babu BK, Pandey D, Agarwal PK, Sood S, Kumar A (2014c) In-silico mining, type and frequency analysis of genic microsatellites of finger millet (Eleusine coracana (L). Gaertn): a comparative genomic analysis of NBS-LRR regions of finger millet with rice. Mol Biol Rep 41:3081–3090

Bajaj D, Srivastava R, Nath M, Tripathi S, Bhardwaj C, Upadhyaya HD, Tyagi AK, Parida SK (2016) EcoTILLING-based association mapping efficiently delineates functionally relevant natural allelic variants of candidate genes governing agronomic traits in chickpea. Front Plant Sci 7:450

Baker A, Ceasar SA, Palmer AJ, Paterson JB, Qi W, Muench SP et al (2015) Replace, reuse, recycle: improving the sustainable use of phosphorus by plants. J Exp Bot 66:3523–3540

Bandyopadhyay T, Mutamilarasan M, Prasad M (2017a) Millets for next generation climate –smart agriculture. Front Plant Sci 8:1266. https://doi.org/10.3389/fpls.2017.01266

Bandyopadhyay T, Jaiswal V, Prasad M (2017b) Nutritional potential of foxtail millet in comparison to other millets and major cereals. In: The foxtail millet genome. Springer, Berlin/Heidelberg, Germany, pp 123–135

Bansal M, Yang J, Karan C, Menden MP, Costello JC, Tang H, Xiao G, Li Y, Allen J, Zhong R, Chen Barkla BJ, Vera-Estrella R, Hernandez-Coronado M, Pantoja O (2009) Quantitative proteomics of the tonoplast reveals a role for glycolytic enzymes in salt tolerance. Plant Cell 21:4044–4058

Barkley NA, Wang ML (2008) Application of TILLING and EcoTILLING as reverse genetic approaches to elucidate the function of genes in plants and animals. Curr Genom 4:212–226

Barnawal D, Bharti N, Pandey SS, Pandey A, Chanotiya CS, Kalra A (2017) Plant growth-promoting rhizobacteria enhance wheat salt and drought stress tolerance by altering endogenous phytohormone levels and TaCTR1/TaDREB2 expression. Physiol Plantarum 161:502–514

Bartwal A, Pande A, Sharma P (2016) Intervarietal variations in various oxidative stress markers and antioxidant potential of finger millet (Eleusine coracana) subjected to drought stress. J Environ Biol 37:517–522

Bayer GY, Yemets A, Blume Y (2014) Obtaining the transgenic lines of finger millet Eleusine coracana (L.) Gaertn. with dinitroaniline resistance. Cytol Genet 3:139–144

Bennetzen JL, Schmutz J, Wang H, Percifield R, Hawkins J, Pontaroli AC, Estep M, Feng L, Vaughn JN, Grimwood J (2012) Reference genome sequence of the model plant Setaria. Nat Biotechnol 30:55–561

Beyer P, Al-Babili S, Ye X, Lucca P, Schaub P, Welsch R, Potrykus I (2002) Golden rice: introducing the β-carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency. J Nutr 132:506S–510S. https://doi.org/10.1093/jn/132.3.506s

Bharathi A (2011) Phenotypic and genotypic diversity of global finger millet (Eleusine coracana (L) Gaertn.) composite collection. Tamil Nadu Agricultural University. Ph.D. Thesis, http://oar.icrisat.org/113/

Bhat S, Srinivasan S (2002) Molecular and genetic analyses of transgenic plants: considerations and approaches. Plant Sci 163:673–681

Bhatt D, Negi M, Saxena SC, Sharma P (2011) Responses to drought induced oxidative stress in five finger millet varieties differing in their geographical distribution. Physiol Mol Biol Plants 4:347–353

Bista DR, Heckathorn SA, Jayawardena DM, Mishra S, Boldt JK (2018) Effects of drought on nutrient uptake and the levels of nutrient-uptake proteins in roots of drought sensitive and tolerant grasses. Plants 7:1–16

Bokhari SA, Wan XY, Yang YW, Zhou L, Tang WL, Liu JY (2007) Proteomic response of rice seedling leaves to elevated CO_2 levels. J Proteome Res 6:4624–4633

Bray EA (1997) Plant responses to water deficit. Trends Plant Sci 2:48–54

Brhane H, Haileselassie T, Kassahun T (2017) Genetic diversity and population structure of Ethiopian finger millet (*Eleusine coracana* (L.) Gaertn) genotypes using inter simple sequence repeat (ISSR) markers. Afr J Biotechnol 21:1203–1209

Cannarozzi G, Plaza-Wüthrich S, Esfeld K, Larti S, Wilson YS, Girma D, de Castro E, Chanyalew S, Blösch R, Farinelli L, Lyons E (2014) Genome and transcriptome sequencing identifies breeding targets in the orphan crop tef (Eragrostis tef). BMC Genomics 15:581

Canovas FM, Gaudot ED, Recorbet G, Jorrin J, Mock HP, Rossignol M (2004) Plant proteome analysis. Proteomics 4(2):285–298

Cappa EP, EI-Kassaby YA, Garcia MN, Acuna C, NMG B, Grattapaglia D, ANM P (2013) Impacts of population structure and analytical methods in genome-wide association studies of complex traits in forest trees: a case study in Eucalyptus globules. PLoS One 8(11):e81267. https://doi.org/10.1371/journal.pone.0081267

Caruso G, Cavaliere C, Foglia P, Gubbiotti R, Samperi R, Laganà A (2009) Analysis of drought responsive proteins in wheat (*Triticum durum*) by 2D-PAGE and MALDITOF mass spectrometry. Plant Sci 177:570–576

Cassán F, Bottini R, Schneider G, Piccoli P (2001) *Azospirillum brasilense* and Azospirillum lipoferum hydrolyze conjugates of GA20 and metabolize the resultant aglycones to GA1 in seedlings of rice dwarf mutants. Plant Physiol 125:2053–2058

Castillo P, Molina R, Andrade A, Vigliocco A, Alemano S, Cassán FD (2015) Phytohormones and other plant growth regulators produced by PGPR: the genus Azospirillum. In: Handbook for azospirillum. Springer, Berlin/Heidelberg, Germany, pp 115–138

Ceasar SA, Ignacimuthu S (2011) Agrobacterium-mediated transformation of finger millet (Eleusine coracana (L.) Gaertn.) using shoot apex explants. Plant Cell Rep 30:1759–1770

Ceasar SA, Ignacimuthu S (2014) Genetic engineering of millets: current status and future prospects. Biotechnol Lett 31:779–788

Ceasar SA, Hodge A, Baker A, Baldwin SA (2014) Phosphate concentration and arbuscular mycorrhizal colonisation influence the growth, yield and expression of twelve PHT1 family phosphate transporters in foxtail millet (*Setaria italica*). PLoS One 9(9):e108459

Ceasar SA, Rajan V, Prykhozhij SV, Berman JN, Ignacimuthu S (2016) Insert, remove or replace: a highly advanced genome editing system using CRISPR/Cas9. Biochem Biophys Acta 1863: 2333–2344. https://doi.org/10.1016/j.bbamcr.2016.06.009

Ceasar SA, Baker A, Ignacimuthu S (2017) Functional characterization of the PHT1 family transporters of foxtail millet with development of a novel Agrobacterium-mediated transformation procedure. Sci. Rep 7:14064

Ceasar SA, Maharajan T, Krishna TPA, Ramakrishnan M, Roch GV, Satish L, Ignacimuthu S (2018) Finger millet [Eleusine coracana (L.) Gaertn.] improvement: current status and future interventions of whole genome sequence. Front Plant Science 9:1054

Chandrasekara A, Shahidi F (2010) Content of insoluble bound phenolics in millets and their contribution to antioxidant capacity. J Agric Food Chem 11:6706

Char SN, Neelakandan AK, Nahampun H, Frame B, Main M, Spalding MH, Becraft PW, Meyers BC, Walbot V, Wang K (2017) An agrobacterium-delivered CRISPR/Cas9 system for high-frequency targeted mutagenesis in maize. Plant Biotechnol J 15:257–268

Chen X, Lu X, Shu N, Wang S, Wang J, Wang D, Guo L, Ye W (2017) Targeted mutagenesis in cotton (*Gossypium hirsutum* L.) using the CRISPR/Cas9 system. Scientific Rep 7:44304

Chinchole M, Pathak RK, Singh UM, Kumar A (2017) Molecular characterisation of EcCIPK24 gene of finger millet (*Eleusine coracana*) for investigating its regulatory role in calcium transport. 3 Biotech 4:267

Cho SM, Kang BR, Han SH, Anderson AJ, Park JY, Lee YH, Cho BH, Yang KY, Ryu CM, Kim YC (2008) R, 3R-butanediol, a bacterial volatile produced by Pseudomonas chlororaphis O6, is involved in induction of systemic tolerance to drought in Arabidopsis thaliana. Mol Biol Plant Microbe Interact 21:1067–1075

Das S, Misra RC, Rout GR, Pattanaik MC, Aparajita S (2009) Relationship of status of polymorphic RAPD bands with genotypic adaptation in early finger millet genotypes. African Crop Sci J. 17 (2). https://doi.org/10.4314/acsj.v17i2.54199

Dasanayaka N (2016) Characterization of some ex situ conserved finger millet (*Eleusine coracana* (L.)) germplasm accessions in Sri Lanka. Int J Multidiscipl Studies. 3(2):141–150

Dechorgnat J, Francis KL, Dhugga KS, Rafalski JA, Tyerman SD, Kaiser BN (2018) Root ideotype influences nitrogen transport and assimilation in maize. Front Plant Sci 9:531

Degenkolbe T, Do PT, Zuther E, Repsilber D, Walther D, Hincha DK, Kohl KI (2009) Expression profiling of rice cultivars differing in their tolerance to long-term drought stress. Plant Mol Biol 69(2):133–153

Devarumath R, Hiremath SC, Rao SR, Kumar A, Bewal S (2005) Genome analysis of finger millet *E. coracana* by interspecific hybridization among diploid wild species of *Eleusine* (Poaceae). Cytologia 70:427–434

Dhanalakshmi T, Ramesh S, Upadhyaya H, Rao A, Gangappa E, Priyadarshini S (2014) Genetic variability for morpho-agronomic traits in core germplasm collections of finger millet (*Eleusine coracana* (L.) Gaertn.) based on third and fourth degree statistics and their origin. Int J Trop Agric 32(1/2):239–242

Dida MM, Srinivasachary RS, Bennetzen JL, Gale MD, Devos KM (2007) The genetic map of finger millet, *Eleusine coracana*. Theor Appl Genet 2:321–332

Dida M, Wanyera N, Dunn MLH, Bennetzen JL, Devos K (2008) Population structure and diversity in finger millet (Eleusine coracana) germplasm. Trop Plant Biol 2:131–141

Dimkpa C, Weinand T, Asch F (2009) Plant–rhizobacteria interactions alleviate abiotic stress conditions. Plant Cell and Environ 32:1682–1694

Disseka WK, Faulet MB, Koné FMB, Gnanwa MJ, Kouamé LP (2018) Phytochemical composition and functional properties of millet (*Pennisetum glaucum*) flours fortified with sesame (*Sesamum indicum*) and moringa (*Moringa oleifera*) as a weaning food. J Adv Res 15:1–11

Dita MA, Rispail N, Prats E, Rubiales D, Singh KB (2006) Biotechnology approaches to overcome biotic and abiotic stress constraints in legumes. Euphytica 147:1–24

Dramadri Onziga I (2015) Characterizing the genetic diversity of finger millet in Uganda. https://repository.ruforum.org/sites/default/files/Dramdri%20Isaac.pdf.

Dwivedi S, Upadhyaya H, Senthilvel S, Hash C, Fukunaga K, Diao X et al (2012) Millets: genetic and genomic resources. In: Janick J (ed) Plant breeding reviews, vol 35. John Wiley & Sons, Hoboken, NJ, pp 247–375

Ediga A, Hemalatha S, Meriga B (2013) Effect of salinity stress on antioxidant defense system of two finger millet cultivars (*Eleusine coracana* (L.) Gaertn) differing in their sensitivity. Adv Biol Res 7:180–187

Egamberdieva D, Kucharova Z (2009) Selection for root colonising bacteria stimulating wheat growth in saline soils. Biol Fertil Soil 45:563–571

Egamberdieva D, Davranov K, Wirth S, Hashem A, Abd Allah EF (2017) Impact of soil salinity on the plant-growth—promoting and biological control abilities of root associated bacteria. Saudi J Biol Sci 24:1601–1608

Eid A, Alshareef S, Mahfouz MM (2018) CRISPR base editors: genome editing without double-stranded breaks. Biochem J 475:1955–1964

Fakrudin B, Kulkarni RS, Shashidhar HE, Hittalmani S (2004) Genetic diversity assessment of finger millet, *Eleusine coracana* (Gaertn), germplasm through RAPD analysis. Plant Genet Resources Newslett 138:50–54

Farag MA, Zhang H, Ryu CM (2013) Dynamic chemical communication between plants and bacteria through airborne signals: induced resistance by bacterial volatiles. J Chem Ecol 39: 1007–1018

Fuller DQ, Hildebrand E (2013) Domesticating plants in Africa. In: Mitchell P, Lane PJ (eds) The Oxford hand book of African archeology. Oxford Academic, Oxford. https://doi.org/10.1093/oxfordhb/9780199569885.013.0035. 5 Sept. 2013, accessed 3 Oct.2022

Furbank RT, Tester M (2011) Phenomics-technologies to relieve the phenotyping bottleneck. Trends Plant Sci 16(12):635–644

Gamalero E, Berta G, Massa N, Glick BR, Lingua G (2010) Interactions between Pseudomonas putida UW4 and *Gigaspora rosea* BEG9 and their consequences for the growth of cucumber under salt-stress conditions. J Appl Microbiol 108:236–224

Gao W, Long L, Tian X, Xu F, Liu J, Singh PK, Botella JR, Song C (2017) Genome editing in cotton with the CRISPR/Cas9 system. Front Plant Sci 8:1364

Gaur VS, Kumar L, Gupta S, Jaiswal JP, Pandey D, Kumar A (2018) Identification and characterization of finger millet OPAQUE2 transcription factor gene under different nitrogen inputs for understanding their role during accumulation of prolamin seed storage protein. 3 Biotech 3:163

Gimode D, Odeny DA, de Villiers EP, Wanyonyi S, Dida MM, Mneney EE, Muchugi A, Machuka J, de Villiers SM (2016) Identification of SNP and SSR markers in finger millet using next generation sequencing technologies. PLoS One 11(7):e0159437. https://doi.org/10.1371/journal.pone.0159437

Ginzberg I, Barel G, Ophir R, Tzin E, Tanami Z, Muddarangappa T, de Jong W, Fogelman E (2009) Transcriptomic profiling of heat-stress response in potato periderm. J Exp Bot 60:4411–4421

Gliblin JD, Fuller DQ (2011) First and second emillennium A.D. agriculture in Rwanda: archaeobotanical finds and radiocarbon dates from seven sites. Vegetation Hist Achaeobot 20: 253–265

Glick BR (2005) Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol Lett 251:1–7

Goron TL, Raizada MN (2015) Genetic diversity and genomic resources available for the small millet crops to accelerate a New Green Revolution. Front Plant Sci 6:157

Gupta P, Raghuvanshi S, Tyagi AK (2001) Assessment of the efficiency of various gene promoters via biolistics in leaf and regenerating seed callus of millets, *Eleusine coracana* and *Echinochloa crus-galli*. Plant Biotechnol 18:275–282

Gupta R, Verma K, Joshi DC, Yadav D, Singh M (2010) Assessment of genetic relatedness among three varieties of finger millet with variable seed coat color using RAPD and ISSR markers. J Genet Eng Biotechnol 2:1–9. Available online at: http://astonjournals.com/manuscripts/Vol2010/GEBJ- 2_Vol2010.pdf

Gupta N, Gupta AK, Singh NK, Kumar A (2011) Differential expression of PBF Dof transcription factor in different tissues of three finger millet genotypes differing in seed protein content and color. Plant Mol Biolo 1:69–76

Gupta N, Gupta AK, Gaur VS, Kumar A (2012) Relationship of nitrogen use efficiency with the activities of enzymes involved in nitrogen uptake and assimilation of finger millet genotypes grown under different nitrogen inputs. Scientific World J 2012:1–10

Gupta AK, Gaur VS, Gupta S, Kumar A (2013) Nitrate signals determine the sensing of nitrogen through differential expression of genes involved in nitrogen uptake and assimilation in finger millet. Funct Integr Genom 13:179–190

Gupta S, Gupta SM, Gupta AK, Gaur VS, Kumar A (2014) Fluctuation of Dof 1/Dof 2 expression ratio under the influence of varying nitrogen and light conditions: Involvement in differential regulation of nitrogen metabolism in two genotypes of finger millet (*Eleusine coracana* L.). Gene 546(2):327–335

Gupta SM, Arora S, Mirza N, Pande A, Lata C, Puranik S, Kumar J, Kumar A (2017) Finger millet: a certain crop for an uncertain future and a solution to food insecurity and hidden hunger under stressful environments. Front Plant Sci 8:643

Gururani K, Kumar A, Tiwari A, Agarwal A, Gupta S, Pandey D (2020) Transcriptome wide identification and characterization of regulatory genes involved in EAA metabolism and validation through expression analysis in different developmental stages of finger millet spikes. 3 Biotech 10(8):347

Gusain YS, Singh U, Sharma A (2015) Bacterial mediated amelioration of drought stress in drought tolerant and susceptible cultivars of rice (Oryza sativa L.). Afr J Biotechnol 14:764–773

Gygi SP, Rist B, Gerber SA, Turecek F, Gelb MH, Aebersold R (1999) Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. Nat Biotechnol 10:994–999

Hatakeyama M, Aluri S, Balachadran MT, Sivarajan SR, Patrignani A, Gruter S, Poveda L, Inatsugi RS, Baeten J, Francoijs KJ, Nataraja KN, Reddy YAN, Phadnis S, Ravikumar RL, Schlapbach R, Sreeman SM, Shimizu KK (2017) Multiple hybrid de novo genome assembly of finger millet, an orphan allotetraploid crop. DNA Res 1:39–47

Hema R, Vemanna RS, Sreeramulu S, Reddy CP, Kumar MS, Udayakumar M (2014) Stable expression of mtlD gene imparts multiple stress tolerance in finger millet. PLoS ONE 9(6): e99110. https://doi.org/10.1371/journal.pone.0099110

Hiremath SC, Salimath SS (1992) The 'A' genome donor of Eleusine cracana (L.) Gaertn. (Gramineae). Theor Appl Genet 84:754

Hittalmani S, Mahesh H, Shirke MD, Biradar H, Uday G, Aruna Y, Lohithaswa H, Mohanrao AJBG (2017) Genome and trascriptome sequence of finger millet (*Eleusine coracana* (L.) Gaertn.) provides insights into drought tolerance and nutraceutical properties. BMC Genome 18:465

Holopainen JK, Gershenzon J (2010) Multiple stress factors and the emission of plant VOCs. Trends Plant Sci 15:176–184

Hossain MA, Uddin SN (2011) Mechanisms of waterlogging tolerance in wheat: Morphological and metabolic adaptations under hypoxia or anoxia. Aust J Crop Sci 5:1094

Ignacimuthu S, Ceasar SA (2012) Development of transgenic finger millet (*Eleusine coracana* (L.) Gaertn.) resistant to leaf blast disease. J Biosci 37:135–147

Islam F, Yasmeen T, Ali Q, Ali S, Arif MS, Hussain S, Rizvi H (2014) Influence of *Pseudomonas aeruginosa* as PGPR on oxidative stress tolerance in wheat under Zn stress. Exotoxicol Enviorn Safety 104:285–293

Jaganathan D, Ramasamy K, Sellamuthu G, Jayabalan S, Venkataraman G (2018) CRISPR for crop improvement: an update review. Front Plant Sci 9:985

Jagga-Chugh S, Kachhwaha S, Sharma M, Kothari Chajer A, Kothari S (2012) Optimization of factors influencing microprojectile bombardment mediated genetic transformation of seed-derived callus and regeneration of transgenic plants in *Eleusine coracana* (L.) Gaertn. Plant Cell Tissue Organ Cult 109:401–410

Jayasudha BG, Sushma AM, Prashantkumar HS, Sashidhar VR (2014) An efficient in-vitro Agrobacterium-mediated transformation protocol for raising salinity tolerant transgenic plants in Finger Millet [*Eleusine coracana* (L.) Gaertn.]. Plant Arch 2:823–829

Ji J, Yuan D, Jin C, Wang G, Li X, Guan C (2020) Enhancement of growth and salt tolerance of rice seedlings (Oryza sativa L.) by regulating ethylene production with a novel halotolerant PGPR strain Glutamicibacter sp. YD01 containing ACC deaminase activity. Acta Physiol Plantarum 42:42

Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E (2012) A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. Science 337:816–821

Jogaiah S, Abdelrahman M, Tran LSP, Ito SI (2018) Different mechanisms of Trichoderma virens-mediated resistance in tomato against Fusarium wilt involve the jasmonic and salicylic acid pathways. Mol Plant Pathol 19:870–882

Johnson M, Deshpande S, Vetriventhan M, Upadhyaya HD, Wallace JG (2019) Genome-wide population structure analyses of three minor millets: Kodo Millet, Little Millet, and Proso Millet. Plant Genome 12:190021

Joshi B, Chaudhary A, Singh H, Kumar PA (2020) Prospective evaluation of individual and consortia plant growth promoting rhizobacteria for drought stress amelioration in rice (*Oryza sativa* L.). Plant Soil 457:225–240

Kai M, Haustein M, Molina F, Petri A, Scholz B, Piechulla B (2009) Bacterial volatiles and their action potential. Appl Microbiol Biotechnol 81:1001–1012

Kannan S (2010) Fingermillet in nutrition transition: an infant weaning food ingredient with chronic disease preventive potential. Br J Nutr 104:1733–1734

Kanwal P, Gupta S, Arora S, Kumar A (2014) Identification of genes involved in carbon metabolism from Eleusine coracana L. for understanding their light-mediated entrainment and regulation. Plant Cell 33(8):1403–1411

Kashtwari M, Wani AA, Rather RN (2019) TILLING: an alternative pathway for crop improvement. J Crop Improvement 33(1):83–109

Khatoon H, Singh V (2016) Impact of water stress on physiological and biochemical parameters of finger millet (Eleusine coracana L). Res Environ Life Sci 9:1474–1477

Kissoudis C, Wiel CVD, Visser RGF, Linden CGVD (2014) Enhancing crop resilience to combined abiotic and biotic stress through the dissection of physiological and molecular crosstalk. Front Plant Sci 5:207

Klap C, Yeshayahou E, Bolger AM, Arazi T, Gupta SK, Shabtai S, Usadel B, Salts Y, Barg R (2017) Tomato facultative parthenocarpy results from Sl AGAMOUS-LIKE 6 loss of function. Plant Biotechnol J 15:634–647

Krishna TPA, Maharajan T, Victor RG, Ramakrishnan M, Ceasar SA, Ignacimuthu S (2020) Hybridization and hybrid detection through molecular markers in finger millet (Eleusine coracana(L.) Gaert.). J Crop Improve 34:335–355

Krishnamurthy L, Upadhyaya HD, Purushothaman R, Gowda CLL, Kashiwagi J, Dwivedi SL, Singh S, Vadez V (2014) The extent of variation in salinity tolerance of the minicore collection of finger millet (*Eleusine coracana* L. Gaertn.) germplasm. Plant Sci 227:59

Kumar A, Gaur VS, Goel A, Gupta AK (2015a) De novo assembly and characterization of developing spikes transcriptome of finger millet (Eleusine coracana): a minor crop having nutraceutical properties. Plant Mol Biol Rep 33:905–922

Kumar A, Yadav S, Panwar P, Gaur VS, Sood S (2015b) Identification ofanchored simple sequence repeat markers associated with calcium content infinger millet (Eleusine coracana). Proc Natl Acad Sci India Sect B: Biol Sci 85:311–317

Kumar A, Kumar S, Bains S, Vaidya V, Singh B, Kaur R, Kaur J, Singh K (2016a) De novo transcriptome analysis revealed genes involved in flavonoid and vitamin C biosynthesis in phyllanthus emblica (L.). Front Plant Sci 7:1610

Kumar A, Metwal M, Kaur S, Gupta AK, Puranik S, Singh S, Singh M, Gupta S, Babu KB, Sood S (2016b) Nutraceutical value of finger millet [*Eleusine coracana* (L.) Gaertn.], and their improvement using omics approaches. Front Plant Sci 7:934

Kumari K, Pande A (2010) Study of genetic diversity in finger millet (Eleusine coracana L. Gaertn) using RAPD markers. Afr J Biotechnol 9:4542–4549

Lata C, Prasad M (2013) Association of an allele-specific marker with dehydration stress tolerance in foxtail millet suggests *SiDREB2* to be an important QTL. J Plant Biochem Biotechnol 23: 119–122

Lata C, Jha S, Dixit V, Prasad M, Sreenivasulu N (2011) Differential antioxidative responses to dehydration-induced oxidative stress in core set of foxtail millet cultivars [*Setaria italica* (L.)]. Protoplasma 4:817–828

Lata C, Gupta S, Prasad M (2013) Foxtail millet: a model crop for genetic and genomic studies in bioenergy grasses. Crit Rev Biotechnol 33:328–343

Latha A, Rao KV, Reddy VD (2005) Production of transgenic plants resistant to leaf blast disease in finger millet (Eleusine coracana (L.) Gaertn.). J Plant Sci 169:657–667

Leng P-F, Lübberstedt T, Xu M-L (2017) Genomics-assisted breeding—a revolutionary strategy for crop improvement. J Integr Agric 16(12):2674–2685

Lenne JM, Takan J, Mgonja MA, Kaloki P, Okwadi J, Brown AE, Sreenivasaprasad S, Manyasa EO, Wanyera N, Muthumeenakshi S, Tamale M (2007) Finger millet blast disease management: a key entry point for fighting malnutrition and poverty in East Africa. Outlook Agric 2:101–108

Li W, Pang S, Lu Z, Jin B (2020) Function and mechanism of WRKY transcription factors in abiotic stress responses of plants. Plants 9:1515

Lifshitz R, Kloepper JW, Scher FM, Tipping EM, Laliberté M (1986) Nitrogen-fixing pseudomonads isolated from roots of plants grown in the Canadian high arctic. Appl Environ Microbiol 51:251–255

Liu JX, Howell SH (2010) Endoplasmic reticulum protein quality control and its relationship to environmental stress responses in plants. Plant Cell 22:2930–2942

Liu Q, Jiang B, Wen J, Peterson PM (2014) Low-copy nuclear gene and McGISH resolves polyploidy history of Eleusine coracana and morphological character evolution in Eleusine. Turkish J Bot 38:1–12

López MA, Bannenberg G, Castresana C (2008) Controlling hormone signaling is a plant and pathogen challenge for growth and survival. Curr Opin Plant Biol 11:420–427

Loreto F, Schnitzler JP (2010) Abiotic stresses and induced BVOCs. Trends Plant Sci 15:154–166

Lule D, De Villiers S, Fetene M, Odeny DA, Rathore A, Das RR, Tesfaye K (2018) Genetic diversity and association mapping of Ethiopian and exotic finger millet accessions. Crop Pasture Sci 69:879–891

Ma HW, Silva MRD, Sun JB, Kumar B, Zeng AP (2007) Reconstruction and structural analysis of metabolic and regulatory networks. Syst Biol:124–146

Mahalakshmi S, Christopher GSB, Reddy TP, Rao KV, Reddy VD (2006) Isolation of cDNA clone (PcSrp) encoding serine-rich-protein from Porteresia coarctata T. and its expression in yeast and finger millet (Eleusine coracana L.) affording salt tolerance. Planta 224:347–359

Maharajan T, Ceasar SA, Krishna TPA, Ramakrishnan M, Duraipandiyan V, Naif Abdulla AD, Ignacimuthu S (2018) Utilization of molecular markers for improving the phosphorus efficiency in crop plants. Plant Breed 137:10–26

Maharajan T, Ceasar SA, Krishna TPA, Ignacimuthu S (2019) Phosphate supply influenced the growth, yield and expression of PHT1 family phosphate transporters in seven millets. Planta 250:1433–1448

Malzahn A, Lowder L, Qi Y (2017) Plant genome editing with TALEN and CRISPR. Cell Biosci 7: 21

Mamidi S, Healey A, Huang P, Grimwood J, Jenkins J, Barry K, Sreedasyam A, Shu S, Lovell JT, Feldman M (2020) A genome resource for green millet *Setaria viridis* enables discovery of agronomically valuable loci. Nat Biotechnol 38:1203–1210

Mantelin S, Touraine B (2004) Plant growth-promoting bacteria and nitrate availability: Impacts on root development and nitrate uptake. J Exp Bot 55:27–34

Manyasa EO, Tongoona P, Shanahan P, Mgonja MA (2015) Genetic diversity in East African finger millet (*Eleusine Coracana* (L.) Gaertn) landraces based on SSR markers and some qualitative traits. Plant Genet Resources: Charact Util 13:45–55

Maqsood M, Ali SNA (2007) Effects of drought on growth, development, radiation use efficiency and yield of finger millet (*Eleucine Coracana* (L.) Gaertn). Pak J Bot 39:123–134

Martignago D, Rico-Medina A, Blasco-Escámez D, Fontanet-Manzaneque JB, Caño- Delgado AI (2020) Drought resistance by engineering plant tissue-specific responses. Front Plant Sci 10: 1676

Matsuura A, An P, Murata K, Inanaga S (2016) Effect of pre-and post –heading waterlogging on growth and grain yield of four millets. Plant Prod Sci 19:348–359

Mayak S, Tirosh T, Glick BR (2004) Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. Plant Sci 166:525–530

Mgonja M, Audi P, Mgonja AP, Manyasa E, Ojulong H (2011) Integrated blast and weed management and microdosing in finger millet. A HOPE project manual for increasing finger millet productivity in Eastern Africa. ICRISAT-Kenya, pp. 1–40.

Miao J, Guo D, Zhang J, Huang Q, Qin G, Zhang X, Wan J, Gu H, Qu L-J (2013) Targeted mutagenesis in rice using CRISPR-Cas system. Cell Res 23:1233–1236

Miglani GS (2017) Genome editing in crop improvement: present scenario and future prospects. J Crop Improve 31(4):453–559

Mirza N, Marla S (2019) Finger Millet (Eleusine coracana L. Gartn.). Breeding 10:1007

Mirza N, Taj G, Arora S, Kumar A (2014) Transcriptional expression analysis of genes involved in regulation of calcium translocation and storage in finger millet (*Eleusine coracana* L. Gaertn.). Gene 550(2):171–179

Molina-Favero C, Creus CM, Simontacchi M, Puntarulo S, Lamattina L (2008) Aerobic nitric oxide production by *Azospirillum brasilense* Sp245 and its influence on root architecture in tomato. Mol Plant Microbe Interact 21:1001–1009

Moreno- Risueno MA, Norman JMV, Moreno A, Zhang J, Ahnert SE, Benfey PN (2010) Oscillating gene expression determines competence for periodic Arabidopsis root branching. Science 5997:1306–1311

Mukami A, Ngetich A, Syombua E, Oduor R, Mbinda W (2020) Varietal differences in physiological and biochemical responses to salinity stress in six finger millet plants. Physiol Mol Biol Plants 26:1569–1582

Mundada PS, Barvkar VT, Umdale SD, Kumar SA, Nikam DT, Ahire LM (2021) An insight into the role of silicon on retaliation to osmotic stress in finger millet (Eleusine coracana (L.) Gaertn). J Hazard Mater 403:124078

Muthamilarasan M, Bonthala VS, Mishra AK, Khandelwal R, Khan Y, Roy R, Prasad M (2014) C_2H_2 type of zinc finger transcription factors in foxtail millet define response to abiotic stresses. Funct Integr Genom 3:531–543

Nagarjuna KN, Parvathi MS, Sajeevan RS, Pruthvi V, Mamrutha HM, Nataraja KN (2016) Full length cloning and characterization of abiotic stress responsive CIPK31- like gene from finger millet, a drought tolerant crop. Curr Sci 5:890–894

Nakarani UM, Singh D, Suthar KP, Karmakar N, Faldu P, Patil HE (2021) Nutritional and phytochemical profiling of nutracereal finger millet (*Eleusine coracana* L.) genotypes. Food Chem 341:128271

Nawaz A, Shabaz M, Asadullah AL, Marghoob MU, Imtiaz M, Mubeen F (2020) Potential of salt tolerant PGPR in growth and yield augmentation of wheat (Triticum aestivum L.) under saline conditions. Front Microbiol. 11:2019

Nirgude M, Babu BK, Shambhavi Y, Singh UM, Upadhyaya HD, Kumar A (2014) Development and molecular characterization of genetic molecular markers for grain protein and calcium content in finger millet (Eleusine coracana (L.) Gaertn.). Mol Biol Rep 3:1189–1200

Nithiyanantham S, Kalaiselvi P, Mahomoodally MF, Zengin G, Abirami A, Srinivasan G (2019) Nutritional and functional roles of millets. A review. J Food Biochem 43:e12859

Numan M, Khan AL, Asaf S, Salehin M, Beyene G, Tadele Z, Ligaba-Osena A (2021) From traditional breeding to genome editing for boosting productivity of the ancient grain Tef [Eragrostis tef (Zucc.) Trotter]. Plants 10:628

Onziga DI (2015) Characterizing the genetic diversity of finger millet in Uganda. Makererer University. http://makir.mak.ac.ug/handle/10570/6606

Pandian S, Sivasankar C, Muthuramalingam C, Ramesh M (2017) An amazing nutritional value in wonderful finger millet makes this "The Most Lovable Food Crop" to the World. Sci J Food Sci Nutr 3:34–36

Pandian S, Satish L, Rameshkumar R, Muthuramalingam P, Rency AS, Rathinapriya P, Ramesh M (2018a) Analysis of population structure and genetic diversity in an exotic germplasm collection of *Eleusine Coracan*a (L.) Gaertn. Using genic-SSR markers. Gene 653:80–90

Pandian S, Marichelvam K, Satish L, Caesar SA, Pandian SK, Ramesh M (2018b) SPAR markers assisted assessment of genetic diversity and population structure in finger millet (Eleusine coracana (L.) Gaertn.) mini-core collection. J Crop Sci Biotechnol 21:469–481

Pandian S, Marichelvam K, Satish L, Ceasar SA, Pandian SK, Ramesh M (2018c) SPAR marker-assisted assessment of genetic diversity and population structure in finger millet (Eleusine Coracana (L.) Gaertn) mini-core collection. J Crop Sci Biotechnol 21:469–481

Panwar P, Nath M, Yadav VK, Kumar A (2010) Comparative evaluation of genetic diversity using RAPD, SSR and cytochrome P450 gene based markers with respect to calcium content in finger millet (Eleusine Coracana L. Gaertn.). J Genet 89:121–133

Panwar P, Jha AK, Pandey PK, Arun K, Gupta AK (2011a) Functional markers based molecular characterization and cloning of resistance gene analogs encoding NBS-LRR disease resistance proteins in finger millet (Eleusine coracana). Mol Biol Rep 38:427–3436

Panwar P, Jha AK, Pandey PK, Gupta AK, Kumar A (2011b) Functional markers based molecular characterization and cloning of resistance gene analogs encoding NBS-LRR disease resistance proteins in finger millet (Eleusine coracana). Mol Biol Rep 38:3427–2436

Parvathi MS, Nataraja KN (2017) Discovery of stress responsive TATA-box binding protein associated Factor6 (TAF6) from finger millet (*Eleusine coracana* (L.) Gaertn). J. Plant Biol 60:335–342. https://doi.org/10.1007/s12374-016-0574-6

Parvathi M, Nataraja KN, Yashoda B, Ramegowda H, Mamrutha H, Rama N (2013) Expression analysis of stress responsive pathway genes linked to drought hardiness in an adapted crop, finger millet (*Eleusine coracana*). J. Plant Biochem Biotechnol 22:193–201. https://doi.org/10.1007/s13562-012-0135-0

Parvathi MS, Nataraja KN, Reddy YAN, Naika MBN, Gowda MVC (2019) Transcriptome analysis of finger millet (Eleusine coracana (L.) Gaertn.) reveals unique drought responsive genes. J Genet. 98:46

Peng R, Zhang B (2020) Foxtail millet: a new model for C4 plants. Trends Plant Sci 26:199–201

Pereyra M, Zalazar C, Barassi C (2006) Root phospholipids in Azospirillum-inoculated wheat seedlings exposed to water stress. Plant Physiol Biochem 44:873–879

Polko JK, Kieber JJ (2019) 1-aminocyclopropane 1-carboxylic acid and its emerging role as an ethylene-independent growth regulator. Front Plant Sci 10:1602

Pudake RN, Mehta CM, Mohanta TK, Sharma S, Varma A, Sharma AK (2017) Expression of four phosphate transporter genes from Finger millet (*Eleusine coracana* L.) in response to mycorrhizal colonization and Pi stress. 3 Biotech 7:1–13

Rahman H, Jagadeeshselvam N, Valarmathi R, Sachin B, Sasikala R, Senthil N, Sudhakar D, Robin S, Muthurajan R (2014a) Transcriptome analysis of salinity responsiveness in contrasting genotypes of finger millet (Eleusine coracana L.) through RNA- sequencing. Plant Mol Biol 5: 485–503

Rahman H, Selvam J, Ramasamy S, Natesan S, Sudhakar D, Robin SI, Muthurajan R, Ramanathan V, Bhor S (2014b) Transcriptome analysis of salinity responsiveness in contrasting genotypes of finger millet (*Eleusine coracana* L.) through RNA- sequencing. Plant Mol Biol 85: 485–503

Rahman H, Ramanathan V, Nallathambi J, Duraialagaraja S, Muthurajan R (2016) Over- expression of a NAC 67 transcription factor from finger millet (*Eleusine coracana* L.) confers tolerance against salinity and drought stress in rice. BMC Biotechnol 1:35

Rajaram V, Napoleon T, Senthilvel S, Varshney RK, Vadez V, Srivastava RK, Shah TM, Supriya A, Kumar S, Kumari BR, Bhanuprakash A, Narasu ML, Riera-Lizarazu O, Hash CT (2013) Pearl millet (Pennisetum glaucum(L)R.Br.) consensus linkage map constructed using four RIL mapping populations and newly developed EST- SSRs. BMC Genomics 14:159

Ramakrishna C, Singh S, Sanagala R, Solanke A (2018) The membrane tethered transcription factor EcbZIP17 from finger millet promotes plant growth and enhances tolerance to abiotic stresses. Scientific Rep 8:2148. https://doi.org/10.1038/s41598-018-19766-4

Ramakrishnan M, Ceasar SA, Duraipandiyan V, Al-Dhabi NA, Ignacimuthu S (2015) Using molecular markers to assess the genetic diversity and population structure of finger millet (*Eleusine coracana* (L.) Gaertn.) from various geographical regions. Genet Resour Crop Evol. https://doi.org/10.1007/s10722-015-0255-1

Ramakrishnan M, Ceasar SA, Duraipandiyan V, Dhabi NA, Ignacimuthu S (2016) Assessment of genetic diversity, population structure and relationships in Indian and non-Indian genotypes of finger millet (Eleusine coracana (L.) Gaertn.) using genomic SSR markers. SpringerPlus 5(1): 1–11

Ramakrishnan M, Caesar SA, Vinod KK, Duraipandiyan V, Krishna TPA, Upadhyaya HD, Dhabi NA, Ignacimuthu S (2017) Identification of putative QTLs for seedling stage phosphorus starvation response in finger millet (Eleusine coracana L. Gaertn.) by association mapping and cross species synteny analysis. PLoS One 12(8):e0183261. https://doi.org/10.1371/journal.pone.0183261

Ramanjulu S, Bartels D (2002) Drought- and desiccation-induced modulation of gene expression in plants. Plant Cell Eniviron 25:141–151

Ramashia SE, Gwata ET, Meddows-Taylor S, Anyasi TA, Jideani AIO (2018) Some physical and functional properties of finger millet (Eleusine coracana) obtained in sub-Saharan Africa. Food Res Int 104:110–118. ISSN 0963-9969. https://doi.org/10.1016/j.foodres.2017.09.065

Ramegowda V, Kumar MS, Nataraja KN, Reddy MK, Mysore KS, Udaykumar M (2012) Expression of a finger millet transcription factor, EcNAC1, in tobacco confers abiotic stress- tolerance. PLos One 7:e40397

Ramegowda V, Gill US, Sivalingam PN, Gupta A, Gupta C, Govind G, Senthil-Kumar M (2017) GBF3 Transcription factor imparts drought tolerance in arabidopsis Thaliana. Scientific Rep 7(1):1–13. https://doi.org/10.1038/s41598-017-09542-1

Reddy DS, Bhatnagar-Mathur P, Vadez V, Sharma KK (2012) Grain legumes (Soybean, Chickpea, and Peanut): Omics approaches to enhance abiotic stress tolerance. In: Tuteja N, Gill SS, Tiburcio AF, Tuteja R (eds) Improving crop resistance to abiotic stress. Wiley-VCH Verlag GmbH & Co. KGaA. https://doi.org/10.1002/9783527632930.ch39

Ricroch A, Clairand P, Harwood W (2017) Use of CRISPR systems in plant genome editing: Toward new opportunities in agriculture. Emerg Top Life Sci 1:169–182

Ryu CM, Farag MA, Hu CH, Reddy MS, Kloepper JW, Paré PW (2004) Bacterial volatiles induce systemic resistance in Arabidopsis. Plant Physiol 134:1017–1026

Saha D, Rana RS, Arya L, Verma M, Gowda MVC, Upadhyaya HD (2017) Genetic polymorphisms among and between blast disease resistant and susceptible finger millet, Eleusine coracana (L.) Gaertn. Plant Genet Resources 15(4):355–365

Sakamma S, Umesh KB, Girish MR, Ravi SC, Satishkumar M, Bellundagi V (2018) Finger millet (Eleusine coracana L. Gaertn.) production system: status, potential, constraints and implications for improving small farmers welfare. J Agric Sci 1:1916

Samayoa LF, Malvar RA, Olukolu BA, Holland JB, Butrón A (2015) Genome-wide association study reveals a set of genes associated with resistance to the Mediterranean corn borer (*Sesamia nonagrioides* L.) in a maize diversity panel. BMC Plant Biol 15:35

Santoro MV, Zygadlo J, Giordano W, Banchio E (2011) Volatile organic compounds from rhizobacteria increase biosynthesis of essential oils and growth parameters in peppermint (*Mentha piperita*). Plant Physiol Biochem J 49:1177–1182

Satish L, Ceasar SA, Ramesh M (2017) Improved Agrobacterium-mediated transformation and direct plant regeneration in four cultivars of finger millet (Eleusine coracana (L.) Gaertn.). Plant Cell Tissue Organ Culture (PCTOC) 131:547–565

Scofield SR, Nelson R (2009) Resources for virus-induced gene silencing in the grasses. Plant Physiol 149:152–157

Seki M, Narusaka M, Ishida J, Nanjo T, Fujita M, Oono Y, Kamiya A, Nakajima M, Enju A, Sakurai T, Satou M, Akiyama K, Taji T, Shinozaki KY, Carninci P, Kawai J, Hayashizaki Y, Shinozaki K (2002) Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high- salinity stresses using a full- length cDNA microarray. Plant J 3:279–292

Sen S, Kundu S, Dutta SK (2016) Proteomic analysis of JAZ interacting proteins under methyl jasmonate treatment in finger millet. Plant Physiol Biochem 108:77–89

Serba DD, Yadav RS (2016) Genomic tools in pearl millet breeding for drought tolerance: status and prospects. Front Plant Sci. https://doi.org/10.3389/fpls.2016.01724

Sharma S, Kaur R, Singh A (2017) Recent advances in CRISPR/Cas mediated genome editing for crop improvement. Plant Biotechnol Rep 11:193–207

Sharma D, Tiwari A, Sood S, Jamra G, Singh NK, Meher PK, Kumar A (2018) Genome wide association mapping of agro-morphological traits among a diverse collection of finger millet (*Eleusine coracana* L.) genotypes using SNP markers. PLoS One 13. https://doi.org/10.1371/journal.pone.0199444

Shi J, Gao H, Wang H, Lafitte HR, Archibald RL, Yang M, Hakimi SM, Mo H, Habben JE (2017) ARGOS 8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. Plant Biotechnol J 15:207–216

Shrawat AK, Lörz H (2006) Agrobacterium-mediated transformation of cereals: a promising approach crossing barriers. Plant Biotechnol J 4:575–603. https://doi.org/10.1111/j.1467-7652.2006.00209.x

Shulaev V, Cortes D, Miller G, Mittler R (2008) Metabolomics for plant stress response. Physiol Plantarum 2:199–208

Singh UM, Chandra M, Shankhdhar C, Kumar C (2014) Transcriptome wide identification and validation of calcium sensor gene family in the developing spikes of finger millet genotypes for elucidating its role in grain calcium. PLoS One. Accumulation. https://doi.org/10.1371/journal.pone.0103963

Singh RK, Sanagala R, Phanindra MLV, Raman KV, Solanke A, Kumar PA, Sharma T, Singh VK (2015a) Expression of finger millet EcDehydrin7 in transgenic tobacco confers tolerance to drought stress. Appl Biochem Biotechnol 177:207–216

Singh UM, Metwal M, Singh M, Taj G, Kumar A (2015b) Identification and characterization of calcium transporter gene family in finger millet in relation to grain calcium content. Gene 566(1):37–46

Singh N, David J, Thompkinson D, Seelam BS, Rajput H, Morya S (2018) Effect of roasting on functional and phytochemical constituents of finger millet (*Eleusine coracana* L.). Pharma Innov J 7:414–418

Smith MW, Mudge SR, Rae AL, Glassop D (2003) Phosphate transport in plants. Plant Soil 248: 71–83

Sood S (2019) Phenomics and genomics of finger millet: current status and future prospects. Planta 250:731–751

Sood S, Kumar A, Babu BK, Gaur VS, Pandey D, Kant L, Pattnayak A (2016) Gene discovery and advances in finger millet [*Eleusine coracana* (L.) Gaertn.] genomics: an important nutri-cereal of future. Front Plant Sci 7:1634

Sreenivasaprasad S, Takan JP, Obilana AB, Manyasa E, Qub B, Bandyopadhyaya R, Muthumeenakshi S (2004) Finger millet blast in East Africa: pathogen diversity and disease management strategies. DFID. R8030. Crop Protection Programme

Sudan J, Adhikari BN, Arora S (2015) Oxidative stress induced expression of monodehydroascorbate reductase gene in Eleusine coracana. Physiol Mol Biol Plants 21(4): 551–558

Sueldo R, Invernati A, Plaza S, Barassi C (1996) Osmotic stress in wheat seedlings: Effects on fatty acid composition and phospholipid turnover in coleoptiles. Cereal Res Commun 24:77–84

Suman A, Surin S, Ahmad E (2019) Finger millet germplasm characterization and evaluation using principal component analysis. Int J Chem Studies 7:1002–1005

Svitashev S, Schwartz C, Lenderts B, Young JK, Cigan AM (2016) Genome editing in maize directed by CRISPR–Cas9 ribonucleoprotein complexes. Nat Commun 7:13274

Talwar H, Kumar S, Madhusudhana R, Nanaiah GK (2020) Variations in drought tolerance components and their association with yield components in finger millet (Eleusine coracana). Funct Plant Biol 7:1071

Tesfaye K, Mengistu S (2017) Phenotypic characterization of Ethiopian finger millet accessions (*Eleusine coracana* (L.) Gaertn), for their agronomically important traits. Acta Univ Sapientiae, Agric Environ 9:107–118. https://doi.org/10.1515/ausae-2017-0010

Timmusk S, Abd El-Daim IA, Copolovici L, Tanilas T, Känaste A, Behers L, Nevo E, Seisenbaeva G, Stenström E, Niinemets Ü (2014) Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: enhanced biomass production and reduced emissions of stress volatiles. PLoS One 9:e96086

Trivedi AK, Verma SK, Tyagi RK (2018) Pheno physiological evaluation of finger millet germplasm of Central Himalayan Region. J Environ Biol 39. http://www.jeb.co.in/index.php?page=abstract&issue=201801_jan18&number=5

Tumwesigye W, Wambi W, Nagawa G, Daniel N (2019) Climate-smart agriculture for improving crop production and biodiversity conservation: opportunities and challenges in the 21st century—a narrative review. J Water Resources Ocean Sci 8:56–62

Ulaganathan V, Nirmalakumari A (2015) Finger millet germplasm characterization and evaluation using principal component analysis. Sabrao J Breed Genet 47:79–88

Upadhyaya HD, Pundir RPS, Hoisington DA, Gowda CLL, Sube S, Reddy VG, Hash CT, Chandra S (2005) Genotyping finger millet germplasm for developing composite collection. http://oar.icrisat.org/id/eprint/5808.

Upadhyaya HD, Gowda CL, Pundir P, Sube S, Reddy VG (2006) Development of core subset of finger millet germplasm using geographical origin and data on 14 quantitative traits. Genet Resources Crop Evol 4:679–685

Vadez V, Hash T, Bidinger FR, Kholova J (2012) Phenotyping pearl millet for adaptation to drought. Front Physiol 11:1–5

Van Buren R, Wai CM, Wang X, Pardo J, Yocca AE, Wang H, Chaluvadi SR, Han G, Bryant D, Edger PP (2020) Exceptional subgenome stability and functional divergence in the allotetrapoloid Ethiopian cereal teff. Nat Commun 2020(11):884

Varshney RK, Shi C, Thudi M, Mariac C, Wallace J, Qi P, Zhang H, Zhao Y, Wang X, Rathore A (2017) Pearl millet genome sequence provides a resource to improve agronomic traits in arid environments. Nat Biotechnol 35:969–967

Veeranagamallaiah G, Jyothsnakumari G, Puli COR, Surabhi GK, Sriranganayakulu G, Rajasekhar B, Sudhakar C, Thippeswamy M, Yanamandra M, Madhurarekha C (2008) Proteomic analysis of salt stress responses in foxtail millet (Setaria italica L. cv. Prasad) seedlings. Plant Sci 175(5):631–641

Villiers SMD, Michael VN, Manyasa EO, Saiyiorri AN, Deshpande S (2015) Compilation of an informative microsatellite set for genetic characterisation of East African finger millet (*Eleusine coracana*). Electr J Biotechnol 18(2):77–82

Waltz E (2018) With a free pass, CRISPR-edited plants reach market in record time. Nat Biotechnol 36(1):6–8

Wang Y, Frei M (2011) Stressed food—the impact of abiotic environmental stresses on crop quality. Agric Ecosyst Environ 141:3–4

Wang M, Wang S, Liang Z, Shi W, Gao C, Xia G (2018) From genetic stock to genome editing: Gene exploitation in wheat. Trends Biotechnol 36:160–172

Watkinson JI, Hendricks L, Heath L, Bohnert HJ, Grene R, Sioson A (2008) Tuber development phenotypes in adapted and acclimated, drought-stressed Solanum tuberosum ssp. andigena have distinct expression profiles of gene associated with carbon metabolism. Plant Physiol Biochem 1:34–45

Yadav RS, Seghal D, Vadez V (2011) Using genetic mapping and genomics approaches in understanding and improving drought tolerance in pearl millet. J Exp Bot 2:397–408

Yadav DK, Shukla D, Tuteja N (2014a) Isolation, in silico characterization, localization and expression analysis of abiotic stress- responsive rice G- protein Bita subunit (RGB1). Plant Signal Behav 9(5):e28890

Yadav S, Gaur VS, Jaiswal JP, Kumar A (2014b) Simple sequence repeat (SSR) analysis in relation to calcium transport and signaling genes reveals transferability amongst grasses and a conserved behaviour within finger millet genotypes. Plant Syst Evol 300:1561–1568. https://doi.org/10.1007/s00606-014-0982-3

Yadav S, Singh UM, Naik SM, Venkateshwarlu C, Ramayya PJ, Raman KA, Sandhu N, Kumar A (2017) Molecular mapping of QTLs associated with lodging resistance in dry direct-seeded rice (*Oryza sativa* L.). Front Plant Sci 8:1431

Yamunarani R, Govind G, Ramegowda HV, Shankar AA (2016) Genetic diversity for grain Zn concentration in finger millet genotypes: potential for improving human Zn nutrition. Crop J 4(3):229–234

Yang M, Ding G, Shi L et al (2011) Detection of QTL for phosphorus efficiency at vegetative stage in *Brassica napus*. Plant Soil 339:97–111. https://doi.org/10.1007/s11104-010-0516-x

Yang W, Duan L, Chen G, Xiong L, Liu Q (2013) Plant phenomics and high-throughput phenotyping: accelerating rice functional genomics using multidisciplinary technologies. Curr Opin Plant Biol 16:180–187

Yang Z, Zhang H, Li X, Shen H, Gao J, Hou S, Zhang B, Mayes S, Bennett M, Ma J (2020) A mini foxtail millet with an Arabidopsis-like life cycle as a C 4 model system. Nat Plants 6:1167–1178

Yemets A, Radchuk B, Bayer GY, Pakhomov A, Blume Y, Bayer O, Baird WB (2008) Development of transformation vectors based upon a modified plant a- tubulin gene as the selectable marker. 5:566–570

Zhang X, Shabala S, Koutoulis A, Shabala L, Zhou M (2016) Meta- analysis of major QTL for abiotic stress tolerance in barley breeding. Planta 2:283–295

Zhang H, Hall N, Goertzen LR, Chen CY, Peatman E, Patel J, McElroy JS (2019) Transcriptome analysis reveals unique relationships among eleusine species and heritage of Eleusine coracana. G3: Genes Genom Genet 6:2029–2036

Zou C, Li L, Miki D, Li D, Tang Q, Xiao L, Rajput S et al (2019) The genome of broomcorn millet. Nat Commun 10:436

Breeding Finger Millet for Biotic Stress Resistance

12

Gutha Venkata Ramesh, Santosh Gudi, Navdeep Singh, and Divya Bhandhari

Abstract

Finger millet (*Eleusine coracana* L.), commonly cultivated in countries with semiarid and tropical climate like India, can be a potential contributor in alleviating global hunger and malnutrition. The remarkable nutritional and agronomic characteristics of finger millet are of growing importance under the changing global climate scenario. Besides its nutritional benefits and climate resilience, the area, production, and productivity are lagging behind in global agriculture. In addition, there are several biotic and abiotic stresses that are restraining the genetic potential of finger millet. Biotic stresses including pests (pink stem borer, root aphid, etc.) and disease (blast, foot rot, etc.) further hinder the production potential of FM. Therefore, breeding for increased resistance will alleviate the damage caused by these pests and diseases. However, the narrow genetic base and lack of germplasm resources coupled with lesser application of advanced technologies constrained the development of resistance cultivars. Therefore, to meet the global nutritional security, there is urgent calls to enhance the genetic resistance in finger millet. This can be achieved by integrating conventional breeding approaches with advanced genomic and biotechnological approaches such as marker-assisted selection (MAS), marker-assisted recurrent selection (MARS), marker-assisted backcrossing (MABC), genome-wide association studies (GWAS), genomic selection, haploid breeding, speed breeding and

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genome editing, etc. This chapter will focus on how these breeding techniques/approaches can be used for improving the biotic stress resistance in finger millet.

Keywords

Finger millet · Biotic stress · Conventional breeding · Advanced genomic approaches

12.1 Introduction

Finger millet (*Eleusine coracana* (L.) Gaertn.) (Gaertner 1788) is one of the promising nutri-cereal crops widely cultivated in arid and semiarid tropics and sub-tropics of Asia and sub-Saharan Africa. The name of finger millet has been adopted from its appearance of finger-like panicle (Fakrudin et al. 2004). In addition, it is also called as ragi, bird's foot millet, coracana, kurukkan, and African millet (Mirza and Marla 2019). Finger millet is an allotetraploid ($2n = 4X = 36$), which ranks fourth among millets, following sorghum, pearl millet, and foxtail millet (Upadhyaya et al. 2007). The genus, *Eleusine*, includes eight species of diploid and tetraploid annual and perennial herbs. The cultivated species also includes several races and subraces, which offered the greater genetic diversity for key agronomically traits (Mirza and Marla 2019). Ragi is superior to the commercially growing cereals like rice and wheat for nutraceutical and stress-resilient characters. The remarkable nutritional and agronomic properties of finger millet, such as higher Ca, K, and Fe content, as well as drought hardiness, are becoming increasingly important due to shifting food demand in a changing global climate scenario (Verma and Patel 2013). Finger millet is also a good source of dietary fiber and essential amino acids (like cystine, methionine, and tryptophan) (Jideani 2012; Mirza and Marla 2019), and it can be stored for longer periods (Mbinda and Masaki 2021). This combination of nutritional properties alongside climate resilience makes finger millet a potential contributor in alleviating global hunger and malnutrition, especially in poor and developing countries. Owing to their excellent nutritional properties, finger millet has been considered a *wonder grain* as well as a *famine crop* where it was included in the ICRISAT mandate as the sixth crop in 2015 (Gupta et al. 2017; Mgonja et al. 2007). Besides its nutritional benefits and climate resilience, the area and production and productivity lag behind in global agriculture. Globally, finger millet covers an area of 4.5 m ha with an annual production of 29 metric ton during 2019–2020 (FAOSTAT 2020). In India, major finger-millet-growing areas are Karnataka, Uttarakhand, Maharashtra, Tamil Nadu, Odisha, Andhra Pradesh, and Gujarat. In India, during cropping season, 2020–2021, it occupied an area of 11.59 lakh ha and an annual production around 19.98 lakh ton with productivity of 1724 kg/ha. Karnataka accounts for 67.71% of total finger millet area and contributes roughly 68.55% production, with Tamil Nadu having the highest productivity (3481 kg/ha) (INDIASTAT 2022).

Despite its importance and production, there are several biotic and abiotic constraints that are restraining the genetic potential of finger millet. Important pests (pink stem borer, root aphid, etc.) and diseases (blast, foot rot, etc.) have a significant impact on production by interfering at various developmental stages (Table 12.1). Among them, blast, a fungal disease caused by *Magnaporthe grisea*, and an insect, pink stem borer (*Sesamia inferens*), are the major devastating constraints and can be found in all the ragi growing regions that are penalizing the potential of finger millet productivity. Emergence of new biotypes/races of pests and diseases further hinder the production potential of finger millet. Work has been done since decades on the management of finger millet pests and diseases, yet framing efficient management strategies based on chemical and biological aspects is rather difficult due to the continuous arms-race evolution of insect pests and diseases. Therefore, breeding for increased resistance will alleviate the damage caused by these pests and diseases.

However, the narrow genetic base and lack of germplasm resources coupled with lesser application of advanced genomic and molecular technologies constrained the development of resistance cultivars. Therefore, to meet the global nutritional security, there is urgent calls to incorporate the genetic resistance in finger millet. Increasing of genetic variability, exploiting advancement of biotechnological approaches, and available whole genome sequence (WGS) information may help in overcoming the limitations of conventional breeding methods. Genetic improvement of finger millet can be achieved by integrating conventional breeding approaches with advanced genomic and biotechnological approaches such as marker-assisted selection (MAS), marker-assisted recurrent selection (MARS), marker-assisted backcrossing (MABC), genome-wide association studies (GWAS), genomic selection (GS), haploid breeding, speed breeding, genome editing, etc. This chapter attempts to present the information and methodologies for finger millet breeding and also focuses on how these advanced breeding tools can be utilized in improving the biotic stress resistance in finger millet. This will also provide a perspective for future research.

12.2 Finger Millet Breeding

Availability of genetic resources are the treasure for continuous genetic improvement of economically important crops to cater to the needs of present and future generations. Considering the importance of finger millet for food security, especially in production systems with frequent drought spells, concerted efforts have led to collection and conservation of a large number of germplasm accessions at different institutes/universities. The largest collection of finger millet genetic resources is held in institutes located in India, like NBPGR, ICRISAT, and the University of Agricultural Sciences (UAS), Bengaluru. Major efforts to breed finger millet were concentrated in India. Breeding finger millet in Africa is rather limited. Breeding is predominantly focused on improving grain yield and its components and resistance to blast disease (Mirza and Marla 2019).

Table 12.1 List of major biotic stress affecting the finger millet

Diseases/insect pests	Causal organism	Key symptoms
Diseases		
Blast	*Magnaporthe grisea* (IS: *Pyricularia grisea*)	Elliptical or diamond-shaped lesions on leaves with greyish centers, water soaked, and surrounded by chlorotic halo are characteristic symptoms
Foot rot or Ragi wilt	*Athelia rolfsii* (IS: *Sclerotium rolfsii*)	Basal portion of the infected plant appears water soaked that later turns brown and eventually dark brown with a concomitant shrinking of the stem in the affected region
Downy mildew/ green ear/crazy top disease	*Sclerophthora macrospora*	Shredding of the infected leaves, malformation of the floral organs, and conversion of spikelet into leafy structure is characteristic symptoms
Leaf blight/seedling blight	*Cochliobolus nodulosus* (IS: *Drechslera nodulosum*)	Typical symptoms appear as brown to dark brown spots on leaf and sheath. Early infection may result in seedling blight
Smut	*Melanopsichium eleusinis*	Initial greenish color of sorus gradually turns pinkish green and finally to dirty black on drying. Ovaries are transformed into velvety gall-like sori
Cercospora leaf spot	*Cercospora eleusinis*	Symptoms appear as reddish-brown specks with yellow halo on older leaves initially. Later, such specks coalesce to form large lesions giving burnt appearance
Bacterial leaf spot	*Xanthomonas eleusinae*	Initial spots are light yellowish-brown on both side of leaves, but soon become dark brown. Under severe conditions, the leaf splits along the streak giving a shredded appearance
Bacterial leaf stripe	*Pseudomonas eleusinae*	Symptoms appear as brown coloration of the leaf sheath from base upward. Infected portion of the lamina and midrib appears straw colored
Bacterial leaf streak	*Xanthomonas axonopodis* pv. *coracanae*	Water soaked, translucent, linear, pale yellow to dark greenish-brown streaks, running parallel to the midrib of the lamina. Initial hyaline streaks gradually turn into a broad yellowish lesion and turns brown
Ragi mottle streak disease	*Ragi mottle streak virus*	Symptoms are of mottle type in the form of white specks along leaf veins when 4–6 weeks old, and the affected plants are generally stunted bearing small ears
Insect pests		
Pink stem borer	*Sesamia inferens*	Larval feeding first leads to leaf scarification and then to make small pin holes in the opening of leaves. Larva bore into stem and kills central shoot forming dead hearts
Root aphid	*Tetraneura nigriabdominalis*	Aphids feed persistently on entire plants at the collar region turning roots to black. Cortical

(continued)

Table 12.1 (continued)

Diseases/insect pests	Causal organism	Key symptoms
		tissue dries u,p and secondary roots shows burning appearance
Cut worm	*Spodoptera exigua*	Larva feeds on the entire plant during earlier crop growth by forming feeding holes on leaf lines parallel to midrib
White borer	*Saluria inficita*	It attacks the plant at soil line. Oozing of excreta from bored holes and shows symptoms of drying
Ear head caterpillar	*Euproctis subnotata*	Larva feeds on lemma of flowers scraping the chlorophyll and later on the milky grains. It damages by constructing webs on ragi ear heads

Note: *IS* imperfect stage

12.3 Conventional Breeding Methods and Their Application in Finger Millet

Conventional breeding is the improvement of crop germplasm using conservative tools for manipulating plant genome within the natural genetic boundaries of species (Acquaah 2015). While breeding, the breeder has to consider a number of traits, viz., traits that govern plant yield, resistance to biotic and abiotic stresses, quality, and shelf life of the produce. These traits are classified as qualitative or quantitative, depending on the level of environmental influence and the number of genes affecting the traits. Qualitative traits are oligogenic in nature with least environmental influence, and hence are easier to breed than quantitative traits. The general breeding steps include deciding breeding objectives; creating genetic variability (via hybridization); and doing artificial selection (in segregating generations), evaluation (replicated and multilocation trails), and release of cultivar (at state and central level). Finger millet is a highly self-pollinated crop, and hence pure line selection, mass selection, pedigree selection (hybridization followed by selection), single-seed descent, bulk, and backcrossing are regarded as the most promising approaches to make its genetic improvements. Until the 1950s, most crop improvement programs were focused on varietal development by selection from landraces or cultivars using pure line selection (Ganapathy 2017). In pure line selection, a new variety is developed by selecting a single superior progeny from landraces or cultivars. The earliest report on breeding of finger millet was from India in 1913 by Leslie C. Coleman, who initiated the work on pure line selection from indigenous cultivars at the Zonal Agricultural Research Station, Bangalore, and developed the first finger millet cv. H-22 in 1918. Afterward several pure line varieties with improved agronomic characters have been developed. Some of them include Champavathi (VR 708), PR 230 (Maruthi), GN 4, PRM 1, VR 762 (Bharathi), GN 5, PRM 2, and KOPN 235 (Phule Nachni) (Seetharam 2013). In addition, pure line selection also resulted in the development and release of several finger millet varieties resistant to blast (KOPN-235, BM-2, PRM-1, GN-5, and GNN-6), blight (PR-230), ear

caterpillars and aphids (VR-762), and viral diseases (INDAF-1) (Seetharam 2013; AICSMIP 2014) (Table 12.2).

Later, the initiation of All India Coordinated Millets Improvement Project (AICRP-Millets) in 1965 as well as the advancement of emasculation and pollination techniques (Mirza and Marla 2019) paved the way for hybridization programs. Hybridization techniques include contact method (panicles of selected plants are tied and bagged together before flowering); manual hybridization (hand emasculation); hot water (52°C for 5 min) (Gupta et al. 2011); cold water; and plastic bag method (uses cold temperature to induce anthesis and high humidity prevents anther dehiscence) (House 1985). Hybridization between genetically diverse parents, such as African and Indian germplasm, creates genetic variability for selection of desired traits from segregating generations. The African germplasm lines with high grain density, vigorous growth, large ears, and blast resistance were utilized in hybridization with Indian varieties to develop "Indaf" hybrids (Naik et al. 1993). The selections from "Indaf" hybrids with high-yielding potential have been released as varieties and also being used as parents in hybridization programs (Seetharam 2013). In 1990, AICSMIP developed GPU28, a variety showing durable resistance to blast by crossing Indaf5 with IE1012, which gained popularity among farmers in the major finger-millet-growing regions of the country (Nagaraja et al. 2008). In India, about 45% of the released varieties have come from hybridization and selection (AICSMIP 2014).

Conventional breeding approaches facilitates the genetic improvement of crop plants by utilizing the available genetic variability present in the gene pool. However, continued selection for desired traits and using the same parental lines in breeding programs resulted in a narrow genetic base, which ultimately makes the crop plants vulnerable to minor pests and diseases (Basey et al. 2015). In addition, changing climatic conditions creating favorable environments for the emergence of new pests and diseases, which affects the production potential of crops plants. Since, conventional breeding approaches are slow and time-consuming (require 8–10 years to develop a variety), they are not able to deliver the resistant cultivars for fast-evolving pests and diseases. Therefore, there is an urgent call to integrate conventional breeding approaches with advanced breeding tools to accelerate the breeding process and introduce resilient cultivars into the farmer's field.

12.4 Mutation Breeding

Mutation is a sudden heritable change that occurs in the genome of living entities. It can either be spontaneous or induced. Mutation breeding is also known as variation breeding. It is the artificial manipulation of the plant genome through the use of physical or chemical mutagens. Mutation breeding is of immense importance in breeding for the traits for which genetic variation is not available in the entire germplasm of particular crop. In this approach, the plant or its parts (i.e., seeds, anther) are exposed to chemicals or radiation to produce desirable mutations. It is a promising approach to develop new varieties with improved agronomic

Table 12.2 Varieties released using different breeding approaches in finger millet in India

Method	Variety/ improved line	Year of release	Trait	References
Conventional breeding				
Pure line selection	KOPN-235	2011	Resistant to blast	AICSMIP (2014)
	PR-230 (Maruthi)	1998	Resistant to blast and blight	AICSMIP (2014)
	BM-2, PRM-1	1995, 2006	Resistant to neck and finger blast	AICSMIP (2014)
	GN-5, GNN-6	2016	Moderately resistant to leaf blast, resistant to neck and finger blast	IIMR
	VR-762 (Bharathi)	2006	Moderately resistant to blast, tolerant to ear caterpillars and aphids	IIMR
	INDAF-1	1976	Moderately susceptible to blast and viral diseases	AICSMIP (2014)
Pedigree selection	KMR-340	2016	Resistant to blast and blight diseases, tolerant to stem borer and aphids	AICSMIP (2014)
	Chilika (OEB-10), Indira Ragi-1	2001, 2012	Moderately resistant to blast, resistant to stem borer	Seetharam (2013)
	OEB-526, OEB-532, Srichaitanya (VR-847)	2011, 2012, 2009	Moderately resistant to blast diseases, highly tolerant to myllocerus weevil, ear head caterpillars, stem borer, and grass hopper	AICSMIP (2014)
Mutation breeding				
Physical and chemical mutants	DibyaSinha (mutant of AKP-7)	1971	Moderately resistant to blast	Sinha and Sahoo (1971)
	BM-9-1 (mutant from BudhaMandia)	1999	Moderately resistant to blast and brown spot	Seetharam (2013)
	OUAT-2 (SUVRA) Mutant of Co-9	1999	Moderately resistant to neck, finger blast, and sheath blight	Seetharam (2013)
	GPU28-2082 (mutant of GPU-28)		Resistance to neck and finger blast	AICRP on Small millet Annual report 2021
Biotechnological approaches				
Tissue culture	Dapoli-2	2017	Moderately resistant to blast and tolerant to aphids and tobacco cutworm (*Spodoptera littura*)	Mirza and Marla (2019)
Transgenics	GPU-45			

(continued)

Table 12.2 (continued)

Method	Variety/ improved line	Year of release	Trait	References
			Agrobacterium-mediated transformation of GPU45 to develop leaf blast resistance	Ignacimuthu and Ceasar (2012)
	Local cultivar PGEC-2 and PGEC-19 accessions		Biolistics was used for transformation of leaf blast resistance	Latha et al. (2005)
Marker-assisted selection				
To the best of our knowledge, application of MAS technique for varietal development in finger millet is very limited up-to-date				Pandey et al. (2013), Mbinda and Masaki (2021)
Genome editing		Not available		

characteristics including biotic and abiotic stress tolerance (Chaudhary et al. 2019). The physical and chemical mutagens widely used for developing mutant lines include gamma-rays, X-rays, EMS (ethyl methane sulfonate), and DES (diethyl sulfate). Lower doses of gamma rays and chemical mutagens (EMS, MMS, nitrosomethyl urea, and 1-methyl-3-nitrol-nitrosoguanidine) have been shown to be effective for inducing mutations (Mahishi and Seetharam 1983). Different mutagens and their combinations were also tested. It was reported that the treatments with 0.30 and 0.45% EMS, 0.03% nitrosoguanidine (NG), and combination of gamma ray (300 Gy) with EMS (0.3%) were more effective in inducing useful mutations (Muduli and Misra 2007). Ambavane and associates (2014) found that exposure to gamma radiation (500 and 600 Gy) were effective in the development of early maturing mutant lines with high yield potential. The first finger millet variety, Hagari-1, was developed by exposing seeds to X-ray irradiation (Krishnaswami and Ayyangar 1941). Sinha and Sahoo (1971) developed the early-maturing (90 days) and blast-resistant cultivar, Dibya Sinha, by treating seeds of finger millet cv. AKP-7 with chemical mutagens, EMS and NG. Similarly, the high-yielding and blast-resistant mutant variety, M21, was created by irradiating the seeds of cv. HES 927 with gamma rays (Shivashankar et al. 1973). Later, in 1999, two mutant lines BM-9-1 (from Budha Mandia) and OUAT-22 (from Co9) with high level of resistance to multiple diseases were released (Seetharam 2013).

12.5 Molecular Breeding

Breeding through conventional approaches is time-taking, especially for quantitative traits. Hence, the use of advanced breeding techniques enables timely and accurate response by overcoming the conventional barriers in crop improvement (Sorrells et al. 2003). These techniques also provide added opportunities to develop crop

cultivars with multiple stress tolerance (Mirza and Marla 2019). Molecular breeding is the genetic manipulation at DNA level to improve the targeted phenotypes of an organism using linked molecular marker. The marker-assisted breeding (MAB) is the major concern areas of molecular breeding. The MAB is the combination of molecular markers with genomic and linkage maps to alter or modify the basis of genotypic characters in target organisms. With the advent of molecular breeding, several MAB techniques have been developed and modified in accordance with the requirements of breeding purposes. Some of them include marker-assisted selection (MAS), marker-assisted recurrent selection (MARS), marker-assisted backcrossing (MABC), and genome-wide selection (GWS).

Molecular markers are small segments of nucleic acid sequence that map or identify targeted loci. These are heritable in nature and can be used as probes or tags to check, target, and locate particular loci responsible for targeted phenotype. Genetic markers are broadly classified into classical and DNA-based markers. Classical markers include cytological, biochemical, and morphological markers, whereas the DNA markers have been classified as hybridization, sequence, and PCR-based markers. Some of them are RFLP (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism), SSR (simple sequence repeats), SNP (single nucleotide polymorphism), DArT (diversity array technology), KASP (Kompetitive allele-specific PCR), etc. (Gudi et al. 2020). Selection of markers is determined by several factors, viz., breeding objective, breeding population, target organism, target gene, level of polymorphism, type of marker expression (dominant and codominant), marker specificity, cost-effectiveness, and resource availability.

The MAB is aimed for developing resistance against biotic and abiotic stresses and yield/quality potential of crops. The MAB has set a benchmark for genetic improvement and plays a vital role in sustaining food security. It aims to develop nutritionally rich, high-yielding cultivars resistant to biotic stresses (Gudi et al. 2020). Molecular markers were utilized for the first time in finger millet by Madhavi and co-authors to identify transgenic plants having high level of resistance to leaf blast in segregating population. For that purpose, the used the molecular markers from the artificially synthesized antifungal protein gene, *PIN*. Using MAB, Sen and Dutta (2011) achieved another milestone by cloning and characterizing the *RBI* (Ragi bifunctional inhibitor) gene. This *RBI* gene is responsible for the inhibition of both α-amylase and trypin. This study acts as basis for several researchers to use marker-assisted selection to map this particular gene conferring resistance against biotic stresses. In 2014, Babu and his associates used 58 SSR markers to screen 190 accessions of finger millet collected worldwide for blast resistance and other agronomic traits (Babu et al. 2014). Babu et al. (2018) developed two SSR markers (*FMBLEST35*, *FMBLEST36*) from *Pi21* gene (blast resistant) sequence of rice which were also found to be associated with finger millet blast resistance.

12.6 Marker-Assisted Recurrent Selection (MARS)

Recurrent selection is the repeated cycles of selection and hybridization to make the genetic improvement of the population for the targeted traits. It is the major breeding technique of cross-pollinated crops. However, in self-pollinated crops, it has been considered as an effective practice for the genetic improvement of polygenic traits by increasing the frequency of targeted alleles and also helps in maintaining high levels of genetic variability in heterozygous populations. There are number of modifications of recurrent selection, such as simple recurrent selection, recurrent selection for general combining ability, recurrent selection for specific combining ability, and reciprocal recurrent selection. It involves rigorous phenotypic selection of individuals over a population for a particular trait or traits followed by self-mating of selected individual by following years to increase the number of desired individuals. However, the major limitation of conventional recurrent selection is that it requires at least two cropping seasons to complete one selection procedure.

The marker-assisted recurrent selection (MARS) is a valuable MAB tool, which facilitates the genetic improvement of population for several quantitative traits (i.e., QTLs) by using molecular markers (Asima Gazal et al. 2015). With the advent of MARS technique, various researchers have used it for different purposes in different cereals. For instance, Ragot and co-authors used MARS to identify different QTLs in maize for cold stress tolerance (Ragot et al. 2000). Similarly, Eathington et al. (2007) conducted a study to show efficiency of MARS over conventional method in European sunflower for grain yield and oil content. To the best of our knowledge, MARS has yet to be adapted in the finger millet. Utilizing MARS in finger millet to enrich their population with resistance genes to several biotic stresses may bring a huge breakthrough in this crop.

12.7 Marker-Assisted Backcrossing (MABC)

Marker-assisted backcrossing (MABC) is an effective tool for introgressing the targeted genomic region from the donor parent by retaining the essential characteristics of the recipient parent with the aid of linked molecular markers. This method helps to recover the recurrent parent genome with only two or three backcrosses' instead of 5–6 in conventional backcrossing. MABC is being used for both biotic and abiotic stresses. With the advent in technology and breeding science, backcrossing has been modified to the greater extent. During backcrossing, three major types of selections have been carried out, viz., foreground selection, recurrent selection, and background selection. In foreground selection, the individual F1 plants carrying the gene of interest from donor parent are selected using gene-specific markers. In the recurrent selection, markers flanking the gene of interest were used to isolate the desired recombinants from the backcross population. This will help to purge-out the associated linkage drag. Whereas, in background selection, genome-wide markers are employed to recover the recurrent parent genome in both carrier and noncarrier chromosomes. A number of studies have already used MABC

to introgress genetic resistance to biotic and abiotic stress tolerance. For instance, MABC has been successfully utilized in transferring the rust-resistant genes in the cultivar background (Randhawa et al. 2019; Sharma et al. 2021). Attempts to use MABC to introgress the gene of interest in the cultivar background for biotic stress tolerance need to be achieved in finger millet.

12.8 Advanced Biotechnological Approach

Transgenic plants were successfully developed in finger millet for first time by Latha et al. (2005) successfully developed transgenics for first time in finger millet using synthesized gene based on prawn antimicrobial peptide (*PIN*) gene against *Pyricularia grisea* (blast disease). Later, Ignacimuthu and Ceasar (2012) developed finger millet transgenics against leaf blast disease by introducing the rice chitinase (*chi11*) gene through *Agrobacterium*-mediated transformation. Screening of potential pathogenicity-related (PR) genes and gene pyramiding will help in developing transgenic plants for a wider spectrum of diseases (Mirza and Marla 2019).

12.9 Genome Editing (GE) Approach

Genome editing (GE) is the targeted modification of a specific gene/sequence. It has broadened the scope of crop improvement since its arrival by fine-tuning the expression of key gene. It uses engineered/artificial nucleases such as mega nucleases, zinc-finger nucleases (ZFNs), transcription factor-like effector nucleases (TALENs), CRISPR (clustered regularly interspaced palindromic repeats)-Cas system, etc. to introduce the targeted mutations in the genome. DSBs stimulates the repair machinery of the cell by homology-directed repair (HDR) or nonhomologous end joining (NHEJ) (Lieber et al. 2003). Owing to its importance and wider adaptability, GE became a tool of every laboratory around the world. Despite its importance, the use of GE in millet crops especially in small millets is very limited. The major use of GE technology has been restricted to the commercial crops like major cereals such as rice, wheat, maize, etc. This might be due to a lack of funding facilities or the fact that only a small portion of Asia and Africa are home to millets cultivation. GE tools have not yet been exploited for most the small millets although they possess variety of resilient characteristics. Foxtail millet (*Setaria italica* L.) is the only millet utilized up-to-date for GE which is due to limited genomic resources and lack of efficient transformation systems in millets. As millets possess many important agro-ecological traits, high-resolution studies with GE tools will help to understand the specific mechanism and transfer of traits in the future (Ceasar 2022).

This technique has been implemented to achieve desired results by targeting various traits, viz., traits controlling yield, nutrient composition, tolerance to biotic stresses, etc. (Matres et al. 2021). Though genome editing is initiated in millets, its utilization is restricted only to foxtail millet (Lin et al. 2018; Cheng et al. 2021; Liang et al. 2022). For instance, CRISPR/Cas9 technology has been successfully used to

induce the targeted mutants in *SiPDS* for (Lin et al. 2018), *SiMTL* for haploid induction (Cheng et al. 2021), and *SiALS* for herbicide tolerance (Liang et al. 2022). However, studies reporting GE in finger millet is not available for any traits including biotic stress tolerance.

12.10 Genome-Wide Association Study (GWAS)

QTL mapping using biparental mapping populations is constrained by the limited number of recombination events, which failed to identify the minor QTLs. However, these constraints were overcome by the linkage disequilibrium (LD)-based genome-wide association study (GWAS). GWAS uses the genome-wide markers to exploit the historical recombinant events that reside in the germplasm collection and helps to unveil the genetic nature of quantitative traits. GWAS was utilized in several crop species, including minor millets, to identify the marker trait associations (MTAs) associated with important agronomic traits (Srivastava et al. 2020). Similarly, GWAS also helps to identify the MTAs for agro-morphological (Sharma et al. 2018) and grain nutritional (i.e., Ca, Fe, protein, etc.) traits (Tiwari et al. 2020; Puranik et al. 2020; Sharma et al. 2022) in finger millet. Efforts were made to find the new source of resistance to finger millet blast by evaluating participatory varietal selections using DArT markers (Dida et al. 2021). This study identified 19 markers associated with the blast. These MTAs will become the basis for marker-assisted introgression for blast resistance. Though the studies reporting the MTAs using GWAS were initiated, they have not been exploited completely in the finger millet as in other crops. This might be owing to the lack of high-throughput genomic resources to be used for GWA studies.

12.11 Genomic Selection (GS)

Genomic selection (GS) has emerged as the most promising breeding strategy to predict the value of an individual from its relatives. The GS provides a great avenue for accelerating genetic gain by eliminating laborious phenotyping in the breeding population. It uses the whole genome marker and phenotype data of the training population to design prediction models and to extract the genome-estimated breeding values (GEBVs) (Varshney et al. 2013). These GEBVs will be further used to predict the value of an individual from the breeding population without demanding phenotyping. The GS has been successfully utilized in many crops plants make the prediction models for biotic stress tolerance. Some of them include rust and *Septoria* blotch of wheat (Ornella et al. 2012; Rutkoski et al. 2015; Mirdita et al. 2015), head blight of barley (Lorenz et al. 2012), NCLB and ear rot of maize (Technow et al. 2013; Riedelsheimer et al. 2013), etc. However, its application to minor millets, specifically to the finger millet, is still lacking. Despite the many difficulties (viz., lack of high-throughput genome sequencing and phenotyping techniques) associated

with GS in finger millet, GS must be utilized to assess the large repository of unexplored diversity in the gene banks.

12.12 Speed Breeding

Accelerating the generation advancement through fast-track breeding methods helps to reduce the breeding cycle by cutting the generation time. Several rapid generation advancement (RGA) techniques have been developed in different crop species to reduce the generation time and to increase the genetic gain. Some of them include shuttle breeding, single-seed descent method (by using a greenhouse), doubled haploids, Biotron breeding, in vitro nurseries, and speed breeding. Speed breeding is the technique of growing crops under controlled growth chambers with extended photoperiod coupled with embryo rescue to shorten the generation time (Watson et al. 2018). This technique enabled the researchers to complete six generations of spring wheat, durum wheat, barley, chickpea, and pea and four generations of canola in a single year. Furthermore, the technique has been successfully utilized in phenotyping for stripe rust (Hickey et al. 2012), leaf rust (Riaz et al. 2016), stem rust (Riaz and Hickey 2017), yellow spot (Dinglasan et al. 2016), and *Fusarium* head blight (Watson et al. 2018). In addition, speed breeding has also been employed in transferring the multiple disease resistance genes (viz., leaf rust, net blotch, and spot blotch) into the background of barley cultivars (Hickey et al. 2017). Efforts to utilize speed breeding or RGA techniques have already been attempted in minor millets to reduce the generation time. For instance, split-culm coupled with embryo rescue was used by Rizal and co-authors to reduce the generation time of sorghum to 88 days from the usual 120 days (Rizal et al. 2014). However, in comparison to other crops, the success of speed breeding or other RGA techniques in millets is negligible. To the best of our knowledge, no attempts were made to accelerate the generation advancement in finger millet. Using RGA in finger millet not only reduces generation time, but it also accelerates trait introgression for biotic stresses.

12.13 Future Finger Millet: Integrating Conventional Breeding with Advanced Breeding Tools

As discussed earlier in this chapter, conventional breeding approaches will fail to achieve timely delivery of cultivars with desired characteristics. Furthermore, any of the advanced breeding tools, including GWAS, GS, speed breeding, and genome editing, will unlikely accelerate the rate of genetic, when they are used separately. However, the successful integration of different technologies will accelerate the genetic gain by identifying and transferring the novel resistant genes, which will facilitate the timely delivery of cultivars to the farmer's field. For instance, instead of conventional screening strategies, utilizing high-throughput phenomics with NGS tools in finger millet breeding helps to identify the novel genomic regions or haplotypes that reside in the crop germplasm. Once the novel QTLs or haplotypes

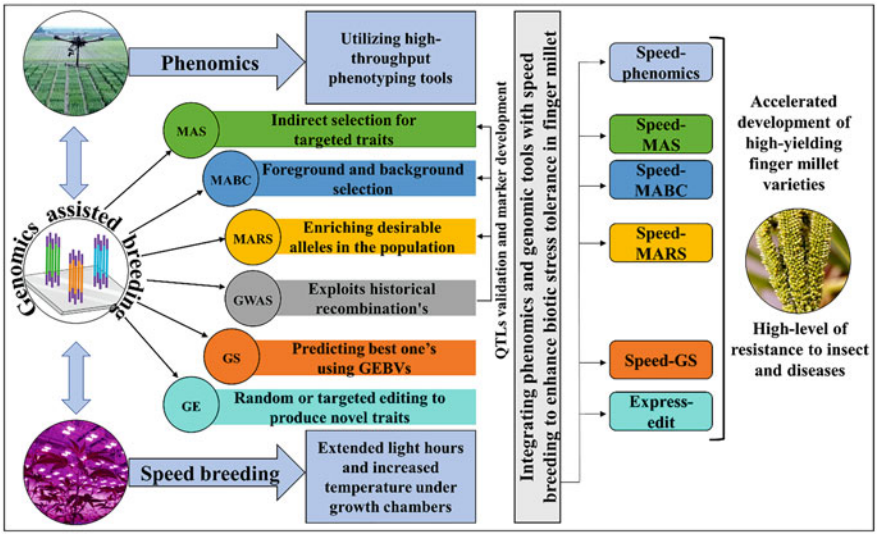

Fig. 12.1 Integrating advanced breeding strategies to accelerate trait introgression and development of high-yielding finger millet varieties with high level of resistance to prevailing insects and diseases. *MAS* marker-assisted selection, *MABC* marker-assisted backcrossing, *MARS* marker-assisted recurrent selection, *GWAS* genome-wide association studies, *GS* genomic selection, *GE* genome editing, *QTLs* quantitative traits loci

are identified, they can be easily transferred into the cultivar background by employing marker-assisted gene introgression under the controlled growth chambers (i.e., Speed-MAS) (Fig. 12.1).

Speed breeding can also be used in recurrent selection to enrich the frequency of resistance alleles in the breeding population (speed-MARS). Combining speed breeding with genomic selection will facilitate the selection of resistant plants and their generation advancement (Voss-Fels et al. 2019). If the resistance sources for a particular pest or disease are not available in the finger millet gene pool, then either the novel variants will be created (by using artificial mutagens or advanced genome editing tools), or the foreign gene will be transferred (using genetic engineering). Once the resistant alleles are available, then speed-MAS can be employed for their rapid introgression in the varietal background. Overall, integrating different breeding methods will save the breeder time with respect to germplasm characterization, gene identification and transfer, and cultivar development. Most importantly, under changing climatic conditions, it will ensure the timely delivery of nutritionally enriched, high-yielding, pest- and disease-resistant finger millet cultivars.

References

Acquaah G (2015) Conventional plant breeding principles and techniques. In: Advances in plant breeding strategies: breeding, biotechnology and molecular tools. Springer, Cham, pp 115–158

AICSMIP. Report on compendium of released varieties in small millets Bangalore, India, 2014
Ambavane AR, Sawardekar SV, Gokhale NB et al (2014) Studies on mutagenic effectiveness and efficiency of finger millet [*Eleusine coracana* (L.) Gaertn] in M1 generation and effect of gamma rays on its quantitative traits during M2 generation. Int J Agric Sci 10:603–607
Asima Gazal ZA, Dar AA, Lone I, Abidi AG (2015) Molecular breeding for resilience in maize. J Appl Nat Sci 7(2):1057–1063
Babu BK, Agrawal PK, Pandey D, Kumar A (2014) Comparative genomics and association mapping approaches for opaque2 modifier genes in finger millet accessions using genic, genomic and candidate gene-based simple sequence repeat markers. Mol Breed 34(3): 1261–1279
Babu GA, Vinoth A, Ravindhran R (2018) Direct shoot regeneration and genetic fidelity analysis in finger millet using ISSR markers. Plant Cell Tissue Organ Cult (PCTOC) 132:157–164. https://doi.org/10.1007/s11240-017-1319-z
Basey AC, Fant JB, Kramer AT (2015) Producing native plant materials for restoration: 10 rules to collect and maintain genetic diversity. Nativ Plants J 16:37–53
Ceasar A (2022) Genome-editing in millets: current knowledge and future perspectives. Mol Biol Rep 49(1):773–781. https://doi.org/10.1007/s11033-021-06975-w
Chaudhary J, Deshmukh R, Sonah H (2019) Mutagenesis approaches and their role in crop improvement. Plan Theory 8(11):467. https://doi.org/10.3390/plants8110467
Cheng Z, Sun Y, Yang S et al (2021) Establishing in planta haploid inducer line by edited *SiMTL* in foxtail millet (*Setaria italica*). Plant Biotechnol J 19:1089–1091. https://doi.org/10.1111/pbi.13584
Dida MM, Oduori CA, Manthi SJ et al (2021) Novel sources of resistance to blast disease in finger millet. Crop Sci 61(1):250–262
Dinglasan E, Godwin ID, Mortlock MY, Hickey LT (2016) Resistance to yellow spot in wheat grown under accelerated growth conditions. Euphytica 209:693–707. https://doi.org/10.1007/S10681-016-1660-Z
Eathington SR, Crosbie TM, Edwards MD, Reiter RS, Bull JK (2007) Molecular markers in commercial breeding. Crop Sci 47:154–163
Fakrudin B, Shashidhar H, Kulkarni R, Hittalmani S (2004) Genetic diversity assessment of finger millet. Eleusine coracana (Gaertn.), germplasm through RAPD analysis. PGR Newslett 138:50–54
FAOSTAT (2020) Food and Agriculture Data. 2020. Food and Agriculture Organization, Rome. Available online at: http://www.fao.org/statistics/en/
Ganapathy KN Improvement in finger millet: status and future prospects. Millets and sorghum: Biology and genetic improvement; 2017. p. 87–111
Gudi S, Atri C, Goyal A et al (2020) Physical mapping of introgressed chromosome fragment carrying the fertility restoring (*Rfo*) gene for Ogura CMS in *Brassica juncea* L. Czern & Coss. Theor Appl Genet 133(10):2949–2959
Gupta A, Sood S, Agarwal PK, Bhatt JC (2011) Floral biology and pollination system in small millets. Eur J Plant Sci Biotech 6(2):81–86
Gupta SM, Arora S, Mirza N, Pande A, Lata C, Puranik S, Kumar A (2017) Finger millet: a "certain" crop for an "uncertain" future and a solution to food insecurity and hidden hunger under stressful environments. Front Plant Sci 8:643
Hickey LT, Wilkinson PM, Knight CR et al (2012) Rapid phenotyping for adult-plant resistance to stripe rust in wheat. Plant Breed 131:54–61. https://doi.org/10.1111/j.1439-0523.2011.01925.x
Hickey LT, Germán SE, Pereyra SA et al (2017) Speed breeding for multiple disease resistance in barley. Euphytica 213. https://doi.org/10.1007/s10681-016-1803-2
House LR (1985) A guide to sorghum breeding, 2nd edn. ICRISAT, Patancheru
Ignacimuthu S, Ceasar SA (2012) Development of transgenic finger millet (*Eleusine coracana* (L.) Gaertn.) resistant to leaf blast disease. J Biosci 37(1):135–147
INDIASTAT (2022). https://www.indiastat.com/data/agriculture

Jideani IA (2012) Digitaria exilis (acha/fonio), Digitaria iburua (iburu/fonio) and Eleusine coracana (tamba/finger millet) non-conventional cereal grains with potentials. Sci Res Essays 7 (45):3834–3843

Krishnaswami N, Ayyangar GNR (1941) Adventitious roots of ragi (*Eleusine coracana* Gaertn.). Curr Sci 10(2):79–80

Latha AM, Rao KV, Reddy VD (2005) Production of transgenic plants resistant to leaf blast disease in finger millet (*Eleusine coracana* (L.) Gaertn.). Plant Sci 169(4):657–667

Liang Z, Wu Y, Ma L, Guo Y, Ran Y (2022) Efficient genome editing in *Setaria italica* using CRISPR/Cas9 and base editors. Front Plant Sci 12:815–946. https://doi.org/10.3389/fpls.2021.815946

Lieber MR, Ma U, Pannicke Y, Schwarz K (2003) Mechanism and regulation of human non-homologous DNA end-joining. Nat Rev Mol Cell Biol 4:712–720

Lin CS, Hsu CT, Yang LH et al (2018) Application of protoplast technology to CRISPR/Cas9 mutagenesis: from single-cell mutation detection to mutant plant regeneration. Plant Biotechnol J 16:1295–1310. https://doi.org/10.1111/pbi.12870

Lorenz AJ, Smith KP, Jannink JL (2012) Potential and optimization of genomic selection for Fusarium head blight resistance in six-row barley. Crop Sci 52:1609–1621

Mahishi DM, Seetharam A (1983) Mutagenic efficiency and effectivene55ss of some physical and chemical mutagens in finger millet. In: National seminar on finger millet genetics and breeding, Bangalore, p. 32

Matres JM, Hilscher J, Datta A et al (2021) Genome editing in cereal crops: an overview. Transgenic Res 4:461–498. https://doi.org/10.1007/s11248-021-00259-6

Mbinda W, Masaki H (2021) Breeding strategies and challenges in the improvement of blast disease resistance in finger millet. A current review. Front Plant Sci 11:602–882. https://doi.org/10.3389/fpls.2020.602882

Mgonja MA, Lenné JM, Manyasa E, Sreenivasaprasad S (eds) (2007) Finger millet blast management in East Africa. Creating opportunities for improving production and utilization of finger millet. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru

Mirdita V, He S, Zhao Y, Korzun V, Bothe R et al (2015) Potential and limits of whole genome prediction of resistance to Fusarium head blight and Septoria tritici blotch in a vast central European elite winter wheat population. Theor Appl Genet 128:2471–2481

Mirza N, Marla SS (2019) Finger millet (*Eleusine coracana* L. Gartn.) breeding. Adv Plant Breed Strategies: Cereals 5:83–132

Muduli KC, Misra RC (2007) Efficacy of mutagenic treatments in producing useful mutants in fnger millet (*Eleusine coracana* Gaertn.). Indian J Genet Plant Breed 67:232–237

Nagaraja A, Gowda J, Krihnappa M, Gowda KTK (2008) GPU 28: a finger millet variety with durable blast resistance. J Mycopathol Res 46:109–111

Naik BJ, Gowda BTS, Seetharam A (1993) Pattern of variability in relation to domestication of finger millet in Africa and India. In: Riley KW, Gupta SC, Seetharam A, Moshanga J (eds) Recent advances in small millets. Proceedings of the second international small millets workshop. Oxford-IBH Publishing Company, pp 347–364

Ornella L, Singh S, Perez P, Burgueno J, Singh R et al (2012) Genomic prediction of genetic values for resistance to wheat rusts. Plant Genome 5:136–148

Pandey MK, Rani NS, Sundaram RM, Laha GS, Madhav MS, Rao KS et al (2013) Improvement of two traditional Basmati rice varieties for bacterial blight resistance and plant stature through morphological and marker-assisted selection. Mol Breed 31:239–246. https://doi.org/10.1007/s11032-012-9779-7

Puranik S, Sahu PP, Beynon S et al (2020) Genome-wide association mapping and comparative genomics identifies genomic regions governing grain nutritional traits in finger millet (*Eleusine coracana* L. Gaertn.). Plants People Planet 2(6):649–662

Ragot M, Gay G, Muller JP, Durovray J (2000) Efficient selection for the adaptation to the environment through QTL mapping and manipulation in maize. In: Ribaut J-M, Poland D

(eds) Molecular approaches for the genetic improvement of cereals for stable production in water-limited environments
Randhawa MS, Bains NS, Sohu VS et al (2019) Marker assisted transfer of stripe rust and stem rust resistance genes into four wheat cultivars. Agronomy 9(9):497
Riaz A, Hickey LT (2017) Rapid phenotyping adult plant resistance to stem rust in wheat grown under controlled conditions. Methods Mol Biol 1659:183–196. https://doi.org/10.1007/978-1-4939-7249-4_16
Riaz A, Periyannan S, Aitken E, Hickey L (2016) A rapid phenotyping method for adult plant resistance to leaf rust in wheat. Plant Methods 12:1–10. https://doi.org/10.1186/s13007-016-0117-7
Riedelsheimer C, Endelman JB, Stange M et al (2013) Genomic predictability of interconnected biparental maize populations. Genetics 194:493–503
Rizal G, Karki S, Alcasid M et al (2014) Shortening the breeding cycle of Sorghum, a model crop for research. Crop Sci 54:520–529. https://doi.org/10.2135/CROPSCI2013.07.0471
Rutkoski J, Singh RP, Huerta-Espino J et al (2015) Genetic gain from phenotypic and genomic selection for quantitative resistance to stem rust of wheat. Plant Genome. https://doi.org/10.3835/plantgenome2014.10.0074
Seetharam A (2013) Genetic improvement of small millets in India during Pre and Post Crop Coordinated Project era. Indian Institute of Millets Research (IIMR)
Sen S, Dutta SK (2011) Cloning, expression and characterization of biotic stress inducible Ragi bifunctional inhibitor (RBI) gene from *Eleusine coracana* Gaertn. J Plant Biochem Biotechnol 21:66–76. https://doi.org/10.1007/s13562-011-0082-1
Sharma D, Tiwari A, Sood S et al (2018) Genome wide association mapping of agro-morphological traits among a diverse collection of finger millet (Eleusine coracana L.) genotypes using SNP markers. PLoS One 13(8):e0199444
Sharma A, Srivastava P, Mavi GS et al (2021) Resurrection of wheat cultivar PBW343 using marker-assisted gene pyramiding for rust resistance. Front Plant Sci 12:570408
Sharma D, Tiwari A, Sood S et al (2022) Identification and validation of candidate genes for high calcium content in finger millet [*Eleusine coracana* (L.) Gaertn.] through genome-wide association study. J Cereal Sci 107:103517
Shivashankar G, Viswanath SR, Ramakrishnaiah KC (1973) M-21, a short duration induced promising mutant in ragi (*Eleusine coracana*). Curr Res 2(6):37
Sinha SK, Sahoo D (1971) A note on ragi improvement. Orissa J Res 13(2):20–135
Sorrells ME, La Rosa M, Bermudez-Kandianis CE et al (2003) Comparative DNA sequence analysis of wheat and rice genomes. Genome Res 13:1818–1827
Srivastava RK, Singh RB, Pujarula VL et al (2020) Genome-wide association studies and genomic selection in Pearl Millet: advances and prospects. Front Gen 10:1389
Technow F, Burger A, Melchinger AE (2013) Genomic prediction of northern corn leaf blight resistance in maize with combined or separated training sets for heterotic groups. G3 (Bethesda) 3:197–203
Tiwari A, Sharma D, Sood S et al (2020) Genome-wide association mapping for seed protein content in finger millet (*Eleusine coracana*) global collection through genotyping by sequencing. J Cereal Sci 91:102888
Upadhyaya HD, Gowda CLL, Reddy VG (2007) Morphological diversity in finger millet germplasm introduced from southern and eastern Africa. J SAT Agric Res 3. http://ejournal.icrisat.org
Varshney RK, Mohan SM, Gaur PM et al (2013) Achievements and prospects of genomics-assisted breeding in three legume crops of the semi-arid tropics. Biotechnol Adv 31:1–55
Verma V, Patel S (2013) Value added products from nutri-cereals, finger millet (Eleusine coracana). Emir J Food Agric 25(3):169–176
Voss-Fels KP, Herzog E, Dreisigacker S et al (2019) "SpeedGS" to accelerate genetic gain in spring wheat. Elsevier Ltd
Watson A, Ghosh S, Williams MJ et al (2018) Speed breeding is a powerful tool to accelerate crop research and breeding. Nat Plants 4:23–29. https://doi.org/10.1038/s41477-017-0083-8

Recent Advances of Using Innovative Strategies in Management of Millet Plant Pathogens

13

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Abstract

Finger millet is a nutritious cereal crop cultivated traditionally in Africa, Asia, and America. It is the second most important cereal crop in India, where it is grown on more than 2.6 million hectares and produces 3.0 million tonnes annually. Finger millet grains are rich in protein, fiber, minerals (calcium, iron, zinc), and amino acids (tryptophan, cysteine, and methionine), and have potential health benefits. However, finger millet production is threatened by various pests and diseases, which can cause significant yield losses and quality deterioration. Climate change may increase these challenges by creating favorable conditions for pest and disease outbreaks or creating unsuitable conditions during production leading to a decrease in cereal yield. Therefore, there is a need to compare and evaluate different pest management strategies for finger millet cultivation and their impact

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on yield, quality, and sustainability. In this chapter, we aim to review the current literature on finger millet pests and disease management and to identify the most effective and environmentally friendly methods and solutions. Our chapter contributes to the knowledge and practice of sustainable finger millet production and food security.

13.1 Introduction

Millet, wheat, sorghum, and pearl millet are important cereal crops that provide food and nutrition security for millions of people around the world. Millet is a drought-tolerant cereal crop that is widely grown in Asia and Africa for food and fodder. Millet has a high nutritional value and can be consumed by people with gluten intolerance, India is the world's largest producer of millet followed by China and Niger. However, these crops are also susceptible to several diseases caused by fungi, bacteria, viruses, nematodes, and insects. Some of the most important diseases affecting these crops are Rusts, which are fungal diseases that affect the leaves, stems, and grains of wheat, millet, and sorghum. Rusts cause a reduction in crop yield and quality and can spread rapidly through wind and rain. Some of the major rusts are stem rust, leaf rust, and stripe rust in wheat. other important diseases are downy mildew and ergot in millet; and long smut and grain mold in sorghum (Gessese 2019). Blast is a fungal disease that affects the leaves, nodes, panicles, and grains of millet and sorghum. Blast causes lesions, blights, and rotting of the plant parts and can reduce the yield by up to 80%. Blast is favored by high humidity, low temperature, and dense planting (Poonacha et al. 2023). Banana bunchy top disease (BBTD), is a viral disease that affects banana and pearl millet. BBTD is caused by the banana bunchy top virus (BBTV), which is a member of the genus Nanovirus. BBTD causes stunting, distortion, and bunching of the leaves and prevents the formation of fruits. BBTD is transmitted by the banana aphid, *Pentalonia nigronervosa,* and by infected suckers (Halbert and Baker 2015). Modern strategies for managing cereal diseases include many processes such as Breeding for resistance, which involves developing and deploying varieties and hybrids that have genetic resistance to the diseases. This is the most effective, economical, and environmentally friendly way of controlling cereal diseases. Breeding for resistance requires screening of germplasm, identification of resistance genes, and incorporation of resistance into improved cultivars (Steiner et al. 2017). Cultural practices, involve modifying the agronomic and environmental factors that influence the development and spread of the diseases. Some of the cultural practices include crop rotation, intercropping, sanitation, weed control, irrigation management, and optimal planting density and date. Chemical control involves applying fungicides, bactericides, nematicides, and insecticides to protect the crops from diseases. Chemical control can be effective in reducing the disease severity and increasing the yield, but it also has some drawbacks, such as high cost, environmental pollution, and the development of resistance. Biological control involves using natural enemies, such as predators, parasitoids, and

antagonists, to suppress the disease agents. Biological control can be an alternative or a complement to chemical control, as it can reduce the use of pesticides and enhance the natural balance of the ecosystem. However, biological control also has some limitations, such as variability, specificity, and availability.

This review highlights the potential for various applications such as modified classical mating designs and hybrid breeding programs, and how to employ all of these applications to gain significant disease resistance. Millet, a cereal grain used for human consumption and livestock feed, is susceptible to various types of pathogens that can infect the plants, causing either foliar or root diseases. These pathogens can be categorized into different groups based on their morphological characteristics, genetic characteristics, and metabolic activities. The type of diseases caused by the pathogens that infect millet can be divided either into foliar or root diseases depending on their mode of infection and host range.

13.2 Foliar Diseases

13.2.1 Fungi

Blast is the most important disease that causes more than 50% yield losses. Field studies have recorded losses of 10–90% in Uganda, 64% in Kenya, and full losses in India in wet seasons. Environmental conditions of rainfall, temperature (25–30 °C), and humidity (90%) are the most important predisposing factors for blast severity, and affects finger millet at all stages of plants. Also millet is infected with seedling and leaf blight caused by *Helminthosporium nodulosum;* this disease has been recorded in India, Japan, and Africa (Jayo 2021; Kumar and Singh 2010; Kumar and Srivastava 2020). Cercospora leaf spot is caused by the airborne fungal pathogen *Cercospora beticola*, and *Phyllachora eleusines* is a fungal pathogen that causes a tar spot of finger millet. This disease was first detected in Uganda on finger millet (Elobu and Adipala 1993). The symptoms of Phyllachora leaf spot include small, black, sunken spots that may be developed to form large necrotic areas. The symptoms of Cercospora leaf spots are circular spots, usually 2-4 mm in diameter, that are light brown to dark tan with a brown to purple border.

*Sclerosporagraminicola*causes powdery mildew in pearl millet. Pearl millet plants are attacked by this Obligate parasite fungi.. Under wet conditions, the fungus may completely cover the afflicted leaf and generate brown blotches of varied sizes, entirely inhibiting spore generation. These young plants were obliterated. It has been unsuccessful to find genotypes that might sustain *S. graminicola* but not *F. longipes* or to use fungicides to selectively inhibit fungal parasites (Navi and Singh 1993).

13.2.2 Viruses

Wheat streak mosaic virus (WSMV) and other viruses that can infect pearl millet or sorghum were identified from the literature. These include the black-streaked dwarf virus, guinea grass mosaic virus, Indian peanut clump virus, maize dwarf mosaic virus, maize streak virus, panicum mosaic virus, and satellite panicum mosaic virus. These viruses have different modes of transmission, host ranges, and geographic distributions, and can cause various symptoms such as mosaic, dwarfing, streaking, and head malformation. The occurrence and impact of these viruses on pearl millet and sorghum production need further investigation (Jones et al. 2021). Pearl millet and sorghum plants grown in Hays, Kansas, exhibited symptoms such as stunting, chlorotic streaks, and leaf splitting. ELISA tests confirmed the presence of wheat streak mosaic virus (WSMV)in the symptomatic plants. while other viruses such as maize dwarf mosaic, sugarcane mosaic virus strain MDMV-B, and johanson grass mosaic virus were not detected. WSMV was transmitted by wheat curl mites from pearl millet and sorghum to wheat plants, causing similar symptoms. the virus also infected several sorghum lines by mechanical inoculation, but the severity of the symptoms varied among the different isolates of WSMV (Seifers et al. 1996)

13.2.3 Bacteria

Bacterial disease was first reported in India in 1937 foliage is pale green and turned brown caused by Xanthomonas coracana. The main leaf bacterial diseases that have been recorded on sorghm in Australia is leaf stripe, Burkholderiaandropogonis, bacterial leaf spot Pseudomonas syringe, Burkholderiatheobromae, and leaf streak Xanthomonas campestris pv. Holcicola(Elliott) Dye (Ryley et al. 2002).

13.3 Root Diseases

Millet is said to be the world's sixth most important grain, since early 2700 BC in China at first (Akanmu et al. 2013), and then in Africa, Japan, Manchuria, and Egypt (Akanmu et al. 2013). According to a recent examination of the list of plant diseases by the APS (American Phytopathological Society) (Leslie et al. 2005.), Fusarium species have dominated the fungal genera in terms of the importance of economic loss. Fusarium species can cause various diseases, such as stem/root rots, cankers, wilt, fruit or seed rots, and leaf diseases, depending on the severity of the infection (Leslie et al. 2005).

Wilt or foot rot is caused by *Sclerotium rolfsii* and is mainly a soilborne disease. The disease occurs in most finger millet growing areas but is more common during the rainy season. The infection of foot rot occurs at the base of the plants, And spread until it reaches to the leaf sheaths. Smut caused by *Melanopsichium eleusinis*. This disease occurs at the time of grain formation (Kumar et al. 2021).

13.3.1 Fungal

According to Li et al., binucleate Rhizoctonia (BNR) AG-A causes foxtail millet damping-off in China (Li et al. 2014). Binucleate Rhizoctonia AG-A, an anamorph of *Ceratobasidium sp*., was found to infect foxtail millet during the planting stage, resulting in significant crop losses. As a result, while considering disease control and generating disease-resistant foxtail millet varieties in the future, we should keep this in mind. Marasas et al. discovered *F. nygamai* on millet in Southern Africa in 1988.

Leslie et al. (2005) stated that Fusarium isolates are mostly identified as *F. moniliforme*, but recently more than one species has been identified and their ability to cause disease (*F. andiyazi, F, pseudonygamai, F. nagamai, F. sorghum*, and *F. thapsinum)* has been identified in pathogenicity tests of sorghum seeds, as *Fusarium moniliforme.* But they differ in terms of toxin profile test that misidentification might account for discrepancies in the literature and variations occurs by researchers who all thought they were working with the same fungus species.

13.3.2 Nematode

In a test using *M. arenaria*, egg mass and J2 counts on Japanese millet (Echinochloafrumentacea) were comparable to those on tomato. Each of the nematode isolates tested positive for Japanese millet. However, some legumes (cowpea, crotalaria, jointvetch, sunn hemp) with potential uses in both nematode and nitrogen management were either poor hosts or non-hosts of the nematode isolates studied.

Jain (2009) reported that Nematodes that parasitize plants are one of the causes that restrict agricultural yield globally. *Heterodera delvii*, *Heterodera avanae, Heterodera gambiensis, Heterodera zeae, Rotylenchulus reniformis, Meloidogyne incognita, Meloidogyne arenaria*, *Meloidogyne graminicola*, *Meloidogyne acronea, Aphelenchoides besseyii, Pretylenchus penetrance, Tylenchorhynchus vulgaris, T. mashhoodi, P. brachyurus, P. zeae,* and *P. indicus* Small millet crops have been linked to *Macroposthonia ornate*, *Criconemoidesornatus, Criconemella* ornate, *Hoplolaimusindicus*, *Caloosiaexilis*, *Hemicriconemoidescocophilus*, *Hirschmanniellaoryzae*, *Hemicycliophora*sp., and *Trichodorus* sp., However, facts on their incidence, distribution, biology, and interactions with other plant diseases are lacking. The current paper summarizes studies on nematode pests of micro millet crops.

De Waele and colleagues (1998) reported that in soil and root samples taken in 20 maize and five pearl millet fields in Namibia, 28 plant-parasitic nematode species from 17 taxa were discovered. Eighteen of these species are Namibian firsts. Three agricultural regions were studied: Hardap Dam, Grootfontein-Tsumeb, and Kavango-CapnvL. The most common Ecto-parasites were *Mesocriconemacurvatum* and *M. sphaerocephalum*, which were present in 32% and 28% of all soil samples, respectively. *Pratylenchuszeae* and *P. penetfans* were the dominant endoparasites, occurring in 64% and 56% of all root samples, respectively. The prominence value of *P. zeae* was higher in the Grootfontein-Tsumeb (5047 5 g-l roots) area than in the Kavango-Caprivi (788 5 g-l roots) and Hardap Dam (67 5 g-l roots) areas, while the prominence value of *P. penetfans* was higher at Kavango-Caprivi (3086 5 g-l roots)

than at Grootfontein-Tsumeb (1696 g-l roots) and Hardap Dam (23 5 g-l roots). In Kavango-Caprivi, the prominence values of *M. curvatum, P. zeae,* and *P. pene*tfans were always higher for maize than for pearl millet.

13.4 Disease Description, and Types of Control

Disease of millet	Description	Area	Management
Blast *Pyriculariagrisea*	– Symptoms observed on seedling or leaves peduncle, finger – Lesion elliptical shape with grey centers – Water soaking, clorotic halo – Become brown	Occurs in all areas of Africa, India, Nepal and Asia where millet is grown and the United States. 25–30 °C and humidity of 90%	– Plant-resistant varieties where available – Resistance varieties against blast are rare, GPU28, GPU 48 is used to neck and finger blast. – Seed treated with recommended fungicide. – Sprays with carbendazim by 0.1% or mancozeb 63% blusCarbendazim 12% or tricyclazole.
Cercosporapenniseti	On leaves lesions are small and dark, usually oval but sometimes oblong to rectangular, centers of lesions are gray to tan in color with visible black dots and may be covered in spores during wet seasons also appear on the stem	When high temperatures degree coincide with high humidity disease emergence occurs.	Prevent planting millet highly susceptible varieties to Cercospora – Control weeds. – Crop rotation and practices good sanitation.
Millet mold *Fusarium* spp. *Aspergillus* sp. *Curvularialunata* *Alternaria alternata* *Phomasorghina* *Bipolaris* spp.	Pigmentation on spikelet tissues of lemma or glume – appear fungal mycelium growth – poor small seeds – pinkish or whitish fungal growth Finger millet seen	Africa, Asia, North America, South America During the rainy season Production losses 30–100%	– Tolerant cultivar s for mold CSH16- CSV20-CSH(27,30) and PVK801) – Maturity point more critical for harvesting. – Chemical sprays – Spraying

(continued)

Disease of millet	Description	Area	Management
	as discoloration brown to black of grains		with fluorescent psedomonad
Downy mildew – *Sclerosporagraminicola* – *S. sorghi* – *Plasmoparapenniset* – *S. macrospora* – *S. graminicola*	– Chlorosis of leaves -beginning lower down on the plant and moving up towards. – White to gray fungal growth on undersides of leaves; brown, – Necrotic leaves; – The distinct margin between diseased leaf tissue and healthy tissue – Also known crazy top in finger millet	Economic importance in India Asia and Africa Infection needs 18 °C and high humidity. But in soil oospores required 10 °C and low moisture	–Adjusting sowing date – Crop rotation – Disease-resistant cultivars e.g. HHB67, ICMH356) – Chemical fungicide treatment as seeds treatment with systemic fungicide Redomyl –MZ 6 g /kg followed by spray every 25 days – Priming the seeds with chitosan can protect millet systemically – Apron star 42Ws (Anaso and Anaso2010) – Metalaxyl
Rust *Pucciniasubstriata* *P. purpurea*	– Small yellow – Raised spots on upper and lower leaf surfaces – Spots tend to be more numerous on lower leaf surface – Spots enlarged and turned to red browen pustules – Surrouned by yellow halo	Africa, north South America, India and Asia. Spores spread by wind or survive in crop debris in soil	– Millet rust-resistant cultivars Planting millet near solanaceous crops such as eggplant. – Chemical controls for rust not more effective Rust: Foliar spray of mancozeb at 0.2% to control the disease.
Seedling and leaf blight-Helminthosporiumnodulosum – *H. turcicum* – Alternaria sp.-*Trichometasphaeriaturcica* – *Bipolaristurcica*	Death of seedlings before they breach the soil surface; – leaf blight – on seedling light brown oval lesions	-appears in India, Japan, and Africa - transmission by infected seeds	– Avoid planting millet varieties that are highly susceptible to this fungi – Control weeds in field;

(continued)

Disease of millet	Description	Area	Management
– *Dreschslerauricica Exserohilumturcicum*	– large dark brown patches – withering of plants – spread on leaf blade, junction and sheath. – tissue necrosis		rotate crops and practice good sanitation – Chemical control like Metalaxyl or captan. – Rotation with crop non-host – Resistant cultivars barnyard millet genotypes (PRB402 TNAU92, VL216) have resistance versus grain and head smut as well as brown spot diseases – Foxtail millet lines "GPUS 27, SiA 3039, SiA 3059, SiA 3066, SiA 3088, TNAU 213" and TNAU 235 cultivar free from brown spot
Bipolarissetariae *-Curvulariapenniseti* *-Drechslerademati oidea* *Ramulisporasorghi* *Pseudomonas syringae* *Xanthomonasaxonopodis* *Gleosercosporasorghi* *Dactuliophoraelongata*	Bipolaris leaf spot Sooty stripes Leaf streak Rough leaf spot	When high temperatures degree coincide with high humidity disease emergence occurs	– Crop rotation – Destruction weeds – Chemical control
Ergot *Claviceps fusiformis* *C. sorghi C. africana*	– Viscous droplet From infected floret. -sclerotia light pink to dark brown – Black. Round or elongated in shape. Hard in texture	Occurs in many countries in Asia, South America and North America, Africa	Ergot: Use of tolerant cultivar the development of disease-resistant synthetics has been suggested using four populations of pearl millet "ICMP1, ICMP2, ICMP3, and ICMP4" – Various inbred lines (ICMPES 1, 2, 23, 27, 28, and 32), were generated as

(continued)

Disease of millet	Description	Area	Management
			sib-bulks of several chosen lines with combined resistance to the three diseases. The ICMPES lines ICMPES28, ICMPES29, and ICMPES45 produced at ICRISAT were found to be resistant to ergot, although their yields were inferior to those of native African cultivars Infection in inbreds in Zimbabwe varied from 0 (ICMPE13–6-13) to 10.9% 10 days after inoculation, but was 95% in the hybrid BJ104. In fact, varieties with functional field resistance may yield more under disease load than varieties without it. – Spraying panicles with fungicides (0.1% Bavistin or 0.2% Tilt or 0.2% Mancozeb) at flowering minimizes ergot incidence and its subsequent spread. (Mushonga 1983; Thakur et al. 1985, 1988; Mbwaga and Mdolwa 1995).

(continued)

Disease of millet	Description	Area	Management
Beniowskiasphaeroidea	False mildew White sporodochia on the leaf.	Occurs in United States and Texas; India; Japan; Africa; Zimbabwe Mtisi and de Milliano (Mtisi and de Milliano 1993)	– Resistance cultivars (ICMPES 28) Also, Forage millet varieties were identified as resistance (Gupta 1992)
Blight Rhizoctonia solani and R. Zeae	Light gry Dark brown lesion on the lower leaf and leaf sheath. – copper color bands across the leaves	Occurs in all Africa growing millet and Asia	– Seed treatment with propiconazole by 1 mL per kg seeds
Sclrotium rolfsii	Southern blight-foot rot – occurs around the collar region – cottony fungal growth and sclerotia attached with host tissue.	Occurs in all Africa growing millet and Asia	– Cultivars resistance like CSV19R, CSV216R and DSV6
Fusarium moniliforme	Top rot	Occurs in all Africa growing millet and Asia	– Plant extract (neem – Garlic-lemon grass) and some oils – Chemical control (numerous fungicides) – Biological control (Javaid et al. (2018)
Smut Tolyposporiumpenicillariae (Moesziomycespenicillariae) Melanopsichiumeleusinis Ustilagocrameri U. panici-frumentacei Sorosporiumpaspali-thunbergii	Floret converts to bright or shiny green in color and turns brown. The sorus appears as an enlarged body in place of the grain. Mature sorus ruptures and releases teletospores balls. Survives as teletospores in infected seed or in soil	Occurs in all areas of India western Africa and the United States -seed born	– Seed dressing with 4 g sulfur/kg seed – Collecting smutted heads and kill the inoculum pathogen helps to reduce the pathogen next season
Pyriculariagrisea	Pyricularia leaf spot	– Eastern and southern Africa	Barnyard millet genotypes"PRB

(continued)

Disease of millet	Description	Area	Management
		and South Asia (Mgonja et al. 2007; Babu et al. 2013)	402, TNAU 92 and VL216" have resistance against the grain and head smut as well as brown spot diseases, while foxtail millet lines"GPUS 27, SiA 3039, SiA 3059, SiA 3066, SiA 3088,TNAU 213 and TNAU 235″ remain free from brown spot
Anthracnose Colletotrichumgraminicola	– causing seedling blight – damages stalk tissue – leaf anthracnose circular spots with center straw-colored –and gran yield loses	First appeared in Togo West Africa in 1902 And then observe in most of the regions of the world in Africa and North America Asia	– Cultural practices – Crop rotation – Cultivar resistant

All data in table collected from resources Patil (2016), Das et al. (2016) and https://www.cabi.org/isc/datasheet/13788#D5AD1C0A-20AA-432A-A3F0-

Researchers from all around the world have made several attempts to stop the threat of plant diseases. Physical, chemical, biological, and cultural techniques have all been used to manage different plant infections. Synthetic pesticides are typically used to effectively and efficiently manage crop disease (Kiran et al. 2006), However, the widespread and indiscriminate use of fungicides poses a serious threat to human health and existing human ecogeographical conditions, since some have already been shown to be mutagenic, carcinogenic, or teratogenic (Babu et al. 2013).

13.5 New Strategy for Control Application and Nanotechnology

The most significant biotic stress factors that negatively affect its production are downy mildew (Sclerosporagraminicola), blast (Pyriculariagrisea; teleomorph: Magnaporthegrisea), rust (Pucciniasubstriata var. indica), ergot (Claviceps fusiformis), and smut (Moesiziomycespenicillariae) (Sharma et al. 2021).

Several microorganisms, including fungi and bacteria, have been demonstrated to inhibit the growth of the blast fungus *M. orizae*. Bacterial species such as *Bacillus, Streptomyces, Psedomonas,Cryseobacterium and Rhizobacteria* have been shown to reduce blast diseases in vitro (Chakraborty et al. 2021).

Pseudomonas spp. and *Bacillus spp*. have been demonstrated in studies to control the foxtail millet and finger millet blast fungus. Future studies should focus on how these antagonists fare against the recently emerged wheat blast fungus *M. oryzae* (MoT). In addition, *Trichoderma spp.* prevented blast pathotypes that infected millets (Goyal and Manoharachary 2014).

Various bacteria produce about 23,000 bioactive secondary metabolites, more than 10,000 of which are antifungal. Streptomyces, for example, may create roughly 7600 chemicals, including geldanamycin and nigericin, which prevent plant diseases (Gashaw et al. 2014).

Breeders are searching via several germplasm sources for novel genes to further raise the threshold level of biotic and abiotic stress tolerance.Crop wild relatives (CWRs) may be a source of new genes vital to the diversity of the pearl millet genetic basis. The effective administration of pre-breeding initiatives employing CWRs as a source of improved and diverse germplasm is suggested using a stage-gate method. The many breeding methods for collecting and using genes from resistant resources to improve features related to disease resistance that described in Sharma et al. (2020). He reported that the free resistant resources for blast (*Pennisetum. hordeoides*Steud. - *P. pedicellatum*Trin. - *P. polystachion* subsp. *atrichum* Stapf & C.E. Hubb. - *P. setosum* (Sw.) Rich. - *P violaceum* (Lam.) Rich. IP 21525, 21,531, 21,536, 21,540, 21,594, 21,610, 21,640, 21,706, 21,711, 21,716, 21,719, 21,720, 21,721, 21,724, 21,987, 21,988, 22,160) (Sharma et al. 2021). Downey mildew (*P. glaucum* monodii (Maire) Brunken PS 202 - *P. pedicellatum* Trin. - *P. polystachion* L. Schult. IPW 407- *P. schweinfurthii* (= *P. tetrastachyum*) Pilg. IPW 151, 152, 153, 155) Rust (*P. glaucum* subsp. *monodii* (Maire) Brunken - *P. pedicellatum*Trin. - *P. polystachion* (L.)Schult. - *P. subangustum* (Schumach.) Stapf & C. E. Hubbard - *P. violaceum* (Lam.) Rich. IP 21629, 21,645, 21,658, 21,660, 21,662, 21,711, 21,974, 21,975, 22,038) (Sharma et al. 2021). The developed pearl millet for resistance to striga infection under striga- infested field conditions by five cycles of recurrent selection according to (Kountche et al. 2013)

13.5.1 Leaf Spot (*P. glaucum subsp. monodii* (Maire) Brunke)

The developed resources for resistance are Striga-resistance (*P. glaucum subsp. monodii* (Maire) Brunken PS 64, 132, 190, 202, 208, 212, 287, 427, 428, 459, 549, 555, 622, 637, 639, 727, 755 - *P. glaucum* ssp. *stenostachyum* (Klotzsch ex Müll. Berol.) Brunken - *P. hordeoidesSteud*. - *P. pedicellatum*Trin) (Sharma et al. 2021).

13.5.1.1 Abiotic Stress Tolerance

Planting drought-tolerant crop varieties, such as improved millet hybrids that can withstand water stress and high temperatures. For Example the developed resources that resistant for Salinity (*P. clandestinum Hochst*. Ex Chiov. - *P. purpureum* Schumach.), Drought (*P. ciliare* L. Mant. - *P. mezianum* Leeke. - P. orientale L.C. Rich.), Freezing and winter hardiness (*P. flaccidum* Griseb. -; *P. orientale* L. C. Rich) (Sharma et al. 2021).

13.5.1.2 Male Sterility (MS) and Fertility Restoration Sources

Cytoplasmic diversity (*P. schweinfurthii*), MS source A4 (*P. glaucum subsp. monodii* (Maire) Brunken), MS source A4 and Av (*P. violaceum* (Lam.) Rich.), Fertility restoration (*P. purpureum* Schumach) (Sharma et al. 2021).

13.5.1.3 Fodder/Forage Characters

Forage yield, quality, and related traits (*P. cenchroides* Rich. - *P. hordeoides* Steud. - *P. pedicellatum* Trin. - *P. purpureum* Schum. - *P. setosum* (Sw.) Rich) (Sharma et al. 2021).

Ornamentals: (*Palopecuroides* (L.) Spreng. - *P. flassidum* Griseb. - *P. orientale* L.C. Rich. - *P. setaceum* (Forssk.) Chiov.) (Sharma et al. 2021).

Apomictic gene [apospory-specific genomic region (ASGR)]: Apomixis (*P. ciliare* L. Mant. (= Cenchrusciliaris L. Mant.) - *P. orientale* L.C. Rich. - *P. setaceum* (Forssk.) Chiov.- *P. squamulatum* Fresen.) (Sharma et al. 2021).

13.5.1.4 Other Agricultural-Related Uses

Growth rate and yield (*P. glaucum subsp. monodii* (Maire) Brunken), Large seed size (*P. schweinfurthii* Pilg. - Serba et al. 2017), Soil bioengineering approach (*P. pedicellatum* Trin. (PPd) - *P. polystachion* (L.) Schult. (PPl)) (Sharma et al. 2021).

As incorporating millet crops into food security programs becomes more popular, scientists are considering improving millet through the use of CRISPR/Cas9 and other genetic modification techniques. For example, it has been reported that foxtail grass genome construction can be used to find important loci for traits such as openness and loss of leaf angle, which are considered important predictors of yield in many grass crops. They used CRISPR/Cas9 to validate the Less Shattering1 (SvLes1) gene to reduce seed breakage. Previous studies used Agrobacterium-mediated transformation of millet (Setariaitalica) to downregulate phosphate transporters (SiPHT1; 2, SiPHT1; 3, and SiPHT1; 4). They found significant reductions in inorganic and total phosphate in root and shoot tissues, and increased numbers of roots and hairy roots for non-redundant tasks. Furthermore, millet was the first millet crop to be sequenced.

The genome sequence of pearl millet has also been published. Another study found that Agrobacterium-mediated production of transgenic crabgrass (*Eleusine coracana* (L.) Gaertn.) plants was successful. An Agrobacterium-mediated transformation of crabgrass has been developed, and four cultivars have been successfully regenerated using the Agrobacterium transformation technique. Whole-genome population studies were performed on three major millet crops: proso millet, millet millet, and kodo millet. 3461 SNIPs were found in kodo millet, 2245 in small millet, and 1882 in proso millet. These results may aid genome editing to improve stress resistance and plant growth. Recently, millet mutants were generated by EMS-induced mutagenesis. A "millet" point mutation was created in the photoreceptor gene phytochrome c (PHYC), which is required for photoperiodic flowering and has a short period (Numan et al. 2021).

Once identified, putative genes involved in high grain calcium (Ca^{2+}) accumulation in crabgrass can be confirmed by transgenic overexpression, and candidate genes can then be used to genetically engineer crops to help them increase calcium levels in grains, thereby increasing grains. The discovery of genome-wide variation in crops such as crabgrass is the first step in linking genotypic variation to phenotype. Converting these genetic variants, most commonly SNPs, into genetic markers is particularly important for crops of agronomic value, as it enables efficient marker-assisted selection strategies, map-based gene cloning, genome-wide fingerprinting, association studies, and population analysis. Based on analysis by (Sharma et al. 2017) that can be exploited for crop improvements.

Downy Mildew

Several mannitol-added oligosaccharides, *N*-acetylchitooligosaccharide, aminobutyric acid, and 3,5-dichloroanthranilic acid have been investigated as inducers of pearl millet downy mildew. As a result, the current situation needs a concerted effort to design a panel of effective inducers of downy mildew disease resistance in pearl millet (Govind et al. 2016).

Trehalose's effects on sporangial development in *S. graminicola* and sporangia zoospore release infected leaves of "HB3" were treated with various concentrations of trehalose and SDW to determine whether trehalose treatment affected *S. graminicola* sporangia development, and the comparative effects of trehalose on in vitro sporangia per cm^2 formation and zoospore release were microscopically studied. This means that there was no significant difference in sporangia generation or zoospore release in vitro between trehalose and SDW treatments. Under our experimental conditions, trehalose treatment exhibited no significant inhibitory effects on *S. graminicola* sporangia development (Govind et al. 2016).

At 3, 6, and 9 h, HB3" seeds treated with 200 mM trehalose provided disease protection of 43.22, 54.50, and 70.25%, respectively; hence, 200 mM trehalose was shown to be the most effective of all concentrations tested. At 3, 6, and 9 h, treatment with 25 mM trehalose provided the lowest disease protection rates of 11.42, 16.85, and 27.40%, respectively. Furthermore, the disease protection efficiency of the 200 mM trehalose treatment was equivalent to that of the Apron 35SD, which attained a disease protection rate of 73.55% at 9 h. Plants produced from SDW-treated seeds were completely resistant to *S. graminicola* infection (Govind et al. 2016).

In both greenhouse and field conditions, trehalose proved that it may be successful in protecting pearl millet against downy mildew. The levels of protection provided by pearl millet against downy mildew were demonstrated to be time and dose-dependent. The maximal downy mildew disease protection level of 70.25% was reached under greenhouse conditions with a 9-h 200 mM trehalose seed treatment, whereas shorter duration periods with lower dose treatments indicated lower downy mildew disease protection. As a result, the amount of trehalose that penetrates the plant cuticle is significant, as seen by higher doses and longer periods of trehalose treatment providing better protection, which was shown to be the greatest across all concentrations (25, 50, 100, and 200 mM) and time durations (3, 6, and 9 h) tested in a greenhouse. Furthermore, in Arabidopsis trehalose

phosphate synthase, exogenous trehalose improved resistance to green peach aphid. In response to pathogen invasion, plants activate defense mechanisms through coordinated changes in gene expression. *S. graminicola* and other biotrophic pathogens feed on living plants. To satisfy their needs, these pathogens interact with host cells and affect a variety of metabolic processes. A ROS-based defense system protects plants from biotrophic diseases. The most studied system, which serves as the first line of defense, includes genes that code for POX, PAL, and PPO. However, the rate at which these genes and associated enzymes react upon pathogen infection varies depending on the type of plant-pathogen interactions. POX catalyzes the redox reaction of many substrates, resulting in cell wall protein lignification and cross-linking. By converting L-phenylalanine to trans cinnamic acid and ammonia, PAL produces a variety of defense-related secondary compounds such as phenols and lignin's. PPOs are copper-metalloproteins that have gotten a lot of interest because of their role in plant defense. In a number of plants, PPO genes and PPO activities in response to defense signals and plant resistance induction have been studied. SAR is in charge of priming the host defense system, which results in the buildup of PAL, PPO, POX, and other pathogenesis-related proteins in uninfected tissues to protect them from any imminent pathogen attack (Govind et al. 2016).

In both laboratory and field settings, microorganisms that produce antibiotics and enzyme inhibitors, such as *Bacillus spp.*, *Pseudomonas spp*., and some other antagonistic bacteria, as well as Actinomycetes fungus, have been used to reduce blast infections in rice. Furthermore, some plant secondary met abolites suppressed *M. oryzae* development and have a high potential for future blast disease reduction caused by new pathotypes in cereals. Many research looked at a variety of possible antagonistic bacteria and natural products for blast disease management in rice, wheat, ragi, and millets. Despite the publication of several assessments for the biological control of various plant diseases on diverse crops, *M. oryzae* may infect the host at any developmental or organ stage, including leaves, internodes, necks, spikes, and panicles. The fungal infection results in a buildup of reactive oxygen species, which causes host cell death or necrotic lesions, allowing hyphal growth and nutrition absorption to proceed (Nandini et al. 2017a, b).

Antibiosis is a mechanism in which antagonists secrete antibiotics or metabolites that are directly toxic to pathogens; parasitism is a mechanism in which the biocontrol agent affects the pathogen; competition is a mechanism in which the antagonist and pathogen compete for limited resources such as nutrients and space, and predation is a mechanism in which the pathogen is directly destroyed by the biocontrol agent (Nandini et al. 2017a, b).

In an in vitro investigation, oligomycin B and F, generated by Streptomyces spp. strains B8739 and A171, decrease the mycelial development of *MagnaportheoryzaeTriticum* (MoT) at 0.05 and 0.005 mg/mL, respectively. These compounds also reduce the development of blast disease in plants by reducing MoTcnidogenesis, germination, and appressoria synthesis (Chakraborty et al. 2020). Also Chakraborty and Islam (Chakraborty and Islam 2022) *P. cepacia* RB425-derived pyrrolnitrin suppressed rice blast disease by 81%. Chitinase obtained from *P. fluorescens* isolate 1 substantially inhibited the mycelial growth of the finger millet blast fungus MoE. (Pfl). Pseudomonas species also produced the

cyclic lipopeptides N3, entolysin or orfamide, lokisin, and WLIP, which inhibited the appressorial growth of the rice blast fungus Magnaportheoryzae. Allicin, also known as diallyl-thiosulfate and isolated from garlic tissue, reduced 90–99% spore germination of rice blast fungus, respectively. Garlic extract, neem extract, and Calotropisprocera L. extract were tested in vitro against rice blast fungus, and garlic extract completely inhibited fungal growth. Garlic clove extracts significantly reduced sporulation and mycelial growth, as well as the severity of neck blast in finger millet produced by *M. oryzae* in both in vitro and in vivo testing. Tulsi, mehendi, and datura leaf extracts, as well as mehogoni, black cumin, and garlic clove seed extracts, were recently tested for their antifungal activity against the wheat blast fungus MoT at various concentrations. According to the findings, garlic clove extract was most effective in reducing mycelial growth in an in vitro test (Nandini et al. 2017a, b; Akanmu et al. 2013; Chakraborty et al. 2021).

In greenhouse and field contexts, some abiotic elicitors employed as seed treatments lowered disease incidence. Treatment of seeds with 50 mM -aminobutyric acid (BABA), a rare non-protein amino acid, provided up to 75% disease protection. The resistance acquired in seedlings remained throughout the vegetative and reproductive development of pearl millet plants. The increase in defense-related proteins such as −1,3-glucanase, phenylalanine ammonia lyase, peroxidase, and hydroxyproline-rich glycoproteins was suggested to be responsible for the enhanced resistance (Goyal and Manoharachary 2014).

Farmers rotate the cash crop of cumin with brown mustard or Raya (Brassica juncea) and wheat in the winter season and with pearl millet in the rainy season to reduce the occurrence of fusarial wilt (Gashaw et al. 2014).

Environmental conservation with *Gliocladium virens:* The lack of long-term resistance, as well as the prevalence of pathogenic variants, and concerns about fungicide resistance, have made such disease-management techniques ineffective, so Environmental conservation is becoming more popular, such as the Effects of Raw Cow Milk and *Gliocladium virens* on Pearl Millet Downy Mildew (Gashaw et al. 2014).

The most virulent isolates reduced finger millet output the greatest isolates with a typical concentration (Bayleton 71.9 ± 5.6c, Curzate 70.7 ± 10.7c, Ridomil 78.1 ± 4.3b, Sancozeb 87.1 ± 2.1a). The findings from the in vivo test under greenhouse conditions revealed the greatest yield decrease. In vitro testing of biological agents on the mycelia development of the isolates revealed that Pseudomonas fluorescence was less effective than the two Trichoderma species. The fungicides were shown to inhibit the growth of the various isolates by 67% to 88.40%. Sancozeb was determined to be the most effective fungicide, followed by Ridomil, Bayleton, and Curzate (Gashaw et al. 2014).

A method was tried through seed priming to increase pearl millet disease resistance by using biotic elicitors for eco-friendly management of the downy mildew pathogen. Four unique *Trichoderma spp*. crude oligosaccharides boost pearl millet disease resistance. Seed priming with *T. asperellum* and the osmopriming chemical mannitol resulted in greater seedling protection and vigour than controls. Modification of defensive enzymes like peroxidase and lipoxygenase confirms the elicitation

of resistance responses in the host with increased enzyme activity at different time interval patterns. In comparison to previous research, *T. virens* with 1% mannitol showed increased defense enzyme activity compared to other treatments, as well as substantial disease prevention (*P* 0.05). Mannitol, when combined with crude elicitors, functions as an osmopriming agent. Roopa et al. show a similar result when osmopriming with mannitol, which enhances seed quality parameters and planting value in PM. When compared to individual isolates of *T. harzianum*, *T. lignorum*, *Gliocladiumvirens*, and *Bacillus subtilis*, Trichoshield, a talc formulation containing spores of *T. harzianum*, *T. lignorum*, *Gliocladiumvirens*, and *Bacillus subtilis*, improved seed germination factors, vegetative and reproductive growth parameters, and provided better protection (Basavaraj et al. 2019).

Using plant extracts, the effects of *Moringaoleifera*, *Manihotesculenta* (peels), and Sennaalata at 5, 10, and 15% g/mL concentrations on *Fusarium anthophilum, Fusarium verticillioides, Fusarium oxysporum,* and *Fusarium scirpi* are investigated by Akanmu et al. (2013) (in vitro and in vivo). Moringa oleifera, Manihot esculenta (peels), and *S. alata* extracts demonstrated a substantial (p 0.05) antagonistic effect *on F. anthophilum, F. verticillioides*, and *F. oxysporum* in vitro and in vivo. *Moringa oleifera* and *S. alata* at 5 and 10% g/mL significantly (p 0.05) reduced the incidence and severity of *F. scirpi*disease on millet seedlings. *F. anthophilum, F. verticillioides, F. oxysporum,* and *F. scirpi,* the most pathogenic *Fusarium spp*., were subjected to biological control approaches to establish a possible control of diseases caused by these Fusarium species on millet kinds. Extracts of *Moringa oleifera*, *Manihot esculenta* (peels), and *Salvia alata* were used as phytofungicides against *Fusarium* infections (Akanmu et al. 2013).

Similarly, the effect of *Manihot esculenta* on *F. scirpi* was confirmed as significantly reduced disease severity in millet seedlings, although *F. anthophilum* and *F. verticillioides* treated with the three extracts at a concentration of 10% g/mL showed no significant reduction. (Akanmu et al. 2013).

The antifungal activity of *S. alata* water flower extracts was tested against three different groups of fungi, namely the aflatoxin-producing fungi *Aspergillus flavus* and *Aspergillus parasiticus*, the phytopathogenic fungi *F. oxysporum and Helminthosporium oryzae*, and the human pathogenic fungi *Candida albicans* and *Microsporum audouinnn*, at 10g/100ml and recorded complete inhibition of growth. The antifungal properties of *S. alata* extract were found to be highly effective against four pathogenic *Fusarium* species in vivo experiments, partially controlling *F. anthophilum and F. verticillioides* at a concentration of 5% g/mL and increasing antagonizing activity in all isolates (Akanmu et al. 2013).

The efficacy of seed priming with *P. fluorescens, T. virens,* and neem leaf extract in increasing plant development and protecting pearl millet against blast disease was demonstrated by Basavaraj et al. (2019). Disease protection investigations were verified by increased activity of defense enzymes (PAL, POX, LOX, and--1,3-glucanase). The results suggest that *P. fluorescens* can be used to establish systemic resistance in pearl millet against *M. grisea* blast disease. Four defensive enzymes were investigated in pearl millet for their response to *M. grisea* challenge inoculation in this study. The results of the study showed that enhanced defense

enzymes in pearl millet seedlings treated with biotic inducers were equivalent to resistant seedlings, indicating that these biotic inducers are effective at generating resistance to pathogen infection (Basavaraj et al. 2019).

Both mycelial extract and cell wall glucans of *Trichoderma hamatum* UOM 13 enhanced seed quality measurements of pearl millet and significantly increased seed germination and seedling vigor when compared to the untreated control. Seed priming with *T. hamatum* UOM 13 cell wall glucan elicitors reduced downy mildew on sensitive pearl millet seedlings in the greenhouse by eliciting systemic host resistance. The combination of transplant root dip + seed treatment + foliar spray exceeded the individual delivery methods significantly. Increased resistance after pathogen inoculation was related to the over-expression of genes encoding critical defense proteins. The UOM elicitor action of *T. hamatum* significantly increased transcripts of the defense enzyme genes glucanase, phenylalanine ammonia-lyase, peroxidase, and polyphenol oxidase. The treatment of *T. hamatum* UOM cell wall glucans boosted the expression of hydroxyproline-rich glycoprotein genes, which are known to play a role in cell wall cross-linking. This study emphasizes the importance of *T. hamatum* UOM as a potential elicitor of downy mildew resistance in pearl millet and gives novel insights into the role of important defense proteins that mediate resistance, such as resistance triggers (Lavanya et al. 2017).

Susceptible pearl millet seeds (cv 7042S) which treated with the plant growth-promoting fungus Penicilliumchrysogenum (PenC-JSB9) at 1×10^{8}sporesml)1 examine the mRNA expression profiles of five defense-responsive genes and test their ability to induce resistance to Sclerosporagraminicola-caused downy mildew. PenCJSB9 treatment at 1×10^{8} CFU $mL)^{-1}$ for 6 h substantially enhanced seed germination (9.8–89%), root length (4.08% to 5.1 cm), shoot length (18.9% to 7.77 cm), and disease incidence compared to untreated controls (28%). PenC-JSB9 in planta colonization demonstrated that all three root segments (0–6 cm) and soil dilutions cultivated on PDA displayed significant mycelial growth, despite the fact that the frequency of colonization of PenC-JSB9 was substantially higher in soil than in root segments. CHS (3.5-fold increase) and Pr-1a (threefold increase) transcript accumulation in pathogen-challenged resistant seedlings occurred at 24 and 12 h, respectively. While non-pretreated susceptible seedlings showed limited expression of hybridized defensive genes after pathogen inoculation, PenC-JSB9 pretreatment susceptible seedlings displayed rapid and enhanced synthesis of LOX and POX at 48 h and CHT at 24 h. The activation of defense genes by PenC-enhanced JSB9 suggests a role in increasing resistance to *S. graminicola*. After 3, 4, and 5 days, there was a significant (P 0.005) decrease in disease incidence in plants of 29.2, 36.3, and 39.5%, respectively. Although plants were protected throughout all five time periods, the highest protection was seen after 3 days and was maintained between the inducer treatment and the challenge inoculation (Murali et al. 2013).

13.5.2 Plant Growth-Promoting Fungus (PGPF)

Penicillium oxalicum is isolated from the rhizosphere soil of pearl millet and tested for its ability to boost growth and generate systemic resistance to downy mildew disease in pearl millet. Plants pretreated with CS of *P. oxalicum* offered significant disease protection of 62% and 58%, respectively, against downy mildew disease under greenhouse and field conditions. *P. oxalicum* inducers required at least 3 days to produce peak disease resistance, which was thereafter maintained, according to the spatiotemporal studies. At 24 h, seedlings treated with CS of PGPF *P. oxalicum* had the maximum Peroxidase (POX) activity (62.7 U), and the activity rapidly dropped at later time points following pathogen inoculation. Chitinase (CHT) activity was significantly higher in inducer-treated seedlings than in pathogen-infected control seedlings after 48 h and remained constant throughout. The inducers of PGPF *P. oxalicum* were tested in the field for their capacity to inhibit downy mildew disease. Despite significant inoculum pressure, all inducers of PGPF *P. oxalicum*-treated pearl millet plants displayed disease resistance greater than 50%. The CS treatment offered the maximum disease protection (58%) among the inducer treatments of PGPF *P. oxalicum*, followed by the LCF and CF treatments, which provided 54 and 51% protection against downy mildew disease, respectively. However, in terms of downy mildew disease prevention, PGPF treatments did not outperform Apron 35 SD, with Apron 35 SD offering 92% disease protection (Murali and Amruthesh 2015).

13.5.3 Nanotechnology

Millet is a cereal grain usually consumed as a cereal or a grain. However, its most famous use is for animal feed. Due to the high protein and gluten content found in millet, it is also used to make bread. Nanotechnology involves building structures with materials smaller than 100 nanometers. By using nanotechnology, we can help farmers fight millet diseases and boost their food production.

Farmers use nanotechnology to improve their millet yields by using it to control pests and weeds. Nano detectors are thin plates coated with silver or gold nanoparticles. These particles attract and kill insects or spores, preventing them from damaging the crop. Nanotechnology is even being used to create genetically modified seeds that resist diseases and diseases caused by pests. This allows farmers to produce high-quality and more consistent crops. In addition, farmers can use nanotechnology to analyze crops to determine nutritional needs and prepare the soil properly. By protecting the technology, scientists can ensure that it is effective and ready for use by farmers.

Nanotechnology gained further attention and recognition in the 1980s and 1990s with the invention of the scanning tunneling microscope. Recent advances in the utilization of innovative approaches for millet plant disease treatment Chitosan NPs were created by acetylating low-molecular-weight chitosan and investigated for their efficacy against the *Sclerospora graminicola*-caused downy mildew disease of pearl

millet. In laboratory investigations, CNP seed treatment significantly increased pearl millet seed germination rate and seedling vigor compared with control. Treatment of seeds with CNP induces systemic and long-lasting resistance as well as considerable downy mildew protection in greenhouse environments as compared to the untreated control. CNP therapy changed gene expression patterns, dramatically increasing phenylalanine ammonia lyase, peroxidase, polyphenol oxidase, catalase, and superoxide dismutase. The pathogenesis-related proteins PR1 and PR5 were upregulated in response to CNP therapy. CNP's anti-downy mildew activity was revealed to be mediated by nitric oxide, and treatment with CNP coupled with NO inhibitors cPTIO completely suppressed the gene expression of defense enzymes and PR proteins. Furthermore, a CNP versus Chitosan comparison revealed that a very small amount of CNP worked similarly to the recommended dose of Chitosan for downy mildew treatment. Converting chitosan to nano chitosan offers several advantages, including biocompatibility, biodegradability, and reduced toxicity, making it perfect for successful elicitor delivery. Nano chitosan differs from chitosan in physiochemical properties such as size, surface area, and cationic nature, which influence biological activity. Many host–pathogen interactions have indicated nano chitosan's capacity to induce resistance to a variety of plant diseases. Cu-chitosan nanoparticles efficiently protected tomatoes from early blight and Fusarium wilt. Chitosan nanoparticles efficiently suppressed the Pyriculariagrisea blast fungus in rice and finger millet. The effect of CNP on seed germination and seedling vigor of pearl millet was studied in the laboratory. CNP was investigated for its effect on pearl millet seed germination and seedling vigor by preparing various concentrations of CNP and comparing them to untreated controls and chitosan. Seed treatment with a CNP concentration of 250 mg kg1 generated the best outcomes in terms of seed growth parameters. CNP at 250 mg kg1 resulted in 97% pearl millet seed germination and 1937 seedling vigor, both of which were significantly higher than the chitosan and untreated controls. The chitosan treatment increased seed germination by 89% and seedling vigor by 1917%. The untreated control germination rate was 82% and seedling vitality was 1866 (Siddaiah et al. 2018).

Chitosan NP's capacity have been shown to induce resistance against downy mildew, a fungal disease that affects pearl millet, a cereal crop. CNP seed treatment enhances seed germination, seedling vigor, and gene expression of defense enzymes and pathogenesis-related proteins in pearl millet plants. The mechanism of CNP-induced resistance involves the generation of nitric oxide, a signaling molecule that modulates plant immunity. Chitosan-NPs have higher efficacy and lower dosage than chitosan for downy mildew management. Chitosan NPs have been shown to develop downy mildew resistance in a greenhouse context. Because when the 250 mg/ kg seed concentration of CNP was found to be the most effective for seed treatment, it was tested for downy mildew disease response in greenhouse conditions, with chitosan, apron, and untreated control. CNP had the lowest incidence of downy mildew disease among the evaluated treatments, at 18.1%. Chitosan treatment reduced the frequency of downy mildew by 19.6%. The incidence of downy mildew disease in apricots and untreated controls was 8.3 and 97.5%, respectively (Siddaiah et al. 2018). This showed that CNP therapy-induced

resistance to pearl millet seeds is systemic. When the duration between seed treatment and pathogen inoculation was one day, CNP treatment resulted in 63% downy mildew protection. The percentage of protection climbed to 73% on the second day and remained consistent throughout the experiment, indicating that a minimum of 2 days were required for full resistance build-up. In the second set of tests, the inducer treatment was delivered as root dip inoculation, and the pattern was comparable. Initially, the protection offered during a 1-day gap was 64%. On the second day, the difference increased to 75%. This level of protection was maintained throughout the trial (Siddaiah et al. 2018).

According to Nandini et al. (2017a, b), silver nanoparticles and selenium nanoparticles are used as growth-promoting and anti-fungal –antibacterial against also Trichoderma-selenium nanoparticles are used to decrease the pearl millet downy mildew,

The ultrasonic emulsification technique was used to create a Nano emulsion from *Trichoderma spp.* membrane lipids and the non-ionic surfactant Tween 80. A nanoemulsion with droplet diameters ranging from 5 to 51 nm was created. To establish an eco-friendly disease management approach, the potential impacts of membrane lipid Nano emulsion on pearl millet (PM) seed development characteristics and elicitation of downy mildew (DM) disease resistance in PM were investigated. According to the current findings, the droplet size of *Trichoderma spp*. membrane lipid Nanoemulsion is negatively associated with disease resistance in PM. Furthermore, *T. brevicompactum* membrane lipid Nano emulsion helps to reduce PM DM disease. As a result, this study indicates a feasible integrated approach for future diabetes care in PM. The results of this investigation suggest that a *Trichoderma spp*. lipid Nano emulsion might be effective in treating the DM pathogen. Furthermore, it opens up a new channel for the effective use of Trichoderma membrane lipid Nanoemulsion formulations for plant disease control (Nandini et al. 2019).

13.6 Crop Losses in the World and Challenges for Planting

Eleusine coracana (L.)Gaertn., sometimes known as finger millet, is a species of Eleusine coracana (L.) Gaertn is an essential and incredibly nutritious cereal grown in semi-arid regions of South Asia, Eastern Africa, and Southern Africa. Information about the world's area devoted to finger millet and other millet, such as pearl millet, is in great detail in the FAO database. Furthermore, the Consultative Group on International Agricultural Research (CGIAR) reports that finger millet accounts for around 10% of the total area (34.6 million hectares) planted to millets. With 2.6 million hectares under cultivation, finger millet is only second to pearl millet in terms of productivity in India (www.indiastat.com).

13.7 Crop Rotation and Intercropping as a Control Strategy

Crop rotation was invented by a world-renowned environmentalist. Crop rotation is the practice of cultivating multiple crops on the same plot of land at different periods of the year. It has various benefits (Li et al. 2019). It helps to preserve soil productivity, reduce pests, reduce chemical consumption, maximize yields, reduce reliance on a single set of nutrients, and promote weed growth. Previously, two field systems were used for rotation; however, this was upgraded to four field systems (Chamberlain et al. 2020). Crop interdependence is described by how it contributes to soil and produces hybrid offspring while interbreeding with other crop management practices that reduce soil erosion and have a large environmental impact (Brankatschk and Finkbeiner 2017). Crop rotation entails growing numerous crops in a single season, whereas intercropping entails growing multiple crops throughout many growing seasons (Maitra et al. 2021).

Intercropping promotes biodiversity, which in turn improves pest control. It also aids in soil fumigation by boosting soil organic matter and inhibiting weed development. As a result, intercropping is a local technique based on ancient practices that is still prevalent today (Iqbal et al. 2019). Mixed or intercropped crops planting many crops at once is known as intercropping or mixed cropping. A single harvest may not always make the optimum use of available resources (Hong et al. 2019). Intercropping is therefore used to boost the productivity of a plot of land. Intercropping can be accomplished through a variety of means. Mixed intercropping is the most basic type of intercropping (Bybee-Finley and Ryan 2018). Row farming involves growing crops in many rows. When the fast-growing crop is harvested before the slow-growing crop develops, this is known as temporal intercropping. Bdliya and Muhammad (2007) investigated the efficacy of intercropping millet and groundnut in avoiding Cercospora leaf spot of groundnut in the Sudan savanna of northeastern Nigeria. It is estimated that 95% of groundnuts grown in Nigeria are blended with cereals such as millet, sorghum, or maize (Okigbo and Greenland 1976). In addition to the productivity gains associated with such mixed cropping systems, there may be fewer severe pest and disease challenges than in monoculture systems.

13.8 Fungal Toxins and Management

Chala et al. (2014) mentioned that all of the finger millet and sorghum samples that were mycotoxin analyzed to have *Fusarium* and *Aspergillus* species contamination. *Epicoccum*, rootstock, and other *Penicillium* species were also included in both granules, but in fewer amounts. Sorghum and finger millet were found to have been contaminated with 84 and 62 metabolites, respectively, according to LC-MS/MS mycotoxin analyses. Except for zearalenone, which was present in a third of the samples and averaged 44 g/kg, the incidence of significant mycotoxins in sorghum was less than 15%. Sorghum samples had aflatoxins B1, B2, G1, G2, and M1, whereas finger millet samples were the only ones to have aflatoxins B1 and G1. The

United States Georgia*Fusarium pseudonygamai* species was discovered in the *Gibberillafujikuroi* complex and fumonisin of pearl millet (Nirenberg and O'Donnell 1998), marking the first reported isolation of this species in the United States (Jurjevic et al. 2005). Also, *Fusarium longipes* were identified on pearlmillet (Navi and Singh 1993).

Fumonisin and moniliform are mycotoxins that are toxic to people and pets and have been studied in Nigeria. Eighteen potentially hazardous fusarium strains were identified from maize ($n = 10$) and sorghum ($n = 7$) growing in the same field. All 17 maize and sorghum strains were 17 strains generated under favorable circumstances. Fumonisin was created by ten strains. Regardless of the host from which they were obtained, all strains could grow and generate poisons using any grain. Because of the interactions of the genetic microenvironment, the isolates generated variable amounts of the toxin on each substrate, with the generation of toxins distinctive to the strain rather than the host from which the strain was extracted. The capacity of *F. proliferatum* strains to generate fumonisins and moniliform on maize, sorghum, and millet varies significantly. If the quantity of toxin generated on the various grains represents real-world situations, such as poor storage, consumers of these infected grains may be exposed to mycotoxin levels much above the recommended daily consumption (Vismer et al. 2019). The occurrence and toxicity of mycotoxigenic Fusarium were examined in 24 proso millet samples collected from fields on Jeju Island, Korea. At maximum levels of 117.7, 861.8, and 433.2 ng/g, the samples were contaminated with zearalenone (62.5%), deoxynivalenol (45.8%), and nivalenol (41.7%). T-2/HT-2 toxins were generated by isolates of *F. asiaticum*, *F. acuminatum*, and others. *Fusarium isolates* from the millet samples comprised *F. asiaticum* (19.5%), *F. acuminatum* (12.4%), *F. graminearum* (11.8%), *F. incarnatum* (10.7%), *F. equiseti* (10.7%), *F. tricinctum* (8.9%), and 12 other Fusarium species (Choi et al. 2021). From Nigeria, Lesotho, and Zimbabwe, seeds of three types of millet were gathered: foxtail millet (Setariaitalica), prosomillet (*Panicummiliaceum*), and pearl millet (*Pennisetumtyphoides*). The samples came from grain grown on experimental farms, maintained in households, left unharvested in the field, or sold in markets. The most common species were *Fusarium equiseti* (34%), *Fusarium nygamai* (26%), *Fusarium moniliforme* (24%), *Fusarium semitectum* (10%), and *Fusarium chlamydosporum* (4%). Additional Fusarium species isolated were *F. napiforme*, *F. subglutinans*, *F. graminearum*, *F. oxysporum*, and *F. solani*, accounting for around 3% of the recovered Fusarium species. While *Fusarium equiseti* and *Fusarium semitectum* predominated in Zimbabwean samples, *Fusarium nygamai* and *Fusarium moniliforme* were most typically recovered from Nigerian seed, while *F. equiseti* was the only species isolated from Lesotho (Onyike et al. 1991).

M6 is a unique plant defense mechanism discovered as a result of an unusual symbiotic relationship between finger millet and a root-dwelling bacterial endophyte (*Enterobacter sp.*). The RHESt is a physical barrier that blocks access and/or captures and kills *F. graminearum*. Other findings suggest that M6's anti-fusarium action may be transferrable to maize and wheat. The seed-coated M6 swarms toward the root-invading Fusarium and is related to root hair development, which bends

parallel to the root axis, resulting in biofilm-mediated micro-colonies and a stunning multi-layered root hair stack (RHESt).

13.9 Abiotic Stress and Climate Changes

Climate change must be urgently addressed as it could seriously threaten human survival if left unchecked as global temperatures rise due to a warming planet resulting from greenhouse gas emissions contributed mainly by industrialized societies such as those in Europe and North America. This shows how serious climate change really is and why it should be urgently addressed by all countries to avoid serious economic consequences. Although some believe that increasing pest and disease levels require adjustments in farming practices like free-range farming or rotating crops, others disagree and think farming will adapt without needing outside help from governments or corporations.

Global climate change is the result of human activity that affects the atmosphere. This is due to increased greenhouse gasses in the atmosphere due to industrialization, urbanization, and farming. This has led to a significant rise in temperature and has had devastating effects on the environment and human health. We should increase our efforts to combat climate change and reduce greenhouse gasses to improve our health, agriculture, and natural resources.

In agriculture, the most climatic changing conditions effectiveness are temperature and wetness. These conditions make it easier for pests and diseases to survive and become more widespread. Plus, they expose crop production to these threats. These effects have been felt worldwide; countries like the United States of America and China have been severely affected by climatic changes in millet production (Fig. 13.1) Source: FAOSTAT, in the period 2010–2022. When we compared the months of millet cultivation in June and July in the main countries for its cultivation, namely India, Africa, South America, and Algeria, we found that in these areas there was a change in climate in the period from 2000 to 2020, which was reflected in a large fluctuation in production, especially in India, the United States, and Kenya, while there was an increase in production in Japan and Ethiopia. Therefore, climate change is a serious threat to agricultural production worldwide and must be addressed immediately (Fig. 13.2).

13.10 Prospects and Challenges of New Formulation and Application in Plant Pest Disease Management

Sudisha et al. (2005) compared the effect of fungicides consisting of strobin compounds against downy mildew and showed that Azoxystrobin had a high disease-curing potential. All strobilurin fungicide treatments significantly boosted grain production compared to controls, with the highest increase in yield (1673 kg ha^{-1}) occurring in treatments that included seed treatment with azoxystrobin foliar spray.

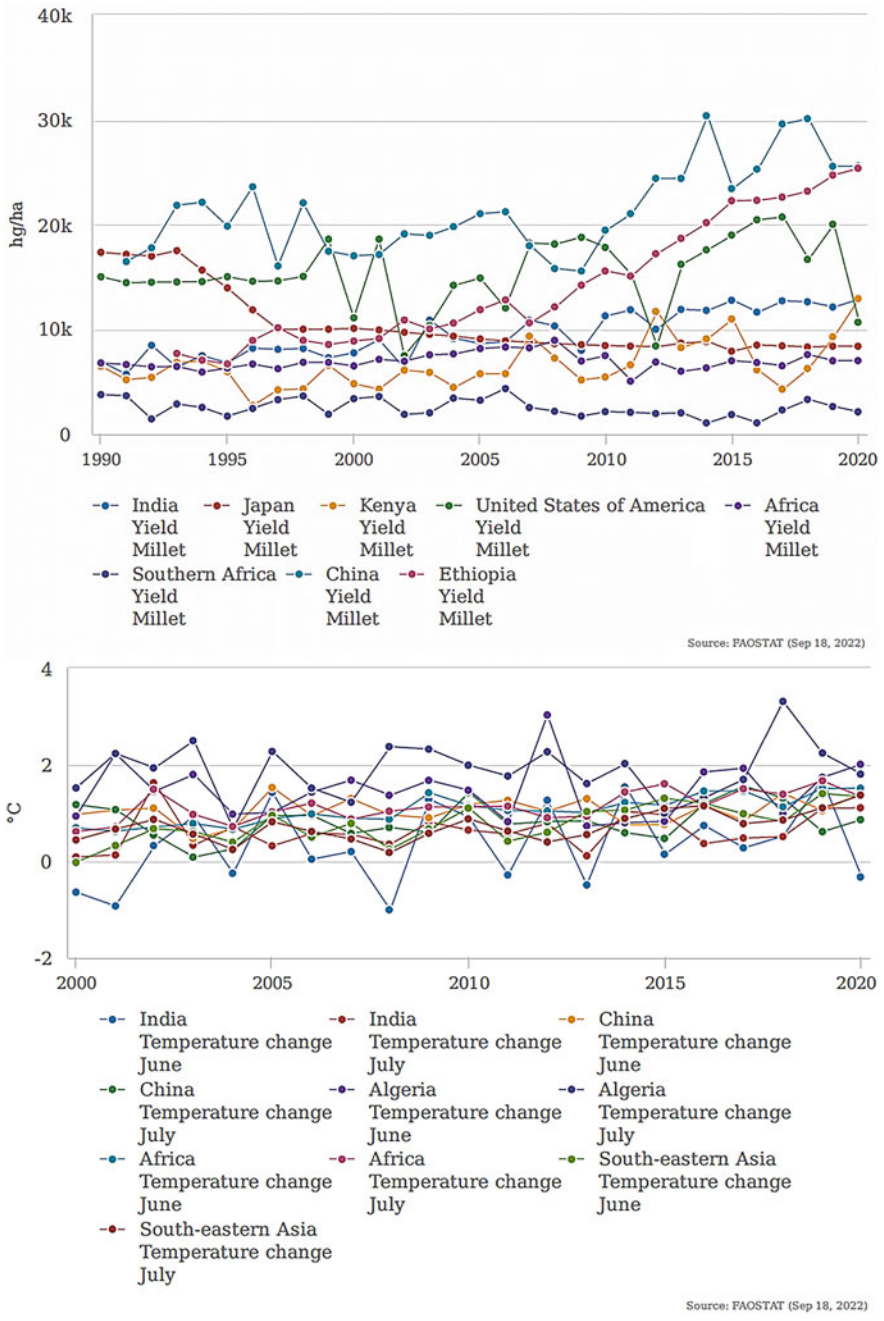

Fig. 13.1 Comparison between months of millet cultivation and production.https://www.fao.org/faostat/en/#compare

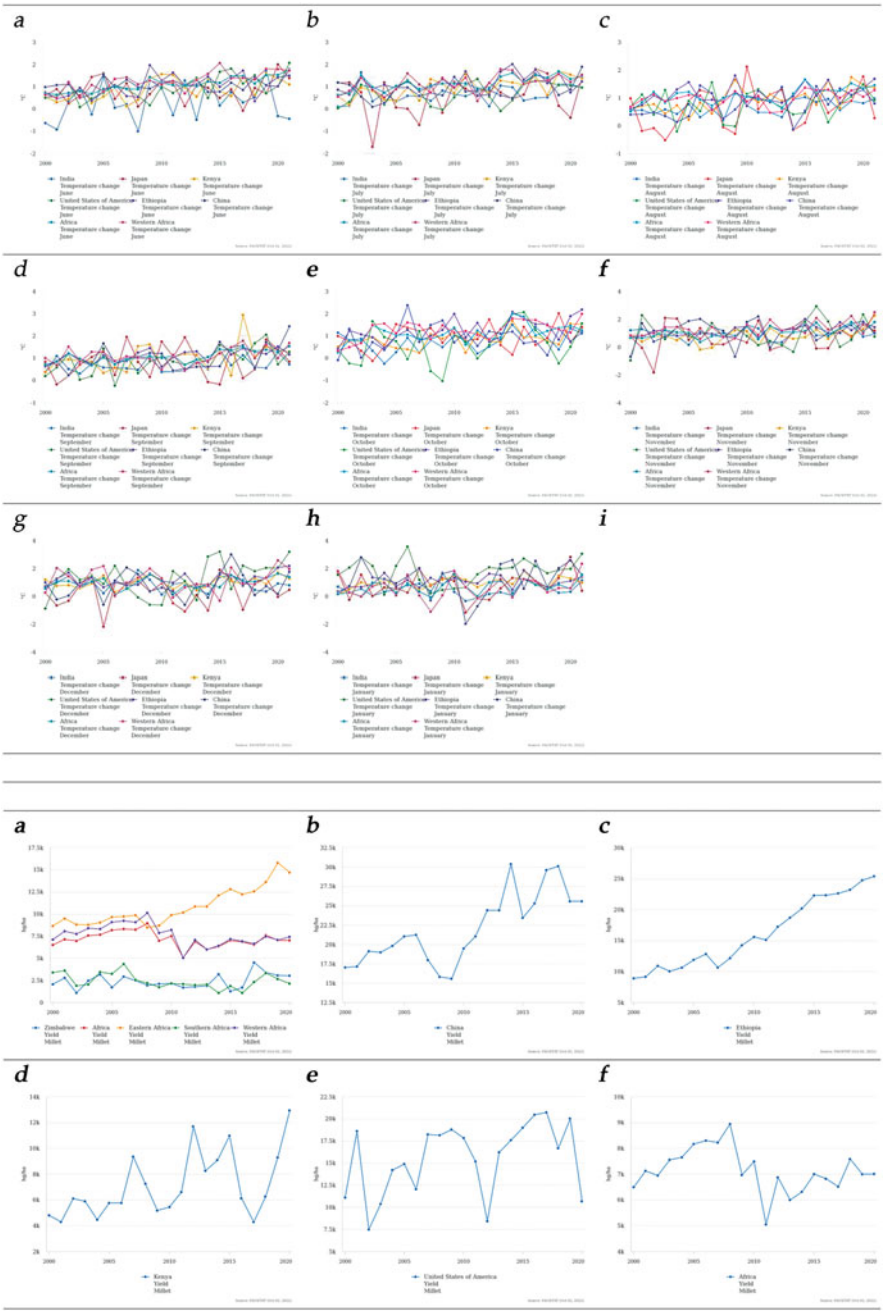

Fig. 13.2 Comparison between changes of temperature in production countries during production months of millet and their yield

Jogaiah et al. (2007) Studied the efficacy of cyazofamid against Sclerospora graminicola Schroet's pearl millet downy mildew disease. At 0.3 mg mL^{-1}, a significant reduction of sporangial sporulation, zoospore release, and motility was seen, as well as a significant fungicidal impact. Cyazofamid's effect on infected plants was studied via seed treatment followed by a single foliar spray. The combination of seed treatment and two foliar treatments performed much better than foliar application alone, with 10 mg mL^{-1} providing 97.9% disease control.

On the other hand, Akanmu et al. (2013) mentioned that *Manihot esculenta* and *Senna alata* at concentrations of 5, 10, and 15% g/mL were the two extracts that seemed to be the most effective, followed by *Moringa oleifera, to reduce fusarium wilt and root rot*. Therefore, the management approach focused on sustainable millet growth in Nigeria was validated by the efficiency of plant extracts against pathogenic Fusarium of pearl millet.

13.11 Inheritance of Disease Resistance

The world faces a huge problem with food, whether due to pollutants from heavy metals or global warming. With increasing population numbers and food scarcity, resources may one day decline resulting in famine, and new approaches to agriculture are necessary to provide as much food as possible to meet the needs of populations around the world. Hence, the role of biotechnology and genetically modified crops, which may help fill the shortage of food, and reduce losses resulting from diseases, whether fungal, bacterial, insect, and nematode, which cause a lack of production. With the development of technology day by day, comes the role of developing control methods to obtain high-quality crops that can face difficult environmental conditions and provide a safe treatment for diseases. On the other hand, artificial intelligence has begun to improve agriculture through mechanisms such as remote sensing techniques, hyperspectral imaging, and 3D laser scanning, which are necessary to develop agricultural standards on an area of thousands of acres. Soil management is essential to understanding diverse soil types and conditions, increasing crop production while conserving soil resources, and giving the expected profit and production to farmers. Genetic diversity is necessary for a population to adapt to changing conditions. To have increased the production of millet significantly. The research efforts in this field have been directed toward understanding the molecular mechanisms involved in the resistance to several abiotic and biotic stresses such as temperature, salinity, drought, and predators. These molecular studies have identified several key molecular components that confer resistance to pathogens. The first is a unique combination of proteins called R-groups which are found in all varieties of millet. The R-groups play a very important role in protecting the plant from stress caused by abiotic and biotic stresses. They also play a key role in the plant's resistance to pathogen attack. The second important molecular component is a group of proteins called defense response proteins (DPRs).

The most cost-effective, secure, and practicable technique of blast disease control (*Pyriculariagrisea*), one of the most serious diseases of finger millet, is through the host plant resistance (HPR) mechanism. To ascertain the blast resistance and

production efficiency of finger millet, four experiments were carried out at the Agricultural Training Centers (ATCs) at the Bomet, Koibatek, Nakuru, and Egerton University Research Stations (Jayo 2021). Also, Hanna and Wells (1989) found three genes of resistance to *pyriculariagrisea*(Cke)sarc. The leafspot of pearl millet was discovered in the weedy relative of pearl millet by Backcrossing and selection.

13.12 Conclusion and Economic for Management Application

Millet is a crop of global importance owing to its high yield and adaptation to challenging growing conditions. However, millet is susceptible to several plant diseases, which have a significant impact on production. Millet is one of the world's most significant crops for food security and economic growth. However, many people are concerned about its susceptibility to plant diseases. Recently, innovations in crop management have been made possible thanks to technological advances. These innovations have the potential to improve global food security and human health by eliminating or reducing the need for pesticides in millet production.

Number one, innovative strategies are useful for managing diseases because they are proactive. Disease management strategies used in millet production can be proactive because they can be used before diseases become an issue. For example, planting millet near corn and sorghum crops can help reduce the risk of bacterial wilt. It is also possible to use integrated pest management (IPM) techniques that combine different methods of controlling plant pests. By using these techniques early, producers can avoid damaging their crops or losing money when disease outbreaks occur. Producers who use these techniques can also earn money from selling the resulting harvest because their crops are in peak condition.

Another advantage of using innovative strategies is that they are less expensive than traditional methods of managing diseases. For example, incorporating livestock manure into millet fields reduces the need for chemical pesticides. This is beneficial because it requires less labor and resources than traditional methods such as crop rotation. It is also possible to use bio-controls such as *Bacillus thuringiensis* (Bt) and neem to control plant pathogens naturally – without the need for chemicals. The effectiveness of these bio-controls has been proven in many studies and is useful for reducing chemical usage while maintaining high production levels. Using innovative strategies can reduce the financial burden of managing diseases by allowing more resources to be allocated toward growing crops instead.

Although these strategies are useful for fighting plant diseases, many experts believe that these strategies cannot always be successful due to natural fluctuations in ecosystems. Many disease outbreaks are naturally occurring and cannot be controlled by humans. For example, blights kill millet plants without leaving visible marks on the plants themselves like aphids do when they feed on plants causing wilt. In addition, some diseases cannot be controlled by any method if they were not present at all times in the first place. For this reason, it is important to invest time in understanding your local ecosystems so that you can anticipate problems before they occur and implement appropriate solutions quickly enough to prevent damage or loss of yields.

Another disadvantage is that using innovative strategies requires more resources than traditional methods of disease management. When using these techniques, farmers must plan so that they can grow multiple crops at once and properly rotate fields between crops every year. This requires time and experience from farmers so that they can properly implement these techniques for maximum yield potential from their fields. The most important current solutions can be summarized as follows:

- One of the most effective ways to manage pathogens is by intercropping plants.
- Using bio-farming is another way to protect plants from pests and diseases.
- Using organic pesticides can damage the environment and become harmful to people's health.
- Creating a diverse environment: Growing diversified crops, such as intercropping millets with legumes, vegetables, or other cereals to enhance soil fertility, pest resistance, and crop resilience. Planting different types of plants creates symbiotic relationships between these plants. some plants can share nutrients and water through their roots, or attract pollinators and protectors with their flowers and fruits. Some plants can also host beneficial fungi or bacteria that help them absorb minerals or protect against diseases. These symbiotic relationships can improve the health and productivity of the plants, as well as the biodiversity and stability of the ecosystem

References

Akanmu AO, Abiala MA, Akanmu AM, Adedeji AD, Mudiaga PM, Odebode AC (2013) Plant extracts abated pathogenic fusarium species of millet seedlings. Arch Phytopathol Plant Protect 46(10):1189–1205

Babu TK, Thakur RP, Upadhyaya HD, Reddy PN, Sharma R, Girish AG, Sarma NDRK (2013) Resistance to blast (Magnaporthegrisea) in a mini-core collection of finger millet germplasm. Eur J Plant Pathol 135(2):299–311

Basavaraj GL, Murali M, Lavanya SN, Amruthesh KN (2019) Seed priming with biotic agents invokes defense response and enhances plant growth in pearl millet upon infection with Magnaporthegrisea. Biocatal Agric Biotechnol 21:101279

Bdliya BS, Muhammad AS (2007) Effect of inter-cropping millet with groundnut on the control of cercospora leaf spot of groundnut in The Sudan savanna of North-Eastern Nigeria. J Sustain Agric 29(2):19–41

Brankatschk G, Finkbeiner M (2017) Crop rotations and crop residues are relevant parameters for agricultural carbon footprints. Agron Sustain Dev 37(6). https://doi.org/10.1007/s13593-017-0464-4

Bybee-Finley KA, Ryan MR (2018) Advancing intercropping research and practices in industrialized agricultural landscapes. Agriculture (Switzerland). https://doi.org/10.3390/agriculture8060080

Chakraborty M, Islam T (2022) Antifungal secondary metabolites against blast fungus. In: Antifungal metabolites of rhizobacteria for sustainable agriculture. Springer, Cham, pp 23–51

Chakraborty M, Mahmud NU, Muzahid ANM, Rabby SF, Islam T (2020) Oligomycins inhibit Magnaportheoryzae Triticum and suppress wheat blast disease. PLoS One 15(8):e0233665

Chakraborty M, Mahmud NU, Ullah C, Rahman M, Islam T (2021) Biological and biorational management of blast diseases in cereals caused by Magnaportheoryzae. Crit Rev Biotechnol 41(7):994–1022

Chala A, Taye W, Ayalew A, Krska R, Sulyok M, Logrieco A (2014) Multimycotoxin analysis of sorghum (Sorghum bicolor L. Moench) and finger millet (Eleusine coracana L. Garten) from Ethiopia. Food Control 45:29–35

Chamberlain LA, Bolton ML, Cox MS, Suen G, Conley SP, Ané JM (2020) Crop rotation, but not cover crops, influenced soil bacterial community composition in a corn-soybean system in southern Wisconsin. Appl Soil Ecol 154. https://doi.org/10.1016/j.apsoil.2020.103603
Choi JH, Nah JY, Lee MJ, Jang JY, Lee T, Kim J (2021) Fusarium diversity and mycotoxin occurrence in proso millet in Korea. LWT 141:110964
Das IK, Nagaraja A, Tonapi VA (2016) Diseases of millets-a ready reckoner. 67pp.
De Waele D, McDonald AH, Jordaan EM, Orion D, Van den Berg E, Loots G (1998) Plant-parasitic nematodes associated with maize and pearl millet in Namibia. Afr Plant Protect 4(2):113–117
Elobu P, Adipala E (1993) Prevalence of finger millet diseases in Kaberamaido Subcounty, Soroti District, Uganda. Uganda J Agric Sci 1(1):13–19
Gashaw G, Alemu T, Tesfaye K (2014) Evaluation of disease incidence and severity and yield loss of finger millet varieties and mycelial growth inhibition of Pyriculariagrisea isolates using biological antagonists and fungicides in vitro condition. J Appl Biosci 73:5883–5901
Gessese MK (2019) Description of wheat rusts and their virulence variations determined through annual pathotype surveys and controlled multi-pathotype tests. Adv Agric 2019:1–7
Govind SR, Jogaiah S, Abdelrahman M, Shetty HS, Tran LSP (2016) Exogenous trehalose treatment enhances the activities of defense-related enzymes and triggers resistance against downy mildew disease of pearl millet. Front Plant Sci 7:1593
Goyal A, Manoharachary C (eds) (2014) Future challenges in crop protection against fungal pathogens. Springer, New York, p 364p
Gupta SC (1992) SADCC ICRISAT Sorghum and Millets Improvement Program. In: Integrated Agricultural Research: proceedings of the SACCAR/Winrock Workshop held in Lilongwe, Malawi, 26 Nov.–1 Dec. 1989. IDRC, Ottawa, ON, CA
Halbert SE, Baker CA (2015) Banana bunchy top virus and its vector Pentalonia nigronervosa (Hemiptera: Aphididae). Pathol Circ 417:1–7
Hanna WW, Wells HD (1989) Inheritance of Pyricularia leaf spot resistance in pearl millet. J Hered 80(2):145–147
Hong Y, Berentsen P, Heerink N, Shi M, van der Werf W (2019) The future of intercropping under growing resource scarcity and declining grain prices - a model analysis based on a case study in Northwest China. Agric Syst 176. https://doi.org/10.1016/j.agsy.2019.102661
Iqbal MA, Hamid A, Ahmad T, Siddiqui MH, Hussain I, Ali S et al (2019) Forage sorghum-legumes intercropping: effect on growth, yields, nutritional quality and economic returns. Bragantia 78(1):82–95. https://doi.org/10.1590/1678-4499.2017363
Jain AK (2009) Nematode pests of small millets—a review. Agric Rev 30(2):132–138
Javaid ARSHAD, Latif UMAIR, Akhtar N, Ahmed D, Perveen SHAGUFTA (2018) Molecular characterization of fusarium moniliforme and its management by methanolic extract of Coronopusdidymus. Pak J Bot 50(5):2069–2075
Jayo, T. M. (2021). Host plant resistance and characterization of blast disease (PyriculariaGrisea) in selected finger millet (Eleusine Coracana L.) genotypes in Kenya (Doctoral dissertation, Egerton University)
Jones et al (2021) Virus diseases of cereal and oilseed crops in Australia: current position and future challenges. Viruses. https://doi.org/10.3390/v13102051
Jogaiah S, Mitani S, KesturNagaraj A, HuntrikeShekar S (2007) Activity of cyazofamid against Sclerosporagraminicola, a downy mildew disease of pearl millet. Pest Manag Sci 63(7):722–727
Jurjevic Z, Wilson DM, Wilson JP, Geiser DM, Juba JH, Mubatanhema W et al (2005) Fusarium species of the Gibberellafujikuroi complex and fumonisin contamination of pearl millet and corn in Georgia, USA. Mycopathologia 159(3):401–406
Kiran K, Linguraju S, Adiver S (2006) Effect of plant extract on Sclerotium rolfsii, the incitant of stem rot of ground nut. J Mycol Plant Pathol 36(1):77–79
Kountche BA, Hash CT, Dodo H, Laoualy O, Sanogo MD, Timbeli A, Haussmann BI (2013) Development of a pearl millet Striga-resistant genepool: response to five cycles of recurrent selection under Striga-infested field conditions in West Africa. Field Crops Res 154:82–90
Kumar B, Singh KP (2010) Important small millets diseases in India and their management. Plant Pathology Section, College of Forestry and Hill Agriculture, Hill Campus, Ranichauri, TehriGarhwal, Uttarakhand.

Kumar B, Srivastava JN (2020) Barnyard millet/Japanese millet or Sawan (Echinochloafrumentacea L.)diseases and their management strategies. In: Diseases of field crops: diagnosis and management. Apple Academic Press, pp 195–204

Kumar PL, Cuervo M, Kreuze JF, Muller G, Kulkarni G, Kumari SG et al (2021) Phytosanitary interventions for safe global germplasm exchange and the prevention of transboundary pest spread: the role of CGIAR germplasm health units. Plan Theory 10(2):328

Lavanya SN, Niranjan-Raj S, Nayaka SC, Amruthesh KN (2017) Systemic protection against pearl millet downy mildew disease induced by cell wall glucan elicitors from Trichoderma hamatum UOM 13. J Plant Protect Res

Leslie JF, Zeller KA, Lamprecht SC, Rheeder JP, Marasas WF (2005) Toxicity, pathogenicity, and genetic differentiation of five species of fusarium from sorghum and millet. Phytopathology 95(3):275–283

Li ZY, Dong ZP, Wang N, Dong L, Bai H, Quan JZ, Liu L (2014) First report of foxtail millet seedling damping-off caused by binucleate rhizoctonia AG-A in China. Plant Dis 98(11): 1587–1587

Li J, Huang L, Zhang J, Coulter JA, Li L, Gan Y (2019) Diversifying crop rotation improves system robustness. Agron Sustain Dev 39(4). https://doi.org/10.1007/s13593-019-0584-0

Maitra S, Hossain A, Brestic M, Skalicky M, Ondrisik P, Gitari H et al (2021) Intercropping—a low input agricultural strategy for food and environmental security. Agronomy 11(2):343. https://doi.org/10.3390/agronomy11020343

Mbwaga AM, Mdolwa SI (1995) Diseases and parasitic weeds of pearl millet in Tanzania with emphasis on screening for ergot resistance. In: breeding for disease resistance with emphasis on durability. Proceedings of a regional workshop for eastern, central and southern Africa, held at Njoro, Kenya, October 2–6, 1994. (pp. 239–243). LandbouwuniversiteitWageningen (Wageningen Agricultural University)

Mgonja MA, Lenne JM, Manyasa E, Sreenivasaprasad S (eds.) (2007). Finger millet blast management in East Africa: creating opportunities for improving production and utilization of finger millet: proceedings of the first International finger millet stakeholder workshop, Nairobi (pp. 1–192). (International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324, AP, India) ISBN: 978–92–9066-505-2

Mtisi E, de Milliano WAJ (1993) False mildew on pearl millet and other hosts in Zimbabwe. East Afr Agric Forestry J 59(2):145–153

Murali M, Amruthesh KN (2015) Plant growth-promoting fungus Penicilliumoxalicum enhances plant growth and induces resistance in pearl millet against downy mildew disease. J Phytopathol 163(9):743–754

Murali M, Sudisha J, Amruthesh KN, Ito SI, Shetty HS (2013) Rhizosphere fungus Penicilliumchrysogenum promotes growth and induces defence-related genes and downy mildew disease resistance in pearl millet. Plant Biol 15(1):111–118

Mushonga JN (1983) Screening for ergot in pearl millet of five inbreds and one hybrid for a breeding programme. Zimbabwe Agric J 80(6):239–241

Nandini B, Hariprasad P, Prakash HS, Geetha N (2017a) Trichoderma oligosaccharides priming mediates resistance responses in pearl millet against downy mildew pathogen. J Appl Biol Biotechnol 5(2):97–103

Nandini B, Hariprasad P, Prakash HS, Shetty HS, Geetha N (2017b) Trichogenic-selenium nanoparticles enhance disease suppressive ability of Trichoderma against downy mildew disease caused by Sclerosporagraminicola in pearl millet. Sci Rep 7(1):1–11

Nandini B, Puttaswamy H, Prakash HS, Adhikari S, Jogaiah S, Nagaraja G (2019) Elicitation of novel trichogenic-lipid nanoemulsion signaling resistance against pearl millet downy mildew disease. Biomol Ther 10(1):25

Navi SS, Singh SD (1993) Fusarium longipes: a mycoparasite of Sclerosporagraminicola on pearl millet. Indian Phytopathol 46(4):365–368

Nirenberg HI, O'Donnell K (1998) New Fusarium species and combinations within the Gibberellafujikuroi species complex. Mycologia 90(3):434–458

Numan M, Serba DD, Ligaba-Osena A (2021) Alternative strategies for multi-stress tolerance and yield improvement in millets. Genes 12(5):739

Okigbo BN, Greenland DJ (1976) Intercropping systems in tropical Africa. Mult Crop 27:63–101. https://doi.org/10.2134/asaspecpub27.c5

Onyike NB, Nelson PE, Marasas WFO (1991) Fusarium species associated with millet grain from Nigeria, Lesotho, and Zimbabwe. Mycologia 83(6):708–712

Patil JV (ed) (2016) Millets and sorghum: biology and genetic improvement. John Wiley & Sons. 463 pp

Poonacha TT, Bhavana CS, Ramesh GV, Gavayi N, Koti PS, Palanna KB, Rajashekara H, Rajesh G, Das IK (2023) Blast disease of millets: present status and future perspectives. https://doi.org/10.5772/intechopen.111392

Ryley MJ, Persley DM, Jordan DR, Henzell RG (2002). Status of sorghum and pearl millet diseases in Australia. Sorghum and millets diseases, pp. 441–448.

Serba DD, Perumal R, Tesso TT, Min D (2017) Status of global pearl millet breeding programs and the way forward. Crop Sci 57(6):2891–2905. https://doi.org/10.2135/cropsci2016.11.0936

Seifers DL, Harvey TL, Kofoid KD, Stegmeier WD (1996) Natural infection of pearl millet and sorghum by wheat streak mosaic virus in Kansas. Plant Dis 80:179–180. https://www.apsnet.org/publications/PlantDisease/BackIssues/Documents/1996Abstracts/PD_80_179.htm

Sharma D, Jamra G, Singh UM, Sood S, Kumar A (2017) Calcium biofortification: three pronged molecular approaches for dissecting complex trait of calcium nutrition in finger millet (Eleusine coracana) for devising strategies of enrichment of food crops. Front Plant Sci 7:2028

Sharma R, Sharma S, Gate VL (2020) Tapping Pennisetum violaceum, a wild relative of pearl millet (Pennisetum glaucum), for resistance to blast (caused by Magnaporthe grisea) and rust (caused by Puccinia substriata var. indica). Plant Dis 104(5):1487–1491. https://doi.org/10.1094/PDIS-08-19-1602-RE

Sharma S, Sharma R, Govindaraj M, Mahala RS, Satyavathi CT, Srivastava RK et al (2021) Harnessing wild relatives of pearl millet for germplasm enhancement: challenges and opportunities. Crop Sci 61(1):177–200

Siddaiah CN, Prasanth KVH, Satyanarayana NR, Mudili V, Gupta VK, Kalagatur NK et al (2018) Chitosan nanoparticles having higher degree of acetylation induce resistance against pearl millet downy mildew through nitric oxide generation. Sci Rep 8(1):1–14

Steiner B, Buerstmayr M, Michel S, Schweiger W, Lemmens M, Buerstmayr H (2017) Breeding strategies and advances in line selection for Fusarium head blight resistance in wheat. Trop Plant Pathol 42:165–174

Sudisha J, Amruthesh KN, Deepak SA, Shetty NP, Sarosh BR, Shetty HS (2005) Comparative efficacy of strobilurin fungicides against downy mildew disease of pearl millet. Pestic Biochem Physiol 81(3):188–197

Thakur RP, Rao VP, Williams RJ, Chahal SS, Mathur SB, Pawar NB, Nafade SD, Shetty HS, Singh G, Bangar SG (1985) Identification of stable resistance to ergot in pearl millet. Plant Dis 69(11):982–985

Thakur RP, Singh SD, King SB (1988) Registration of four populations of pearl millet germplasm with multiple disease resistance. Crop Sci 28(2):381–382

Vismer HF, Shephard GS, Van der Westhuizen L, Mngqawa P, Bushula-Njah V, Leslie JF (2019) Mycotoxins produced by fusarium proliferatum and F. Pseudonygamai on maize, sorghum and pearl millet grains in vitro. Int J Food Microbiol 296:31–36

Advanced Biotechnological Tools for Genetic Improvement of Finger Millet

14

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Abstract

Growing human population demands a doubling of food production by 2050 necessitating significant increase in agricultural production, a challenge that is exacerbated by the changing climatic conditions. To tackle this, new lines of crops are continuously being identified, varieties bred, and transgenic technology developed, made possible by the deployment of modern molecular tools. Finger millet (*Eleusine coracana* (L.) Gaertn.) is a nutritious millet, assuming high significance as a rich source of good quality protein, micro-nutrients like calcium and iron and fiber, apart from being drought-hardy. This crop has spectacular adaptive abilities, and the grain has superior keeping quality. The plethora of micronutrients consisting of an abundance of calcium and moderately high contents of iron, manganese, and phosphorus that are present in this grain in comparison to other cereals makes it a promising crop for nutritional studies. The crop's status as an "orphan millet" has been remodelled by the recent attempts on genome sequencing and various transcriptomics studies in the crop making the time ripe for harnessing this information for genomic and functional genomic applications. The nutritional supremacy and extreme adaptability make the crop an interesting candidate for nutri-genomics and climate resilience studies. Modern genetic tools will aid in unravelling the untapped gene pool of the crop which might contribute towards agronomic and nutritional improvement of other crops. This chapter discusses the advancements in the areas of molecular marker

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technology, gene mapping, NGS based genomics, and modern breeding tools and genetic transformation in finger millet and how these tools are accelerating crop improvement.

Keywords

Finger millet · Ragi · Genotyping · SSRs · SNP · Genome · Transcriptome · Transgenics

14.1 Introduction

Finger millet (*Eleusine coracana* (L.) Gaertn.) is a nutritious millet that offers good quality protein, micro-nutrients like calcium, manganese, and iron as well as dietary fiber and antioxidants, apart from being drought-hardy. The crop has recently assumed high significance as a promising nutri-cereal for the future even in the policy level. Finger millet or ragi (*Eleusine coracana*) is an allotetrapoloid species ($2n = 4x = 36$) belonging to the genus *Eleusine* grown primarily in semi-arid regions of India, Africa, Nepal, and South America. The genus *Eleusine* is divided into two subspecies namely subsp. *coracana* (that includes cultivated finger millet) and subsp. *africana*. This crop has its origin from the diversity center of East Africa (Bisht and Mukai 2002). *E. africana* is proposed as the wild progenitor of this species. Domestication of finger millet is believed to have happened approximately 5000 years ago in Africa and was introduced to India around 2000 years from then. India is regarded as a secondary center of diversity of this crop. In India it is commonly known as ragi, mandua, nachni etc. The crop is an allotetraploid (AABB) and while the diploid species *E. indica* is identified as the A genome progenitor (maternal genome donor), B genome contributor is believed to be extinct and is unknown (Hilu 1988; Bisht and Mukai 2001; Liu et al. 2014). The genome *Eleusine* has around eight species that exists, namely *E. africana*, *E. coracana*, *E. kigeziensis*, *E. indica*, *E. floccifolia*, *E. intermedia*, *E. multiflora*, and *E. jaegeri* out of which *E. coracana* is the only domesticated species.

Finger millet has spectacular adaptive abilities and thrives well from the arid plains in India to up to 2300 m elevation in the Himalayas. Finger millet grains are one of the richest vegetative sources of calcium and it has moderately high contents of iron, manganese, and phosphorus in comparison to other cereals. Its grains are rich in protein (7–8%) and its methionine rich storage protein possesses high biological value and superior quality. With its rich nutrient profile, it is considered as a promising solution for malnutrition and hidden hunger. The grains are rich in dietary fiber and has antioxidant, anti-diabetic, and anti-microbial properties. Its straw serves as an important cattle fodder and grain malt, a nutritious baby food and the grain has superior keeping quality. Finger millet is regarded as a stress hardy crop and can withstand drought and salinity to a greater extent than many other crops. The crop has good survival ability at high temperatures making it an ideal crop for changing climatic conditions. The valuable and unique stress tolerant genes and

gene networks harbored by finger millet need to be explored for utilization in other crops and in finger millet itself. Altogether finger millet makes a wise component of sustainable agriculture for the future generations owing to its inherent ability to thrive well in marginal lands with poor nutrient levels and ability to withstand frequent droughts.

Finger millet so far was considered as an orphan crop having only local significance and lacked well documented scientific information. There was scarcity of germplasm and genetic resources as well as genomic information in comparison to other field crops. The crop is highly self-pollinated with tiny flowers and emasculation of small flowers is often injurious to floral parts. In addition, the crop has only a narrow genetic base of germplasm all of which were impediments in breeding improvement in this crop. Molecular marker technology has revolutionized breeding programs through various tools such as molecular markers, QTL mapping, association mapping, and so on. Molecular tools help in identifying genomic variation and marker-trait association more efficiently and they have become valuable tools in crop improvement. This chapter covers the recent advances in the application of biotechnological tools in this crop and the impact that they had brought in.

14.2 Advances in Molecular Marker Studies

One of the ways to enhance plant productivity is to expand the genetic base by introducing genes from the germplasm pool. Molecular tools will expedite the process and help in developing superior varieties. To make utilization of the germplasm resources manageable and more effective, a "core subset" of finger millet germplasm accessions was developed (Upadhyaya et al. 2006) just like in sorghum, barley maize, wheat etc. Based on the geographical origin and data on 14 quantitative traits, 622 accessions were selected for core subset generation from a total of 5940 global collection of accessions available in ICRISAT, Patancheru. The representation of the five races and the types of genotypes (improved cultivars, breeding materials, landraces, and wild types) were in similar proportion in the core subset as in the entire collection. Much of the diversity of the entire set has been captured in the core, as substantiated by various statistical analyses, and they can be used as a good starting point for any research programs. From this core collection, a "minicore" comprising of 80 accessions was developed later based on multi-location evaluation data of the core collection (Upadhyaya et al. 2010). It was ensured that the minicore captured the entire diversity of the core collection and it serves as an ideal pool of diverse germplasm for exploring new sources of variation and thereby enhancing the genetic potential of the crop.

Development of a genetic map is a steppingstone in any crop improvement program. The first finger millet genetic map was constructed using RFLP, AFLP, EST, and SSR markers (Dida et al. 2007). The map was constructed in an F_2 population raised from a cross between *E. coracana* subsp. *africana* (accession MD-20) and *E. coracana* subsp. *coracana* cv. Okhale-1 and the population consisted of 151 progenies. They developed genomic SSRs by isolating di- and

trinucleotide SSRs from genomic libraries of finger millet. The first genetic map covered all 18 finger millet chromosomes, at least partially. In addition, to be used for marker assisted selection, a set of 82 SSR markers was also developed and some of them were mapped. A finger millet-rice comparative map analysis was carried out to create a better finger millet genetic map and interestingly gene orders between rice and finger millet were found to be highly conserved (Srinivasachary et al. 2007). Linkage groups in finger millet had high collinearity to respective rice chromosomes (Srinivasachary et al. 2007). Table 14.1. gives the important marker systems used in finger millet for various purposes.

An essential part of germplasm conservation and utilization in breeding programs is genetic variability studies. Three different marker systems namely RFLP, RAPD, and ISSR were employed for the analysis of 22 accessions of 5 different species of *Eleusine* (Salimath et al. 1995). The study revealed a very low level of DNA sequence variability in finger millet and among the three, ISSR was identified as the most promising marker system for diversity analysis. Later, RAPD was successfully utilized to understand the genetic variability and relationships between various genotypes in finger millet (Das et al. 2007; Fakrudin et al. 2007; Babu et al. 2007). A variety of 18 RAPD primers, 10 SSR primers, and 10 pairs of cytochrome P450 gene-based markers were employed for a trait based comparative evaluation of genetic diversity in finger millet genotypes collected from different districts of Uttarakhand where the trait considered was calcium content. Although all these marker systems were effective in identifying polymorphism, SSR gave the maximum Polymorphism Information Content (PIC) of 0.505 (Panwar et al. 2010). Using these markers, genotypes could be effectively grouped into high, medium and low calcium categories. Genome-wide association mapping identified 16 significant associations between 13 SSR markers and six agronomic traits in 138 Ethiopian and exotic accessions (Lule et al. 2018). Pandian et al. (2018) developed 56 new genic SSRs from drought responsive ESTs and used them in population structure and genetic diversity analysis.

Appraisal of relationships at the phylogenetic level is important as wild relatives often are repositories of several valuable untapped traits. The first phylogenetic relationship study in finger millet used species-specific chloroplast deoxyribonucleic acid (cpDNA) polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) and chloroplast simple sequence repeat (cpSSR) markers/sequences (Agrawal et al. 2013). This study also helped in re-affirming the hypothesis that *E. indica* is the maternal parent of *E. coracana*.

Blast disease caused by the fungus, *Magnaporthe grisea,* is the major limiting factor for finger millet production worldwide. It causes huge financial losses when the fungus affects the economically important grains by infecting neck and fingers. Before finger millet genome was sequenced, efforts were underway in gathering information available in rice and other grass species where blast was a serious problem and utilizing the information in identifying disease resistant genes or resistance related markers in finger millet. Finger millet germplasm for blast disease showed a continuous variation in resistance trait indicating a quantitative gene control of resistance. A comparative genomics analysis for blast resistance was

Table 14.1 Summary of molecular markers developed and utilized for finger millet crop improvement

Marker system used (number of markers used in brackets)	Objective of the study	Reference
cpDNA-RFLP	Phylogenetic relationships between subspecies of *E. coracana*	Hilu (1988)
Nuclear ITS and plastid *trn*T-*trn*F	Phylogenetic relationships in the genus *Eleusine*	Neves et al. 2005
RAPD (13)	Genetic variability study	Das et al. (2007)
1st genetic map using RFLP, AFLP, SSR (82 SSRs developed from finger millet).	1st genetic map using RFLP, AFLP, EST and SSRs. This map spans 721 cM on the A genome and 787 cM on the B genome and covers all 18 finger millet chromosomes.	Dida et al. (2007)
SSR (45)	Phylogenetic study and population structure analysis	Dida et al. (2008)
RAPD (18), SSR (10), cytochrome P450 based markers (10 pairs)	Study on genetic diversity in finger millet with respect to calcium content	Panwar et al. (2010)
cpPCR-RFLP and cpSSR (8 numbers)	Phylogenetic relationship between finger millet and its wild relatives by species-specific chloroplast deoxyribonucleic acid (cpDNA) polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) and chloroplast simple sequence repeat (cpSSR) markers/sequences for the first time	Agrawal et al. (2013)
Functional SSRs (58) and SSRs (104)	Blast resistance in finger millet (comparative genomics and association mapping)	Babu et al. (2014a)
Genomic SSRs (46)	Association mapping for agronomic traits	Babu et al. (2014b)
EST-SSRs (146)	Developed from Ca^{2+} transporters and sensors of rice and sorghum and studied their cross-transferability and polymorphism in finger millet	Yadav et al. 2014
Anchored SSRs (23) (designed from calcium transporters and sensors)	Association mapping for calcium content	Kumar et al. (2015)
SSRs (23)	Association study in 340 accessions from Kenya, Tanzania, and Uganda for 5 qualitative traits	Manyasa et al. (2015)
SSRs (101) SNPs (92)	Development of SSRs and SNPs using NGS technologies	Gimode et al. (2016)
SRAP (12) SSR (12)	Genetic diversity assessment	Saha et al. 2016
SSRs (87)	Association mapping for agronomic traits including leaf blast	Ramakrishnan et al. (2016)

(continued)

Table 14.1 (continued)

Marker system used (number of markers used in brackets)	Objective of the study	Reference
SSRs (72)	Association mapping for phosphorus starvation response	Ramakrishnan et al. (2017)
SNP (109; developed from GBS data)	Association mapping to identify reliable marker(s) linked to grain yield and its component traits	Sharma et al. (2018)
SSR (20)	Association mapping in Ethiopian finger millet accessions for agronomic traits	Lule et al. (2018)
EST-SSR (56 developed from drought related ESTs)	Population structure and genetic diversity study	Pandian et al. (2018)
RAPD (28) ISSR (20) SSR (101)	Hybrid detection using molecular markers	Krishna et al. (2020)

carried out by Babu et al. (2014a) using 190 genotypes and 58 functional SSRs developed from blast resistance genes of rice/finger millet. Half of the markers were polymorphic, and they yielded 65 scorable alleles with a mean of 2.4 alleles per marker. Association mapping analysis was also carried out using the phenotypic data (leaf blast, neck blast, finger blast) and the genotypic data of 104 microsatellite markers spread across the chromosomes. The association of SSR marker data with the leaf blast, neck blast, and finger blast data identified four significant QTLs for finger blast and one for neck blast. This is the first report in the development of functional SSR markers for finger millet blast resistance genes. A few of the Nucleotide Binding Site (NBS) Leucine-rich Repeat (LRR) resistance (R) genes were cloned from finger millet and were utilized in developing molecular markers linked to resistance (Panwar et al. 2011). Genetic diversity analysis of 190 global finger millet accessions for blast resistance using 58 genic functional SSR markers identified a few markers that could effectively differentiate the genotypes based on their response to blast pathogen (Babu et al. 2014a). Association mapping has identified some QTLs for blast resistance using a few genomic and genic SSR markers (Babu et al. 2014b; Ramakrishnan et al. 2016). Evaluation of 128 genotypes using 87 genomic SSRs along with phenotyping for agronomic traits identified 7 QTLs associated with agronomic traits including leaf blast (Ramakrishnan et al. 2016). Putative candidate genes associated with these QTLs were identified through cross-species validation. The association of SSR marker data with leaf blast, neck blast, and finger blast data identified five significant QTLs for finger blast and neck blast which are efficient tools for cloning resistant genes and candidates for transgenic development. Various marker systems like SRAP and SSRs were also used to know the extent of genetic polymorphism between resistant and susceptible genotypes to select diverse parents for use in breeding programs (Saha et al. 2016).

Finger millet grains are rich sources of protein and are exceptionally rich in Calcium which is almost five times of that in wheat and ten times of that in rice. Nirgude et al. 2014 developed 36 EST-SSR primers for the opaque2 modifiers and

20 anchored-SSR primers for calcium transporters and calmodulin for analysis of the genetic diversity of 103 finger millet genotypes for grain protein and calcium contents. The opaque2 modifiers specific EST-SSRs could differentiate finger millet genotypes into high, medium, and low protein containing genotypes. However, calcium-dependent candidate gene-based EST-SSRs could only broadly differentiate the genotypes based on the calcium content with a few exceptions. Finger millet being a heavy accumulator of calcium and since its genome was not sequenced, Yadav et al. (2014) explored the possibility of using microsatellite markers designed from calcium signalling and transport genes of rice and sorghum for assessing cross-transferability among grass species. Primers were designed for around 146 genic SSR markers which showed on an average 68% cross transferability to finger millet but failed to generate polymorphism between genotypes, may be indicating the highly conserved behavior of calcium related genes in plants. In a similar line, 23 anchored SSR markers were designed specifically from calcium transporters and sensors to be used in association mapping of finger millet accessions with high or low seed calcium content (Kumar et al. 2015a). They identified nine marker-trait associations related with calcium content that explained up to 41% of the total variation in calcium content. Association mapping approach identified putative QTLs for seedling stage phosphorus starvation response in finger millet (Ramakrishnan et al. 2017).

Many EST-SSRs and SNPs loci belonging to genes involved in calcium transport and sensing were identified from the spike transcriptome data which could be valuable tools for mapping, marker-assisted breeding, and comparative genomics studies in finger millet (Kumar et al. 2015b). As these are gene specific markers, they can be ideal for genotyping finger millet lines for seed calcium and protein traits. SNPs are much more effective than SSRs in revealing the genetic diversity among genotypes. With the advances in Next Generation Sequencing (NGS) technologies, SNP discovery has been more efficient. Around 10,327 SSRs and 23,285 non-homologous SNPs were identified from two finger millet genotypes (Gimode et al. 2016) of which 101 SSRs and 92 SNPs were tested across germplasm for polymorphism. The polymorphic markers gave a mean polymorphism information content (PIC) of 0.42 for the SSRs and 0.29 for SNPs. One hundred and nine SNPs generated through genotyping by sequencing (GBS) were used for association mapping to identify reliable marker(s) linked to grain yield and its component traits (Sharma et al. 2018).

Being a highly self-pollinated plant with tiny flowers and floral parts, makes crossing as well as identification of true hybrids tedious in finger millet. Krishna et al. (2020) used molecular markers for the assessment of the genetic purity of F_1 hybrids. RAPD, ISSR, and SSR markers were used in assessing parental polymorphism in a reciprocal cross between PR202 and IE2606. This study found that molecular markers are effective to compare the efficiency of emasculation methods and in identification of true hybrids in the early stages of seedling growth.

14.3 Progress in Identification and Functional Analysis of Candidate Genes

Ragi being a stress hardy crop is a prospective candidate for isolation of genes governing stress tolerance. These genes, so identified, could be recruited as potential candidates for crop improvement through transgenesis, not only in ragi accessions, but even in distant species, also. Identification and characterization of a stress responsive transcription factor, *EcNAC1,* from finger millet was such a step. Its enhanced expression under low moisture and salinity stress imparted resistance to these stresses in transgenic tobacco plants proving its applicability (Ramegowda et al. 2012). A salinity responsive NAC transcription factor gene from finger millet, EcNAC67, was introduced to rice through agrobacterium mediated transformation and transgenic lines had enhanced tolerance against drought and salinity and maintained a higher relative water content during drought and displayed lesser drought-induced spikelet sterility (Rahman et al. 2016). Transgenic expression of *EcGBF3* (G-box Binding Factor) in *Arabidopsis* imparted enhanced tolerance to abiotic stresses like drought, high salinity, and osmotic stress (Ramegowda et al. 2017). EcbZIP17 transcription factor overexpressed transgenic tobacco plants had better vegetative growth and seed yield and were tolerant to many abiotic stresses by modulating ER (Endoplasmic Reticulum) pathways (Ramakrishna et al. 2018).

Another transcription factor, Ec*Dof* (DNA binding with one finger) from *Eleusine coracana,* was identified as the key regulators of protein accumulation in the grains of ragi even at zero nitrogen input (Gupta et al. 2011, 2012). The ratio of the two transcripts *Dof1/Dof2* was also identified as a pointer to the NUE (Nitrogen Use Efficiency) of crops (Gupta et al. 2014). *Dof1* also consistently expressed itself in high grain protein ragi genotypes along with light inducible genes involved in carbon metabolism and can even be used as a biomarker of traits like grain protein content and yield (Kanwal et al. 2014). Amplification of five genes namely, *EcHNRT2*, *EcLNRT1*, *EcNADH-NR*, *EcGS*, and *EcFd-GOGAT* involved in nitrate uptake and assimilation helped in understanding the molecular basis of high nitrogen use efficiency of finger millet (Gupta et al. 2013). Candidate gene approach also elucidated how light regulated the expression of genes involved in photosynthesis and carbon metabolism in two finger millet genotypes differing in grain protein content (Kanwal et al. 2014). Co-expression pattern of seven carbon metabolism related genes and a transcription factor, *Dof1*, showed that *Dof1* differentially regulated the carbon metabolism genes and thereby controlled grain protein content in finger millet genotypes. OPAQUE2 transcription factor gene was characterized in finger millet (EcO_2) (Gaur et al. 2018) and its expression levels were found different in genotypes that differed in their seed protein content. Depending on the nitrogen status in soil and thereby in plant, nitrogen responsive regulatory elements modulated EcO_2 expression and in turn regulated seed protein status. Four *Pht* genes (inorganic phosphate transporter) were also identified from finger millet which helps in phosphate acquisition especially in symbiotic relationship with arbuscular mycorrhizae (Pudake et al. 2017). One of the genes, *EcPT1-4,* was consistent in its expression levels even under Pi (inorganic phospohorus) stress in

seedlings showing promise in utilizing them in enhancing the phosphorus use efficiency of the crop.

From among the many genes involved in calcium absorption and transport in the plant, a calmodulin gene that is strongly expressed during grain development was cloned and characterized at the molecular and protein level and the study could infer that a high expression of this gene might cause enhanced calcium accumulation in the grains (Nath et al. 2010; Kumar et al. 2014). Yet another gene involved in calcium metabolism, *EcCIPK24* was cloned and characterized (Chinchole et al. 2017). It is a calcium sensor gene having a role in calcium transport regulation by binding with a calcium exchanger, *CAX*. Based on the expression studies in two contrasting finger millet genotypes for calcium content and in silico study, *EcCIPK24* was found activating *EcCAX1b* protein and thereby playing an important role in high seed Ca^{2+} accumulation.

Finger millet is known to be tolerant to abiotic stress such as heat and drought and the molecular machinery behind it needs to be worked out completely. A stress responsive *bHLH* (basic helix-loop-helix) transcription factor gene was isolated and characterized in finger millet whose transcript was induced by ABA, NaCl, PEG, methyl viologen, and drought stress (Babitha et al. 2015). An abiotic-stress responsive protein kinase, EcCIPK31-like, was also cloned and characterized which was upregulated in multiple stress conditions such as salinity, desiccation, oxidative, and high temperature stress as well as drought (Nagarjuna et al. 2016). Overexpression of *EcbHLH57* in tobacco imparted tolerance to salinity and drought stress and the transgenic plants showed improved root growth, higher photosynthetic rate, and altered stomatal conductance under stress as well as enhanced the expression of several stress responsive genes including antioxidant genes. Antioxidant genes of finger millet were also shown to be involved in controlling blast fungus growth in finger millet plants (Jacob et al. 2019). Differential expression of these genes was found to determine the extent of ROS (reactive oxygen species) accumulation in the tissues and compatibility of a plant-pathogen interaction deciding the fate of the plant, whether to be resistant or susceptible. Candidate gene identification and characterization has remarkable impact on trait characterization and improvement through breeding. As in many studies described above, many genes from finger millet when expressed in other species, imparted tolerance to various kinds of stresses implying cross-species utility of candidate genes.

14.4 Progress in Genome Sequencing

Advances in NGS (Next generation Sequencing) techniques have facilitated whole genome sequencing (WGS) of many plant species starting from Arabidopsis, the first plant whose genome was completely sequenced (Kaul et al. 2000). Finger millet was considered as an orphan crop genomically till recently and genetic as well as genomic studies were missing in the crop due to non-availability of sufficient genome sequence information. Even when genome of other millets like sorghum and foxtail millet have been sequenced, there wasn't much progress in finger millet

genomics which was an impediment in breeding and crop improvement efforts in the crop. The polyploidy of finger millet was another hindrance in its genome sequencing and annotation efforts. Genome size in finger millet is around 1500 Mb. First de novo genome assembly of finger millet was reported in 2017 in a short duration, high yielding, and drought tolerant ML-365 variety (Hittalmani et al. 2017). It was accomplished using a combination of Illumina and SOLiD sequencing technologies. There were 525,759 scaffolds of ~200 bp length with an N50 value of around 24 kb. A total of 85,000 genes were predicted from the sequenced data, majority of which could be functionally annotated. It had shared gene families with other major grass species and had highly conserved genomic regions with these species. Collinearity of finger millet genome was highest with that of foxtail millet and rice, followed by sorghum, and maize. Synteny relationships of finger millet will help in comparative genomics studies and in utilizing the QTLs and genes of interest from related plant species. Like most other plant genomes, finger millet genome also contained repetitive DNA elements, which accounted for ~50% of the total genome dominated by LTRs.

In an attempt to overcome the issues of polyploid genome sequencing and to develop a high-quality assembled genome, Hatakeyama et al. (2018) sequenced finger millet genome using diverse technologies with sufficient coverage and assembled it using a multiple hybrid assembly workflow. The high yielding national check from India, that is resilient to high heat and drought, PR202 was sequenced, and the sequenced genome was 1.2 Gb in size with around 63,000 predicted genes. They observed a significant number of single-copy genes which was not expected considering the tetraploid status of the crop. This may indicate that polyploidization happened prior to domestication and many of the duplicated genes might be lost. Sequenced genome had 2387 scaffolds having an N50 value >2.5 Mb. Genome sequence information generated in finger millet is expected to expedite identification of many SSRs, SNPs, candidate genes, alleles, and molecular breeding programs in general. Having the genomic information enables us to understand the molecular and genetic basis of traits better. The sequence information of finger millet can be accessed at https://phytozome-next.jgi.doe.gov/info/Ecoracana_v1_1.

14.5 Advances in Transcriptomics, Proteomics, and Metabolomics

High throughput techniques have enabled the development and utilization of multiple omics modules in plant biology research. Functional genomics involving multiple omics techniques have enabled the dissection of traits and their understanding more effective. There have been a few omics based trait characterization in finger millet lately and Table. 14.2 lists the major omics studies undertaken in the crop.

The next-generation sequencing platform has been used in finger millet in a genome-wide transcriptional analysis of two finger millet genotypes differing in their level of salinity tolerance (Rahman et al. 2014). The leaf transcripts were sequenced, and reads were mapped and annotated against rice gene models leading

Table 14.2 List of omics studies in finger millet

Trait under study	Methodology	References
Drought stress	Transcriptomics	Hittalmani et al. (2017)
Differential calcium accumulation in spikes	Transcriptomics	Singh et al. (2014)
Drought stress	Transcriptomics	Parvathi et al. (2019)
CAMPTA (calmodulin-binding transcription activator) transcription factor family identification	Using publicly available transcriptome data	(Kadri et al. 2022
Salinity stress	Transcriptomics	(Rahman et al. 2014)
Nutrient transport pathways (N, ammonia, phosphorus, sulfur and micronutrient pathways)	In silico analysis	Maharajan et al. (2022)
Phylogenetic relationship between six species in *Eleusine* genus	Illumina Hiseq transcriptome sequencing	Zhang et al. (2019)
Ascorbate-glutathione pathway (antioxidant pathway)	Transcriptomics	Avashthi et al. (2018)
Grain-filling stage transcriptome of contrasting finger millet genotypes differing in grain calcium and protein content.	Illumina transcriptomics	Kumar et al. (2015)
Characterization of oxalic acid biosynthetic pathway in finger millet spikes	Illumina HiSeq	Akbar et al. (2018)
Identification and characterization of calcium transporter gene family	Illumina paired end sequencing	Singh et al. (2015)
Drought and rehydration	Global transcriptome & proteome	Li et al. (2021)
Calreticulin identification	Nano LC-MS Peptide mass finger printing	Singh et al. (2016)
Osmotic stress alleviation through silicon amendment	Transcriptomics and metabolomics	Mundada et al. (2021)

to the identification of several useful candidate salinity responsive genes. Two genotypes differing in their sensitivity to salinity were used (tolerant "Trichy" and sensitive "CO12") and the study found groups of genes like transcription factors, transporters, aquaporins, genes involved in osmotic homeostasis and biosynthesis of compatible solutes to be upregulated in the tolerant genotype. This study helped in the identification of various salinity responsive transcription factors and signalling elements in finger millet.

Drought responsive genes were identified from ML-365 genotype of finger millet using transcriptomics approach (Hittalmani et al. 2017). The transcripts for ATP-binding and zinc ion types were plenty and transcripts coding for membrane integral components were found to be enriched in the cellular and biological process component. The upregulated genes in low moisture conditions included protein

kinases and phosphatases, Myb-like protein binding and zinc binding genes, pectinacetylesterase, protein tyrosine kinase, and late embryogenesis abundant proteins. Cytochrome P450, NB-ARC, UDP-glucoronosyl, and UDP-glucosyl transferase proteins were among downregulated genes. In a later study using GPU-28, several pathways were found to be regulated under water stress in finger millet (Parvathi et al. 2019). Several drought stress signalling genes were found to act in the process including serine threonine protein phosphatase 2A (PP2A), calcineurin B-like interacting protein kinase31 (CIPK31), farnesyl pyrophosphate synthase (FPS), signal recognition particle receptor α (SRPR α) etc. Interestingly, basal regulatory genes like TATA-binding protein associated factors were also responsive to drought indicating the crucial involvement of housekeeping genes too in stress regulation. In a combined transcriptome and proteome study (Li et al. 2021), several pathways associated with photosynthesis, response to water stress, translation, ribosome process, and carbon metabolism. Significantly differentially expressed proteins belonged to glycosyl hydrolase family 17 (GHL17), the thaumatin family, aquaporins, glutathione sulpha-transferase and peroxidase gene families, all of which are involved in various abiotic stress responses.

Finger millet is a treasure trove of calcium. It is one of the richest plant-based sources of calcium in nature. The exceptionally high calcium accumulation in ragi grains is intriguing as it is almost 10 to 20 times more than that in other cereals and millets. Hence it is interesting to find out the exact function of this mineral in this crop. Calcium uptake and transport are genetically and epigenetically regulated traits and there are various transporters and calcium sensor proteins that are involved in calcium content regulation in plants. One of the earliest studies in this line used transcriptome sequencing of spike tissue in two genotypes of finger millet (GP-1 and GP-45) differing in their calcium content (Singh et al. 2014). They identified and annotated calcium sensor gene families using the transcript data and were classified in to eight calcium sensor gene families such as CaM, CaMLs, CBLs, CIPKs, CRKs, PEPRKs, CDPKs, CaMKs, and CCaMK. There was differential expression of the genes belonging to these classes and most of them belonged to stress adaptation, hormonal changes, and biotic stress, mostly pathogen resistance. Differential expression of a few chosen genes (Ca^{2+}/H^{+} antiporter (CAX1), two pore channel (TPC1), CaM-stimulated type IIB Ca^{2+} ATPase and two CaM dependent protein kinase (CaMK1 and 2) homologs) involved in calcium metabolism was studied in the same genotypes (Mirza et al. 2014) and for most of the genes the high accumulating genotype had a higher expression. Kumar et al. (2015b) reported complete grain-filling stage transcriptome of GP-1 and GP-45, two contrasting genotypes for grain calcium and protein content. This study helped in identifying the genes responsible for high grain calcium and protein content. Several gene families involved in calcium transport and signalling such as calcium channel, calcium ATPase, calcium exchanger (CaX), calcium-dependent protein kinase (CDPKs), and calcium-binding proteins (CBPs) during grain development were identified. Transcripts and expression levels for main storage proteins like prolamin, globulin, gliadin, kafirin, albumin, glutemin, and legumin were also identified. The contrasting genotypes were

found to express many of the gene family members differentially and the information will be of great help in breeding programs aiming at biofortification in the crop.

Through transcriptomics, an extensive analysis of Ca^{2+} transporter gene families in relation to grain calcium was done from the developing spikes of genotypes contrasting for their grain calcium content (Singh et al. 2015). A total of 19 transporter genes were identified and a high correlation was established between the expression of *EcCAX3* gene, a Ca^{2+}/H^{+} exchanger, and the amount of calcium accumulated in spike. Anti-nutritional compounds such as oxalates are reported in finger millet that will adversely affect calcium availability and absorption by humans. Developing spike transcriptomics was employed to study the molecular mechanism of high calcium accumulation in line with oxalate metabolism (Akbar et al. 2018). More than one pathway for oxalate synthesis was established in finger millet and the crop could be a prospective candidate for studying the nutrient-anti-nutrient interactions.

A combined transcriptome and metabolome approach to know the effect of silicon (Si) amendment in overcoming osmotic stress in finger millet proved that Si improved seed germination as well as growth parameters under stress (Mundada et al. 2021). Enhanced silicon mediated the diversion of an enhanced pool of acetyl coA to lipid biosynthesis and membrane lipid damage was reduced significantly. The metabolite abundance was in line with relative expression of transcripts, the study found. A genome-wide identification of nutrient transporters in finger millet was undertaken computationally to have a better understanding of the nutrient transport pathways and to improve the pathways using the genomic information (Maharajan et al. 2022). Many of the health benefits of finger millet grains and the stress tolerance of the crop is related to its antioxidant properties. To have a systematic analysis of the ROS producing and scavenging genes in this crop, Avashthi et al. (2018) undertook a transcriptome analysis to identify the genes of the ascorbate-glutathione cycle (Halliwell-Asada pathway) and related pathways. Several key genes of this pathway were identified (such as *APX, DHAR, MDHAR, GR*, and *SOD*) from a low and high Ca^{2+} genotype (GP1 and GP45 respectively). The key genes identified using rice as a reference genome are hoped to open new avenues for the systematic functional analysis of antioxidant genes in finger millet which has direct/indirect effects in human health and nutrition.

14.6 Status of Genetic Transformation and Transgenics Development

In the literature reports available on genetic transformation in finger millet, *Agrobacterium* mediated method is found to be more widely used as compared to ballistic method. It could be due to the development of superior strains of agrobacterium and tissue culture protocols developed in the recent years. Effective transformation protocols have been developed and standardized by different research groups.

Co-cultivation of shoot apex explants obtained on the 16th day after callus induction with hygromycin phosphotransferase (hptII) as selectable marker was used to obtain 3.8% stable transformation in two finger millet genotypes, GPU45 and CO14 (Ceasar and Ignacimuthu 2011). A rapid protocol using shoot apical meristems (SAMs) as explants enabled transgenic plantlet production in the greenhouse within 45 days through recovery of transgenic plants via direct plant regeneration without a callus phase (Satish et al. 2017). Forty-five-day old calli obtained from the scutellum of the mature seeds was used by Hema et al. (2014). Calli from mature seeds was used for infection with *Agrobacterium* (Anjaneyulu et al. 2014).

Optimum conditions for particle bombardment of finger millet were defined as 1100 psi rupture disk pressure with 3 cm distance from rupture disk to macrocarrier and 12 cm microprojectile travel distance, double bombardment with gold particles of 1.0 μm size and osmotic treatment of callus with 0.4 M sorbitol (Jagga-Chugh et al. 2012). Calli were bombarded and placed on regeneration medium containing hygromycin as the selection marker that led to 45.3% transformation efficiency.

Blast disease affects the leaf, neck, and spikes of finger millet and sometimes it causes average yield losses of 20–50% and even complete crop loss. *Rice chitinase* (*chi11*) gene was introduced in the genotype GPU45 for resistance to leaf blast disease (Ignacimuthu and Ceasar 2012). Chitinase was transformed through *Agrobacterium*-mediated transformation under the control of maize ubiquitin promoter. Somatic embryogenesis and regeneration of shoot apex explant was employed. Bioassay showed fewer lesions in transgenic plants compared to control plants. A gene coding for an antifungal protein (PIN) of prawn driven by CaMV 35S promoter was chemically synthesized and introduced into the callus of shoot-tip explant (Latha et al. 2005). Transgenic plants expressing *pin* gene exhibited marked resistance to leaf blast disease (0–4 scale) while control plants showed heavy damage (5–9 scale) in bioassay.

Mutant alpha-tubulin gene (TUAm 1) isolated from R-biotype goosegrass (*Eleusine indica* L.) conferred resistance to dinitroaniline herbicides. It is a modified tubulin gene that can be used as a selectable marker. Trifluralin, the key constituent of dinitroaniline herbicides, is used as selection agent for differentiating genetically transformed cells. Trifluralin at a concentration of 10 microM was found optimum as selection agent in the production of transgenic finger millet for dinitroaniline-resistance (Bayer et al. 2014). Transgenic finger millet plants expressing the mannitol biosynthetic pathway gene from bacteria, mannitol-1-phosphate dehydrogenase (*mtlD*) were developed through *Agrobacterium tumefaciens*-mediated genetic transformation (Hema et al. 2014). These progenies had better growth under drought and salinity stress compared to wild type. A vacuolar proton pyrophosphatase from *Sorghum bicolor* (*SbVPPase*) was introduced into embryogenetic calli through Agrobacterium and it enhanced the plant's performance under salt stress (Anjaneyulu et al. 2014). Relative water content (RWC), plant height, leaf expansion, finger length and width, and grain weight were more compared to control plants and relative changes in enzyme activities were identified in transgenic plants. Salinity tolerant finger millet transgenic plants were developed using a double gene construct consisting of PgNHX1 from pearl millet and AVP1 from Arabidopsis

using Agrobacterium mediated transformation (Jayasudha et al. 2014). This was the first time a double gene construct was used for producing finger millet transgenic.

*XvAld1*gene that encodes aldose reductase was introduced into finger millet via Agrobacterium-mediated transformation and the transgenic events regenerated through direct organogenesis using shoot apical meristems to impart resistance to drought and salinity stress, where the transgenic plants were found tolerant as compared to wild plant types (Mukami 2019). *OsSOS1* gene from *Oryza sativa* was overexpressed through *Agrobacterium tumerfaciens* – mediated transformation using direct plant regeneration by culturing shoot apical meristems that lead to high salt tolerance, promoted seed germination, and increased root length, shoot length, chlorophyll, membrane stability index, and reduction in reactive oxygen species (ROS) relative to wild type plants (Pushpa et al. 2020).

14.7 Research Gaps and Future Strategies

In the light of increasing world population, climate change and reduction in ground water table as well as soil nutrient levels, there is a dire need to improve minor/orphan crops like finger millet as they have proven their drought hardiness and nutritional superiority. As there is a gradual decline in the cultivated area under finger millet in recent years, the only alternative is to increase the productivity which is possible only through improved breeding tools. Since the crop has been genetically and genomically under-exploited, concerted efforts are needed in the development of advanced molecular breeding tools for crop improvement. There is huge potential in identification of QTLs associated with characteristic features of millets like drought, biotic stress resistance, and high calcium and mineral contents. This would further facilitate the marker assisted back cross transfer of mapped QTLs to the genetic background of economically important parental lines. Finger millet is an ideal candidate for basic studies on the biochemical and molecular mechanisms underlying the ability of this crop and millets in general, to survive under low soil moisture and low soil fertility to be used further in other crops that are susceptible to these conditions. The cellular pathways leading to their high physiological efficiency to make them the hardy crops that they are is a potential area yet to be explored. There is huge scope for genomic technologies and high throughput phenotyping in breeding strategies to better utilize natural and induced genetic variations existing in germplasm collections and wild relatives. The genome sequencing has given a fresh boost to the genetic research in finger millet. The accessibility of the genome sequence in the public domain is a potential tool for more research into the structural and functional genetics of the crop and this area needs to be exploited more. Considering the severity and economic significance of blast disease in finger millet, there is immediate need for elaborate studies on resistance gene identification against the fungus and host-pathogen interactions which might yield unique and useful insights.

14.8 Conclusion

Advances in molecular techniques and their effective application in breeding programs have made crop improvement rapid and much more efficient in comparison to conventional methods. Even though it took several years to utilize the modern techniques in the orphan crop like finger millet, the recent developments have been promising. Whole genome sequencing has given a new direction to genomics-aided breeding programs in finger millet. Still, there are several research gaps that need to be addressed starting from the characterization of the germplasm resources, mapping population development, identification of candidate genes for important traits, SNP identification, association mapping, and NGS based allele discovery. Improvements in molecular breeding can make the exploitation of valuable traits owned by this nutritionally superior and stress tolerant crop for its own development as well as for the betterment of other crop species.

References

Agrawal R, Agrawal N, Tandon R, Raina SN (2013) Chloroplast genes as genetic markers for inferring patterns of change, maternal ancestry and phylogenetic relationships among Eleusine species. AoB Plants. https://doi.org/10.1093/aobpla/plt056. Print 2014

Akbar N, Gupta S, Tiwari A, Singh KP, Kumar A (2018) Characterization of metabolic network of oxalic acid biosynthesis through RNA seq data analysis of developing spikes of finger millet (*Eleusine coracana*): deciphering the role of key genes involved in oxalate formation in relation to grain calcium accumulation. Gene 649:40–49

Anjaneyulu E, Reddy PS, Sunita MS, Kavi Kishor PB, Meriga B (2014) Salt tolerance and activity of antioxidative enzymes of transgenic finger millet overexpressing a vacuolar H+-pyrophosphatase gene (*SbVPPase*) from *Sorghum bicolor*. J Plant Physiol 171:789–798. https://doi.org/10.1016/j.jplph.2014.02.001

Avashthi H, Pathak RK, Pandey N et al (2018) Transcriptome-wide identification of genes involved in ascorbate–glutathione cycle (Halliwell–asada pathway) and related pathway for elucidating its role in antioxidative potential in finger millet (*Eleusine coracana* (L.)). 3 Biotech 8:499. https://doi.org/10.1007/s13205-018-1511-9

Babitha KC, Vemanna RS, Nataraja KN, Udayakumar M (2015) Overexpression of EcbHLH57 transcription factor from *Eleusine coracana* L. in tobacco confers tolerance to salt, oxidative and drought stress. PLoS One 10(9):e0137098

Babu BK, Senthil N, Gomez SM, Biji KR, Rajendraprasad NS, Kumar SS et al (2007) Assessment of genetic diversity among finger millet (*Eleusine coracana* (L.) Gaertn.) accessions using molecular markers. Genet Resour Crop Evol 54:399–404. https://doi.org/10.1007/s10722-006-0002-8

Babu BK, Dinesh P, Agrawal PK, Sood S, Chandrashekara C, Bhatt JC, Kumar A (2014a) Comparative genomics and association mapping approaches for blast resistant genes in finger millet using SSRs. PLoS One 9(6):e99182

Babu BK, Agrawal PK, Pandey D, Jaiswal JP, Kumar A (2014b) Association mapping of agro-morphological characters among the global collection of finger millet genotypes using genomic SSR markers. Mol Biol Rep 41:5287–5297. pmid:24861452

Bayer GY, Yemets AI, Blume YB (2014) Obtaining the transgenic lines of finger millet *Eleusine coracana* (L.). With dinitroaniline resistance. Cytol Genet 48:139–144. https://doi.org/10.3103/S0095452714030025

Bisht MS, Mukai Y (2001) Genomic in situ hybridization identifies genome donor of finger millet (*Eleusine coracana*). Theor Appl Genet 102:825–832

Bisht MS, Mukai Y (2002) Genome organization and polyploid evolution in the genus *Eleusine* (Poaceae). Plant Syst Evol 233:243–258

Ceasar SA, Ignacimuthu S (2011 Sep) Agrobacterium-mediated transformation of finger millet (*Eleusine coracana* (L.) Gaertn.) using shoot apex explants. Plant Cell Rep 30(9):1759–1770. https://doi.org/10.1007/s00299-011-1084-0

Chinchole M, Pathak RK, Singh UM, Kumar A (2017) Molecular characterization of EcCIPK24 gene of finger millet (*Eleusine coracana*) for investigating its regulatory role in calcium transport. 3 Biotech 7(4):1–10

Das S, Mishra RC, Rout GR, Aparajita S (2007) Genetic variability and relationships among thirty genotypes of finger millet (*Eleusine coracana* L. Gaertn.) using RAPD markers. Z Naturforsch C 62(1-2):116–122

Dida MM, Ramakrishnan SS, Bennetzen JL, Gale MD, Devos KM (2007) The genetic map of finger millet, *Eleusine coracana*. Theor Appl Genet 114:321–332

Dida MM, Wanyera N, Harrison Dunn MLN, Bennetzen JL, Devos KM (2008) Population structure and diversity in finger millet (*Eleusine coracana*) germplasm. Trop Plant Biol 1:31–141

Fakrudin B, Kulkarni RS, Shashidhar HE, Hittalmani S (2007) Genetic diversity assessment of finger millet, *Eleusine coracana*, germplasm through RAPD analysis. PGR Newslett 138:52–54

Gaur VS, Kumar L, Gupta S, Jaiswal JP, Pandey D, Kumar A (2018) Identification and characterization of finger millet OPAQUE2 transcription factor gene under different nitrogen inputs for understanding their role during accumulation of prolamin seed storage protein. 3 Biotech 8(3):1–11

Gimode D, Odeny DA, de Villiers EP, Wanyonyi S, Dida MM, Mneney EE et al (2016) Identification of SNP and SSR markers in finger millet using next generation sequencing technologies. PLoS One 11(7):e0159437. https://doi.org/10.1371/journal.pone.0159437

Gupta N, Gupta AK, Singh NK, Kumar A (2011) Differential expression of PBF Dof transcription factor in different tissues of three finger millet genotypes differing in seed protein content and color. Plant Mol Biol Report 29:69–76

Gupta N, Gupta AK, Kumar A (2012) Spatial distribution pattern analysis of Dof1 transcription factor in different tissues of three *Eleusine coracana* genotypes differing in their grain protein, yield and photosynthetic efficiency. Mol Biol Rep 39:2089–2095

Gupta AK, Gaur VS, Gupta S, Kumar A (2013) Nitrate signals determine the sensing of nitrogen through differential expression of genes involved in nitrogen uptake and assimilation in finger millet. Funct Integr Genom 13(2):179–190

Gupta S, Gupta SM, Gupta AK, Gaur VS, Kumar A (2014) Fluctuation of Dof1/Dof2 expression ratio under the influence of varying nitrogen and light conditions: involvement in differential regulation of nitrogen metabolism in two genotypes of finger millet (*Eleusine coracana* L.). Gene 546:327–335

Hatakeyama M, Aluri S, Balachadran MT, Sivarajan SR, Patrignani A, Grüter S et al (2018) Multiple hybrid de novo genome assembly of finger millet, an orphan allotetraploid crop. DNA Res 25:39–47. https://doi.org/10.1093/dnares/dsx036

Hema R, Vemanna RS, Sreeramulu S, Reddy CP, Senthil-Kumar M, Udayakumar M (2014) Stable expression of mtlD gene imparts multiple stress tolerance in finger millet. PLoS One 9(6):e99110

Hilu KW (1988) Identification of the "a" genome of finger millet using chloroplast DNA. Genetics 11:163–167

Hittalmani S, Mahesh H, Shirke MD, Biradar H, Uday G, Aruna Y et al (2017) Genome and transcriptome sequence of finger millet (*Eleusine coracana* (L.) Gaertn.) provides insights into

drought tolerance and nutraceutical properties. BMC Genomics 18:465. https://doi.org/10.1186/s12864-017-3850-z

Ignacimuthu S, Ceasar SA (2012 Mar) (2012) development of transgenic finger millet (*Eleusine coracana* (L.) Gaertn.) resistant to leaf blast disease. J Biosci 37(1):135–147. https://doi.org/10.1007/s12038-011-9178-y

Jacob J, Madhu P, Balakrishna D, Das IK (2019) *Magnaporthe grisea* infection modifies expression of antioxidant genes in finger millet. J Plant Pathol 101(1):129–134

Jagga-Chugh S, Kachhwaha S, Sharma M et al (2012) Optimization of factors influencing microprojectile bombardment-mediated genetic transformation of seed-derived callus and regeneration of transgenic plants in *Eleusine coracana* (L.) Gaertn. Plant Cell Tissue Organ Cult 109:401–410. https://doi.org/10.1007/s11240-011-0104-7

Jayasudha BG, Sushma AM, Prashantkumar S. Hanjagi, Sashidhar VR (2014) An efficient in-vitro agrobacterium-mediated transformation protocol for raising salinity tolerant transgenic plants in finger millet [*Eleusine coracana* (L.) Gaertn.].

Kadri SU, Mulla SI, Suchithra B, Bilal M, Ameen F, Bharagava RN, Saratale GD, Ferreira LF, Américo-Pinheiro JH (2022) Transcriptome-wide identification and computational insights into protein modeling and docking of CAMTA transcription factors in *Eleusine coracana* L (finger millet). Int J Biol Macromol 206(2022):768–776., ISSN 0141-8130. https://doi.org/10.1016/j.ijbiomac.2022.03.073

Kanwal P, Gupta S, Arora S, Kumar A (2014) Identification of genes involved in carbon metabolism from *Eleusine coracana* (L.) for understanding their light-mediated entrainment and regulation. Plant Cell Rep 33:1403–1411

Kaul S, Koo HL, Jenkins J, Rizzo M, Rooney T, Tallon LJ, Feldblyum T, Nierman W, Benito MI, Lin XY et al (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408:796–815

Krishna TA, Maharajan T, Roch GV, Ramakrishnan M, Ceasar SA, Ignacimuthu S (2020) Hybridization and hybrid detection through molecular markers in finger millet [*Eleusine coracana* (L.) Gaertn.]. J Crop Improv 34(3):335–355

Kumar A, Mirza N, Charan T, Sharma N, Gaur VS (2014) Isolation, characterization and immunolocalization of a seed dominant CaM from finger millet (*Eleusine coracana* L. Gartn.) for studying its functional role in differential accumulation of calcium in developing grains. Appl Biochem Biotechnol 172:2955–2973

Kumar A, Yadav S, Panwar P et al (2015a) Identification of anchored simple sequence repeat markers associated with calcium content in finger millet (*Eleusine coracana*). Proc Natl Acad Sci India Sect B Biol Sci 85:311–317. https://doi.org/10.1007/s40011-013-0296-1

Kumar A, Gaur VS, Goel A et al (2015b) De novo assembly and characterization of developing spikes transcriptome of finger millet (*Eleusine coracana*): a minor crop having nutraceutical properties. Plant Mol Biol Report 33:905–922. https://doi.org/10.1007/s11105-014-0802-5

Latha MK, Venkateswara Rao V, Reddy D (2005) Production of transgenic plants resistant to leaf blast disease in finger millet (*Eleusine coracana* (L.) Gaertn.). Plant Sci 169(4):657–667

Li J, Wang Y, Wang L, Zhu J, Deng J, Tang R et al (2021) Integration of transcriptomic and proteomic analyses for finger millet [*Eleusine coracana* (L.) Gaertn.] in response to drought stress. PLoS One 16(2):e0247181

Liu Q, Jiang B, Wen J, Peterson PM (2014) Low-copy nuclear gene and McGISH resolves polyploid history of *Eleusine coracana* and morphological character evolution in Eleusine. Turkish J Bot 38(1–12):3

Lule D, de Villiers S, Fetene M, Odeny DA, Rathore A, Das RR, Tesfaye K (2018) Genetic diversity and association mapping of Ethiopian and exotic finger millet accessions. Crop Pasture Sci 69(9):879–891

Maharajan T, Stanislaus Antony Ceasar, Thumadath Palayullaparambil Ajeesh Krishna (2022) Finger millet (*Eleusine coracana* (L.) Gaertn): nutritional importance and nutrient transporters, Crit Rev Plant Sci, 41:1, 1-31. DOI: https://doi.org/10.1080/07352689.2022.2037834

Manyasa E, Tongoona P, Shanahan P, Mgonja M, De Villiers S (2015) Genetic diversity in east African finger millet (*Eleusine coracana* (L.) Gaertn) landraces based on SSR markers and some qualitative traits. Plant Genetic Resources 13(1):45–55. https://doi.org/10.1017/S1479262114000628

Mirza N, Taj G, Arora S, Kumar A (2014) Transcriptional expression analysis of genes involved in regulation of calcium translocation and storage in finger millet (*Eleusine coracana* L. Gartn.). Gene 550:171–179. https://doi.org/10.1016/j.gene.2014.08.005

Mukami NA (2019) Genetic engineering of finger millet (*Eleusine coracana*) with aldose reductase gene isolated from *Xerophyta viscosa* to enhance drought and salinity tolerance. Post graduate thesis submitted to South Eastern. Kenya University, Kenya

Mundada PS, Barvkar VT, Umdale SD, Kumar SA, Nikam TD, Ahire ML (2021) An insight into the role of silicon on retaliation to osmotic stress in finger millet (*Eleusine coracana* (L.) Gaertn). J Hazard Mater 403

Nagarjuna KN, Parvathi MS, Sajeevan RS, Pruthvi V, Mamrutha HM, Nataraja KN (2016) Full-length cloning and characterization of abiotic stress responsive CIPK31-like gene from finger millet, a drought-tolerant crop. Curr Sci:890–894

Nath M, Goel A, Taj G, Kumar A (2010) Molecular cloning and comparative in silico analysis of calmodulin genes from cereals and millets for understanding the mechanism of differential calcium accumulation. Journal of Proteomics Bioinformatics 3:294–301

Neves SS, Swire-Clark G, Hilub KW, Baird WV (2005) Phylogeny of *Eleusine* (Poaceae: Chloridoideae) based on nuclear ITS and plastid *trnT–trnF* sequences. Mol Phylogenet Evol 35:395–419

Nirgude M, Babu BK, Shambhavi Y, Singh UM, Upadhyaya HD, Kumar A (2014) Development and molecular characterization of genic molecular markers for grain protein and calcium content in finger millet (*Eleusine coracana* (L.) Gaertn.). Mol Biol Rep 41(3):1189–1200

Pandian S, Satish L, Rameshkumar R, Muthuramalingam P, Rency AS, Rathinapriya P, Ramesh M (2018) Analysis of population structure and genetic diversity in an exotic germplasm collection of *Eleusine coracana* (L.) Gaertn. Using genic-SSR markers. Gene 653:80–90

Panwar P, Nath M, Yadav VK, Kumar A (2010) Comparative evaluation of genetic diversity using RAPD, SSR and cytochrome P450 gene-based markers with respect to calcium content in finger millet (*Eleusine coracana* L. Gaertn.). J Genet 89(2):121–133

Panwar P, Jha AK, Pandey PK, Gupta AK, Kumar A (2011) Functional markers based molecular characterization and cloning of resistance gene analogs encoding NBS-LRR disease resistance proteins in finger millet (*Eleusine coracana*). Mol Biol Rep 38:3427–3436. https://doi.org/10.1007/s11033-010-0452-0

Parvathi MS, Nataraja KN, Reddy YAN et al (2019) Transcriptome analysis of finger millet (*Eleusine coracana* (L.) Gaertn.) reveals unique drought responsive genes. J Genet 98:46. https://doi.org/10.1007/s12041-019-1087-0

Pudake RN, Mehta CM, Mohanta TK et al (2017) Expression of four phosphate transporter genes from Finger millet (*Eleusine coracana* L.) in response to mycorrhizal colonization and Pi stress. 3 Biotech 7:17. https://doi.org/10.1007/s13205-017-0609-9

Pushpa BN, Kiranmai K, Shankar AG (2020) Development of finger millet (*Eleusine coracana* (L.) Gaertn.) transgenic for salt tolerance by overexpressing antiporter gene OsSOS1 involved in sodium extrusion. Ind J Pure Appl Biosci 8(6):598–610

Rahman H, Jagadeeshselvam N, Valarmathi R, Sachin B, Sasikala R, Senthil N, Sudhakar D, Robin S, Muthurajan R (2014) Transcriptome analysis of salinity responsiveness in contrasting genotypes of finger millet (*Eleusine coracana* L.) through RNA sequencing. Plant Mol Biol 85:485–503

Rahman H, Ramanathan V, Nallathambi J et al (2016) Over-expression of a NAC 67 transcription factor from finger millet (*Eleusine coracana* L.) confers tolerance against salinity and drought stress in rice. BMC Biotechnol 16(Suppl. 1):35. https://doi.org/10.1186/s12896-016-0261-1

Ramakrishna C, Singh S, Raghavendrarao S et al (2018) The membrane tethered transcription factor EcbZIP17 from finger millet promotes plant growth and enhances tolerance to abiotic stresses. Sci Rep 8:2148. https://doi.org/10.1038/s41598-018-19766-4

Ramakrishnan M, Antony Ceasar S, Duraipandiyan V, Vinod KK, Kalpana K, Al-Dhabi NA et al (2016) Tracing QTLs for leaf blast resistance and agronomic performance of finger millet (*Eleusine coracana* (L.) Gaertn.) genotypes through association mapping and *in silico* comparative genomics analyses. PLoS One 11(7):e0159264. https://doi.org/10.1371/journal.pone.0159264

Ramakrishnan M, Ceasar SA, Vinod KK, Duraipandiyan V, Ajeesh Krishna TP, Upadhyaya HD et al (2017) Identification of putative QTLs for seedling stage phosphorus starvation response in finger millet (*Eleusine coracana* L. Gaertn.) by association mapping and cross species synteny analysis. PLoS One 12(8):e0183261. https://doi.org/10.1371/journal.pone.0183261

Ramegowda V, Senthil-Kumar M, Nataraja KN, Reddy MK, Mysore KS, Udayakumar M (2012) Expression of a finger millet transcription factor, EcNAC1, in tobacco confers abiotic stress tolerance. PLoS One 7(7):e40397

Ramegowda V, Gill US, Sivalingam PN et al (2017) GBF3 transcription factor imparts drought tolerance in *Arabidopsis thaliana*. Sci Rep 7:9148. https://doi.org/10.1038/s41598-017-09542-1

Saha D, Rana RS, Arya L, Verma M, Gowda MC, Upadhyaya HD (2016) Genetic polymorphisms among and between blast disease resistant and susceptible finger millet, *Eleusine coracana* (L.) Gaertn. Plant genetic. Resources 15(4):355–365

Salimath SS, Olivera ACD, Godwin ID, Bennetzen JL (1995) Assessment of genome origins and diversity in the genus *Eleusine* with DNA markers. Genome 38:757–763

Satish L, Ceasar SA, Ramesh M (2017) Improved agrobacterium-mediated transformation and direct plant regeneration in four cultivars of finger millet (*Eleusine coracana* (L.) Gaertn.). Plant Cell Tissue Organ Cult 131:547–565. https://doi.org/10.1007/s11240-017-1305-5

Sharma D, Tiwari A, Sood S, Jamra G, Singh NK, Meher PK, Kumar A (2018) Genome wide association mapping of agro-morphological traits among a diverse collection of finger millet (*Eleusine coracana* L.) genotypes using SNP markers. PLoS One 13(8):e0199444

Singh UM, Chandra M, Shankhdhar SC, Kumar A (2014) Transcriptome wide identification and validation of calcium sensor gene family in the developing spikes of finger millet genotypes for elucidating its role in grain calcium accumulation. PLoS One 9(8):e103963. https://doi.org/10.1371/journal.pone.0103963

Singh UM, Metwal M, Singh M, Taj G, Kumar A (2015) Identification and characterization of calcium transporter gene family in finger millet in relation to grain calcium content. Gene 566(1):37–46

Singh M, Metwal M, Kumar VA, Kumar A (2016) Identification and molecular characterization of 48 kDa calcium binding protein as calreticulin from finger millet (*Eleusine coracana*) using peptide mass finger printing and transcript profiling. J Sci Food Agric 96:672–679. https://doi.org/10.1002/jsfa.7139

Srinivasachary DMM, Gale MD, Devos KM (2007) Comparative analyses reveal high levels of conserved colinearity between the finger millet and rice genomes. Theor Appl Genet 115:489–499

Upadhyaya HD, Gowda CL, Pundir RP, Reddy VG, Singh S (2006) Development of core subset of finger millet germplasm using geographical origin and data on 14 quantitative traits. Genet Resour Crop Evol 53(4):679–685

Upadhyaya HD, Sarma NDRK, Ravishankar CR, Albrecht T, Narasimhudu Y, Singh SK, Varshney SK, Reddy VG, Singh S, Dwivedi SL, Wanyera N (2010) Developing a mini-core collection in finger millet using multilocation data. Crop Sci 50(5):1924–1931

Yadav S, Gaur VS, Jaiswal JP et al (2014) Simple sequence repeat (SSR) analysis in relation to calcium transport and signaling genes reveals transferability among grasses and a conserved behavior within finger millet genotypes. Plant Syst Evol 300:1561–1568. https://doi.org/10.1007/s00606-014-0982-3

Zhang H, Hall N, Goertzen LR, Chen CY, Peatman E, Jinesh Patel J, McElroy S (2019) Transcriptome analysis reveals unique relationships among *Eleusine* species and heritage of *Eleusine coracana*. G3 Genes Genomes Genet 9(6):2029–2036. https://doi.org/10.1534/g3.119.400214

Origin, Diversity, Floral Biology, Pollination, and Genetics in Foxtail Millet

15

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Abstract

Foxtail millet (*Setaria italica* (L.) P. Beauv.; $2n = 2x = 18$; Poaceae) is the second oldest cultivated species of millet. It is thought to be derived from the progenitor, *Setaria viridis* (L.) P. Beauv. upon cultivation and domesticated around 7400 years ago in northern China while some archeological remains were also recovered from Europe and the Middle East. Foxtail millet is a C_4 crop that has the ability to survive in harsh agroecological conditions with immense nutraceutical properties. The characteristic features of this millet include the presence of the smallest genome among the Panicoid grasses, a smaller number of the repetitive genome, a short generation cycle, and is

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self-incompatible making it a wonder millet. Therefore, this crop is considered to be an excellent experimental model for studying architectural traits, evolutionary history, and other physiological characteristic features. The floral biology of this millet is interesting as there are many ecotypes found depending on the variation in the number of inflorescences, color, height, and other attributes. The chapter will briefly discuss the origin, genetic diversity, distribution, taxonomy, and botany of foxtail millet. It will also provide a scope for exploring opportunities for employing in crop improvement and breeding programs.

Keywords

Foxtail millet · Origin · Diversity · Taxonomy · Botany · *Setaria italica*

15.1 Introduction

Millets belong to the family *Poaceae* and are low-yielding, nutrient-rich, agronomically, and economically important crops (Babele et al. 2022; Wilson and VanBuren 2022). The millets are often considered "orphan crops" and placed second in the series of staple (primary cereals) crops (Babele et al. 2022; Wilson and VanBuren 2022). Climate change including the outbreak of Coronavirus disease 2019 (COVID-19) disrupted agriculture, caused a huge loss of both property & lives and the economy (Ahmad et al. 2021; Khan et al. 2021; Babele et al. 2022), crops like millets are one such plausible solution for tackling problems of human health, nutrition, and environmental sustainability (Babele et al. 2022). They rank sixth among the major cereal grains (Shahidi and Chandrasekara 2013). They are annual C_4 grasses while foxtail millet is considered to be the model C4 cereal (Ceasar 2022). Small-seeded millets are produced worldwide for feed, meals, fuel, and forage (Changmei and Dorothy 2014). Millets act as food for both animals and humans. Millets except for pearl millet (*Pennisetum glaucum* (L.) R.Br.) and sorghum (*Sorghum bicolor* (L.) Moench) are known as minor millets, whereas pearl and sorghum millets are categorized as the major millets. Millets are addressing the need for fuel and feeds. It has the potential to produce biofuel (Singh et al. 2017). As the demand for millets increases, it creates more business opportunities for entrepreneurs and increases the employability of farmers like women (Chakraborty and Chakraborty 2021). One such agronomically and economically important millet is the foxtail millet.

Foxtail millet (*Setaria italica* (L.) P. Beauv.) (thereon *S. italica* or FM) is counted as the first crop that was domesticated. It is a multipurpose plant having immense potential and contains nine pairs of chromosomes ($2n = 2x = 18$) (Babele et al. 2022; Ceasar 2022). It is the second oldest cultivated species of millet. *Setaria viridis* (L.) P. Beauv. (thereon *S. viridis*) also green foxtail (GM), a wild species is presumed to be the progenitor of modern-day foxtail millet upon cultivation and domestication around 7400 years ago in northern China while some archeological remains were also recovered from Europe and the Middle East (Vavilov 1926; Sakamoto 1987;

Doust et al. 2009). FM is grown throughout the majority of China, the United States, Canada, the Korean Peninsula, Japan, Indonesia, Australia, and northern parts of Africa and India. This millet is mostly grown in the western and northern Great Plains, the Midwest, Colorado, the Dakotas, Kansas, and Wyoming in the United States (Huang et al. 2014). In some regions of southern Europe, it is planted mainly as a feed crop. India is the highest producer of millets in the globe and the fifth largest exporter of millets. Karnataka, Rajasthan, Andhra Pradesh, Madhya Pradesh, and Tamil Nadu are the main producer states. Its exports are increasing exponentially as the demand for millets is increasing at a fast rate (Diao and Jia 2017a, b; Meena et al. 2021). According to the productivity of small millets, India is the leading producer among other countries (Maitra and Shankar 2019). During the period of the green revolution in India, there was a focus on the cultivation of fine cereals such as rice, wheat, etc., as a result the cultivation of millets was neglected. Recently, millets have gained popularity because of their role in mitigating metabolism for diseases, nutrition, climate resilience, and agronomic importance. It has been found that millet controls the glycemic index more efficiently in living beings with impaired glucose tolerance (Fu et al. 2021). Millets are gluten-free, making them a more reliable source of meals for patients with celiac disease (Taylor and Emmambux 2008; Rai et al. 2018).

The chapter will describe the origin, genetic diversity, distribution, taxonomy, and genetics of foxtail millet. It will also shed some light on the opportunities that can be employed in breeding and crop improvement programs.

15.2 Origin and Diversity of Foxtail Millet

According to Vavilov's theory, foxtail millet originated from East Asia along with China and Japan based on the presence of species diversity (Vavilov 1926; Sakamoto 1987). Similarly, the early remains of FM were found in the Yellow River valley (Cishan and Peiligang regions) of northern China around 7400 and 7935 years ago (Doust et al. 2009), suggesting that it originated from East Asia including China. According to others, it originated 8000 years ago in China (Li and Wu 1996; Shelach 2000; Zohary and Hopf 2000; Austin 2006; Darmency and Dekker 2011). FM was the predominant crop for many years (~ 4 millennia). The occurrence has been reported at many sites for instance, in the Yiluo valley of northern China, and southern China until joining the present North China farming pattern of millets, wheat, and legume rotations from 3600 to 3300 years ago. Numerous origin and domestication theories have been proposed by various groups to date (Yang et al. 2012). The repeated domestication theory is the most widely accepted and recognized (Yang et al. 2012). However, it is well acknowledged that GM and FM are native to Eurasia, while GM is a diploid perennial possessing the same characteristics that gave rise to further *Setaria* species (Singh et al. 2017). The recent phylogenetic analyses emphasized and suggested that *Setaria* is polyphyletic, with firmly established evolutionary relationships with African, Asian, and South American clades as well as other taxa clades. However, there is no common ancestral

relationship between or within the clades/taxa, thereby making the geographical analysis and conclusive inferences problematic (Singh et al. 2017).

According to previous reports, common millet and FM made a significant contribution to the growth of Chinese civilization because ancient China's political center was typically found in the north. Foxtail millet had its earliest domestication between 23,000 and 9000 years ago (He and Hui 2015). Therefore, it was assumed that GM was harvested from the wild as food and that FM domestication started at about the same time (He and Hui 2015).

FM is abundant and possesses a rich genetic diversity. Based on the comparative morphology of the FM accessions, Prasada Rao et al. (1987) suggested three landraces namely moharia, maxima, and indica which are further divided into 10 subraces namely aristata, fusiformis, and glabra (moharia), in maxima - compacta, spongiosa and assamense while erecta, glabra, nana, and profusa under indica race. Race moharia is commonly found in Europe, Southeast Russia, Afghanistan, and Pakistan, whereas race maxima are abundant in East China, Georgia (Eurasia), Japan, Korea, Nepal, and Northern India, and finally, the race indica is found in the remaining parts of India and Sri Lanka (Prasada Rao et al. 1987). While in Li et al. (1995), Li and Coworkers included the race nana along with the other races as mentioned earlier and depicted the plants that take after the wild GM and are exceptionally small and slim having many tillers with small panicles and early development as an isolated race nana. Around 26,670 accessions have been kept at the Chinese National Gene Bank (CNGB), Institute of Crop Science, and Chinese Academy of Agricultural Sciences, in China (Wang et al. 2012a, b) while in India, there are around 1534, 1279, and 766 accessions available at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), National Institute of Agrobiological Sciences (NIAS), Japan, and Plant Genetic Resources Conservation Unit (PGRCU), US Department of Agriculture respectively (Wang et al. 2012a, b).

15.3 History of Domestication

During the eighteenth century, Linneaus (1753) separated the two species of millets, foxtail, and green namely, *Panicum viride* (or *S. viridis*) and *Panicum italica* (or *S . italica*) respectively (Linneaus 1753; Darmency and Dekker 2011). The foundation of ancient Chinese agriculture was established through the domestication of broomcorn, common millet, foxtail millet, and rice in the Northern and Southern regions of China (Singh et al. 2017). According to numerous studies, common millet and foxtail millet made a significant contribution to the growth of Chinese civilization because ancient China's political center was typically found in the north. FM had its earliest domestication between 23,000 and 9000 years ago.

Amid the Chinese Neolithic Age, between 9000 and 6000 years ago, FM underwent its second domestication phase. The Donghulin site in Beijing yielded the oldest FM grains discovered to date, which were calibrated between 11,000 and 9000 years ago. In the middle and upper regions of the Yellow River in Northern China, numerous older intact FM grains have been found. At the Xinglong Gou site

in eastern Inner Mongolia, FM and other millets were discovered (Diao and Jia 2017a, b). Hundreds of archeological sites in China have yielded carbonized foxtail millet remnants, with several sites yielding considerable amounts. The mid- and late-Yangshao Culture sites (6000–5000 years ago) in Henan Province's Yellow River basin are typical examples of such sites that demonstrated the spread of FM in China's southwest region (Diao and Jia 2017a, b). The archeological pieces of evidence obtained from the Longshan culture (4500 years ago) in Shandong Province, east of the Yellow River region, Hongshan culture (5500–500 years ago), and north of the Yellow River in Inner Mongolia demonstrated the widespread use of FM following its domestication (Diao and Jia 2017a, b).

The history of FM goes through the middle Yellow River regions along with the provinces of Shanxi, Shannxi, Hebei, and Henan, which are believed to have been domesticated (Singh et al. 2017). It then moved along the Yangtze River from Hunan to the Chengdu Plain in Sichuan Province. This scenario explains the spread of foxtail millet in China's southwest. The abundance of millets at archeological sites is evidence that FM was widely used after becoming domesticated in the Yellow River basin (Diao and Jia 2017a, b). The lack of evidence for FM is puzzling. Even now, some semi-arid and arid regions of Northern China continue to rely on FM as a primary grain. Chinese culture and human civilization, in general, have benefited significantly from the long and rich history of FM farming (Diao and Jia 2017a, b).

15.4 Distribution

Many have proposed different concepts regarding the origin and domestication of this crop (De Wet et al. 1979; Li et al. 1995). Also, a multiple domestication hypothesis which is widely accepted suggests the three main centers, viz., Europe, Afghanistan–Lebanon, and China (Li et al. 1995). Hirano et al. (2011) studied 425 landraces of FM and 12 accessions of the foxtail (or green) with the help of transposon display and concluded with the two clear genetic borders. West Europe and East Europe provide solid territorial separations and have a long history of development that supports numerous domestication occasions in foxtail millet. Also, the genetic border increases between East Asia and those of other areas namely Central, South, or Southeast Asia, etc.

15.5 Taxonomy

The millet consists of a single stalk or some tillers, with inflorescences developing almost at the same time. The stems are slender, upright, and green, and the height varies from 120 to 200 cm (Reddy et al. 2006). The culms are slender and upright with empty internodes. The stems are crowned by a bristly panicle that is lengthy (5–30 cm) and reddish or purple (Reddy et al. 2006). The panicle has the appearance of a foxtail, for which the millets are popularly referred to as "foxtail" millets (Reddy

et al. 2006). The spikelets are swarmed and blended with hardened bristles. Each spikelet is complete and contains a yellow pistil (Reddy et al. 2006).

The seeds are approximately 2 mm (sometimes may vary) in size with a papery body. The seeds are smooth and shiny and are of different colors (Dekker 2003; Diao and Jia 2017a, b; Fatima and Rao 2019). FM is typically planted in late spring as a summer crop. After 65–70 days, it can be harvested for green matter, yielding 15,000–20,000 kg/ha, and for roughage, yielding 3000–4000 kg/ha. After 75–90 days, it can be harvested for grain. Early development and great water utilization proficiency make it appropriate for development in dry regions. Currently, FM is disseminated in most of China, a few parts of India, the United States, Canada, the Korean Promontory, Japan, Indonesia, Australia, and the northern portion of Africa (Austin 2006; Doust et al. 2009; Li and Brutnell 2011).

15.6 Pollination Biology

The inflorescence has a chief stalk with shortened facet branches bearing spikes and bristles (Baltensperger 1996). They are terminal spikes, ranging from 8 to 32 cm in length, drooping, dense, cylindrical lobed, borne on a skinny and short pedicel (Sundararaj and Thulasidas 1976). Each spikelet contains two small flowers enclosed by a pair of glumes. The lower flower is sterile while the upper one is fertile and bisexual, with three stamens and a long oval smooth ovary with two long styles that end in feathery stigmas (Kumari and Vetriventhan 2010). The anthers are yellow or white, the ovary is surmounted with the aid of using lengthy patterns and feathery stigmas.

The anthers shed spores after being extruded from the glumes. Once pollinated, the lodicules shrink, and glumes start to close (Siles et al. 2001). Blossoming in foxtail usually takes place close to the middle of the night and in the morning, however, varies considerably with the environment (Jayaraman et al. 1997). The most variety of flowers blooms on the sixth day of appearance. The flower begins to open beneath the apex of the pinnacle when approximately three-fourth of the pedicle emerges from the sheath. Flowering starts from the pedicle and moves downward to the foremost spike. It takes around 8–16 days for a flower to reach maturity (Sundararaj and Thulasidas 1976). A single floret stays open for approximately 30 min, and approximately eighty mins for entire blooming (Malm and Rachie 1971). During pollination, glumes start to spread which is followed by the tips of stigmatic branches along with the anthers protruding through the slits of the incurvate edges of the palea. Once anther emergences, longitudinal dehiscing occurs through slits from the pinnacle to the bottom (Sundararaj and Thulasidas 1976). Sometimes, anthers remained adhered inside the curved edges of the palea (Siles et al. 2001). Humidity and temperature are the primary environmental factors that play important roles during pollination (Li et al. 1935). The FM is highly self-pollinated and the rate of cross-pollination is less and varies from 1.4 to 4% (Rao et al. 1987; Till-Bottraud et al. 1992). The lodicules are 2 in number (Jayaraman et al. 1997). The grain is oval in shape, shiny, 2 mm in length, and tightly enclosed by the

thickened lemma and palea. It varies in colors from cream to orange and yellow-brown to black (Seetharam et al. 2003).

15.7 Genetics and Crop Improvement

The plant transformation era has been a flexible platform that helps essentially in crop development programs thereby leading to the formation of new varieties. Abiotic (drought, excessive temperature, and soil salinity) and biotic (pests and pathogens) stresses constantly act upon crops thereby severely affecting yield and production. Hence, securing a stronger yield balance is predicated on enhancing tolerance in crops towards stresses (Pandey et al. 2017; Gull et al. 2019; Umar et al. 2021). However, yield losses caused by abiotic stress are severe and unpredictable in terms of stress duration, timing, and depth. As a result, millets are relatively resistant to drought heat, and nutrient input requirements. They are suitable for sustaining agriculture and food protection on low fertile and marginal lands in arid and semi-arid India that are not suitable for primary cereal crops (Amadou et al. 2013). Despite their relevance, millets are regarded as small cereals whose adjacent value and characteristics have remained an under-researched crop product. Millets have tempting possibilities for small-scale grain production as a result of climate change (Tadele 2016). They can withstand more stringent and harsh environmental conditions (Dai 2011) and other stresses. Millets are thus important plant genetic assets for the agriculture and food security of poor farmers living in desert, uncultivated, and marginal regions (Dai 2011). Ban et al. (1971) published the first report on an in vitro investigation of foxtail millet and anthers as explants. Later, somatic embryogenesis of immature inflorescences was employed as explants for regeneration (Xu et al. 1984; Yang et al. 2012). Regeneration was studied for wild FM, particularly *S. lutescens* and *S. glauca* (Xu et al. 1983). It has been observed that the regeneration in *Setaria* is primarily based on the genotype and explant. However, for *S. viridis*, Brutnell et al. (2010) obtained callus from mature seeds following the protocol of *S. italica* and *Brachypodium distachyon* (Rao et al. 1988; Brutnell et al. 2010).

With the current changing climate, small millets are an excellent choice for mitigating environmental problems and challenges (Singh et al. 2021). Moreover, with the dynamic environment witnessing frequent and sudden changes, there is an urgent need for developing resistant and tolerant varieties that can withstand such dynamism (Singh et al. 2021). Singh et al. (2021) have summarized the QTLs involved in providing tolerance during abiotic stress. However, the knowledge in this area is very scant and needs further exploration to harness the complete potential of the crop (Singh et al. 2021).

Likewise, male sterility (MS) and heterosis are another set of important parameters that can be explored to produce hybrid varieties and will be helpful in improvement and breeding programs. Reports are there suggesting that the aspect of MS and heterosis have been studied in foxtail millet (Siles et al. 2004; Zhang et al. 2021; recently Ramlal et al. (2022), reviewed the importance of MS and heterosis in

soybean, another agronomically and economically important), however, need serious focus for further identification of MS lines which thereby can be used for crop improvement and stress management programs.

15.8 Economic Uses

Foxtail millet acts as a primary source of food for people in China, India, Korea, Japan, and Nepal (Austin 2006; Dwivedi et al. 2012). Millets are used as feed crops for both humans and animals and also have the potential to produce biofuel (Singh et al. 2017). The millet market size was more than USD 9 billion in 2018 and will witness more in the following years (2018–25) and the value is projected to increase by more than USD 12 billion (Nayana and Kumar 2021).

Food and Nutritional Security—The grains have high iron, zinc, and calcium contents along with protein (14–16%), crude fat (6–8%), and iron (Muthamilarasan et al. 2016a, b; Singh et al. 2017). FM has higher levels of seven of the eight essential amino acids (Zhang et al. 2007). FM is a good source of fiber, which is necessary for stomach health. A single grain of FM contains almost 2.5 times the amount of fiber available in rice. It is rich in linoleic (66.5%) and oleic (13.0%) acids and contains crude oil of about 8–10% (Liang et al. 2010) (Fig. 15.1). The seeds were used to create them through their lengthy cultivation and dietary use. In China, flour is used to make snacks, bread, chapattis, and pancakes (Weber and Fuller 2008). Steamed bread made from composite flour and including foxtail millet, wheat, and soybean has grown more popular in Northern China (Diao et al. 2014). In India, flour is used to make bread, chapattis, cookies, and snacks. However, in many countries, it is still largely produced for hay, silage, fodder, and birdseed (Wrigley et al. 2004).

Therapeutic uses—They are commonly utilized by patients, children, diabetic people and pregnant women (Sema and Sarita 2002; Dwivedi et al. 2012). Consuming foxtail millet is important for those with type II diabetes as it decreases blood glucose, glycosylated hemoglobin, and serum lipids (Thathola et al. 2010). In China, it is used to alleviate rheumatism. Cultivars with yellow seeds contain digestive,

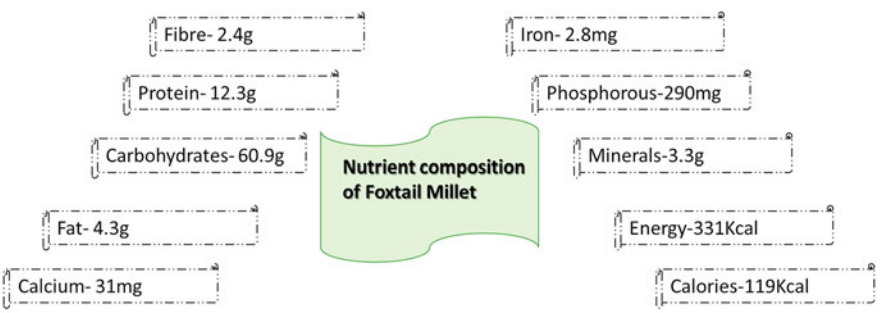

Fig. 15.1 Nutrient Composition of Foxtail Millet

emollient, astringent, and stomachic effects. It includes dyspepsia, poor digestion, and food stagnation in the stomach (Sarita and Singh 2016).

Managing malnutrition and overnutrition—There is a rising awareness of the need to switch to better, more readily available, and more inexpensive diets, including millet, due to the problem of malnutrition and overnutrition (obesity, metabolic syndrome, and lifestyle illnesses) in India (Dayakar Rao et al. 2017). In addition to being naturally gluten-free and nutrient-dense, millets are also a great source of protein, essential fatty acids, dietary fiber, and vitamin B (Srivastava and Arya 2021).

Economic security—Millets are also drought and climate-resistant crops that may thrive in challenging environmental circumstances with minimal inputs. By doing so, greater millets consumption can help ensure the producer/financial farmer's security. To preserve India's unique biodiversity, the Food and Agriculture Organization of the United Nations (FAO) and the government are collaborating to increase crop genetic variety, especially indigenous crops. This is accomplished by assisting smallholder farmers, particularly women, in strengthening their function as stewards of agrobiodiversity diversity (Durgad 2021).

Industrial uses—Apart from Asia and Africa, the goods are gaining popularity in other areas of the world as a regular diet in various forms such as porridge, ready-to-eat snacks, and drinks (Desikachar 1975). Milling produces concentrated functional components, which may subsequently be utilized to mass-produce items such as infant meals, snack foods, dietary foods, and traditional sweets (Subramanian and Viswanathan 2007). Furthermore, this gluten-free flour may be utilized to partially replace wheat flour in food items (Devisetti et al. 2014).

Boosting sustainability—Millets production may aid in promoting a transition to sustainable agriculture by reducing reliance on synthetic fertilizers and pesticides, diversifying crop rotations, and preventing the development of mono-cropping systems (Vinoth and Ravindhran 2017). Crop residues are particularly crucial for maintaining and raising soil carbon levels, necessary for sustainable cropping systems, and, when applicable, crucial for simultaneously providing feed for animals, according to their high carbon content (Nithiyanantham et al. 2019; Verma et al. 2020).

Food and fodder crop—It is a significant grain and fodder crop. It is a short-duration plant that is ideal for arid environments because of its high producing potential, endurance to drought and heat, high photosynthetic efficiency, and adaptability to soil types (Shkodina and Balun 2020). As dried hay in the winter, it is a useful source of nutrition for cattle.

15.9 Scope and Improvement

Millet as a food is an older practice for both animals and humans. Millets except for pearl millet and sorghum are known as minor millets, whereas pearl and sorghum millets are categorized as major millets. During the period of the green revolution in India, there was a focus on the cultivation of fine cereals such as rice, wheat, etc., as a

result the cultivation of millets gets neglected. But as urbanization took place, there was a rise in demand for food, a change in people's food habits, and became the go-to food for poor men. But time has changed and due to a re-evaluation of the nutritional qualities of millet people again have started to consume it (Maitra 2020a). Small millets have rich protein, energy, dietary fiber, and some nutraceutical properties (Banerjee et al. 2020). Nowadays crops are facing some immense problems such as climate change, greenhouse gas emissions, and uncertainties in rainfall but there are small millets that are unaffected by all these problems so it is also known as miracle crops, also due to their variable uses. Millets are planted that come under the category of C4 plants which are capable of converting atmospheric CO_2 into biomass (Brahmachari et al. 2019). Among all the different small millets, foxtail millet has a great history in the Asian region. Earlier it was mostly cultivated in central China (Miller et al. 2016).

In recent times, millet has gained some attention due to its role in the mitigation of metabolism for diseases. It has been found that millet controls the glycemic index more efficiently in living beings with impaired glucose tolerance (Fu et al. 2021). Millet is gluten-free, making it a more reliable food for getting ill. Gluten consumption can cause Coeliac disease. In studies, it has been found that millets possess a good proportion of some phenolics and also act as natural antioxidants and radical scavenging and it is also a reducing agent, and a chelator of metal ions (Talukder and Sharma 2015). During the storage of millet, the antioxidants present in it incorporates millet grains in products which can increase the shelf life of the product (Chandrasekara and Shahidi 2011). Millet is a good source of dietary fiber; its bran has more contribution to it. In millet, the amount of insoluble dietary fiber is 65.55 g/100 g; for soluble dietary fiber, it is 7.63 g/100 g (Talukder and Sharma 2015).

In dryland areas, farmers face low productivity which results in lower income, also there are threats due to monsoon, and some other uncertainty. Being an important crop, FM needs to be improved in its production and flavor including other traits (Maitra 2020b). Millets have a very unique grainy and gritty texture which is an acceptable texture for most food products. Nirmala et al. (2000) reported that there has been an improvement in food flavor profile if it has been added with millets (Nirmala et al. 2000).

15.10 Conclusion

Foxtail millet is known as the oldest cultivated crop in the world, with varieties adapted to a range of climatic conditions from tropical to temperate, including India, China, and America. Despite its importance, there has been limited research on this crop, and it deserves more attention for its development. Heterosis, or hybrid vigor, is a valuable method for improving crops and ensuring food security. Foxtail millet is a nutritious and versatile crop that can help address environmental challenges and provide a resilient food and feed source. It is important to recognize the value of this crop and treat it as a mainstream crop rather than an orphaned one.

References

Ahmad S, Chitkara P, Khan FN, Kishan A, Alok V, Ramlal A, Mehta S (2021) Mobile technology solution for COVID-19: surveillance and prevention. In: Raza K (ed) Computational intelligence methods in COVID-19: surveillance, prevention, prediction and diagnosis. Studies in computational intelligence, vol 923. Springer, Singapore

Amadou I, Gounga ME, Le GW (2013) Millets: nutritional composition, some health benefits and processing: a review. Emirates J Food Agric 25:501–508

Austin DF (2006) Fox-tail millets (*Setaria*: Poaceae)—abandoned food in two hemispheres. Econ Bot 60(2):143–158

Babele PK, Kudapa H, Singh Y, Varshney RK, Kumar A (2022) Mainstreaming orphan millets for advancing climate smart agriculture to secure nutrition and health. Front Plant Sci 13:902536

Baltensperger DD (1996) Foxtail and proso millet. In: Janick J (ed) Progress in new crops. ASHS Press, Alexandria, VA, pp 182–190

Ban Y, Kokubu T, Miyaji Y (1971) Production of haploid plant by anther-culture of Setaria italica. Kagoshima Univ Fac Agr Bull 21:77–81

Banerjee P, Maitra S, Banerjee P (2020) The role of small millets as functional food to combat malnutrition in developing countries. Indian J Nat Sci 10(60):20412–20417

Brahmachari K, Sarkar S, Santra DK, Maitra S (2019) Millet for food and nutritional security in drought prone and red laterite region of eastern India. Int J Plant Soil Sci 26(6):1–7

Brutnell TP, Wang L, Swartwood K, Goldschmidt A, Jackson D, Zhu XG et al (2010) *Setaria viridis*: a model for C4 photosynthesis. Plant Cell 22(8):2537–2544

Ceasar SA (2022) Foxtail millet (*Setaria italica*) as a model system to study and improve the nutrient transport in cereals. Plant Growth Regul 99(1):1–8

Chakraborty SK, Chakraborty S (2021) Rural entrepreneurship development in millet processing. In: Millets and millet technology. Springer, Singapore, pp 345–361

Chandrasekara A, Shahidi F (2011) Antiproliferative potential and DNA scission inhibitory activity of phenolics from whole millet grains. J Funct Foods 3(3):159–170

Changmei S, Dorothy J (2014) Millet-the frugal grain. Int J Sci Res Rev 3(4):75–90

Dai A (2011) Drought under global warming: a review. Wiley Interdiscip Rev Clim Chang 2(1): 45–65

Darmency H, Dekker J (2011) Setaria. In: Kole C (ed) Wild crop relatives: genomic and breeding resources. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-14255-0_15

Dayakar Rao B, Bhaskarachary K, Arlene Christina GD, Sudha Devi G, Vilas AT, Tonapi A (2017) Nutritional and health benefits of millets. ICAR_Indian Institute of Millets Research (IIMR), Hyderabad, India, p 112

De Wet JMJ, Oestry-Stidd LL, Cubero JI (1979) Origins and evolution of foxtail millets (Setaria italica). J Agric Trop Bot Appl 26(1):53–64

Dekker J (2003) The foxtail (*Setaria*) species-group. Weed Sci 51(5):641–656

Desikachar HSR (1975) Processing of maize, sorghum and millets for food uses. J Sci Ind Res 34: 231–237

Devisetti R, Yadahally SN, Bhattacharya S (2014) Nutrients and antinutrients in foxtail and proso millet milled fractions: evaluation of their flour functionality. LWT-Food Sci Technol 59(2): 889–895

Diao X, Jia G (2017a) Foxtail millet germplasm and inheritance of morphological characteristics. In: Doust A, Diao X (eds) Genetics and genomics of *Setaria*. Springer, Cham, pp 73–92

Diao X, Jia G (2017b) Origin and domestication of foxtail millet. In: Doust A, Diao X (eds) Genetics and genomics of *Setaria*. Springer, Cham, pp 61–72

Diao X, Schnable J, Bennetzen JL, Li J (2014) Initiation of *Setaria* as a model plant. Front Agric Sci Eng 1(1):16–20

Doust AN, Kellogg EA, Devos KM, Bennetzen JL (2009) Foxtail millet: a sequence-driven grass model system. Plant Physiol 149(1):137–141

Durgad AG (2021) Consumer preference for foxtail and little millets in north eastern region of Karnataka. Econ Aff 66(1)

Dwivedi S, Upadhyaya H, Senthilvel S, Hash C, Fukunaga K, Diao X, Santra D, Baltensperger D, Prasad M (2012) Millets: genetic and genomic resources. In: Janick J (ed) Plant breeding reviews. Wiley Blackwell, New York, pp 247–237

Fatima Z, Rao A (2019) Development, organoleptic evaluation and acceptability of products developed by incorporating foxtail millet. J Food Sci Nutr Res 2(2):128–135

Fu Y, Yin R, Guo E, Cheng R, Diao X, Xue Y, Shen Q (2021) Protein isolates from raw and cooked foxtail millet attenuate development of type 2 diabetes in streptozotocin-induced diabetic mice. Mol Nutr Food Res 65(6):2000365

Gull A, Lone AA, Wani NUI (2019) Biotic and abiotic stresses in plants. In: de Oliveira AB (ed) Abiotic and biotic stress in plants, IntechOpen, pp 1–19

He H, Hui F (2015) The history of millet cultivation in ancient China. China Agricultural Science and Technology Press, Beijing

Hirano R, Naito K, Fukunaga K, Watanabe KN, Ohsawa R, Kawase M (2011) Genetic structure of landraces in foxtail millet (*Setaria italica* (L.) P. Beauv.) revealed with transposon display and interpretation to crop evolution of foxtail millet. Genome 54:498–506

Huang P, Feldman M, Schroder S (2014) Population genetics of *Setaria viridis*, a new model system. Mol Ecol 23(20):4912–4925

Jayaraman, N., Suresh, S., Nirmala, A., Ganeshan, N.M. (1997). Genetic enhancement and breeding strategies in small millets. In: National seminar on small millets, 23–24 April 1997, Coimbatore, India, pp. 19–21 (extended summaries).

Khan FN, Khanam AA, Ramlal A, Ahmad S (2021) A review on predictive systems and data models for COVID-19. In: Raza K (ed) Computational intelligence methods in COVID-19: surveillance, prevention, prediction and diagnosis. Studies in computational intelligence, vol 923. Springer, Singapore

Kumari AN, Vetriventhan M (2010) Characterization of foxtail millet germplasm collections for yield contributing traits. Electr J Plant Breed 1(2):140–147

Li HW, Meng CJ, Liu TN (1935) Problems in the breeding of millet (*Setaria italica* (L.) Beuav.). J Am Soc Agron 27:426–438

Li P, Brutnell TP (2011) *Setaria viridis* and *Setaria italica*, model genetic systems for the Panicoid grasses. J Exp Bot 62(9):3031–3037

Li Y, Wu S (1996) Traditional maintenance and multiplication of foxtail millet (*Setaria italica* (L.) P. Beauv.) landraces in China. Euphytica 87:33–38

Li Y, Wu S, Cao Y (1995) Cluster analysis of an international collection of foxtail millet (*Setaria italica* (L.) P. Beauv.). Euphytica 83(1):79–85

Liang S, Yang G, Ma Y (2010) Chemical characteristics and fatty acid profile of foxtail millet bran oil. J Am Oil Chem Soc 87(1):63–67

Linneaus C (1753) Species plantarum. Holmiae, L. Salvius, Stockholm.

Maitra S (2020a) Agronomic management of Foxtail millet (*Setaria italica* L.) in India for production sustainability: a review. Int J Bioresour Sci 7(1):11–16. https://doi.org/10.30954/2347-9655.01.2020.3

Maitra S (2020b) The potential horizon of brown-top millet cultivation in drylands : a review. Crop Res 55(1&2):57–63. https://doi.org/10.31830/2454-1761.2020.012

Maitra S, Shankar T (2019) Agronomic management in little millet (*Panicum sumatrense* L.) for enhancement of productivity and sustainability. Int J Bioresour Sci 6(2):91–96

Malm NR, Rachie KO (1971) Setaria millets: a review of the world literature. S.B. 513. University of Nebraska, College of Agriculture, Agricultural Experiment Station, Lincoln, NE, 133 p

Meena RP, Joshi D, Bisht JK, Kant L (2021) Global scenario of millets cultivation. In: Kumar A, Tripathi MK, Joshi D, Kumar V (eds) Millets and millet technology. Springer, Singapore, pp 33–50

Miller NF, Spengler RN, Frachetti M (2016) Millet cultivation across Eurasia: origins, spread, and the influence of seasonal climate. Holocene 26(10):1566–1575

Muthamilarasan M, Dhaka A, Yadav R, Prasad M 534 (2016a) Exploration of millet models for developing nutrient-rich graminaceous crops. Plant Sci 242:89–97

Muthamilarasan M, Mangu VR, Zandkarimi H, Prasad M, Baisakh N (2016b) Structure, organization and evolution of ADP-ribosylation factors in rice and foxtail millet, and their expression in rice. Sci Rep 6:24008

Nayana, H.N., Kumar. U. (2021). Popular article on millets, Kisan World.

Nirmala M, Subba Rao MVSST, Muralikrishna G (2000) Carbohydrates and their degrading enzymes from native and malted finger millet (Ragi, *Eleusine coracana*, Indaf-15). Food Chem 69(2):175–180

Nithiyanantham S, Kalaiselvi P, Mahomoodally MF, Zengin G, Abirami A, Srinivasan G (2019) Nutritional and functional roles of millets: a review. J Food Biochem 43(7):1–10

Pandey P, Irulappan V, Bagavathiannan MV, Senthil-Kumar M (2017) Impact of combined abiotic and biotic stresses on plant growth and avenues for crop improvement by exploiting physio-morphological traits. Front Plant Sci 8:537

Prasada Rao KE, de Wet JMJ, Brink DK, Mengesha MH (1987) Intraspecific variation and systematics of cultivated *Setaria italica*, foxtail millet (Poaceae). Econ Bot 41:108–116

Rai S, Kaur A, Chopra CS (2018) Gluten-free products for celiac susceptible people. Front Nutr 5: 116

Ramlal A, Nautiyal A, Baweja P, Mahto RK, Mehta S, Mallikarjun BP, Vijayan R, Saluja S, Kumar V, Dhiman SK, Lal SK, Raju D, Rajendran A (2022) Harnessing heterosis and male sterility in soybean (*Glycine max* (L.) Merr.): a critical revisit. Front Plant Sci. https://doi.org/10.3389/fpls.2022.981768/

Rao AM, Kishor PB, Reddy LA, Vaidyanath K (1988) Callus induction and high frequency plant regeneration in Italian millet (*Setaria italica*). Plant Cell Rep 7(7):557–559

Rao KEP, De-Wet JMJ, Brink DE, Mangesha MH (1987) Intraspecific variation and systematics of cultivated *Setaria italica* foxtail millet (Poaceae). Econ Bot 41:108–116

Reddy VG, Upadhyaya HD, Gowda CLL (2006) Characterization of world's foxtail millet germplasm collections for morphological traits. Int Sorghum Millets Newslett 47:107–109

Sakamoto S (1987) Origin and dispersal of common millet and foxtail millet. JARQ 21(22):84–89

Sarita ES, Singh E (2016) Potential of millets: nutrients composition and health benefits. J Scientific Innov Res 5(2):46–50

Seetharam A, Gowda J, Halaswamy JH (2003) Small millets. In: Chowdhury SK, Lal SK (eds) Nucleus and breeder seed production manual. Indian Agricultural Research Institute, New Delhi, India, pp 54–67

Sema A, Sarita S (2002) Suitability of millet-based food 560 products for diabetics. J Food Sci Technol (Mysore) 39(4):423–426

Shahidi F, Chandrasekara A (2013) Millet grain phenolics and their role in disease risk reduction and health promotion: a review. J Funct Foods 5(2):570–581

Shelach G (2000) The earliest Neolithic cultures of Northeast China: recent discoveries and new perspectives on the beginning of agriculture. J World Prehist 14:363–413

Shkodina EP, Balun OV (2020) Prospects of millet crops cultivation for forage purposes in the non-black earth zone north-west area. IOP Conf. Ser.: earth. Environ Sci 613:012138

Siles MM, Russell WK, Baltensperger DD, Nelson LA, Johnson B, Van Vleck LD et al (2004) Heterosis for grain yield and other agronomic traits in foxtail millet. Crop Sci 44(6):1960–1965

Siles MM, Baltensperger DD, Nelson LA (2001) Technique for artificial hybridization of foxtail millet (*Setaria italica* (L.) Beauv.). Crop Sci 41:1408–1412

Singh RK, Muthamilarasan M, Prasad M (2021) Biotechnological approaches to dissect climate-resilient traits in millets and their application in crop improvement. J Biotechnol 327:64–73

Singh RK, Muthamilarasan M, Prasad M (2017) Foxtail millet: an introduction. In: Prasad M (ed) The foxtail millet genome, compendium of plant genomes. Springer International Publishing, pp 1–9

Srivastava S, Arya C (2021) Millets: malnutrition and nutrition security. In: Millets and Millet Technology. Springer, Singapore, pp 81–100

Subramanian S, Viswanathan R (2007) Bulk density and friction coefficient of selected minor millet grains and flours. J Food Eng 81(1):118–126
Sundararaj DP, Thulasidas G (1976) Botany of field crops. Macmillan Publisher, India, p 509
Tadele Z (2016) Drought adaptation in millets. In: Shanker AK, Shanker C (eds) Abiotic and biotic stress in plants—recent advances and future perspectives. In Tech, Rijeka, pp 639–662
Talukder S, Sharma BD (2015) Scope of millet grains as an extender in meat products. Crit Rev Food Sci Nutr 55(6):735–739
Taylor JR, Emmambux MN (2008) Gluten-free foods and beverages from millets. In: Arendt EK, Bello FD (eds) Gluten-free cereal products and beverages. Academic Press, p 119-V
Thathola A, Srivastava S, Singh G (2010) Effect of foxtail millet (*Setaria italica*) supplementation on serum glucose, serum lipids and glycosylated hemoglobin in type 2 diabetics. Diabetol Croat 40:23–28
Till-Bottraud I, Reboud X, Brabant P, Lefranc M, Rherissi B, Vedel F, Darmency H (1992) Outcrossing and hybridization in wild and cultivated foxtail millets: consequences for the release of transgenic crops. Theor Appl Genet 83(8):940–946
Umar OB, Ranti LA, Abdulhamid AK, Biola MR, Victor KO (2021) Stresses in plants: biotic and abiotic. In: Ansari MR (ed) Current trends in wheat research. IntechOpen
Vavilov NI (1926) Studies on the origin of cultivated plants. Inst. Bot. Appl. Amel. Plant, Leningrad
Verma VC, Acharya S, Verma BC (2020) Millets for sustainable agriculture and nutritional security. Biot Res Today 2(10):1055–1057
Vinoth A, Ravindhran R (2017) Biofortification in millets: a sustainable approach for nutritional security, vol 8. Front Plant Sci, p 29
Wang C, Jia G, Zhi H, Niu Z, Chai Y, Li W et al (2012a) Genetic diversity and population structure of Chinese foxtail millet [*Setaria italica* (L.) Beauv.] landraces. G3: Genes Genomes Genet 2(7):769–777
Wang Y, Tang H, DeBarry JD, Tan X, Li JP, Wang XY (2012b) MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. Nucleic Acids Res 40:e 49
Weber SA, Fuller DQ (2008) Millets and their role in early agriculture. Pragdhara 18:69–90
Wilson ML, VanBuren R (2022) Leveraging millets for developing climate resilient agriculture. Curr Opin Biotechnol 75:102683
Wrigley C, Corke H, Walker CE (eds) (2004) Millet: in encyclo-paedia in grain science, vol 2. Elsevier, London
Xu Z, Wei Z, Yang L (1983) Tissue culture of *Setaria italica* and *Setaria lutescens*. Plant Physiol Commun 5:40
Xu ZH, Wang DY, Yang LJ, Wei ZM (1984) Somatic embryogenesis and plant regeneration in callus cultured immature inflorescence of *Setaria italica*. Plant Cell Rep 3:149–150
Yang X, Wan Z, Perry L, Lu H, Wang Q, Zhao C et al (2012) Early millet use in northern China. Proc Natl Acad Sci U S A 109(10):3726–3730
Zhang C, Zhang H, Li IX (2007) Advances of miller research on nutrition and application. J Chin Cereals Oils Assoc. 22-51-55
Zhang W, Zhi H, Tang S, Zhang H, Sui Y, Jia G et al (2021) Identification of no pollen 1 provides a candidate gene for heterosis utilization in foxtail millet (*Setaria italica* L.). Crop J 9(6): 1309–1319
Zohary D, Hopf M (2000) Domestication of plants in the old world: the origin and spread of cultivated plants in West Asia, Europe and the Nile Valley (No. Ed. 3). Oxford University Press

Genetic Improvement of Foxtail Millet Through Advanced Biotechnological Methods

16

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Abstract

Foxtail millet has been under cultivation since 5000 years in Asia and is a rich source of nutrients. Foxtail millet has been a well-known crop for its drought and salinity tolerance as well as high water use efficiency. In the last 50 years, various efforts have been made to improve foxtail millet through traditional and modern breeding methods. To feed the ever-raising population and to meet the quality standards of the consumers, emphasis should now be placed on the application of advanced biotechnological methods like transgenics and non-transgenics (ZFNs, TALENs, and CRISPR). In this chapter, various genetic improvement methods were discussed in brief with respect to foxtail millet. Ample scope is available for furthering the research and development in use of such advanced methods for making foxtail millet acceptable to growers and consumers, as well make it safe to environment.

Keywords

CRISPR · Genome editing · *Setaria* · Transgenics

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16.1 Introduction

Millets are small seeded granular cereals grown all over the world. Millets are members of grass (*Poaceae*) family and are classified into large millets namely, pearl millet, foxtail millet, proso millet, and finger millet; and small millets (kodo millet, barnyard millet, little millet, guinea millet, browntop millet, adlay millet, etc.). Millets are a rich source of protein, fiber, iron, and calcium; gluten-free; their antioxidant properties may help in reducing the risk of diabetes. From the agricultural perspective, millets are equally important because of their short generation time, minimal water requirement, survival in harsh environments, and resistance to drought and pests. In developing countries, people in the low economic strata consume millet as a food, because they offer high nutritional value at affordable prices compared to rice and wheat. In developed countries, millets are grown for birdfeed, hay, or as emergency crash crop and for beer production (Baker 2003). Globally, foxtail millet (*Setaria italica* L.) is the second-most widely planted millet after finger millet. Higher water use efficiency (WUE) and drought resistance in foxtail millet make it suitable for arid and semi-arid regions. Historically foxtail millet is one of the major crop and staple foods in many regions of China and India. The vernacular names of foxtail millet are Kora, Ghomi, Kangni, Kang, Hirse, Awa, Jo, Kaguno, and Mogara.

16.1.1 History and Domestication of Foxtail Millets

Foxtail millet is one of the oldest cereals and has grown since 5000 BC in China and 3000 BC in Europe. It was domesticated at least 16,000 years ago (Brink and Belay 2006; de Wet 2006; Hermuth et al. 2016; Sakamoto 1987). Carl Linnaeus in 1753, classified green foxtail (*Panicum viridis*) and foxtail millet (*P. italica*) into independent species, but later they were transferred to genus *Setaria.* The recent morphological, cytological, and molecular evidence suggest similarities between *S. viridis* and *S. italica* as they produce fertile hybrids, and share continuous and overlapping genetic variation (Dwivedi et al. 2012; Schontz and Rether 1998, 1999; Wang et al. 1995). The whole-genome sequencing helped in concluding that *S. italica* is derived from its wild ancestor *S. viridis* (Jia et al. 2013). Despite the controversy in the origin of foxtail millet, it is believed to be originated from Europe to Japan, especially central China and spread to India and Europe thereafter (Hermuth et al. 2016; Rahayu and Jansen 1996). Foxtail millet might have more than one domestication event; however, the most primitive archeological evidence of foxtail millet was found in the Cishan and Peiligang ruins in Northern China around 7400 and 7935 years before, respectively (Doust et al. 2009; Hunt et al. 2008; Li and Wu 1996).

16.1.2 Global and Indian Scenario

It is cultivated in arid and semi-arid regions of the world including Europe, China, India, Indonesia, the Korean peninsula, and the former U.S.S.R and remains a major rainfed crop in China and India to this day (Saxena et al. 2018). Foxtail millet requires warm weather in tropics and sub-tropical regions with low-to-moderate (50–75 cm) annual rainfall. The crop can be grown on well-drained loamy soils up to an altitude of 2000 m (Chapke et al. 2020). The figures for the global production of foxtail millet are not available, but together with other millets, the production is nearly 32.8 million tons (Mt) in 2020 (FAO stat data 2020, http://faostat.fao.org/). In 2014, China ranked first in terms of foxtail millet production with 1.81 Mt. from 0.72 million hectares (Mha) followed by India with 0.05 Mt. from 0.07 Mha (Diao 2017). In India, the area under cultivation has fallen drastically during the 1990s due to the introduction of more remunerative crops. At present, it is grown mainly in Andhra Pradesh, Karnataka, Telangana, Rajasthan, Maharashtra, Tamil Nadu, Madhya Pradesh, Uttar Pradesh, and to a small extent in the North-Eastern states of India.

16.1.3 Nutrition and Health Benefits

Foxtail millet is a rich source of nutrients like carbohydrates, proteins, dietary fibers, vitamins, and minerals (Table 16.1). There are nutritional and anti-nutritional components (phytic acid, tannins, and total phenolic compounds) in foxtail millet (Jan et al. 2022). Various phytochemicals are known to play a role in stimulating the immune system, lowering the division rate tumor cells, and increasing natural insulin levels (Table 16.2). Balanced diet and rational nutrition can lower the risk of type-2 diabetes (Chandalia et al. 2000; Montonen et al. 2003) while increased consumption of whole grains suppresses the level of high glycemic index (Hu et al. 2020; Xi and Liu 2016). Polyphenols of foxtail millet can improve gut microbiota disorders caused by colitis-associated carcinogenesis (Yang et al. 2020).

Table 16.1 Nutritional components of raw foxtail millet (Jan et al. 2022)

Parameters	Weight in g per 100 g of raw foxtail millet
Protein	22.00
Fiber	8.00
Carbohydrates	20.00
Fat	4.00
Calcium	3.10×10^{-3}
Iron	2.17×10^{-4}
Zinc	2.79×10^{-4}
Manganese	5.00×10^{-5}
Thiamine	5.90×10^{-5}
Riboflavin	1.10×10^{-5}
Niacin	3.20×10^{-4}

Table 16.2 Phytochemical compounds of foxtail millet with their molecular weight (Pujari and Hoskeri 2022; Xiang et al. 2019; Zhang and Liu 2014)

Name of the compound	Phytochemical	Molecular weight (g/mol)
Xanthophyll	Carotenoids	586.9
Zeaxanthin		568.88
p-Hydroxybenzoicacid	Phenols	138.12
Vanillicacid		168.14
Syringicacid		198.17
2-Methylisocitricacid		206.15
Ferulictruxillicacid		296.3
Homocitricacid		206.15
2-Methylisocitricacid		206.15
Sinapicacid		224.21
Cis-p-Coumaricacid		164.16
Protecatechulicaldehyde		138.2
p-Hydroxybenzaldehyde		122.12
Caffeicacid		180.16
Caffeoylspermidine		142.5
Kaempferol		286.24
Apigenin		564.5
Di-*p*-coumaroylspermidine		437.23
N′-caffeoyl-N′-feruloylspermidine		483.6
N,N′,N″-diferuloylspermidine-Dihexoside		673.8
Ferulicacid		194.18
Vanillicacid		338.2
Trans-p-Coumaricacid		326.3
Protecatechulicacid		154.12

16.1.4 Agricultural Importance

Foxtail millet is considered as a "smart crop" due to the following benefits that it offers to farmer, consumer, and planet as whole:

(a) Farmer-friendly by virtue of its drought and temperature resistance, and the efficiency to grow well with minimal water and other inputs (Brink 2006; Diao et al. 2014; Lata et al. 2013).
(b) Climate resilient as the grains of foxtail millet require only 26% of their weight in water to germinate (Diao et al. 2014).
(c) The straw of foxtail millet is an important fodder/hay for livestock and can also be used for thatching and bedding purposes.
(d) An excellent catch crop in the event of failure of the main crop (Austin 2006).
(e) A quick-growing species and the stubble left after harvest of fodder protects soil from erosion (FAO 2011; Brink 2006).

(f) A short-duration crop (80–90 days) with high water use efficiency, making it a suitable crop for cultivation in semi-arid, dry, and marginal lands.
(g) Provides shade and food for fauna (Rasnake et al. 2005).
(h) It is recommended in the "Forestry Reclamation Approach", where tree seedlings were grown under the cover of fast-growing grass species (Burger et al. 2009).
(i) Alleviates the effects of global warming as it can be grown in drier areas (Brink 2006).
(j) Requires very minimal quantities of synthetic pesticides and fertilizers.

16.1.5 Strengths and Weaknesses

Foxtail millet is a climate resilient crop with remarkable drought tolerance and water use efficiency (Lata et al. 2011b; Zhang et al. 2007a). In addition, morphological characteristics like dense root system, thick cell wall, epidermal cell arrangements, and miniscule leaf area contribute to drought tolerance (Li 1997). Lata et al. (2011a) have identified two highly drought tolerant cultivars (IC-403579 and Prasad) and two highly sensitive cultivars (IC-480117 and Lepakshi). Unlike drought tolerance, salinity tolerance in foxtail millet is perceived and/or responded through a different gene set, as only 18 genes (10%) were common in cultivar Prasad when exposed separately to drought and salinity (Puranik et al. 2011). Other abiotic stresses that affect foxtail millet are low temperature, water logging, and lodging. China has developed cultivar Liggu No. 26 with extreme cold tolerance; Longgu 28 and Nenxian 13 with lodging resistance; and Lugu No. 7 with tolerance to water logging (Chen and Qi 1993; Dwivedi et al. 2012). In India, sources for dwarfing oligo genes were identified by Dinesh Kumar et al. (1992) and can be used for developing dwarf varieties.

Among the biotic stresses, diseases affecting foxtail millet are blast (*Magnaporthe grisea*), rust (*Uromyces setariae*), brown spot (*Cochliobolus setariae*), grain smut (*Ustilago crameri*), and downy mildew or green ear (*Sclerospora graminicola*). Blast alone causes crop losses up to 60% (Karthikeyan and Gnanamanickam 2008). Notable insect-pests that infest foxtail millet are shoot fly (*Atherigona atripalpis* Wiedemann), cutworms, army worms and leaf-scrapping beetles (Hariprasanna et al. 2018). Cultivars with grains possessing lower moisture, crude protein, and total sugar content were known to be least preferred by shoot fly (Sanjeev et al. 2011).

16.1.6 Scope for Genetic Improvement

Genetic improvements of crops are beneficial to mankind and also essential to feed ever-raising global population. China and India have the largest population (1.426 billion and 1.417 billion, respectively) and hence require developing new crop cultivars/hybrids with higher yield and better nutrition value. The future areas of

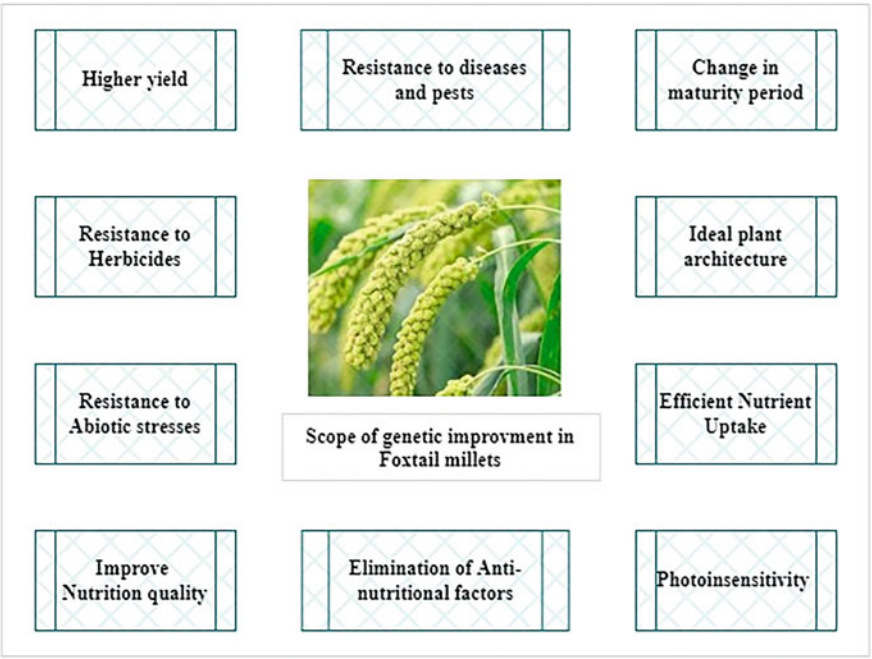

Fig. 16.1 Scope of genetic improvement in foxtail millets. Designed on https://app.diagrams.net/

crop improvement include, functional genomics of biochemical pathways; identification of sources of resistance to diseases and pests; effective utilization of male sterility to develop varieties; and breeding for protein and oil-rich varieties. Plant genetic improvement involves changes in specific portion of gene(s) to advance desired characters. Foxtail millet is nutritionally rich with several health benefits, as described above; however, it is least cultivated crop due to biotic and abiotic stresses; presence of anti-nutritional factors; poor yields and economic returns to farmers. Foxtail millet is drought-resistant crop but can be further improved for the traits like higher seed yield; resistance to diseases/pests and herbicides; and photoinsensitivity, to change maturity time by which plant can sustain in any type of environment (Fig. 16.1). Millets has multiple health benefits, but anti-nutritional compounds interfere in digestion and absorption of nutrients. These stumbling blocks in foxtail millets can be overcome by genetic modification. In the last decade, study of plant cell architecture also became an efficacious tool for crop improvement. Plant architecture facilitates morphological adaptations that can be adjusted to prevailing environmental conditions.

16.2 Genetic Improvement Methods

It is assumed that crop yields will be insufficient to nourish the world's large population by 2050. To overcome this, more consistent crop production must be achieved with the help of genetic improvement methods. In 2012, genome of foxtail millet (~423 Mb and ~ 400 Mb) was sequenced independently by Beijing Genome Initiative of China and Joint Genome Institute of the USA (Zhang et al. 2012; Bennetzen et al. 2012). Compact genome size of highly conserved region of foxtail millet will be useful to investigate plant architecture and genome evolution (Devos et al. 1998). Furthermore, foxtail millet is self-compatible C4 crop which make it ideal experimental crop. Foxtail millets have the ability to convert CO_2 into carbohydrate with higher photosynthetic efficiency, high WUE, abiotic stress tolerance, and minimal requirement of external minerals (Peng and Zhang 2021). However, most of the research work was done at genomic and breeding level. Rare work was published subject to plant tissue culture and genetic engineering. Research on genetic transformation was started in the late 1980s, and in 1995, genetically modified products were commercially available in many developed countries. In the last decade, science has welcomed many easy and less expensive genome editing techniques. Foxtail millet was not explored with such advanced biotechnological methods due to inaccessible optimized experimental methods. In the following sections, different genetic improvements methods were briefed along with accomplishments in foxtail millets regarding gene and genetics. The techniques of genetic improvement are classified into two main groups: breeding and genetic engineering (Fig. 16.2).

16.2.1 Breeding

Breeding is a very traditional method of genetic improvement in which principles of genetics were applied to transfer desired character from one plant to another. Breeding is easy, non-expansive, and genetically modified organism-free. Breeding further classified into classical breeding and modern breeding. Crossing, backcrossing, and mutagenesis are approaches in classical breeding; while marker-assisted breeding, speed breeding, gene pyramiding and genome-assisted breeding are part of modern breeding.

16.2.1.1 Classical Breeding

Countries like China, India, and France started foxtail millets breeding program in 1900s. In China, The Xinxiang Agricultural Research Institute in Henan Province initiated cross-breeding and released the cultivar "Xinnongdong 2" in 1959 (Diao and Jia 2017). In India, many crossed varieties such as TNAU186 (Co5 × SIA326) were nationally released for high seed yield. To enhance the resistance towards rust, high protein content and fodder yield, Co7 was developed by crossing of Co6 and Ise247. HMT100–1 developed for high tillering and stay-green characters by crossing of RS118 × PS3 (Seetharam 2020). France also upgraded foxtail millets for bird-feed production and herbicide-resistant green foxtail was used to breed the

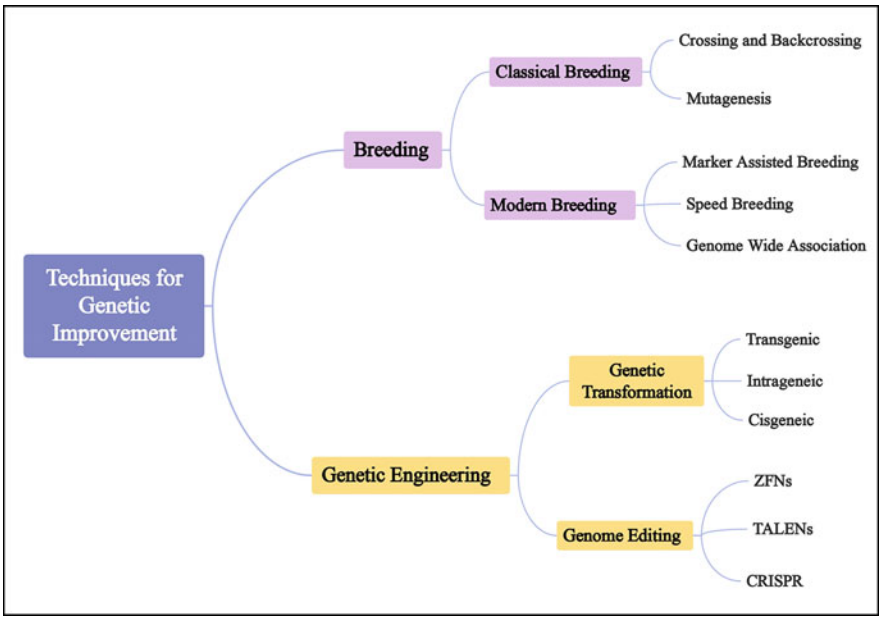

Fig. 16.2 Genetic improvement techniques employed in foxtail millet. Designed on https://gitmind.com/app/doc/796nxq0lvw0cn0ytihsls601t71bo2f8

herbicide-resistant foxtail millet varieties that were later introduced into China in the late 1990s (Wang et al. 2001). In spite of these efforts, floral morphology and anthesis behavior of foxtail millets pose difficulties to crossing.

Mutagenic plants can be generated by exposing the seeds to mutagens and this approach is known as mutation breeding or variation breeding. Conventional mutagens are chemicals, radiation, and enzyme which generate germplasm with desirable characteristics and which can be further used in breeding program. PS4 is a mutant cultivar with mutation in *SiA326* released for wider adaptability, high yield, and profuse tillering in India (Seetharam 2020). Jing et al. (2019) successfully developed white panicle mutant *wp1.b* using ethyl methanesulfonate mutagen. Mutagenesis is not target specific, and random mutagenesis can lead to deleterious modification in plants, therefore is least used approach by plant breeders.

16.2.1.2 Modern Breeding

Modern breeding is a wider term in which many molecular techniques/tools are used to obtain desired trait in the organism of interest. Ratio of success is high in modern breeding compared to classical breeding. Marker-assisted or marker-aided selection is widely used by breeders with classical breeding in which trait of interest is selected based on a marker. Markers can be morphological, cytological, biochemical, or DNA/RNA variations. Non-availability of markers associated with important agricultural traits, limited availability morphological markers, and protein dependencies of biochemical markers restricts their use in modern breeding. DNA-based marker

are widely used for selection and genetic improvement. In marker-assisted breeding, molecular marker technology is used to select independent plants based on their genotype and then match up with their phenotype for selected traits. According to Kushanov et al. (2021), many genetic markers are available: (1) non-PCR-based (restriction fragment length polymorphism/RFLP and variable number tandem repeat/ variable number tandem repeat/VNTR); (2) PCR-based markers (random amplified polymorphic DNA/RAPD, amplified fragment length polymorphism/ AFLP, simple-sequence repeat/SSR, expressed sequence tag/EST, inter simple-sequence repeat/ISSR, sequence-characterized amplified region/SCAR, cleaved amplified polymorphic sequence/CAPS, sequence-tagged site/STS, inter-retrotransposon amplified polymorphism/IRAP, sequence-specific amplification polymorphism/SSAP); and (3) DNA chip and sequencing-based markers (single-nucleotide polymorphism/SNP, diversity arrays technology/DArT).

Not all but many markers have found usefulness in foxtail millet improvement. Gupta et al. (2013) successfully reported 94 genomic SSR markers and generated 64 pairs of primers with 45.3% of polymorphic potential in F2 population of cultivar Prasad. Similarly, 45 SSR markers were developed to provide tools for examining genetic relatedness among foxtail millets and related species (Lin et al. 2011). Jia et al. (2013) studied sequence of 916 diverse foxtail millet varieties and pinned down 0.8 million common SNPs to construct a haplotype map which act as fundamental resource for research and genetic improvement in foxtail millet.

To support and accelerate the breeding process, new techniques emerged that shorten the agricultural breeding cycle and hasten crop improvement through rapid generation advancement, known as speed breeding (Watson et al. 2018). Forster et al. (2014) manipulated photoperiod and temperature for sorghum and achieved six generations per year using speed breeding. This opens up new area in foxtail millet breeding as Sorghum and foxtail millets both are part of the same subfamily, Panicoideae.

Identification of genome-wide genetic variation in population has become popular with the advances in sequencing and data-mining approaches. The genomic sequencing of genetically diverse foxtail millet accessions leads to development of molecular markers and use in genotype calling. Genome-wide association study (GWAS) performed along with the molecular markers and phenotyping data of agronomic traits. Candidate gene(s) for variable traits and their loci can be predicted through gene annotation, expression profiling, and genic variation identification. Recently, genomic regions and SNP loci associated with flowering time and pigmentation in 190 foxtail millet germplasm accessions were identified through genotyping-by-sequencing and GWAS (Upadhyaya et al. 2015). Hence, GWAS can be further utilized to identify the genetic basis of stress resistance, plant maturity, nutrition, seed yield, etc., and help in marker-assisted breeding.

16.2.2 Genetic Engineering

Since 1982, genetic engineering is a well-known method to manipulate plant genome by inserting a fragment of DNA isolated from a foreign organism. The increased demand for food can be solved by genetic engineering. Genetic engineering unblocks the key to develop resistance towards stresses, and herbicides; nutrition and quality; and seed yield. This technique allows direct or indirect manipulation of gene within similar or dissimilar species to achieve desired agronomic trait(s). Herbicide-resistant tobacco was the first genetically modified crop and its field trial was conducted in France and the United States (Fraley et al. 1983).

Compared to traditional breeding, genetic engineering is less time-consuming and more specific. Genetic engineering required prior knowledge of the gene associated with desired trait. Identified gene was copied, cloned into vector, and transferred into plant (identify, copy, insert, grow). There are several gene transfer methods available, some are indirect (*Agrobacterium* mediated and viral vector mediated) and direct (electroporation, microinjection, sonication, lipofection, particle bombardment, and laser microbeam (UV) induced gene transfer). Many of these methods have some limitations and they vary from species to species (Keshavareddy et al. 2018). Non-availability of optimized tissue culture protocol, off-target gene integration, and ploidy level of crop always act as bottleneck for genetic engineering. Simple genomic nature of foxtail millet is an advantage for effective usage of advanced genetic engineering techniques.

Genetic engineering can be further categorized in to transgenics and non-transgenic methods. In transgenic methods, a foreign gene(s) from any organisms, i.e., bacteria, plant, or animal are inserted into a plant. Varietal development using genetic transformation have been limited to pearl millets and Bahia grass. Liu et al. (2005) first reported optimized *Agrobacterium*-mediated gene delivery in foxtail millets. They tested expression of *GUS* gene after transferring T-DNA into callus through *Agrobacterium*-mediated gene transformation with 6.6% transformation efficiency. Transgenic foxtail millet line Si401 was developed by Fang et al. (2008) through *Agrobacterium*-mediated transformation by cloning pollen-specific gene under the control of pollen-specific promoter of maize (Zm13). Woefully, this line showed premature degeneration of tapetum, pre-deposition of fibrous bands in endothelium cells, followed by aborted pollen grains. Using the protocol developed by Fang et al. (2008), Wang et al. (2011, 2014) over-expressed *SiLEA14*, a homolog of the late embryogenesis abundant proteins in foxtail millets. Li et al. (2014, 2017), Pan et al. (2016), and Wang et al. (2011) improved cultivar Jigu11 for drought and salinity tolerance by targeting different functional genes, *SiARDP*, *SiASRP4*, *SiLTP*, *SiLEA14*, respectively.

First time biolistic perspective was used to functionally validate *SiPf40* gene in foxtail millet variety 3661 using florets as explants (Liu et al. 2009). A very limited number of genetic engineering methods including the transgenic method were used to target very few traits. There are a few disadvantages to Agrobacterium-mediated gene transfer: the transformation process is complex and time-consuming, and many a time field trials were restricted subject to GMO regulations. A major concern in

acceptance of transgenic crops by the consumer and general public is mixing of genetic materials between species that cannot hybridize by natural means. To address this concern, new transformation concepts namely, cisgenesis and intragenesis were developed.

Progress in plant genome sequencing cleared the way for identification of genes from crossable species known as cisgenes. Genetic modification achieved by transferring advantageous alleles from crossable species is known as cisgenesis. This is the combined approach of traditional breeding and modern biotechnology to hasten the breeding process. This permits to modify plant genome while the rest of the plant will be in same gene pool, hence, it is not considered as transgenic for environmental impact (Hou et al. 2014). Whereas, intragenesis is a method in which new combination of genes and regulatory sequence was transferred from that particular species. Certain new expression patterns can be obtained as a product of intragenesis that allows the construction of new genetic combinations by introducing flexibility in gene expression (Espinoza et al. 2013). The use of non-plant-based foreign sequence such as selection gene and vector backbone are restricted in both cisgenesis and intragenesis. RNA interference (RNAi) approach is one of the intragenesis techniques as it uses native DNA sequence. Reintroduction of gene of interest either with its own promoter and terminator (cisgenesis) or with a promoter and terminator isolated from the sexually compatible gene pool (intragenesis) lead to higher expression level of a trait. Whereas, for the lower expression level of a trait, different silencing constructs can be employed (intragenesis). A certain degree of disagreement exists in categorization of cisgenesis and intragenesis as different transgenics, as they all employ *Agrobacterium*-mediated transformation (Holme et al. 2013).

Unlike other crops, in spite of the availability of complete genome sequence of foxtail millet, crop improvement using cisgenesis and intragenesis techniques were lacking. Cisgenesis was used for expressing *R*-gene to impart late blight resistance to potato (Haverkort et al. 2009); expressing *VVTL-1*, *NtpII* genes to impart fungal disease resistance to Grapewine (Dhekney et al. 2011); over expressing *HvPAPhy_a* gene to improve grain phytase activity in Barley (Holme et al. 2012). Similarly, intragenesis was used to silence *GBSS*, *Ppo*, *R1*, *PhL*, *StAs1*, and *StAS2* genes in potatoes for high amylopectin, preventing black spot bruise, limiting cold-induced degradation of starch, and limiting acrylamide in French fries (Chawla et al. 2012; de Vetten et al. 2003; Rommens 2004; Rommens et al. 2006, 2008). Apart from gene silencing, intragenesis can also be used for gene expression. Scab resistance was developed in apple by expressing *HcrVf2* gene using intragenesis (Joshi et al. 2011).

Genetic manipulation can be done without introducing foreign gene into genome of the host. CRISPR/CAS9, CRISPR base editors, transcription activator-like effector nucleases (TALENs), and zinc finger nucleases (ZFNs) are popular technologies of contemporary genetic engineering and play key role in modern agriculture for crop optimization. All the artificial sequence-specific nucleases make double-stranded break in genome locus; later, the cleaved site is repaired by either non-homologous end joining (NHEJ) or homology-directed repair (HDR) (Rodríguez-Leal et al. 2017). NHEJ directly joins broken DNA by natural error-prone repair pathway, as a result natural insertion, deletion or frame-shift mutation

takes place. In the HDR, donor DNA functions as template to provide precise information for double-stranded break repair, facilitating DNA insertion or sequence replacement (Yin et al. 2017).

Herbicide resistance in foxtail millet is becoming key factor for effective and economical management of weeds using herbicides. Ran et al. (2018) reported for the first time the application of ZFN-dependent genome editing to develop imidazolinone herbicide-resistant wheat by changing single amino acid of acetohydroxyacid synthase (*AHAS*) gene. Similarly, ZFN-induced mutagenesis was utilized to create mutation in *IPK1* of maize (Shukla et al. 2009); mitochondrial malate dehydrogenase gene of tomato (Shukla et al. 2016); and *DCL4a* and *DCL4b* genes of soybean (Curtin et al. 2013).

Minor differences exist between TALENs and CRISPR; TALENs recognize DNA–protein interaction, while CRISPR recognizes RNA–protein interactions. They both do precise genome editing with many advantages and disadvantages. TALENs do not require PAM sequence to identify the target region; however it requires additional time and finance to address the difficulties in protein engineering. Whereas, CRISPR is able to do multiplexing and can target methylated DNA, it is inexpensive and easy to engineer, but have higher possibility for off-target effects (Malzahn et al. 2017). There were no studies looking into the application of TALENs in foxtail millet. However, some work reported from other crops belonging to Poaceae family. Liang et al. (2014) first reported application of TALENs for targeted mutagenesis in *ZmPDS*, *ZmIPK1A*, *ZmIPK* and *ZmMRP4* of maize with 23.1% efficiency. Similarly, Luo et al. (2019) attempted to reactivate pseudogene (lr21 Ψ) present in Fielder wheat using TALENs.

In the past two decades, CRISPR technique has expanded and now its variants viz., CRISPR/Cas9, CRISPR base editing, CRISPR/Cas12a, CRISPR/Cas12b, CRISPRI, CRISPR activation (CRISPRa), and CRISPRoff/CRISPRon are significantly applied for genome editing in many plant species. Lin et al. (2018) first tested CRISPR/CS9 mutagenesis in foxtail millets by knocking down *PDS* gene through protoplast transfection. Cheng et al. (2021) produced haploid embryo by CRISPR/Cas9-mediated mutation in *SiMTL* gene, which is orthologous to the maize Matrilineal/Not-Like-Dad/Phospholipase A (*MTL*/*NLD*/*ZmPLA*) gene. His study stated that haploid induction can be achieved in foxtail millet by downregulating *SiMTL* gene. Systematic protocols for genetic transformation of foxtail millets are available warranting standardization of the genome editing methods. Till date, only two reports are available of genome editing-based mutagenesis in foxtail millets that are limited to gene knock down. There lies scope for application of genome editing techniques to improve traits by manipulating genes directly to overexpress or downregulate expression.

Diploid genome of foxtail millet has been sequenced and annotated recently, thus increasingly becoming a model for C4 plants (Bennetzen et al. 2012; Jia et al. 2013). Many genes were functionally validated to have vital role in traits like enhancing seed yield, and resistance to drought, and salinity, but still their use is exempted for genetic improvement. There are several potential candidate genes for CRISPR-mediated gene manipulation in foxtail millets (Table 16.3). Developing an efficient

Table 16.3 Potential candidate genes of foxtail millet for CRISPR applications

Gene	Gene Function	Method for functional validation	Reference
A. Plant architecture			
NEKODE1	Tip-branched panicle	High-throughput sequencing, genotyping	Liu et al. (2022a)
SiMADS34	Panicle width, primary branch length, number of primary branches, panicle length and grain weight	High-throughput sequencing, genotyping	Liu et al. (2022a)
Heading date 1 (*Hd1*), *FLAVIN-BINDING KELCH REPEAT F-BOX 1*(*FKF1*), *Roc4* and *Seita.1G242300*	Plant height and heading time	NGS, linkage and bulked segment analyses	He et al. (2021), Liu et al. (2022a), Mauro-Herrera et al. (2013)
B. Salt tolerance			
SiOPR1	Salt tolerance	cDNA and microarray	Zhang et al. (2007b)
SiMYB19	Tolerance to high salt stress	Validate by cloning in yeast	Xu et al. (2022)
C. Drought, heat and salt tolerance			
OsbZIP72	Drought tolerance	NGS, promotor analysis	Mathan et al. (2021)
Aldose reductase	Drought and heat tolerance	Tested in foxtail millet	Veeranagamallaiah et al. (2009)
DNAj	ABA, salinity and drought stress tolerance	Transferring into wheat	Wang et al. (2009)
SiLTP	Salinity and drought tolerance	Tested in tobacco and foxtail millets	Pan et al. (2016)
SiASR4	Drought tolerance	Tested in *Arabidopsis thaliana* and foxtail millets	Li et al. (2017)
D. Seed yield, quality and plant maturity			
Seita.6G250500	Yield-related	High-throughput sequencing, genotyping	Liu et al. (2022a)
SiSWEET proteins	Total 24 SWEET genes	Protein sequencing analysis	Liu et al. (2022b)
Siprr37	Early spring sowing	NGS, QTL	Li et al. (2021)

transformation system could lead to creation of improved cultivars of foxtail millets in a way similar other crops from Poaceae family i.e., rice, wheat, sorghum, and maize. Recently, transgenic expression of zinc transporters resulted in the development of high grain zinc while transcriptomics revealed various calcium sensor genes involved in uptake, translocation, and accumulation of calcium in finger millet (Singh et al. 2014). Biofortification in foxtail millets is still limited by the presence of antinutrients like phytic acid, polyphenols, and tannins, which can be removed or minimized by employing advanced biotechnological methods.

16.3 Conclusions and Future Prospectus

Foxtail millet is one of the nutrient-rich cereal with numerous health benefits. Foxtail millet is a climate-resilient crop with an inbuilt tolerance to drought and salinity and higher water use efficiency. In spite of such good traits, acreage under its cultivation has declined over the years owing to poor seed yield, biotic stresses, water logging, and other anti-nutritional factors. Traditional ways of crop improvement are time taking and costly. Employing the advanced biotechnological methods like genome editing through CRISPR, TALENs, and ZFNs, etc. and use of MAS or GWAS can prove beneficial in creating cultivars that can have better agronomic traits, better nutrition, and remunerative to farmers. In the coming years, research should focus on application of such genetic improvement technologies to improve seed yield, plant architecture, stress resistance and reduce the anti-nutritional factors in the foxtail millet.

References

Austin DF (2006) Fox-tail millets (Setaria: Poaceae)—abandoned food in two hemispheres. Econ Bot 60(2):143–158

Baker RD (2003) Millet production. Guide A-414. New Mexico State University. http://www.hort.purdue.edu/newcrop/nexus/setaria_italica_nex.html. Accessed 12 April 2012

Bennetzen JL, Schmutz J, Wang H et al (2012) Reference genome sequence of the model plant *Setaria*. Nat Biotechnol 30:555–561

Brink M (2006) *Setaria italica* (L.) P. Beauv. Record from Protabase. In: Brink M, Belay G (eds) PROTA (plant resources of tropical Africa). PROTA Foundation, Wageningen, Netherlands

Brink M, Belay G (2006) Plant resources of tropical Africa 1: cereals and pulses. PROTA Foundation, Wageningen, Netherlands, p 298

Burger J, Davis V, Franklin J, et al (2009) Tree-compatible ground-covers for reforestation and erosion control. Forest reclamation advisory, no.6. The Appalachian regional reforestation initiative. https://www.osmre.gov/sites/default/files/inline-files/FRA_No.6.pdf. Accessed 22 September 2022

Chandalia M, Garg A, Lutjohann D et al (2000) Beneficial effects of high dietary fibre intake in patient with type 2 diabetes mellitus. N Engl J Med 342(19):1392–1398

Chapke RR, Shyam Prasad G, Das IK et al (2020) Latest millet production and processing technologies. ICAR-Indian Institute of Millets Research, Hyderabad, p 82

Chawla R, Shakya R, Rommens CM (2012) Tuber-specific silencing of asparagine synthetase-1 reduces the acrylamide-forming potential of potatoes grown in the field without affecting tuber shape and yield. Plant Biotechnol J 10(8):913–924

Chen J, Qi Y (1993) Recent developments in foxtail millet cultivation and research in China. In: Riley KW, Gupta SC, Seetharam A et al (eds) Advances in small millets. Oxford and IBH Publishing Co., New Delhi, India, pp 101–107

Cheng Z, Sun Y, Yang S et al (2021) Establishing *in planta* haploid inducer line by edited *SiMTL* in foxtail millet (*Setaria italica*). Plant Biotechnol J 19(6):1089

Curtin SJ, Anderson JE, Starker CG et al (2013) Targeted mutagenesis for functional analysis of gene duplication in legumes. In: Rose R (ed) Legume genomics: methods in molecular biology. Humana Press, Totowa, NJ, pp 25–42

de Vetten N, Wolters AM, Raemakers K et al (2003) A transformation method for obtaining marker-free plants of a cross-pollinating and vegetatively propagated crop. Nat Biotechnol 21(4):439–442

de Wet JMJ (2006) *Eleusine coracana* (L.) Gaertn. In: Brink M, Belay G (eds) PROTA (plant resources of tropical Africa). PROTA Foundation, Wageningen, Netherlands. Available via http://database.prota.org/search.htm. Accessed 19 March 2012

Devos K, Wang Z, Beales J et al (1998) Comparative genetic maps of foxtail millet (*Setaria italica*) and rice (*Oryza sativa*). Theor Appl Genet 96:63–68

Dhekney SA, Li ZT, Gray DJ (2011) Grapevines engineered to express cisgenic *Vitis vinifera* thaumatin-like protein exhibit fungal disease resistance. In Vitro Cell Dev Biol-Plant 47(4): 458–466

Diao X (2017) Production and genetic improvement of minor cereals in China. Crop J 5:103–114

Diao X, Jia G (2017) Foxtail millet breeding in China. In: Doust A, Diao X (eds) Genetics and genomics of *Setaria*. Springer, Cham, Denmark, pp 93–113

Diao X, Schnable J, Bennetzen J et al (2014) Initiation of *Setaria* as a model plant. Front Agric Sci Eng 1:16–20

Dinesh Kumar SP, Sashidhar VR, Ravikumar RL et al (1992) Identification of true dwarfing genes in foxtail millet (*Setaria italica* Beauv.). Euphytica 60:207–212

Doust AN, Kellog EZ, Devos KM et al (2009) Foxtail millet: a sequence driven grass model system. Plant Physiol 149:137–141

Dwivedi S, Upadhyaya H, Senthilvel S et al (2012) Millets: genetic and genomic resources. In: Janick J (ed) Plant breeding reviews. John Wiley & Sons, Inc., Hoboken, pp 247–375

Espinoza C, Schlechter R, Herrera D et al (2013) Cisgenesis and intragenesis: new tools for improving crops. Biol Res 46(4):323–331

Fang FQ, Qian Z, Ao GM et al (2008) Co-suppression of Si401, a maize pollen *speciWcZm401* homologous gene, results in aberrant anther development in foxtail millet. Euphytica 163:103–111

FAO (2011) Grassland index. In: A searchable catalogue of grass and forage legumes. FAO, Rome, Italy. Available via https://web.archive.org/web/20161017085644/, http://www.fao.org/ag/Agp/agpc/doc/Gbase/DATA/Pf000314.htm. Accessed 22 September 2022

Forster BP, Till BJ, Ghanim AMA et al (2014) Accelerated plant breeding. CAB Rev 9:1–16

Fraley RT, Rogers SG, Horsch RB et al (1983) Expression of bacterial genes in plant cells. Proc Nat Acad Sci 80(15):4803–4807

Gupta S, Kumari K, Muthamilarasan M et al (2013) Development and utilization of novel SSRs in foxtail millet [*Setaria italica* (L.) P. Beauv.]. Plant Breed 132(4):367–374

Hariprasanna K, Sangappa SK et al (2018) Foxtail Millet. ICAR-Indian Institute of Millets Research, Hyderabad, India, p 6

Haverkort AJ, Struik PC, Visser RGF et al (2009) Applied biotechnology to combat late blight in potato caused by *Phytophthora infestans*. Potato Res 52(3):249–264

He Q, Zhi H, Tang S et al (2021) QTL mapping for foxtail millet plant height in multi-environment using an ultra-high density bin map. Theor Appl Genet 134(2):557–572

Hermuth J, Janovská D, Čepková PH et al (2016) Sorghum and foxtail millet—promising crops for the changing climate in Central Europe. In: Konvalina P (ed) Alternative crops and cropping systems. InTech Open, Rijeka, Croatia, pp 3–28

Holme IB, Dionisio G, Brinch-Pedersen H et al (2012) Cisgenic barley with improved phytase activity. Plant Biotechnol J 10(2):237–247

Holme IB, Wendt T, Holm PB (2013) Intragenesis and cisgenesis as alternatives to transgenic crop development. Plant Biotechnol J 11(4):395–407

Hou H, Atlihan N, Lu ZX (2014) New biotechnology enhances the application of cis genesis in plant breeding. Front Plant Sci 5:389

Hu Y, Ding M, Sampson L et al (2020) Intake of whole grain foods and risk of type 2 diabetes: results from three prospective cohort studies. BMJ 370:m2206

Hunt HV, Vander Linden M, Liu X et al (2008) Millets across Eurasia: chronology and context of early records of the genera *Panicum* and *Setaria* from archaeological sites in the old world. Veg Hist Archaeobot 17:5–18

Jan S, Kumar K, Ahmed N et al (2022) Beneficial effect of diverse fermentation treatments on nutritional composition, bioactive components, and anti-nutritional factors of foxtail millet (*Setaria italica* L.). J Postharvest Technol 10(2):35–47

Jia G, Huang X, Zhi H et al (2013) A haplotype map of genomic variations and genome-wide association studies of agronomic traits in foxtail millet (*Setaria italica*). Nat Genet 45:957–961

Jing S, Sinh LN, Zhenhua C et al (2019) Generation and characterization of a foxtail millet (*Setaria italica*) mutant library. Front Plant Sci 10:369

Joshi SG, Schaart JG, Groenwold R et al (2011) Functional analysis and expression profiling of HcrVf1 and HcrVf2 for development of scab resistant cisgenic and intragenic apples. Plant Mol Biol 75(6):579–591

Karthikeyan V, Gnanamanickam SS (2008) Biological control of *Setaria* blast (*Magnaporthe grisea*) with bacterial strains. Crop Protec 27(2):263–267

Keshavareddy G, Kumar ARV, Ramu VS (2018) Methods of plant transformation: a review. Int J Curr Microbiol Appl Sci 7(07):2656–2668

Kushanov FN, Turaev OS, Ernazarova DK et al (2021) Genetic diversity, QTL mapping and MAS technology in cotton (*Gossypium* spp.). Front Plant Sci 12:779386

Lata C, Bhutty S, Bahadur RP et al (2011a) Association of an SNP in a novel DREB2-like gene SiDREB2 with stress tolerance in foxtail millet [*Setaria italica* (L.)]. J Exp Bot 62:3387–3401

Lata C, Gupta S, Prasad M (2013) Foxtail millet: a model crop for genetic and genomic studies in bioenergy grasses. Crit Rev Biotechnol 33:328–343

Lata C, Jha S, Dixit V et al (2011b) Differential antioxidative responses to dehydration-induced oxidative stress in core set of foxtail millet cultivars [*Setaria italica* (L.)]. Protoplasma 248:817–828

Li C, Wang G, Li H et al (2021) High-depth resequencing of 312 accessions reveals the local adaptation of foxtail millet. Theor Appl Genet 134(5):1303–1317

Li C, Yue J, Wu X et al (2014) An ABA-responsive DRE-binding protein gene from *Setaria italica*, *SiARDP*, the target gene of SiAREB, plays a critical role under drought stress. J Exp Bot 65(18): 5415–5427

Li J, Dong Y, Li C et al (2017) *SiASR4*, the target gene of SiARDP from *Setaria italica*, improves abiotic stress adaption in plants. Front Plant Sci 7:2053

Li Y, Wu SZ (1996) Traditional maintenance and multiplication of foxtail millet (*Setaria italica* (L.) P. Beauv.) landraces in China. Euphytica 87(1):33–38

Li Y-M (1997) Breeding for foxtail millet drought tolerant cultivars (in Chinese). In: Li Y (ed) Foxtail millet breeding. Chinese Agriculture Press, Beijing, China, pp 421–446

Liang Z, Zhang K, Chen K et al (2014) Targeted mutagenesis in *Zea mays* using TALENs and the CRISPR/Cas system. J Genet Genomics 41(2):63–68

Lin CS, Hsu CT, Yang LH et al (2018) Application of protoplast technology to CRISPR/Cas9 mutagenesis: from single-cell mutation detection to mutant plant regeneration. Plant Biotechnol J 16(7):1295–1310

Lin HS, Chiang CY, Chang SB et al (2011) Development of simple sequence repeats (SSR) markers in *Setaria italica* (Poaceae) and cross-amplification in related species. Int J Mol Sci 12(11): 7835–7845

Liu T, He J, Dong K et al (2022a) Genome-wide identification of quantitative trait loci for morpho-agronomic and yield-related traits in foxtail millet (*Setaria italica*) across multi-environments. Mol Gen Genomics 297:873–888

Liu Y, Feng X, Xu Y et al (2009) Overexpression of millet ZIP-like gene (*SiPf40*) affects lateral bud outgrowth in tobacco and millet. Plant Physiol Biochem 47(11–12):1051–1060

Liu YH, Yu JJ, Zhao Q (2005) Genetic transformation of millet (*Tetaria italica*) by *agrobacterium*-mediated. J Agric Biotechnol 13(1):32–37

Liu Z, Fan H, Ma Z (2022b) Comparison of SWEET gene family between maize and foxtail millet through genomic, transcriptomic, and proteomic analyses. Plant Genome 15(3):e20226

Luo M, Li H, Chakraborty S et al (2019) Efficient TALEN-mediated gene editing in wheat. Plant Biotechnol J 17(11):2026–2028

Malzahn A, Lowder L, Qi Y (2017) Plant genome editing with TALEN and CRISPR. Cell Biosci 7(1):1–18

Mathan J, Singh A, Ranjan A (2021) Sucrose transport in response to drought and salt stress involves ABA-mediated induction of OsSWEET13 and OsSWEET15 in rice. Physiol Plant 171(4):620–637

Mauro-Herrera M, Wang X, Barbier H et al (2013) Genetic control and comparative genomic analysis of flowering time in *Setaria* (Poaceae). G3: Genes Genomes Genet 3(2):283–295

Montonen J, Knekt P, Järvinen R et al (2003) Whole-grain and fibre intake and the incidence of type 2 diabetes. Am J Clin Nutr 77(3):622–629

Pan Y, Li J, Jiao L et al (2016) A non-specific *Setaria italica* lipid transfer protein gene plays a critical role under abiotic stress. Front Plant Sci 7:1752

Peng R, Zhang B (2021) Foxtail millet: a new model for C4 plants. Trends Plant Sci 26(3):199–201

Pujari NS, Hoskeri JH (2022) Minor millet phytochemicals and their pharmacological potentials. Pharmacog Rev 16(32):100–106

Puranik S, Jha S, Srivastava PS et al (2011) Comparative transcriptome analysis of contrasting foxtail millet cultivars in response to short-term salinity stress. J Plant Physiol 168:280–287

Rahayu M, Jansen PCM (1996) *Setaria italica* (L.) P. Beauvois *cv*. group foxtail millet. In: Grubben GJH, Partohardjono S (eds) Plant resources of South-East Asia, No. 10: Cereals. Backhuya Publishers, Leiden, The Netherlands, pp 127–130

Ran Y, Patron N, Kay P et al (2018) Zinc finger nuclease-mediated precision genome editing of an endogenous gene in hexaploid bread wheat (*Triticum aestivum*) using a DNA repair template. Plant Biotechnol J 16(12):2088–2101

Rasnake M, Lacefield G, Miksch D, et al (2005) Producing summer annual grasses for emergency or supplemental forage. University of Kentucky, cooperative extension service, College of Agriculture, AGR-88. Available via http://www.ca.uky.edu/agc/pubs/agr/agr88/agr88.pdf. Accessed 22 September 2022.

Rodríguez-Leal D, Lemmon ZH, Man J et al (2017) Engineering quantitative trait variation for crop improvement by genome editing. Cell 171(2):470–480

Rommens CM (2004) All-native DNA transformation: a new approach to plant genetic engineering. Trends Plant Sci 9(9):457–464

Rommens CM, Yan H, Swords K et al (2008) Low-acrylamide French fries and potato chips. Plant Biotechnol J 6(8):843–853

Rommens CM, Ye J, Richael C et al (2006) Improving potato storage and processing characteristics through all-native DNA transformation. J Agric Food Chem 54(26):9882–9887

Sakamoto S (1987) Origin and dispersal of common millet and foxtail millet. Japan Agric Res Q 21: 84–89

Sanjeev U, Jagadish PS, Mahendra R et al (2011) Biochemical basis of resistance to shoot fly (*Atherigona atripalpis* Wiede.) in foxtail millet. Int J Agric Environ Biotechnol 4(3):221–222

Saxena R, Vanga SK, Wang J et al (2018) Millets for food security in the context of climate change: a review. Sustainability 10:2228

Schontz D, Rether B (1998) Genetic variability in foxtail millet *Setaria italica* (L.) P. Beauv. — RFLP using a heterologous probe. Plant Breed 117:231–234

Schontz D, Rether B (1999) Genetic variability in foxtail millet, *Setaria italica* (L.) P. Beauv.: identification and classification of lines with RAPD markers. Plant Breed 118:190–192

Seetharam A (2020) Genetic improvement of small millets in India during pre and post coordinated project era. ICAR-Indian Institute of Millets Research, Hyderabad. Available via https://www.millets.res.in/books/Genetic_improvement_of_small_millets_in_India.pdf. Accessed 20 September 2022

Shukla V, Gupta M, Urnov F, et al (2016) Targeted modification of malate dehydrogenase. US patent 9,523,098, 20 December 2016.

Shukla VK, Doyon Y, Miller JC et al (2009) Precise genome modification in the crop species *Zea mays* using zinc-finger nucleases. Nature 459(7245):437–441

Singh UM, Chandra M, Shankhdhar SC et al (2014) Transcriptome wide identification and validation of calcium sensor gene family in the developing spikes of finger millet genotypes for elucidating its role in grain calcium accumulation. PLoS One 9(8):e103963

Upadhyaya HD, Vetriventhan M, Deshpande SP et al (2015) Population genetics and structure of a global foxtail millet germplasm collection. Plant Genome 8(3) eplantgenome2015.07.0054

Veeranagamallaiah G, Ranganayakulu GS, Thippeswamy M et al (2009) Aldose reductase expression contributes in sorbitol accumulation and 4-hydroxynon-2-enal detoxification in two foxtail millet (*Setaria italica* L.) cultivars with different salt stress tolerance. Plant Growth Regul 59(2): 137–143

Wang M, Li P, Li C et al (2014) SiLEA14, a novel atypical LEA protein, confers abiotic stress resistance in foxtail millet. BMC Plant Biol 14(1):1–16

Wang MZ, Pan YL, Li C et al (2011) Culturing of immature inflorescences and agrobacterium-mediated transformation of foxtail millet (*Setaria italica*). Afr J Biotechnol 10(73): 16466–16479

Wang RL, Wendel JF, Dekker JH (1995) Weedy adaptation in *Setaria* spp. I. Isozyme analysis of genetic diversity and population genetic structure in *Setaria viridis*. Am J Bot 82:308–317

Wang TY, Zhao ZH, Yan HB et al (2001) Gene flow from cultivated herbicide resistant foxtail millet to its wild relatives: a basis for risk assessment of the release of transgenic millet. Acta Agron Sin 27:681–687

Wang YF, Zhang J, Cui RL et al (2009) Transformation of wheat with *DNAj* gene from foxtail millet via pollen-tube pathway. Acta Agric Bor Sin 24(2):17–21

Watson A, Ghosh S, Williams MJ et al (2018) Speed breeding is a powerful tool to accelerate crop research and breeding. Nat Plants 4(1):23–29

Xi P, Liu RH (2016) Whole food approach for type 2 diabetes prevention. Mol Nutr Food Res 60(8):1819–1836

Xiang J, Zhang M, Apea-Bah FB et al (2019) Hydroxycinnamic acid amide (HCAA) derivatives, flavonoid C-glycosides, phenolic acids and antioxidant properties of foxtail millet. Food Chem 295:214–223

Xu C, Luo M, Sun X et al (2022) *SiMYB19* from foxtail millet (*Setaria italica*) confers transgenic rice tolerance to high salt stress in the field. Int J Mol Sci 23(2):756

Yang R, Shan S, Zhang C et al (2020) Inhibitory effects of bound polyphenol from foxtail millet bran on colitis-associated carcinogenesis by the restoration of gut microbiota in a mice model. J Agric Food Chem 68(11):3506–3517

Yin K, Gao C, Qiu JL (2017) Progress and prospects in plant genome editing. Nat Plants 3(8):1–6

Zhang G, Liu X, Quan Z et al (2012) Genome sequence of foxtail millet (*Setaria italica*) provides insights into grass evolution and biofuel potential. Nat Biotechnol 30:549–554

Zhang J, Liu T, Fu J et al (2007a) Construction and application of EST library from *Setaria italica* in response to dehydration stress. Genomics 90(1):121–131

Zhang JP, Liu TS, Zheng J et al (2007b) Cloning and characterization of a putative 12-oxophytodienoic acid reductase cDNA induced by osmotic stress in roots of foxtail millet. DNA Seq 18:138–144

Zhang LZ, Liu RH (2014) Phenolic and carotenoid profiles and antiproliferative activity of foxtail millet. Food Chem 174:495–501

Omics-Aided Crop Improvement in Foxtail Millet

17

Kanti Meena, Jinu Jacob, R. Swarna, and C. Deepika

Abstract

One of the oldest cultivated grasses, foxtail millet (*Setaria italica* (L.) P. Beauv.) is a promising model crop for nutritional studies, stress genomics, and in understanding the molecular basis of biofuel crops owing to its short growing cycle, small diploid genome size, excellent seed production, low repetitive DNA content, and its close relation to leading bioenergy grasses. Foxtail millet is a significant source of good quality protein, fiber, and minerals and offers potential antioxidant properties. The grains are rich in proteins and have unique protein composition dominated by prolamines. A crop with a comparatively smaller genome size of 515 Mb, foxtail millet has the earliest sequenced genome among small millets, offering it the leverage for advancement in modern molecular research. This has helped its progress in omics technologies which primarily aim at the collective detection of genes, transcriptomes, proteins, and metabolites in the crop with diverse applications. Known to be a drought-hardy and salinity-tolerant crop, the genetic basis of these traits has been studied through deep sequencing of RNAs and small RNAs and by analyzing their proteomes. Several of the stress-responsive transcriptional factor families have been systematically studied and database established for further use. Lately, there has been a surge in metabolomics studies in this crop that encompassed comprehensive nutritional profiling and species-specific accumulation of metabolites, stress metabolomics as well as metabolome-based GWAS to understand natural variations, all aiding in crop improvement with breeding applications. Among small millets, foxtail

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millet is a forerunner in the omics research arena that will be discussed in detail in this chapter.

Keywords

Foxtail millet · *Setaria italica* · Omics · Genomics · Proteomics · Metabolomics · Transcriptomics · Epigenomics

17.1 Introduction

17.1.1 Omics: The New Era of Technology

Novel advanced tools and technologies have been introduced to study the molecules present in the cell or tissue since the human genome project was initiated. They can be applied to a biological system that is of interest to create a picture of the underlying biology at a resolution previously unattainable (Micheel et al. 2012). Analysis of structural changes between species, as well as the functional activity of biochemical pathways, which were previously unachievable, may now be retrieved in their totality (Debnath et al. 2010). One of the distinct characteristics of omics technologies is their ability to function holistically within the context of the cell, tissue, or organism (Subramanian et al. 2020). The basic principle of these methodologies is that when a complex system is viewed as a whole, it may be better comprehended. Because holistic processes acquire and assess all relevant evidence to develop a hypothesis that can later be validated, they are ideal for hypothesis-generating research. So, in a nutshell, omics refers to a large field of science concerned with high-throughput measurements of biological molecules.

A few of the areas covered by the scientific discipline under "omics" include genomics, proteomics, transcriptomics, metabolomics, lipidomic, and epigenomics, which mainly focus on the genome (gene) the quantitative study of protein-coding genes, regulatory elements and noncoding sequences, proteome (emphasis on proteins), transcriptome (RNA and gene expression), and metabolome (metabolites and its networks in biological sample), as well as epigenome (altered histone proteins and methylation DNA on chromosomes) in an unbiased manner (Mayer 2011). In plant biology, omics technologies can help improve the quality of conventionally used medicines, crop nutrition, single gene analysis, sequence similarity, protein modeling, crop breeding, and insect resistance (Hakeem et al. 2016). Their popularity has risen in recent years across a variety of academic disciplines. The more current methods have improved the sequencing of environmental DNA and RNA at a lower cost (metagenomics). Metagenomic and transcriptomic approaches can be used to explore the genome assembly of species from a pool of organisms, such as soil microbial genomes (Tanveer et al. 2018).

Genomics includes issues such as the evolution of the genome, its structure, and function, as well as the mapping, characterization, and quantification of the numerous genes involved in the manufacture of various proteins using RNA and enzymes.

The proteome refers to the total number of proteins in a cell, tissue, or organism and proteomics studies the biochemical properties, functional roles, and changes in their numbers and modifications during development and in response to internal and external stimuli. The transcriptome is the collection of all messenger RNA molecules in one cell, tissue, or organism. It comprises the quantity or concentration of each RNA molecule along with its identification. The metabolome is the collection of all metabolites (by-products of cellular processes) discovered in a biological cell, tissue, organ, or organism. Metabolomics is the study of chemical fingerprints or metabolite profiles, created by certain biological activities throughout their activity.

17.1.2 Foxtail (*Setaria Italica* (L.) P. Beauv)

Foxtail millet (*Setaria italica* (L.) P. Beauv.), also known as Italian millet, German millet, or hay millet (Kumari et al. 2011), is the second most widely cultivated millet species (CGIAR Factsheet; http://www.cgiar.org/our-research/crop-factsheets/millets) and a key food crop in East Asia, particularly. The genus *Setaria* is part of the subfamily Panicoideae, which has roughly 125 species spread around the world (Muthamilarasan and Prasad 2015). Foxtail millet is mostly grown in Northern China, where it is thought to have originated (Yang et al. 2012) and has been domesticated for centuries. It is a self-pollinating, diploid ($2n = 18$), C4 panicoid crop and has been considered as a model crop for genetic and molecular investigations recently due to its short life cycle, low level of repetitive DNA, compact diploid genome, inbreeding nature, and exceptional abiotic stress-tolerance traits (Jayaraman et al. 2008; Doust et al. 2009; Pan et al. 2018; Lata et al. 2010). It is extensively farmed in dry and semi-arid regions of the world owing to the crop's ability to tolerate extreme environments such as high salinity and drought (Sharma and Niranjan 2017). Furthermore, it is grown as a forage crop in Australia, North America, Africa, India, and Japan, as well as a substitute food source. Green foxtail millet (*Setaria viridis* (L.) Beauv.), which grows in North China, is regarded as the wild parent of this crop, which was primarily domesticated in China (Fukunaga and Kato 2003). Along with switchgrass and pearl millet, it serves as a model crop for additional biofuel species (Zhang et al. 2012).

Foxtail millet has a lot of energy, lipids, proteins, fatty acids, vitamins, minerals, and high contents of dietary fiber (Zhu et al. 2018) and phytochemicals (Zhang and Liu 2015). It thrives in adverse agroclimatic conditions and is grown because it is non-glutinous, acid-free, and easily digested. Except for lysine and threonine, proteins in this crop are good sources of all essential amino acids, especially sulfur-containing amino acids such as methionine and cysteine. Foxtail millet grains are high in vitamins, phytochemicals, phenolic acids, and glycated flavonoids which are highly antioxidant in nature. Its grains have a low glycemic index as its high levels of dietary fiber have been demonstrated to help maintain glucose metabolism and it has high b-glucan content. It has been found that the resistant starch and dietary fiber available in these grains have hypoglycemic and hypolipidemic properties. Millet grains have antioxidative properties due to their greater levels of

phytochemicals such as phenolics, tannins, phytates, and microminerals, among others.

Bioactive compounds present in foxtail millet, like phenolics, bioactive peptides, carotenoids, and tocopherols, have been demonstrated to offer a variety of health and biological benefits, including the prevention of hyperglycemia, hypertension, and proliferative disorders (Hutabarat and Bowie 2022). Increased foxtail millet consumption may bring health benefits such as a lower risk of acquiring chronic illnesses such as abnormal cholesterol metabolism, type 2 diabetes, and hypertension (Sharma and Niranjan 2017; Hou et al. 2018). The positive effects of foxtail millet on human health are mostly attributed to antioxidants such as phenolics, carotenoids, −tocopherol, and specific polysaccharides (Sharma and Niranjan 2017). The most important phenolic compounds in foxtail are reported as phenolic acid and flavonoids (Chandrasekara and Shahidi 2011; Zhang and Liu 2015; Pradeep and Sreerama 2018).

17.2 Advances in Foxtail Millets Omics

Foxtail millet has one of the earliest millet genomes to be sequenced. The decoding of the foxtail genome by Bennetzen et al. (2012) and Zhang et al. (2012) has brought momentum to genomics-related research in the crop and has helped in complete exploitation of the genetic potential of this crop. The progress around various "omics" techniques is also comparatively faster in this crop. The rest of the chapter describes the advances in genomics, transcriptomics, proteomics, metabolomics and epigenomics research in this crop.

17.2.1 Genomics

Genomics is the study of the full set of DNA of an organism encompassing all its genes (genome). Genomics helps in understanding how genes affect specific traits of interest and involve studies focusing on a small number of genes or portions of genes with known functions (Koonin and Galperin 2003). The advances in next-generation sequencing (NGS) technology and computational biology have made acquiring genome-scale data easier than ever and hence have reduced the gap between genotype and phenotype and improved our ability to analyze and understand whole genomes. Genome-wide association studies (GWAS) had a significant role in identifying the candidate genes/regions associated with traits of interest called quantitative trait loci (QTL) (Gondro et al. 2013). Individual genome sequencing is ushering in a new era of research opportunities, and it has helped in the functional analysis of genes, efficient genotype selection, identifying quantitative differences due to environmental factors ultimately helping in designing efficient experimental setups for more successful disease control (Bai et al. 2013) both in plants and animals.

The genome of foxtail millet was independently sequenced by the United States Department of Energy-Joint Genome Initiative (USDOE-JGI) (Bennetzen et al. 2012) and the Beijing Genome Initiative (BGI), China (Zhang et al. 2012), two well-known organizations from different parts of the world. The release of the whole genome sequence of this crop has made a great way to discover high-throughput molecular markers throughout the genome at a large scale for application in crop improvement.

Genome sequence data facilitated comparative genome studies, genome-wide investigations, and characterization and physical mapping of various transcription factors (Muthamilarasan et al. 2014a). Foxtail millet, like other grasses, underwent Whole Genome Duplication (WGD) approximately 70 MYA (Million Years Ago) possibly leading to the expansion of many families of genes (Zhang et al. 2012). Elaborate studies on such gene families would enable a better understanding of the relationship between different grass species and would help in predicting the functions of newly identified genes. In this line, systematic characterization and expression profiling of NAC transcription factors (Puranik et al. 2013), WD40 proteins (Mishra et al. 2014), and ALDH (Alcohol Dehydrogenase) (Zhu et al. 2014) gene families have been undertaken in foxtail millet that aided in understanding the cellular network of these proteins, for comparative genomics of other under-exploited grass species and in understanding the neofunctionalization of duplicated genes over evolution. More such studies would help in digging up foxtail millet-specific gene families that are implicated in molding this species into a stress-tolerant one. After the release of the whole genome sequence and the popular delegation of the crop as a model species for biofuel crops, genomics studies in foxtail millet are gaining momentum which demanded the development of a comprehensive portal for genomic information retrieval. FmMDb, a database of foxtail millet, is the first of its sort for structural and comparative genomics in millet and bioenergy grass species and it provides accessibility to information on a wide range of DNA markers (Suresh et al. 2013).

Before genome sequencing, a lot of markers in foxtail have been developed as reported by the literature. The first foxtail millet restriction fragment length polymorphism (RFLP)-based map was constructed by Wang et al. (1998) and was used for comparative evaluation of genetic maps of foxtail millet and rice (Devos et al. 1998). Later on, EST-based simple sequence repeat (SSR) markers were applied for phylogenetic and transferability studies in foxtail millet (Jia et al. 2007, 2009; Gupta et al. 2012) followed by the development of different marker system such as EST-SSRs (Kumari et al. 2013), intron length polymorphic (ILP) markers (Gupta et al. 2011; Muthamilarasan et al. 2014b), miRNA and TE-based markers (Yadav et al. 2014a, b), and GATA gene family marker (Lai et al. 2022).

In a step forward in the molecular marker scenario in foxtail millet, Solexa sequencing technology was employed to re-sequence an important landrace, Shi-Li-Xiang along with reference genomes which revealed many single nucleotide polymorphisms (SNPs), insertion–deletions (indels), and structural variations (SVs). Interestingly, ~40% of SNPs resided in genes that are related to pathogen disease resistance such as NB-ARC domain, Protein kinases or LRRs which makes

sense as pathogen pressure is one of the main driving forces behind protein diversification. Many of these SNPs and indels could be prized tools in the future for Marker-Assisted Selection (MAS) and Genome-Wide Association Studies (GWAS) in foxtail millet which is a yet-to be exploited/advanced area in this crop (Bai, et al. 2013). In yet another large-scale SNP study (Jia et al. 2013), 2.58 million SNPs were identified by sequencing 916 diverse foxtail millet varieties, and the SNP information was utilized to identify many genomic loci associated with a variety of agronomic traits.

The different molecular systems for different applications have been utilized for genomic study in foxtail millet and a few of them are represented in Table 17.1. Foxtail millet molecular marker studies are still lagging in the areas of marker–trait association analysis and MAS with just a few reports on association mapping related to the mapping of yield contributing agronomic traits (Gupta et al. 2014; Jia et al. 2013).

17.2.2 Transcriptomics

Transcriptome is the collective population of messenger RNAs (mRNAs) in a cell, tissue, or organism, and the study of this population is referred to as transcriptomics (Lata 2015). It is a high-throughput technique having a high sensitivity, and high resolution, that is used to study organisms without needing any reference genome sequences (Guo et al. 2021). Gene expression varies across plant tissues and organs and understanding and comparing these variants reveal genes that are regulating traits, secondary metabolite and resistance gene expression patterns, and plant responses to their environment. The transcriptome technique is a useful tool that allows for in-depth analysis and quantification of changes caused by abiotic stresses at the organismal level (Lata 2015). Genome-wide expression profiling studies make it feasible to identify genes involved in many biological processes and stress regulation networks (Reddy et al. 2012). Transcriptomics has been transformed by the recent development of high-throughput NGS technology, which enables massive-scale RNA analysis using cDNA sequencing (Voelkerding et al. 2010).

Due to its ability to tolerate drought stress naturally, foxtail millet is endowed with higher water use efficiency (WUE) when compared to other cereals such as sorghum, maize, and wheat (Lata et al. 2013). Zhang et al. (2007) identified several genes in foxtail millet that were differentially expressed because of drought using suppression subtractive hybridization (SSH) technique and a cDNA microarray. They reported the gene expression data that revealed that various gene sets have been active in roots and shoots under drought stress and the majority of them were activated during the protein deterioration pathway. Jayaraman et al. (2008) reported that 90 differentially expressed transcripts were identified in response to salt stress using cDNA-AFLP technique in salt-tolerant and sensitive foxtail millet cultivars. Zhang et al. (2012) reported 586 genes that probably play a critical role in response to stress and adaptation in foxtail millet. Transcriptome analysis of drought-stressed foxtail millet showed the involvement of Fbox protein as reported by Yin et al.

Table 17.1 List of different genomic approaches utilized in foxtail millet

S. No.	Application	Approach	Genotype	Marker	References
1	Identification of markers	Whole genome sequence	Zhang gu and A2	SNPs, InDels, and SVs	Zhang et al. (2012)
2	High-density	RIL population	B100 X green foxtail A10	SNPs	Bennetzen et al. (2012)
3	High-density physical map	Whole genome	–	Microsatellites	Pandey et al. (2013)
4	miRNA-based markers	Whole genome sequence	–	microRNAs	Khan et al. (2014)
5	Haplotype map	Sequenced	916 foxtail millet varieties	SNPs	Jia et al. (2013)
6	Identification of markers	Resequencing	Landrace 'Shi-Li-Xiang' (SLX)	Genetic variations	Bai et al. (2013)
7	–	Resequencing	SLX and Yugu1	SNPs, InDels, sequence variants	(Bennetzen et al. 2012)
8	–	–	SLX and Zhang gu	SNPs, InDels, sequence variants	Zhang et al. (2012), Bai et al. (2013)
9	Genetic structure analysis and GWAS	Resequencing	190 foxtail millet accessions	SNPs	Upadhyaya et al. (2015)
10	Repeat junction-based markers	Genome sequence data	–	Transposable elements identification	Yadav et al. (2015)
11	Identification of morphological and agronomical candidate gene linkage map using	Next-generation sequencing	RILs Japanese and a Taiwanese landrace	Flexible ddRAD-seq	Fukunaga et al. (2022)
12	High-density genetic map	RAD-seq	Hongmiaozhangu and Changnong35	SNP	Wang et al. (2017)
13	High-density genetic map and QTL for agronomic and yield traits	–	Yugu1 and Longgu7	SSR	Fang et al. (2016)

(continued)

Table 17.1 (continued)

S. No.	Application	Approach	Genotype	Marker	References
14	Genetic mapping and marker development	–	B100 and A10	SSR	Jia et al. (2009)
15	Constructed a haplotype map of foxtail millet	Resequencing of 916 varieties	–	SNPs	Jia et al. (2013)
16	Developed large scale ILP markers and demonstrated their utility in germplasm characterization, transferability, phylogenetics and comparative mapping studies in millets and bioenergy grass species	From publicly available foxtail millet ESTs	–	ILP (intron length polymorphism) markers	Muthamilarasan et al. (2014b)

(2014). Qi et al. (2013) attempted transcriptomics through deep sequencing and identified about 2824 genes that were responsible for drought tolerance in the crop. Among those, 48.23% of genes were upregulated involving dehydrins, HSPs, aquaporins LEA proteins, and phosphatase 2C protein which might be imparting drought tolerance in foxtail millet.

To understand the molecular processes underlying tolerance to dehydration stress, the transcriptome changes of foxtail millet were analyzed at two time points (early and late) by Lata et al. (2010). At 0.5 and 6 h of PEG-induced dehydration stress, 21-day-old Prasad seedlings (drought tolerant) were utilized to generate two suppression subtractive hybridization (SSH) forward libraries. Both libraries had 327 different ESTs, which were grouped into 11 groups based on their putative functions. The expression profiles of nine randomly selected upregulated genes during dehydration stress were compared between the tolerant cv. Prasad and sensitive cv. Lepakshi using qRT-PCR. The study concluded that plant responses to dehydration stress are complicated, including essential genes involved in metabolism, stress, signaling, transcription regulation, translation, and proteolysis. Similarly, expression analysis of PEG-treated JinGu45 seeds showed that the DEGs were associated with cell stimulation and response as well as metabolism based on GO and plant hormone signal transduction and phenylpropanoid metabolism based on KEGG enrichment analysis. They reported two genes SnRK2 and PAL to be involved in seed germination under drought stress in foxtail (Xu et al. 2018).

Recently, Xu et al. (2019) discovered that genotypes Damaomao and Hongnian exhibited high conservatism in a variety of key biological pathways that respond to drought stress, including hormone biosynthesis (particularly abscisic acid-responsive genes), proline and soluble sugar synthesis, and ROS (reactive oxygen species) metabolism and they were all part of foxtail millet's early drought response strategy. In times of water constraint, the earlier genotype fared better than the latter genotype, with a more moderate relative water content and a slower reduction in chlorophyll. However, some of the genes involved in these pathways showed a variety of expression patterns. KEGG pathway analysis found that the greater the number of active genes in the ascorbate-glutathione cycle, the lower the malondialdehyde in both genotypes.

In one of the latest studies on drought tolerance in foxtail millet, Guo et al. (2022) carried out transcriptome analysis of three cultivars and reported 2954, 1531, and 2344 deferentially expressed genes (DEGs) under drought stress. DEGs were significantly enriched in genes involved in photosynthesis, chlorophyll metabolism, amino acid metabolism, and carbohydrate metabolism in all the cultivars. They shortlisted 46 genes whose transcription changes were consistent with the drought resistance trends among three cultivars of foxtail millet. Pan et al. (2019) screened 14 varieties of foxtail millet for salt tolerance and reported transcriptome analysis by RNA-sequencing in tolerant and susceptible varieties before and after salt treatment. They identified a total of 2786 and 4413 DEGs in tolerant and resistant lines, respectively, which were associated with several processes, including ion transmembrane transport, redox homeostasis, secondary metabolism, organic acid, polyamine, and phenylpropanoid biosynthetic process. The qRT-PCR analysis for genes such as

cation transporter (HKT8), peroxidase (POD), flavanone 3-dioxygenase (FL3H), and MYB transcription factors revealed higher expression variation in tolerant varieties under salinity, suggesting that these genes may play important roles in the salt response process of foxtail millet. Similarly, to understand the molecular responses behind plant responses to short-term salt stress, two SSH cDNA libraries (forward and reverse) of two contrasting cultivars were generated. It resulted in 249 non-redundant ESTs which were classified into 11 functional classes. They reported that these transcripts could be novel gene sources for specialized responses to short-term salt stress in foxtail millet. In response to salt stress, 159 (63.9%) of these clones were differently expressed (two-fold), with 115 upregulated and 44 downregulated. Several transcription factors and signaling genes were preferentially expressed in the tolerant cultivar (Puranik et al. 2011).

Very recently, Han et al. (2022) examined how 104 foxtail millet accessions responded to salt stress (0.17 molL-1 NaCl) and used transcriptome analysis to determine the molecular mechanisms of salt responsiveness in a salt-tolerant (Hong Gu 2000) and a salt-sensitive (Pu Huang Yu) accession. According to their findings, there were 2019 and 736 genes that were differentially expressed when exposed to salt stress in salt-sensitive and salt-tolerant accessions, respectively. The reactions of foxtail millet to salt stress were discovered to be significantly dependent on the transcription factor families bHLH, WRKY, AP2/ERF, and MYB-MYC. Salt-sensitive accessions had restricted growth due to the downregulation of ribosomal protein-related genes. Transcriptome analysis of PEG-treated foxtail millet seeds revealed that DEGs increased more throughout the water uptake phase (phase III) than they did during the rapid initial uptake phase (phase I) and the plateau phase (phase II) under PEG stress; Yu et al. (2020). The highly enriched DEG categories consisted of phase III phenylalanine metabolism, plant hormone signal transduction, and phenylpropanoid biosynthesis. Twenty foxtail millet genes related to phenylpropanoids were shown to be downregulated throughout the period of increasing water intake under PEG stress. The phenylpropanoids-related pathway contained four genes: phenylalanine ammonia-lyase, 4-coumarate-CoA ligase 3, cinnamoyl-CoA reductase 1, and cationic peroxidase SPC4, which were discovered through additional expression analysis and were critical in the response of foxtail millet to PEG stress at different germination times.

Another trait is grain filling, which is an important developmental process for foxtail millet productivity and quality, which has received less attention. Examination of transcription patterns and identification of genes significantly involved in grain filling at five different unique developmental stages using RNA-sequencing showed 11,399 DEGs and 9002 transcription factors (TFs) by Wang et al. (2020). Through functional annotation and investigating temporal expression patterns, several important genes involved in grain filling were discovered including genes in starch biosynthesis, cell-wall invertases, hormone signalling, and polyamine metabolism. The study helped in expanding our understanding of the complex molecular pathways that are associated with panicle formation in foxtail millet.

To study genes associated with leaf tannin content and identification of low tannin resources Li et al. (2022a, b), collected leaves of 4 varieties endowed with

different levels of tannin content and identified 335 DEGs using transcriptomics. GO and KEGG enrichment analyses identified many DEGs linked to tannin biosynthesis pathway. Similarly, another trait of plant height in foxtail was studied by Zhu et al. (2022) and they reported 8918 DEGs from RNA-sequencing analysis. GO analysis revealed DEGs associated with activities earlier reported to be linked with plant height that is gibberellin metabolic process and oxidoreductase activity.

Evidence is mounting on the critical roles played by miRNAs in multiple biological processes of plants by regulating the transcript levels of their target genes. The recent completion of the sequencing of foxtail genome has facilitated the identification of the miRNA sequences, prediction of their target genes, and successive validation. A study by Han et al. (2014) has identified 271 foxtail millet miRNAs belonging to 44 families using a bioinformatics approach. 432 potential target genes for 38 miRNA families were also identified that are involved in various stages of plant development and signal transduction pathways. Studies of this sort on miRNAs lead to a better understanding of the molecular machinery active inside the plant at various stages of growth and development.

17.2.3 Proteomics

Characterization, quantification, expression patterns, structures, and functions of all the proteins present in a cell, organ, or organism at a given time are the main facets of proteomics (Mayer 2011), which provide novel biomarkers, information on post-translational modifications of the proteins, and extent of protein-protein interactions (Chandramouli and Qian 2009) using the high-throughput technique. The core of modern proteomics is mass spectrometry, which is a vital new technology in the field of protein characterization mainly focusing on large-scale quantification of specific proteins expressed in specific cell types under specific conditions (Mayer 2011). In this technique, all chemical components in a sample are ionized and the resulting charged molecules (ions) are analyzed based on their mass-to-charge (m/z) ratios (Aebersold and Mann 2003). The complex mixtures are separated before subjecting them to MS analysis through one- or two-dimensional polyacrylamide gel electrophoresis (1D-PAGE, 2D-PAGE), HPLC, or liquid chromatography. Recognizing the parent protein from peptides digested is carried out in silico using a software of search engine wherein the mass of the experimentally derived protein fragment is matched to what is available in the database. Chemical, metabolic, enzymatic, and label-free labeling are all MS-based quantification approaches that can reliably measure the identified protein (Mayer 2011).

Although proteomics research has come a long way and its advancements are evident, reports on proteomics application in foxtail millet are limited. Abiotic stress is a major stress adversely affecting crop production and productivity all over the world. Stress proteomics is an upcoming area, and it is a promising approach to identify stress-regulated proteins to be used in stress management. A comparative proteome investigation of temporal changes in the total protein profile of seven-day-old salt-stressed seedlings using 2-D electrophoresis reported 175 protein spots with

maximum up-regulation of gene (Veeranagamallaiah et al. 2008). MALDI-TOF/MS analysis and database search identified proteins involved in a variety of processes such as photosynthesis, cell wall biogenesis, signal transduction, and proteins involved in metabolism of energy, lipid, nitrogen, carbohydrate, and nucleotides.

Zhangzagu10, a hybrid generated by a two-line hybrid breeding strategy, endowed with qualities such as high yield, stability, lodging, and disease resistance, was studied for the molecular basis of heterosis in foxtail by Weng et al. (2020). A proteome quantitative examination of the hybrid and its parents identified a total of 4015 proteins, including about 276 (male) and 610 (female) proteins with differential expression between parent and hybrid. The expression profile of the hybrid closely mirrored that of the male parent. The GO assay categorized the differentially expressed proteins into three functional categories namely cellular component, biological process, and molecular function. Differentially expressed proteins (DEPs) in photosynthesis, carbon fixation, and metabolic pathways were found to be significantly enriched in the KEGG metabolic pathway analysis. According to quantitative real-time-PCR (qRT-PCR) verification of the differential expression data, two-on-two hemoglobin-3, G-type lectin S-receptor-like serine/threonine-protein kinase SD2–5, and peroxidase 5-like were among the genes whose expression levels were higher in Zhangzagu10 than in its father. Seven selected genes, including indole-3-acetaldehyde oxidase, cysteine-rich receptor-like protein kinase 45, peroxidase 64-like, and others, were shown to be more expressed in Zhangzagu10 than in its male parent but much lower in females.

Another major abiotic stress factor, drought, was investigated in foxtail seedlings using a quantitative proteomic approach that identified 2474 DEPs by Pan et al. (2018). The proteins belonged to stress and defense responses, ROS scavenging, protein synthesis, hormone metabolism, photosynthesis, carbon metabolism, fatty acid and amino acid metabolism, polyamine biosynthesis, and cell wall modifications based on their GO classification. They reported 252 and 69 proteins as upregulated and downregulated, respectively. Similarly, a 2-D combination with (MALDI-TOF/TOF) proteomic study of landrace Huangjinmiao grain protein under drought found 104 differentially abundant protein spots (DAPs), with 57 up- and 47 downregulated (Li et al. 2019). These proteins have been linked to a variety of biological processes, including storage proteins, starch and sucrose metabolism, glycolysis/gluconeogenesis, amino acid biosynthesis, detoxification and defense, protein degradation, the tricarboxylic acid (TCA) cycle, protein synthesis, energy metabolism, transporters, the pentose phosphate pathway, and signal transduction. They observed an increase in gliadin levels as albumin concentration decreased. Similarly, drought-induced variations in grain protein content were reported by Xu et al. (2020) and the study revealed 83 proteins associated with numerous biochemical and metabolic processes, including storage proteins, amino acid and protein metabolisms, energy metabolism, stress response and defense, and chaperone levels. Drought was found to cause an increase in grain protein content, especially the storage proteins. DEPs implicated in starch metabolism, protein and amino acid production, and drought stress were all enhanced in one line, perhaps increasing the grain protein content of seeds, on the other hand, protein biosynthesis was affected.

Selenium regulates the antenna complex of photosynthesis, enhancing the pigments and protecting chlorophyll in plants thereby having a role in mitigating the adverse effects of the climate change (Lanza and Reis 2021). In this context, Liang et al. (2020) studied foliar application of Selenium at the critical stage and improvement of Se content in mature foxtail millet using proteomic analysis using relative quantification. The study reported 123 DEPs associated with carbohydrate and amino acid metabolism, which were the ones most profoundly affected by Se treatment.

17.2.4 Metabolomics

Metabolomics is a comprehensive, sensitive, and practical tool for learning about the composition of a metabolite pool found in an organism (Patel et al. 2021). In the quickly expanding and enticing field of metabolomics, there is a lot of potential and opportunity for crop enhancement activities (Razzaq et al. 2019). Modern metabolomics techniques are being utilized to reveal hidden regulatory networks impacting crop growth and health as well as to explain complex biological mechanisms (Patel et al. 2021). Metabolite profiling in microorganisms, plants, and mammals is now possible owing to metabolomics research, which has emerged as one of the most significant developments in recent years. Metabolomics, which provides a full understanding of cellular metabolites, such as small organic molecules that participate in numerous cellular activities, can indicate a cell's absolute physiological condition. It also displays regulation and interception between linked pathways, as well as how a given gene influences the metabolic route (Wen et al. 2015). To analyze very complex mixtures of plant extracts, several integrated approaches, like mass spectrometry (MS)-based methods involving gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), capillary electrophoresis-mass spectrometry (CE-MS), Fourier transform ion cyclotron resonance-mass spectrometry (FTICR-MS), matrix-based mass spectrometry (MBMS), and matrix-based mass spectrometry (MBMS) are employed (Patel et al. 2016). Undoubtedly, genomic, transcriptomic, and proteomic data have played a significant role in revealing information about genotypes and complex biological processes. However, they fall short of identifying phenotype, which is ultimately determined by the metabolite of a cell. Very few reports on metabolomics in foxtail millets are available, which provides scope for cellular metabolites study in this crop for the future.

Using Ultra Performance Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry (UPLC-ESI-MS/MS), a total of 116 flavonoid metabolites from 5 foxtail millet varieties were identified by Zhang et al. (2021). Flavonoid metabolites were found in similar amounts in all the investigated types, although different flavonoid metabolites accumulated in each variety. They reported 33 different flavonoid metabolites in cultivars with high- and low-eating qualities, such as luteolin in three varieties with high-eating quality foxtail, quercetin in YG1, and procyanidins in NMB. With the help of KEGG database, many critical enzymes that

may influence the production of these metabolites were subsequently identified. Similarly, a large population of about 150 foxtail millet germplasm was analyzed by Wei et al. (2021) using liquid chromatography-tandem mass spectrometry in a broad-targeted metabolomics approach and identified as well as quantified 330 annotated chemicals. According to their findings, millets from India and the North and South of China contain a wide range of primary and secondary metabolites, including flavonoids, phenol amides, hydroxycinnamoyl derivatives, nucleotides, and lipids, with flavonoids being the most conspicuous.

The metabolite composition of foxtail millet seed from various agricultural zones in the northern and southern Shanxi Province was examined by Yang et al. (2021), using electrospray ionization quadrupole/orbitrap high-resolution mass spectrometry and ultra-high performance liquid chromatography. They were able to characterize 158 compounds by comparing their mass fragmentation patterns to data in databases and publications. Using metabolomic analysis, the significant differences between the foxtail millet from both provinces were found and assigned to 20 distinct compounds from various metabolic pathways and three metabolic pathways being the significant contributors. The study concluded that environmental/climatic changes can cause metabolite variations in foxtail millet.

Metabolic variations in a herbicide-resistant and tolerant foxtail millet varieties were studied by Sun et al. (2022). The group compared the metabolomic profiles of atrazine-resistant (Gongai2) and sensitive foxtail variety (Longgu31) in their leaves. They identified 192 differentially expressed metabolites involving 82 upregulated, 110 downregulated in resistant line and 215 including 95 upregulated and 120 downregulated in the susceptible line. They reported numerous biosynthetic pathways for glutathione metabolism, amino acid biosynthesis, and phenylpropanoid biosynthesis as differentially expressed in their study. They mentioned that scoploletin enrichment may help LG31 plants cope with atrazine stress, and the co-expression investigation found that resistant plants had improved proline biosynthesis and glutathione metabolism, which contributed to their greater atrazine tolerance.

Foxtail millet contains more folate than other cereal grains and a comprehensive metabolome study of 12 folate metabolites in a foxtail millet panicle using LC-MS and discovered that the overall folate and derivative content gradually declined as the panicle matured (Hou et al. 2022). Polyglutamate-5-formyl-tetrahydrofolate was the most prevalent form. The study discovered 28 genes involved in the folate metabolic pathway and gene expression analysis in three phases of panicle development revealed that the key enzymes involved in folate synthesis and degradation in the panicles are SiADCL1 and SiGGH. Their expression levels were found to decrease as the panicle development advanced.

17.2.5 Epigenomics

Histone modifications, cytosine DNA methylation, and small RNA-mediated methylation are only a few of the epigenetic modification methods that can modify

chromatin, a complicated structure composed of genetic material DNA connected to histone proteins. Because of alterations in chromatin structure, affected regulatory proteins, such as transcription factors, are unable to access genomic DNA, affecting gene expression. It is now possible to examine epigenetic mechanisms at the genome-wide level thanks to advances in high-throughput sequencing techniques. In epigenomic research, high-throughput technologies will be used to improve our understanding of the roles and processes of the regulatory pathways found in plant genomes, making it easier to change these pathways genetically and biochemically. This technique may be an effective research tool for highlighting the systematic links between genetic and epigenetic alterations, particularly when applied to the cytosine methylation of a given cell or tissue's genomic area (Yadav et al. 2018). DNA methylation is an important type of epigenetic modification in eukaryotes.

Because grain filling is important for crop yield and quality, it is uncertain whether grain filling causes epigenetic alterations in foxtail millet. The findings of the author suggested that DNA methylation has an influence on the transcriptional regulation of genes involved in grain filling. Wang et al. (2017) carried out global DNA methylation and transcriptome analysis to examine the epigenetic consequences of the grain-filling process in foxtail millet spikelets at various stages by employing whole-genome bisulfite deep sequencing and powerful analytics to sequence and detect every instance of DNA methylation during the filling of foxtail millet grains. They studied patterns of DNA methylation-mediated gene expression, as well as the associated gene network and biological pathway. They reported that gene expression was negatively associated with DNA methylation, especially in the CG and CHG regions.

Pattern and genome-wide methylation level in foxtail millet was studied in Chaogu 58 and Yugu 1 by Methylation Sensitive Amplified Polymorphism analysis. About 32 pairs of MSAP primers produced clearly distinguishable and reproducible bands including 3 types of methylation patterns (Zhang et al. 2019). Similarly, Pandey et al. (2017) used genome-wide methylation analysis with MSAP primers to quantify salt-induced methylation changes in two foxtail millet cultivars with differing levels of salt stress resistance. They discovered that tolerant cultivars had lower DNA methylation levels than sensitive ones. Around 86 polymorphic MSAP fragments were sequenced, and functionally annotated and they had sequence similarities with several genes, including transcription factors, transporters, protein phosphatases, disease resistance genes, oxidoreductases, cell wall-related enzymes, and retrotransposon and transposase-like proteins, indicating salt stress-induced methylation in these genes.

Integrative "omics" and next generation sequencing (NGS) approaches have recently made advances in understanding the complex gene regulatory networks and the molecular processes that underpin growth and development (Panchal et al. 2022). The foxtail millet genome sequencing data is being used to create genetic resources for future research by discovering novel gene families, creating high-density linkage maps, and developing molecular markers. It is possible to introduce abiotic stress tolerance traits in the foxtail millet using genetic engineering techniques, such as its ability to withstand salinity, drought, and nutrient-poor soil.

Stable plant transformation systems and precise gene editing being developed in foxtail millet could revolutionize future crops that can withstand changing climates (Panchal et al. 2022). Very recently, Li et al. (2022a, b) studied 398 foxtail millet accessions using a multi-omics approach and reported the regions of the genome that are linked to the process of domestication and traits associated with the metabolite pathway. They reported the role of *phytoene synthase 1* gene in color and quality of foxtail millet grains using CRISPR-associated gene editing and mentioned about 83 metabolites associated with anti-inflammatory properties. Similarly, Liu et al. (2022) used muti-omics to study the SWEET gene family in maize and foxtail millet and reported the availability of more high sugar transport genes in maize in comparison to foxtail, which is an important criterion for crop yield and biomass. According to them, this could be one of the reasons why this millet has less yield and biomass in comparison to maize.

17.3 Conclusion

Biotechnology is a promising tool for improving biotic and abiotic stress tolerance in millets. Being a climate-smart crop with the ability to act as a powerhouse of nutrients, efforts need to be made positively in the direction of valuing these crops for ensuring food security and climate resilience. Successful application of genomics followed by transcriptomics for abiotic stress tolerance has been reported; however, in the area of proteomics and metabolomics, the investigation has just been initiated. The current tools and technologies and upcoming high-throughput sequencing platforms can provide a wide variety of applications to researchers including the identification of different marker systems and the deciphering of the molecular basis for foxtail millet's nutritional superiority and stress hardiness. It was once thought that the crop's abundance of vitamins, minerals, essential fatty acids, and fibers was responsible for its health benefits, but new research has shown that these nutrients can also work in tandem with other bioactive molecules to have a positive impact. Even though there is a basic understanding of nutrient biosynthetic pathways, an experimental framework and a multi-omics approach are required to investigate specialized nutrient pathways in millets. To meet future demand for this nutrient-rich, climate-smart superfood grain, foxtail millet omics must be successfully developed and applied to the development of supreme foxtail millet varieties.

References

Aebersold R, Mann M (2003) Mass spectrometry-based proteomics. Nature 422(6928):198–207. https://doi.org/10.1038/nature01511

Bai H, Cao Y, Quan J, Dong L, Li Z, Zhu Y, Zhu L, Dong Z, Li D (2013) Identifying the genome-wide sequence variations and developing new molecular markers for genetics research by re-sequencing a landrace cultivar of foxtail millet. PLoS One 8:e73514

Bennetzen JL, Schmutz J, Wang H, Percifield R, Hawkins J, Pontaroli AC, Estep M, Feng L, Vaughn JN, Grimwood J, Jenkins J, Barry K, Lindquist E, Hellsten U, Deshpande S, Wang X,

Wu X, Mitros T, Triplett J, Yang X, Ye CY, Mauro-Herrera M, Wang L, Li P, Sharma M, Sharma R, Ronald PC, Panaud O, Kellogg EA, Brutnell TP, Doust AN, Tuskan GA, Rokhsar D, Devos KM (2012) Reference genome sequence of the model plant Setaria. Nat Biotechnol 30: 555–561

Chandramouli K, Qian PY (2009) Proteomics: challenges, techniques and possibilities to overcome biological sample complexity. Hum Genomics Proteomics 2009:239204. https://doi.org/10.4061/2009/239204

Chandrasekara A, Shahidi F (2011) Determination of antioxidant activity in free and hydrolyzed fractions of millet grains and characterization of their phenolic profiles by HPLC-DAD-ESI-MS. J Funct Foods 3:144–158. https://doi.org/10.1016/j.jff.2011.03.007

Debnath M, Prasad GBKS, Bisen P (2010) Omics technology. In: Molecular diagnostics : promises and possibilities. Dordrech, Heidelberg, London, pp 11–31. https://doi.org/10.1007/978-90-481-3261-4

Devos K, Wang Z, Beales J, Sasaki T, Gale MD (1998) Comparative genetic maps of foxtail millet (Setaria italica) and rice (Oryza sativa). Theor Appl Genet 96:63–68. https://doi.org/10.1007/s001220050709

Doust AN, Kellogg EA, Devos KM, Bennetzen JL (2009) Foxtail millet: a sequence-driven grass model system. Plant Physiol 149:137–141. https://doi.org/10.1104/pp.108.129627

Fang X, Dong K, Wang X, Liu T, He J, Ren R, Zhang L, Liu R, Liu X, Li M, Huang M, Zhang Z, Yang T (2016) A high density genetic map and QTL for agronomic and yield traits in foxtail millet [Setaria italica (L.) P. Beauv.]. BMC Genomics 17:336. https://doi.org/10.1186/s12864-016-2628-z

Fukunaga K, Kato K (2003) Mitochondrial DNA variation in foxtail millet, Setaria italica(L.) P. Beauv Euphytica 129(1):7–13. https://doi.org/10.1023/a:1021589019323

Fukunaga K, Abe A, Mukainari Y et al (2022) Recombinant inbred lines and next-generation sequencing enable rapid identification of candidate genes involved in morphological and agronomic traits in foxtail millet. Sci Rep 12:218. https://doi.org/10.1038/s41598-021-04012-1

Gondro C, van der Werf J, Hayes B (2013) Genome-wide association studies and genomic prediction, vol 1019. Humana Press, Totowa, NJ. ISBN: 978-1-62703-446-3

Guo J, Huang Z, Sun J, Cui X, Liu Y (2021) Research Progress and future development trends in medicinal plant transcriptomics. Front Plant Sci 12:691838. https://doi.org/10.3389/fpls.2021.691838

Guo Y, Hao D, Wang X et al (2022) Comparative transcriptomics reveals key genes contributing to the differences in drought tolerance among three cultivars of foxtail millet (Setaria italica). Plant Growth Regul 99:45. https://doi.org/10.1007/s10725-022-00875-0

Gupta S, Kumari K, Das J, Lata C, Puranik S, Prasad M (2011) Development and utilization of novel intron length polymorphic markers in foxtail millet (Setaria italica (L.) P. Beauv.). Genome 54:586–602

Gupta S, Kajal K, Pranav S, Sudhakar V, Manoj P (2012) Sequence-based novel genomic microsatellite markers for robust genotyping purposes in foxtail millet [Setaria italica (L.) P. Beauv]. Plant Cell Rep 31:323–337. https://doi.org/10.1007/s00299-011-1168-x

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

Hakeem K, Tombuloglu H, Kekec G (2016) Plant omics: trends and applications. Springer, Basel, Switzerland, p 2016

Han F, Sun M, He W, Guo S, Feng J, Wang H, Yang Q, Pan H, Lou Y, Zhuge Y (2022) Transcriptome analysis reveals molecular mechanisms under salt stress in leaves of foxtail millet (Setaria italica L.). Plan Theory 11:1864. https://doi.org/10.3390/plants11141864

Han J, Xie H, Sun Q, Wang J, Lu M, Wang W, Guo E, Pan J (2014) Bioinformatic identification and experimental validation of miRNAs from foxtail millet (Setaria italica). Gene 546(2):367–377. https://doi.org/10.1016/j.gene.2014.05.050

Hou D, Chen J, Ren X, Wang C, Diao X, Hu X, Zhang Y, Shen Q (2018) A whole foxtail millet diet reduces blood pressure in subjects with mild hypertension. J Cereal Sci 84:13–19. https://doi.org/10.1016/j.jcs.2018.09.003

Hou S, Man X, Lian B, Ma G, Sun Z, Han L, Yan L, Gao H, Du W, Wang X, Zhang Y, Li H, Han Y (2022) Folate metabolic profiling and expression of folate metabolism-related genes during panicle development in foxtail millet (Setaria italica (L.) P. Beauv). J Sci Food Agric 102(1): 268–279. https://doi.org/10.1002/jsfa.11355. Epub 2021 Jun 21

Hutabarat DJC, Bowie VA (2022) IOP conference series: earth and environmental science. Bioactive compounds in foxtail millet (Setaria italica)-extraction, biochemical activity, and health functional. IOP Conf Ser: earth. Environ Sci 998:012060

Jayaraman A, Puranik S, Rai NK et al (2008) cDNA-AFLP analysis reveals differential gene expression in response to salt stress in foxtail millet (Setaria italica L.). Mol Biotechnol 40: 241–251

Jia X-P, Shi Y-S, Song Y-C, Wang G, Wang T-Y, Li Y (2007) Development of EST-SSR in foxtail millet (Setaria italica). J Genet Resources Crop Evol 54:233–236. https://doi.org/10.1007/s10722-006-9139-8

Jia X, Zhang Z, Liu Y, Zhang C, Shi Y, Song Y, Wang T, Li Y (2009) Development and genetic mapping of SSR markers in foxtail millet [Setaria italica (L.) P. Beauv.]. Theor Appl Genet 118: 821–829. https://doi.org/10.1007/s00122-008-0942-9

Jia G, Huang X, Zhi H, Zhao Y, Zhao Q, Li W, Chai Y, Yang L, Liu K, Lu H, Zhu C, Lu Y, Zhou C, Fan D, Weng Q, Guo Y, Huang T, Zhang L, Lu T, Feng Q, Hao H, Liu H, Lu P, Zhang N, Li Y, Guo E, Wang S, Wang S, Liu J, Zhang W, Chen G, Zhang B, Li W, Wang Y, Li H, Zhao B, Li J, Diao X, Han B (2013) A haplotype map of genomic variations and genome-wide association studies of agronomic traits in foxtail millet (Setaria italica). Nat Genet 45:957–961

Khan Y, Yadav A, Suresh BV, Muthamilarasan M, Yadav CB, Prasad M (2014) Comprehensive genome-wide identification and expression profiling of foxtail millet [Setaria italica (L.)] miRNAs in response to abiotic stress and development of miRNA database. Plant Cell Tissue Org Cult 118:279–292

Koonin EV, Galperin MY (2003) Sequence—evolution—function: computational approaches in comparative genomics. Kluwer Academic, Boston. Chapter 2, Evolutionary Concept in Genetics and Genomics. Available from: https://www.ncbi.nlm.nih.gov/books/NBK20255/

Kumari R, Dikshit N, Sharma D et al (2011) Analysis of molecular genetic diversity in a representative collection of foxtail millet [Setaria italica (L.) P. Beauv.] from different agro-ecological regions of India. Physiol Mol Biology Plants 17:363. https://doi.org/10.1007/s12298-011-0085-3

Kumari K, Muthamilarasan M, Misra G, Gupta S, Subramanian A, Parida SK, Chattopadhyay D, Prasad M (2013) Development of eSSR-markers in Setaria italica and their applicability in studying genetic diversity, cross-transferability and comparative mapping in millet and non-millet species. PLoS One 8(6):e67742. https://doi.org/10.1371/journal.pone.0067742

Lai D, Yao X, Yan J et al (2022) Genome-wide identification, phylogenetic and expression pattern analysis of GATA family genes in foxtail millet (Setaria italica). BMC Genomics 23:549. https://doi.org/10.1186/s12864-022-08786-0

Lanza MGDB, Reis ARD (2021) Roles of selenium in mineral plant nutrition: ROS scavenging responses against abiotic stresses. Plant Physiol Biochem 164:27–43. https://doi.org/10.1016/j.plaphy.2021.04.026

Lata C (2015) Advances in omics for enhancing abiotic stress tolerance in millets. Proc Indian Natl Sci Acad 81:397–417. https://doi.org/10.16943/ptinsa/2015/v81i2/48095

Lata C, Sahu PP, Prasad M (2010) Comparative transcriptome analysis of differentially expressed genes in foxtail millet (Setaria italica L.) during dehydration stress. Biochem Biophys Res Commun 393(4):720–727. https://doi.org/10.1016/j.bbrc.2010.02.068

Lata C, Gupta S, Manoj P (2013) Foxtail millet: a model crop for genetic and genomic studies in bioenergy grasses. Crit Rev Biotechnol 33:328–343. https://doi.org/10.3109/07388551.2012.716809

Li J, Li X, Yang Q, Luo Y, Gong X, Zhang W, Yingang H, Yang T, Dong K, Feng B (2019) Proteomic changes in the grains of foxtail millet (Setaria italica (L.) beau) under drought stress. Span J Agric Res 17:e0802. https://doi.org/10.5424/sjar/2019172-14300

Li S, Cui Y, Liu D, Zilong Z, Jing Z, Zhengli L (2022a) Transcriptome analysis and characterization of genes associated to leaf tannin content in foxtail millet [Setaria italica (L.) P. Beauv.]. BMC Genom 23:512. https://doi.org/10.1186/s12864-022-08746-8

Li X, Jianhua G, Jingyi S, Kai G, Siyu H, Xingchun W, Qiang H, Yanyan Z, Yakun Z, Yulu Y, Jiaoyan T, Hailang W, Staffan P, Mingquan H, Lishuai X, Linlin Z, Dongqin L, Yongming L, Hua W, Xianmin D, Peng C, Xiaowen W, Yuanhuai H (2022b) Multi-omics analyses of 398 foxtail millet accessions reveal genomic regions associated with domestication, metabolite traits, and anti-inflammatory effects. Mol Plant 15(8):1367–1383. https://doi.org/10.1016/j.molp.2022.07.003

Liang K, Liang S, Zhu H (2020) Comparative proteomics analysis of the effect of selenium treatment on the quality of foxtail millet. LWT 131:109691. https://doi.org/10.1016/j.lwt.2020.109691

Liu Z, Fan H, Ma Z (2022) Comparison of SWEET gene family between maize and foxtail millet through genomic, transcriptomic, and proteomic analyses. Plant Genome 15(3):e20226. https://doi.org/10.1002/tpg2.20226

Mayer B (2011) Omics technologies. In: Methods in molecular biology bioinformatics for omics data, vol 719. Data and Bioinformatics Principles. https://doi.org/10.1007/978-1-61779-027-0. (Chapter 1), 3–30. doi: 10.1007/978-1-61779-027-0_1

Micheel CM, Nass SJ, Omenn GS (2012) Evolution of translational omics: lessons learned and the path forward. National Academies Press (US), Washington (DC). 2, Omics-Based Clinical Discovery: Science, Technology, and Applications. Available from: https://www.ncbi.nlm.nih.gov/books/NBK202165/

Mishra AK, Muthamilarasan M, Khan Y, Parida SK, Prasad M (2014) Genome-wide investigation and expression analyses of WD40 protein family in the model plant foxtail millet (Setaria italica L.). PLoS One 9(1):e86852. https://doi.org/10.1371/journal.pone.0086852

Muthamilarasan M, Prasad M (2015) Advances in Setaria genomics for genetic improvement of cereals and bioenergy grasses. Theor Appl Genet 128:1–14. https://doi.org/10.1007/s00122-014-2399-3

Muthamilarasan M, Bonthala VS, Mishra AK, Khandelwal R, Khan Y, Roy R, Prasad M (2014a) C2H2 type of zinc finger transcription factors in foxtail millet define response to abiotic stresses. Funct Integr Genomics 14:531. https://doi.org/10.1007/s10142-014-0383-2

Muthamilarasan M, Venkata SB, Pandey G, Kumari K, Parida SK, Prasad M (2014b) Development of 5123 intron-length polymorphic markers for large-scale genotyping applications in foxtail millet. DNA Res 21(1):41–52. https://doi.org/10.1093/dnares/dst039

Pan J, Li Z, Wang Q, Garrell AK, Liu M, Guan Y, Zhou W, Liu W (2018) Comparative proteomic investigation of drought responses in foxtail millet. BMC Plant Biol 18(1):315. https://doi.org/10.1186/s12870-018-1533-9

Pan JW, Li Z, Wang Q, Guan Y, Li X, Dai S, Ding G, Liu W (2019) Transcriptomics analysis of NaCl response in foxtail millet (Setaria italica L.) seeds at germination stage. Sci Agric Sin 52(22):964–3975

Panchal A, Singh RK, Prasad M (2022) Recent advancements and future perspectives of foxtail millet genomics. Plant Growth Regul 99:11. https://doi.org/10.1007/s10725-022-00858-1

Pandey G, Misra G, Kumari K, Gupta S, Parida SK, Chattopadhyay D, Manoj P (2013) Genome-wide development and use of microsatellite markers for large-scale genotyping applications in foxtail millet [Setaria italica (L.)]. DNA Res 20(2):197–207. https://doi.org/10.1093/dnares/dst002

Pandey G, Yadav CB, Sahu PP, Muthamilarasan M, Prasad M (2017) Salinity induced differential methylation patterns in contrasting cultivars of foxtail millet (Setaria italica L.). Plant Cell Rep 36(5):759–772. https://doi.org/10.1007/s00299-016-2093-9

Patel MK, Mishra A, Jha B (2016) Untargeted metabolomics of halophytes. In: Kim S (ed) Marine omics: principles and applications. CRC Press, Boca Raton, FL, pp 309–325

Patel MK, Pandey S, Kumar M, Haque MI, Pal S, Yadav NS (2021) Plants metabolome study: emerging tools and techniques. Plants (Basel) 10(11):2409. https://doi.org/10.3390/plants10112409

Pradeep PM, Sreerama YN (2018) Phenolic antioxidants of foxtail and little millet cultivars and their inhibitory effects on α-amylase and α-glucosidase activities. Food Chem 247:46–55. https://doi.org/10.1016/j.foodchem.2017.11.103

Puranik S, Jha S, Srivastava PS, Sreenivasulu N, Prasad M (2011) Comparative transcriptome analysis of contrasting foxtail millet cultivars in response to short-term salinity stress. J Plant Physiol 168(3):280–287. https://doi.org/10.1016/j.jplph.2010.07.005

Puranik S, Sahu PP, Mandal SN, Parida SK, Prasad M (2013) Comprehensive genome-wide survey, genomic constitution and expression profiling of the NAC transcription factor family in foxtail millet (Setaria italica L.). PLoS One 8(5):e64594. https://doi.org/10.1371/journal.pone.0064594

Qi X, Xie S, Liu Y, Yi F, Yu J (2013) Genome-wide annotation of genes and noncoding RNAs of foxtail millet in response to simulated drought stress by deep sequencing. Plant Mol Biol 83(4):459–473

Razzaq A, Sadia B, Raza A, Khalid Hameed M, Saleem F (2019) Metabolomics: a way forward for crop improvement. Meta 9:303. https://doi.org/10.3390/metabo9120303

Reddy DS, Bhatnagar-Mathur P, Vadez V, Sharma KK (2012) Grain legumes (soybean, chickpea, and Peanut): omics approaches to enhance abiotic stress tolerance. In: Tuteja N, Gill SS, Tiburcio AF, Tuteja R (eds) Improving crop resistance to abiotic stress. John Wiley & Sons. https://doi.org/10.1002/9783527632930.ch39

Sharma N, Niranjan K (2017) Foxtail millet: properties, processing, health benefits, and uses. Food Rev Intl 34:329. https://doi.org/10.1080/87559129.2017.1290103

Subramanian I, Verma S, Kumar S, Jere A, Anamika K (2020) Multi-omics data integration, interpretation, and its application. Bioinform Biol Insights 14:1177932219899051. https://doi.org/10.1177/1177932219899051

Sun L, Sun L, Liu L, Wang Y, Feng Y, Yang W, Wang D, Gao S, Miao X, Sun W (2022) Integration of metabolomics and transcriptomics for investigating the tolerance of foxtail millet (Setaria italica) to atrazine stress. Front Plant Sci 10:890550. https://doi.org/10.3389/fpls.2022.890550

Suresh BV, Muthamilarasan M, Mishra G, Prasad M (2013) FmMDb: a versatile database of foxtail millet markers for millets and bioenergy grasses research. PLoS One 8(8):e71418

Tanveer T, Shaheen K, Parveen S, Misbah ZT, Babar MM, Gul A (2018) Omics-based bioengineering in environmental biotechnology. In: Barh D, Azevedo V (eds) Omics technologies and bio-engineering. Academic Press, pp 353–364. ISBN 9780128158708, doi: B978-0-12-815870-8.00019-X.)

Upadhyaya HD, Vetriventhan M, Deshpande SP, Sivasubramani S, Wallace JG, Buckler ES, Hash CT, Ramu P (2015) Population genetics and structure of a global foxtail millet germplasm collection. The plant. Genome 8(3) plantgenome2015-07. https://doi.org/10.3835/plantgenome2015.07.0054

Veeranagamallaiah G, Jyothsnakumari G, Thippeswamy M, Chandra P, Giridara Kumar S, Sriranganayakulu G, Mahesh Y, Rajasekhar B, Madhurarekha C, Chinta S (2008) Proteomic analysis of salt stress responses in foxtail millet (Setaria italica L. cv. Prasad) seedlings. Plant Sci 175:631–641. https://doi.org/10.1016/j.plantsci.2008.06.017

Voelkerding KV, Dames S, Durtschi JD (2010) Next generation sequencing for clinical diagnostics-principles and application to targeted resequencing for hypertrophic cardiomyopathy: a paper from the 2009 William Beaumont Hospital symposium on molecular pathology. J Mol Diagn 12:539–551. https://doi.org/10.2353/jmoldx.2010.100043

Wang Z, Devos K, Liu C, Wang R, Gale MM (1998) Construction of RFLP-based maps of foxtail millet, Setaria italica (L.) P. Beauv Theor Appl Genet 96:31–36. https://doi.org/10.1007/s001220050705

Wang J, Wang Z, Du X, Yang H, Han F, Han Y, Yuan F, Zhang L, Peng S, Guo E (2017) A high-density genetic map and QTL analysis of agronomic traits in foxtail millet [*Setaria italica* (L.) P. Beauv.] using RAD-seq. PLoS ONE 12(6):e0179717. https://doi.org/10.1371/journal.pone.0179717

Wang T, Song H, Li P, Wei Y, Hu N, Chen Z, Wang W, Liu J, Zhang B, Peng R (2020) Transcriptome analysis provides insights into grain filling in foxtail millet (Setaria italica L.). International journal of molecular. Science 21(14):5031. https://doi.org/10.3390/ijms21145031

Wei W, Li S, Wang Y, Wang B, Fan G, Zeng Q, Zhao F, Xu C, Zhang X, Tang T, Feng X, Shi J, Shi G, Zhang W, Song G, Li H, Wang F, Zhang Y, Li X, Wang D, Zhang W, Pei J, Wang X, Zhao Z (2021) Metabolome-based genome-wide association study provides genetic insights into the natural variation of foxtail millet. Front Plant Sci 12:665530. https://doi.org/10.3389/fpls.2021.665530

Wen W, Li K, Alseekh S, Omranian N, Zhao L, Zhou Y et al (2015) Genetic determinants of the network of primary metabolism and their relationships to plant performance in a maize recombinant inbred line population. Plant Cell 27:1839–1856. https://doi.org/10.1105/tpc.15.00208

Weng Q, Song X, Zhao Y et al (2020) Proteomic profiling of foxtail millet hybrid Zhangzagu10 and its parent lines using iTRAQ-based technique. J Plant Biochem Biotechnol 29:439–449. https://doi.org/10.1007/s13562-020-00551-2

Xu B, Yin M, Wen Y, Pei S, Ke Z, Zhang B, Yuan X (2018) Gene expression profiling of foxtail millet (*Setaria italica* L.) under drought stress during germination[J]. Sci Agric Sin 51(8):1431–1447

Xu B, Gao X, Gao J, Li J, Yang P, Feng B (2019) Transcriptome profiling using RNA-seq to provide insights into foxtail millet seedling tolerance to short-term water deficit stress induced by PEG-6000. J Integr Agric 18(11):2457–2471. https://doi.org/10.1016/S2095-3119(19)62576-1

Xu B, Gao X, Dong K, Li X, Yang P, Yang T, Feng B (2020) Grain protein content comparison and proteomic analysis of foxtail millet (Setaria italica L.) seed response to different drought stress levels. Acta Physiol Plant 42(2):20. https://doi.org/10.1007/s11738-019-2999-2

Yadav CB, Muthamilarasan M, Pandey G, Prasad M (2014a) Identification, characterization and expression profiling of dicer-like, Argonaute and RNA-dependent RNA polymerase gene families in foxtail millet. Plant Mol Biol Rep 33:43–55. https://doi.org/10.1007/s11105-014-0736-y

Yadav CB, Muthamilarasan M, Pandey G, Khan Y, Prasad M (2014b) Development of novel microRNA-based genetic markers in foxtail millet for genotyping applications in related grass species. Mol Breed 34:2219. https://doi.org/10.1007/s11032-014-0137-9

Yadav CB, Bonthala VS, Muthamilarasan M, Pandey G, Khan Y, Prasad M (2015) Genome-wide development of transposable elements-based markers in foxtail millet and construction of an integrated database. DNA Res 22(1):79–90. https://doi.org/10.1093/dnares/dsu039

Yadav CB, Pandey G, Muthamilarasan M, Prasad M (2018) Epigenetics and epigenomics of plants. In: Varshney R, Pandey M, Chitikineni A (eds) Plant genetics and molecular biology. Advances in biochemical engineering/biotechnology, vol 164. Springer, Cham. https://doi.org/10.1007/10_2017_51

Yang X, Wan Z, Perry L, Lu H, Wang Q, Zhao C, Li J, Xie F, Yu J, Cui T, Wang T, Li M, Ge Q (2012) Early millet use in northern China. Proc Natl Acad Sci U S A 109:3726–3730. https://doi.org/10.1073/pnas.1115430109

Yang L, Li R, Cui Y, Qin X, Li Z (2021) Comparison of nutritional compositions of foxtail millet from the different cultivation regions by UPLC-Q-Orbitrap HRMS based metabolomics approach. J Food Biochem 45(10):e13940. https://doi.org/10.1111/jfbc.13940

Yin H, Yu Q, An L, Li W (2014) Cloning and expression analysis of an F-box gene (SiFBX) rapidly responsive to drought stress. Acta Agron Sin 40(6):1027–1034

Yu A, Zhao J, Wang Z, Cheng K, Zhang P, Tian G, Liu X, Guo E, Du Y, Wang Y (2020) Transcriptome andmetabolite analysis reveal the drought tolerance of foxtail millet significantly

correlated with phenylpropanoids-related pathways during germination process under PEG stress. BMC Plant Biol 20(1):274. https://doi.org/10.1186/s12870-020-02483-4

Zhang LZ, Liu RH (2015) Phenolic and carotenoid profiles and antiproliferative activity of foxtail millet. Food Chem 174:495–501. https://doi.org/10.1016/j.foodchem.2014.09.089

Zhang J, Liu T, Fu J, Zhu Y, Jia J, Zheng J, Zhao Y, Zhang Y, Wang G (2007) Construction and application of EST library from Setaria italica in response to dehydration stress. Genomics 90(1):121–131. https://doi.org/10.1016/j.ygeno.2007.03.016

Zhang G, Liu X, Quan Z, Cheng S, Xu X, Pan S, Xie M, Zeng P, Yue Z, Wang W, Tao Y, Bian C, Han C, Xia Q, Peng X, Cao R, Yang X, Zhan D, Hu J, Zhang Y, Li H, Li H, Li N, Wang J, Wang C, Wang R, Guo T, Cai Y, Liu C, Xiang H, Shi Q, Huang P, Chen Q, Li Y, Wang J, Zhao Z, Wang J (2012) Genome sequence of foxtail millet (Setaria italica) provides insights into grass evolution and biofuel potential. Nat Biotechnol 30(6):549–554. https://doi.org/10.1038/nbt.2195

Zhang Y, Ruan Y, Zhao C, Xue M, Li B, Wang J, Liu Y, Wang K, Wang H (2019) Analysis of genomic DNA methylation level in foxtail millet by methylation sensitive amplified polymorphism. Sheng Wu Gong Cheng Xue Bao 35(2):263–269. https://doi.org/10.13345/j.cjb.180220

Zhang Y, GaoJ QQ, Yang Y, Hou S, Wang X, Li X, Han Y (2021) Comparative analysis of flavonoid metabolites in foxtail millet (Setaria italica) with different eating quality. Life 11:578. https://doi.org/10.3390/life11060578

Zhu C, Ming C, Zhao-shi X, Lian-cheng L, Xue-ping C, You-Zhi M (2014) Characteristics and expression patterns of the aldehyde dehydrogenase (ALDH) gene superfamily of foxtail millet (Setaria italica L.). PLoS One 9(7):e101136. https://doi.org/10.1371/journal.pone.0101136

Zhu Y, Chu J, Lu Z, Lv F, Bie X, Zhang C, Zhao H (2018) Physicochemical and functional properties of dietary fiber from foxtail millet (Setaria italic) bran. J Cereal Sci 79:456–461. https://doi.org/10.1016/j.jcs.2017.12.011

Zhu M, Qiang H, Mingjie L, Tiantian S, Qian G, Hui Z, Huan W, Guanqing J, Sha T, Xiliu C, Rui W, Andi X, Haigang W, Zhijun Q, Jun L, Xianmin D, Ying G (2022) Integrated genomic and transcriptomic analysis reveals genes associated with plant height of foxtail millet. Crop J 11:593. https://doi.org/10.1016/j.cj.2022.09.003

Floral Biology, Pollination, Genetics, Origin, and Diversity in Proso Millet (*Panicum miliaceum* L.)

18

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Abstract

Proso millet is a diet crop that was domesticated about 10,000 years ago, and it has been rampant throughout the ancient civilization. At present, this crop is cultivated across Asia, Australia, North America, Europe, and Africa. In developed nations, it serves as both bird and livestock feed, while in certain Asian regions, it is consumed as a food source for humans. This marginal crop displays high variation in its morphological features and is appropriate for dry-land agriculture. It exhibits exceptional water-use efficiency, necessitating the minimum water requirement among all cereal crops. Furthermore, it boasts a nutritional profile abundant in protein, vitamins, minerals, and essential micronutrients like Fe, Zn, Cu, and Mn, in comparison to other primary cereal crops. Globally, plant breeders are employed to create upgraded cultivars using traditional and innovative breeding methodologies. The germplasm encompassing a broad range of crop genetic diversity is safeguarded by many countries in gene banks. Nonetheless, the absence of a linkage map information and sufficient genomic tools have impeded the progress of crop enhancement. Therefore, proso millet must receive extra consideration from the scientific community and superior advancement is essential in genetic improvement and associated research.

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Keywords

Proso millet · Germplasm · Origin

18.1 Introduction

Proso millet, scientifically known as *Panicum miliaceum* L., is tetraploid small millet with a chromosome count of $2n = 4x = 36$ and a basic chromosome count of $x = 9$. This ancient cereal crop has been cultivated by humans for centuries. The diversity of its names showcases its worldwide distribution. In the United States, it is known as common millet and hog millet, while in China, it is referred to as broomcorn millet. In Germany, it is called 'hersey', and across Asia Pacific nations, Korea, and Japan, it is recognized as common millet. In France, it is known as French white (Rajput et al. 2014). It goes by diverse names in different languages in India, such as, Panivaragu in Tamil, Cheena in Bengali, Baragu in Kannada, Variga in Telugu, Vari in Marathi, China Bachari-bagmu in Odia, and Cheno in Gujarati (Rajasekaran and Francis 2021). This exceptionally diverse crop possesses remarkable nutritional qualities and is poised to become a crucial element in enhancing food diversity. It is primarily utilized for human consumption across Asia. However, it finds use as both birdseed and livestock feed in the United States.

Proso millet is cultivated on marginal lands with less inputs as minor millet. It is a shallow-rooted, short-duration (70–90 days) growing crop with the lowest water and nourishment prerequisite making it adaptable for rising in warm and dry atmospheres. It displays remarkable drought tolerance and has the ability to emerge in dry, semi-arid areas, producing seeds with an annual precipitation as low as 330–350 mm (Baltensperger 2002; Lyon et al. 2008). From a nutritional standpoint, the grains represent a valuable carbohydrate source, and have abundant protein (>14%), dietary fiber, minerals, vitamins, and a well-balanced profile of crucial amino acids.

18.2 Origin and Domestication of Proso Millet

Proso millet is a primeval identified cereal domesticated roughly 10,000 years ago in semi-arid parts of China (Lu et al. 2009a, b; Hunt et al. 2014). This crop has historical significance and possesses different theories about its origin. Vavilov (1926) stated that China serves as the focal point of variation for this millet, while Harlan (1975) suggested that both China and Europe are probable regions for the domestication of proso millet. Furthermore, instances of charred grains and grain impressions on pottery have been discovered at multiple locations in Eastern Europe dating back to periods before 7000 calibrated years before the present (cal BP), as noted by Hunt et al. (2008) and Zohary and Hopf (2000).

Examining phytoliths discovered in ancient storage pits at the archeological site of Cishan, situated on the border between the Loess Plateau and the North China

Plain, was conducted by Lu et al. (2009a, b). By analyzing carbon-dating results from 47 archeological samples, they deduced that the earliest cultivation of this millet commenced around 10,000 years BC in the semi-arid regions of China. Proso millet was probably cultivated more rapidly than other grains during the early Holocene because of relatively arid conditions. As a result, the oldest records are from the Cishan site in the Yellow River basin in China, which dates to between 10,300 and 8700 cal years BP. Moreover, it was postulated by Lu et al. that proso millet was autonomously cultivated as a primary crop in the Northern Chinese region approximately 10,000 years ago. Over time, it subsequently spread to adjoining areas including Russia, India, the Middle East, and Europe. However, Zhao (2011) contended that due to the degraded state of samples obtained from the Cishan excavation, the actual domestication might have taken place between 7600 and 8100 years ago. Based on analyses of charred remnants of proso millet seeds discovered in Dadiwan, Northwestern China, it is conceivable that the domestication could have transpired around 5900 BC. The dispersal of proso millet to Europe and West Asia might have occurred by the close of initial millennium BC, influenced by alterations in agronomic practices in respective regions (Miller et al. 2016). With the aim of understanding the archeo-botanical evidence of millet discovered in Eastern Europe and exploring the possibility of several domestication locations, Hunt et al. (2011) performed genetic diversity and phylogeographic analyses on proso millet across Eurasia. These two earlier data points point to its separate domestication in central Asia or Eastern Europe, although they might possibly point to domestication within China that later moved across the Eurasian steppe in a westward trajectory (Jones 2004). One western and one eastern gene pool had been detected via their analyses, but further investigation and factual support would be obligatory to pinpoint the second domestication center (Eastern Europe or Central Asia).

18.3 Taxonomic Hierarchy and Botany

Proso millet is a member of the family *Poaceae*, tribe *Paniceae*, and order *Poales* (Christenhusz and Byng 2016; Gomashe 2017). The primary mode of pollination for proso millet is self-pollination, although a natural cross-pollination rate of over 10% has also been observed (Baltensperger 2002). It is generally considered as allotetraploid possessing $2n = 4x = 36$ chromosomes, even though there is insufficient genetic distinction. The ancestral lineages of proso millet remain ambiguous. Nonetheless, an attempt was made to identify potential progenitors that might have acted as paternal contributors to proso millet (Hunt et al. 2014). They utilized nuclear as well as chloroplast DNA sequences from *Panicum miliaceum* as well as various diploid and tetraploid relatives, to conduct an in situ hybridization analysis. Weedy forms of *P. miliaceum*, i.e., witch grass (*P.capillare*) and Torpedo grass (*P. repens*) have been identified to play a role in its origin as an allotetraploid. (Fig. 18.1). The *Panicum* species, especially those from the Old World, need to be studied further.

The *Panicum* genus contains two sub-species (subspp), namely *subspp. Miliaceum*, which has cultivated variants, and *subspp. Ruderale*, with weedy and

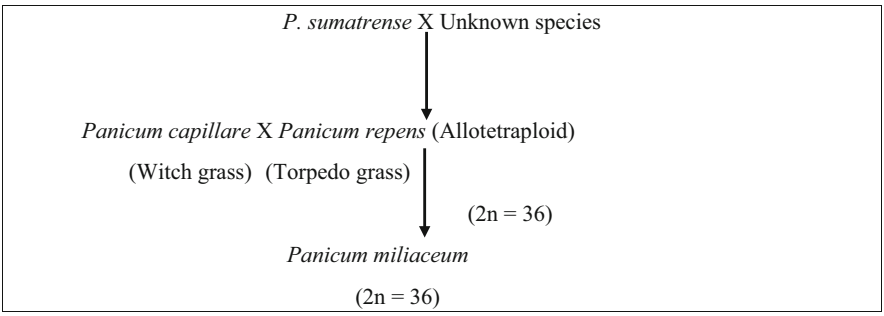

Fig. 18.1 Possible source of *Panicum miliaceum* origin. (Source: Conceptualised from Hunt et al. 2014)

Table 18.1 Various races of proso millet

Race	Inflorescence morphology	Characteristics
Miliaceum	The "inflorescences" are sizeable and open, featuring slightly upright branches that are minimally divided. The form of the inflorescence morphology bears resemblance to wild *P. miliaceum*	These two races befall across the range of broomcorn millet cultivation, from Eastern Europe to Japan
Patentissimum	Its having slender and diffused panicle branches, which are quite similar to race *miliaceum* and is difficult to distinguish	
Contractum	Compact and drooping inflorescences	
Compactum	Cylindrical-shaped erect inflorescences	Highly evolved cultivars of "broomcorn" millet, which are generally have compact inflorescences
Ovatum	Compacted and a little curved inflorescences with ovate shape	

Source: Gomashe (2017)

natural forms (Gomashe 2017; Zhang et al. 2018). Cultivated *P. miliaceum* are further classified into five different races (*miliaceum, patentissimum, contractum, compactum,* and *ovatum*) as per the inflorescence morphology (Lyssov 1975) without any taxonomic validity (see Table 18.1).

The wild proso types have lax panicles, narrow lemmas and spikelet stalks are jointed, and true wild proso types are native to central China from where cultivated ones are aroused. Whereas, cultivated types are characterized by lax or compressed panicles, jointless spikelet stalks and broader lemmas. Yet, the wild variations present in the temperate regions of Europe, Asia, and the United States exhibit distinctions from the wild variant found in China. It is probable that these wild variations originated from cultivated types that have regained the ability for seeds to disperse naturally, subsequently spreading as unwanted plants.

18.4 Botany and Genetic Features

Proso millet is categorized as a short-day C_4 plant that completes its life cycle within a year. It typically grows upright, reaches up to a height of 30 to 100 cm. Its stem and leaves can be hollow, covered in hair, or smooth. The plant displays swollen spaces between its nodes and possesses a shallow root system. It can withstand drought but is vulnerable to frostbite and water-logging conditions. It is usually harvested at physiological maturity to avoid seed-shattering (Kaume 2006). The young plants produce seminal roots that are eventually replaced by adventitious roots. The stem, also known as a culm, is often hollow and cylindrical. At the lower internodes, axillary buds develop into tillers and lateral branches.

Proso millet is primarily self-pollinated, although there is more than 10% of spontaneous cross-pollination (Baltensperger 2002). The inflorescence is a drooping panicle that are typically 10–45 cm long, open or compact and appears like broom with basipetal pattern (from top to bottom) of florets opening (Gomashe 2017; Changmei and Dorothy 2014; Habiyaremye et al. 2017a, b).

The primary branches conclude in spikelets with zero bristles below them. The proso spikelets are solitary, nearly 0.5 cm long and comprises two glumes (lower and upper glume) and two lemmas (lower and upper lemmas), and a single palea (Lu et al. 2009b). The outer and inner glumes are of different lengths, with the inner glume being the same length as the spikelet and shorter outer glume. The lower lemma possess a sterile floret without stamen; however, upper lemma encompasses fertile floret (Gupta et al. 2011) and is shorter than lower lemma. The palea of lower lemma (sterile floret) is very much reduced, while the palea of upper lemma is well present (Seetharam et al. 2003). Upper lemma has three stamen and two feathery stigmas. These anthers are blackish or dark brown in color. The ovary has bifid style and has plumose stigmas (Nanda and Agrawal 2008).

Proso normally blooms between 10:00 and 12:00. The window of time between the blooming and closure of flowers is about 7 min. Cloudy days may delay blossoming, while intense sunshine and reduced humidity may rush the process. From the beginning of anthesis to the end of the last floret on the panicle, it takes around 12–15 days. The anther dehiscence overlaps with stigma receptivity and anthers dehydrate within a few minutes of floral opening (Fig. 18.2).

Achieving successful crossbreeding in proso millet presents a challenge due to a 10% potential for cross-pollination. This poses difficulty in carrying out crossing without causing harm to the stigma and preventing premature pollen shedding before emasculation. A method for emasculation and crossing in proso millet, as outlined by Nandini et al. (2019), involves a cold spray technique. In addition, Nelson et al. (1984) provide a description of crossing procedures utilizing manual emasculation. The conventional approach to emasculation in proso millet includes the use of hot water with temperature of 50 °C for a duration of 5 min to inactivate anthers and pollen grains. By customizing the treatment's temperature and time, this approach may be utilized successfully anywhere. The tiny caryopsis seeds are oval in form which measure around 3 mm in length and 2 mm in breadth. The seeds of proso millet show a range of colors, spanning from white, cream, and yellow to orange, red, black, and brown (Fig. 18.3).

Fig. 18.2 (**a**, **b**) Proso millet crop in field, (**c**) inflorescence, (**d**) panicle, (**e**) seeds, (**f**) seed enclosed in glumes and clasping lemma and palea

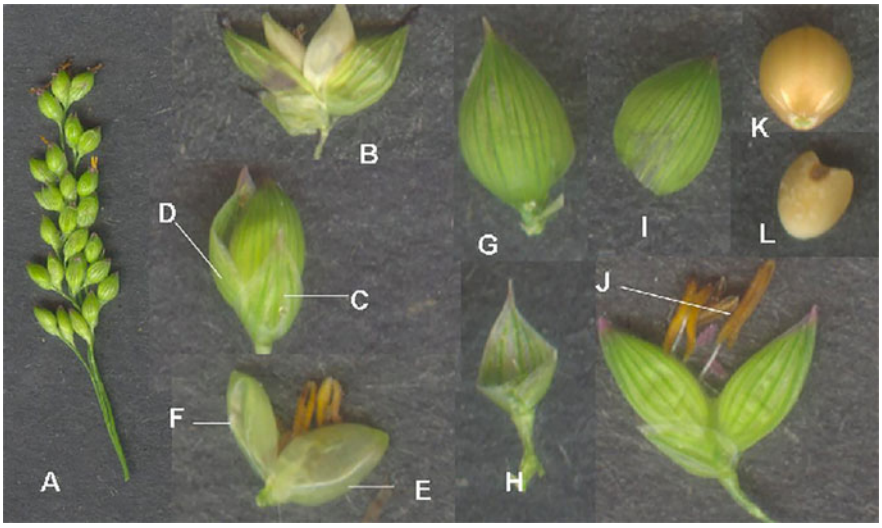

Fig. 18.3 Proso millet inflorescence and its parts. (**a**) Inflorescence; (**b**) Opened spikelet; (**c**) Outer glume; (**d**) Inner glume; (**e**) Inner lemma; (**f**) Palea; (**g**) Inner glume; (**h**) Outer glume; (**i**) Upper lemma; (**j**) Anther; (**k**) Grain enclosed in lemma and palea; (**l**) Grain. (Source- Gupta et al. 2011)

18.5 Germplasm Conservation and Utilization

The success of every breeding program hinges on the germplasm variability. Improvement through conventional plant breeding has decreased both intraspecific variation and interspecific variability among farmed crops by creating and

encouraging genetically homogeneous cultivars (Haussmann et al. 2004). Such as, the six cultivars of proso millet that are most often cultivated in the United States were all derived by classical breeding and selection from landraces. As a result, they all have a limited genetic base (Rajput et al. 2014). Thus, creating and maintaining a collection of plant genetic resources, or germplasm, for all cultivated crops is crucial, especially for underused and understudied crops like millets (Upadhyaya et al. 2014) (Table 18.2).

Study of proso millet has been overlooked because of the priority given to primary crops. Currently, a global inventory of 29,308 proso millet accessions has been compiled and preserved. The most extensive germplasm collection is maintained by Russia, housing 8778 accessions, with China following closely at 8451. Other significant repositories of the crop's genetic diversity can be found in Ukraine, India, and the United States as well (Upadhyaya et al. 2016). The future progress of proso millet genetics will heavily rely on effectively harnessing this genetic variation through breeding. Within India, approximately 3000 accessions are safeguarded across two national institutions: the All India Coordinated Research Project on Small Millets (AICRP-small millets) and the National Bureau of Plant Genetic Resources (NBPGR). In addition, the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) is also involved in the maintenance of plant genetic resources. In 1976, AICRP established a specialized center in Bengaluru (Rajasekaran and Francis 2021), where ongoing endeavors encompass the gathering, examination, and documentation of germplasm for small millets. Remarkably, AICRP oversees 920 accessions of Proso millet, while NBPGR, India's primary institution for managing plant genetic resources of agri-horticultural crops, supervises around 994 proso millet accessions. In addition, ICRISAT, which possesses 849 accessions, is engaged in categorizing and evaluating proso millet germplasm. Using 20 different qualitative and quantitative characteristics, ICRISAT has established a central collection comprising 106 accessions from the 833 proso millet accessions procured from 30 distinct countries. The process of forming this core collection involved randomly selecting 10% of accessions from each of the 101 clusters (Upadhyaya et al. 2011). ICRISAT has disseminated a total of 6047 proso germplasm accessions to 25–37 countries, including two sets of core collections. It is imperative to subject these accessions to thorough evaluation for variability, a crucial step toward the development of high-yielding varieties.

18.6 Germplasm Resource Evaluation and Management

Managing germplasm resources is crucial for enhancing the genetic traits of crop species, as highlighted in Table 18.3. The diverse collection of Proso millet germplasm accessions at the ICRISAT Genebank showcases significant variation in important agronomic and nutritional attributes, as well as resilience against both biotic and abiotic stresses. A study by Wang et al. (2007) evaluated the agronomic viability, disease resistance, and nutritional significance of Proso millet germplasm accessions held at the National Centre for Crop Germplasm Conservation in Beijing,

Table 18.2 Prominent genetic repositories safeguarding proso millet germplasm on a global scale

		Germplasm		
Country	Institution	Cultivated	Wild	Total
Russia federation	N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry (VIR)	8778	–	8878
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
Japan	Department of Genetic Resources I, National Institute of Agrobiological Sciences (NIAS)	516	–	516
Bulgaria	Institute for Plant Genetic Resources "K. Malkov" (IPGR)	489	–	489
Mexico	Estación de Iguala, Instituto Nacional de InvestigacionesAgrícolas (INIA-Iguala)	400	–	400
Poland	Plant Breeding and Acclimatization Institute (IHAR)	354	–	354
	Botanical Garden of Plant Breeding and Acclimatization Institute (BYDG)	359	–	359
Hungary	Institute for Agrobotany (RCA)	243	1	244
Australia	Australian Tropical Crops and Forages Genetic Resources Centre (ATCFC)	228	–	228
Bangladesh	Plant Genetic Resources Centre, BARI (PGRC, BARI)	198	–	198
Czech Republic	GenBank Department, Division of Genetics and Plant Breeding, Research Institute of Crop Production (RICP)	171	–	171
Germany	GenBank, Leibniz Institute of Plant Genetics and Crop Plant Research	165	–	165

Source: Upadhyaya et al. (2016)

China. This assessment led to the identification of superior accessions exhibiting single or multiple advantageous traits. Regarding the germplasm maintained within the ICRISAT collection, it was observed that the majority of early-flowering genotyes were originated from Syria, whereas late-flowering types were traced back to India. In addition, dwarf plant height accessions were found to have roots in Mexico, in contrast to tall plant height accessions that were linked to Sri Lanka.

Table 18.3 Genetic assets pinpointed for diverse characteristics within the Indian program

Character	Promising genotypes	Reference
Days to flower	IC345024, JPM 44,IPM 40, IPM 2125, IPM 2589, IPM 84, IPM 273, IPM-982	NBPGR- Annual Report, (1986, 1987)
Panicle length	Shai Local, Rampur Local	NBPGR- Annual Report, (2011)
Blast, sheath blight, leaf blight, grain smut	TNAU 218, TNAU 164, DHPRMV 2769, TNAU 148	Anonymous (2014)
Rust	TNAU 145, TNAU 204, 218, 220 and CO 5	AICSMIP (2014)
Grain yield (g)	EC 24114,IPM 364, IC 41847, IPM 36, IPM 33, IC 52792, IC 28829, EC 24113	NBPGR- Annual Report (2012)

Source: Gomashe (2017)

The origins of shorter panicle exertion accessions could be traced to the former USSR, whereas longer panicle variants were sourced from Nepal. Notably, accessions demonstrating favorable exertion tendencies were predominantly associated with Australia and China (Reddy et al. 2007).

Graybosch and Baltensperger (2009) assessed 650 different accessions of proso millet to identify instances of endosperm starch with a waxy (amylase free) characteristic. They came to the conclusion that this waxy trait was controlled by duplicate recessive alleles at two distinct loci. Dikshit and Sivaraj (2013) identified accessions with elevated protein content and a wide range of morpho-agronomical attributes. Other investigations, such as those by Natarajan et al. (1978), Manoharan and Sivasubramanian (1982), Hawlader (1991), Prasad et al. (1995), Panwar and Kapila (1992), Reddy et al. (2007), Salini et al. (2010), and Sasamala et al. (2011), have also underscored significant diversity in proso millet concerning both its morphological traits and attributes linked to yield.

In proso millet, only a few diseases have been documented, with the main ones being head smut, sheath blight, bacterial spot, and others. Researchers have focused on enhancing proso millet's resistance to head smut and melanosis (Konstantinov and Grigorashchenko 1987; Maslenkova and Resh 1990; Konstantinov et al. 1989, 1991; Soldatov and Agafonov 1980; Shailaja et al. 2009). Some proso millet germplasm varieties and accessions that show promise in resisting shoot fly and rice moth have also been identified (Shailaja et al. 2009; Murthi and Harinarayana 1989). Abiotic stresses also affect proso millet. Breeding efforts in the United States have given priority to developing strains resistant to lodging (Baltensperger et al. 1995a, b, 2004). In addition, some sources have been reported for demonstrating salinity tolerance (Accession no. 008211, 008214, and 008226) (Sabir et al. 2011).

18.6.1 Core Collection Development

A core collection, which is made up of a small proportion (about 10%) of accessions from an existing germplasm collection, aids in capturing all of the variability for use in breeding operations. At ICRISAT, 833 accessions were classified into five groups

based on race, and Ward's approach was used to cluster the data using information on 20 morpho-agronomic variables (Upadhyaya et al. 2011). A core collection of 106 accessions was generated by randomly selecting 10% (or at least one accession) from each of the 101 clusters. These core collections serve as excellent genetic resources for discovering novel sources of variation and to conduct genomic research.

18.7 Germplasm Molecular Characterization

The extent of exploration has been limited for genetic diversity for proso millet (Goron and Raizada 2015). The genetic diversity analysis presents challenges due to its tetraploid nature and the absence of sequencing data (Hunt et al. 2011). Molecular markers have significantly aided studies concerning genetic diversity, taxonomic relationships, and population structure across various species. Nevertheless, marker information for proso millet remains scarce (Rajput et al. 2014). Research focusing on proso millet's genetic diversity has employed molecular markers like random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeat (SSR) markers (Habiyaremye et al. 2017a).

Using RAPD molecular markers, the diversity of four *Panicum* species and individual accessions of proso millet was assessed (M'Ribu and Hilu 1996). Their research proved that molecular markers might be a useful tool for preserving genetic variation. More than ten years afterward, AFLP markers were employed to examine the genetic variation among three cultivated and nine untamed biotypes from the United States and Canada (Karam et al. 2004). In another study, Lágler et al. (2005) contrasted a medieval millet landrace with 20 prevalent proso millet cultivars using markers like inter simple sequence repeat (ISSR), simple sequence repeat (SSR), and cleaved amplified polymorphic DNA (CAP).

SSR markers, also known as microsatellites, are highly utilized for diversity studies due to their plentiful presence. The scarcity of proper proso millet genomic resources has led researchers to derive SSR markers from genomic sequences of related plant species for proso millet (Habiyaremye et al. 2017a). In a study by Hu et al. (2009), a total of 46 SSR markers sourced from rice, wheat, oat, and barley were employed to study the genetic diversity of 118 Chinese germplasms originating from diverse ecological regions. Similarly, Rajput et al. (2014) evaluated 8 proso millet genotypes using 548 SSR markers derived from switch grass, given that switch grass is the closest genetic relative to proso millet. The initial proso genome SSR markers were introduced by Cho et al. (2010). As a result, numerous researchers have effectively harnessed SSR markers for probing genetic diversity in distinct sets of proso millet germplasm (Hunt et al. 2011; Rajput et al. 2016; Liu et al. 2016).The exploration of proso millet's genetic diversity encompasses various approaches, including DNA markers and additional genomic tools such as polymerase chain reaction (PCR) involving 6 intron splice junctions (ISJ) and long random primers, as demonstrated by Hu et al. (2008) and Araki et al. (2012). Furthermore, genotyping methodologies (Hunt et al. 2011, 2013) and the utilization of Illumina

sequencing along with de novo transcriptome assemblies for generating short-read sequences have also contributed to understanding proso millet's genetic makeup (Hou et al. 2017).

18.8 Genetic Improvement of Proso Millet: Achievements and Status

18.8.1 India

Since the beginning of the study, the All India Coordinated Small Millets Improvement Project (AICSMIP) and SAUs have investigated the germplasm lines for yield and other trait improvement. However, very few improved varieties of proso millet (Table 18.3 and 18.4) have been generated using traditional plant breeding methods.

18.8.2 United States

In 1972, the United States initiated a dedicated breeding initiative for proso millet at the Panhandle Research and Extension Center (PHREC). At Scottsbluff, Lenis Nelson established the inaugural proso millet center for research and breeding, resulting in the release of four cultivars: Sunup, Dawn, Cerise, and Rise. Notably, Dawn emerged as the primary parent in different varietal development endeavors. In 1988, David Baltensperger earned significant recognition for his varieties, namely Early Bird, Hutsman, Sunrise, and Horizon, which remain widely cultivated to this day. More recently, Dipak Santra has embraced breeding programs for alternative crops, aiming to accelerate breeding efforts through genomic tools and enhance genomic resources for marker-aided selection (MAS) applications.

18.8.3 Russia

Russians employed mainly intra-specific hybridization for proso improvement. The remarkable cultivars such as 'Bistrove' and 'Krupnoskoroe' (*ssp. Subcoccineum*), with more than 5 tha^{-1} grain yield and shorter life cycles were released (Kurzeva et al. 2012). The value-added varieties like 'Sputnik' (*ssp. coccineum*), Sojuz (selection line l.1980), and 'Slavjanskoe' (spp. *subflavum*) containing resistance for smut disease were developed in 2006. The new variety 'Regent' was developed using anther-culture technique in 2011. Mutant forms serve as the foundation for novel selection technologies, alongside the production of dihaploid plants, genotype documentation utilizing storage protein electrophoresis, and PCR-based DNA markers. The Proso millet genetic resources from Russia and USA are given in Table 18.5.

Table 18.4 Improved varieties released in proso millet in India

Sl. No.	Variety	Pedigree	Development center	Year	Salient features
1	Shyamcheena	A pure-line selection	–	1960	
2	Ram cheena	A pure-line selection	–	1960	
3	V-306	–	Andhra Pradesh	1965	
4	V-27	–	Andhra Pradesh	1965	
5	K2	A pure-line selection from PV-1685	Tamil Nadu Agricultural University, Coimbatore	1980	Tolerant to major pests and diseases
6	K1	Selection from local	Tamil Nadu Agricultural University, Coimbatore	1982	
7	BR-7	A pure-line selection	–	1982	Moderately resistant to Helminthosporium and shoot fly
8	CO-2	Pure-line selection from a local collection from Manjanaikanur in Annamalai block Coimbatore	TNAU, Coimbatore	1985	Shining yellow bold grain; densely pubescent
9	CO-3	Pure-line selection from the local collection maintained at TNAU	TNAU, Coimbatore	1985	Shining golden yellow grain drought tolerant, leaf pubescent
10	CO-4	Pure-line selection	TNAU, Coimbatore	1989	
11	Nagarjuna	Pure-line selection	ANGRAU, Hyderabad	1989	Early maturity
12	Sagar	Selection from local	ANGRAU, Hyderabad	1989	High seed yield
13	Bhawna	Pure-line selection	CSAUAT, Kanpur	1992	Early maturity
14	GPUP 8	S 7 × L 111	CSAUAT, Kanpur	2001	Resistant to brown spot
15	GPUP 21	GPUP 14 × K 1	PC unit, UAS, Bangalore	2003	High yield, moderately tolerant to shoot fly
16	Pratap chenna-1 (PR-18)	Pure-line selection	MPUA&T, Udaipur	2006	For sub-marginal/ shallow, undulating and hilly agro-ecological

(continued)

Table 18.4 (continued)

Sl. No.	Variety	Pedigree	Development center	Year	Salient features
					situations in Rajasthan
17	CO (PV) 5	Cross derivative of PV 1403 × GPUP 21	TNAU, Coimbatore	2007	Resistant to brown spot and tolerant to rust and grain smut
18	PRC-1	Selection from GPMS 519	GBPUA&T, Pantnagar	2008	Resistant to helminthosporium
19	TNAU 145	Cross-derivate of PV 1454 × TNAU 96	TNAU, Coimbatore	2009	Tolerant to rust and shoot fly
20	TNAU 151	TNAU 96 × PV 1673	TNAU, Coimbatore	2009	Tolerant to rust and shoot fly
21	TNAU 164	Derivate of TNAU 137 × CO4	TNAU, Coimbatore	2010	Resistant to rust and grain smut disease incidence
22	TNAU 202	PV 1453 × GPUP 16	TNAU, Coimbatore	2011	Recommended for cultivation in dry lands of India
23	TNPm-230	TNAU-164 × IPM-19	TNAU, Coimbatore	2017	Short duration, drought tolerant variety
24	DHPM-2769	Selection from IPM-2769	ARS, Hanumanamatti, UAS, Dharwad	2018	Suitable for contingency planting
24	PMV-442	GPMS 109 × GPMS 908	Project Coordinating Unit, UAS, Bengaluru	2019	

18.8.4 China

The Agricultural Experiment Farms of the former Suyuan province (now located in Langshan town, Linhe District, Inner Mongolia) and the Guanghua Farm (which later became the Yan'an Research Institute of Agricultural Sciences) in the Shan-Gan-Ning Region were both engaged in proso millet breeding around 1940. Notably, the initial Agricultural Experiment Farm identified the Langshan 462 and Micang 155 varieties. These two varieties, Langshan 462 and Micang 155, gained prominence as the earliest proso millet types cultivated in the Qianshan region and Tumochuan Plain of Bayan Nur and Ulan Qab, Inner Mongolia, prior to 1965.

Between 1940 and 1970, there was a proliferation of 10 research organizations dedicated to enhancing proso millet varieties. This collective effort resulted in the successful development of 42 distinct varieties, constituting approximately 31% of all varieties bred in China. An influential contribution came from the Crop Breeding Institute of the Helongjiang Academy of Agricultural Sciences, which pioneered

Table 18.5 Proso millet genetic resources in different countries

Cultivar	Important traits	Cultivation location
Dawn	Maturing at an extremely early stage; uniform ripening; large seed	USA
Rise	Demonstrates stability across diverse cultivation conditions; characterized by small seeds and compact panicles.	USA
Sunup	Exhibits resilience across a broad spectrum of cultivation conditions.	USA
Earlybird	Strong straw durability, compact panicle; exceptionally large seed size, maturing at an early stage.	USA
Huntsman	Compact form of panicle, significant seed size, maturing at a later stage.	USA
Sunrise	Significant seed size, densely packed panicle.	USA
Horizon	Significant seed size; panicle with a closed structure.	USA
Bistroye	Produces abundant yield at an early stage of maturity.	Russia
Kruppnoskoroe	High-yielding early maturing, large grain	Russia
Sputnik, Slavjanskoe	Generates substantial harvest, maturing in the mid-early range, resilient against smut, and boasting superb groat quality.	Russia
Alba	Yields a significant harvest, matures in the mid-early phase, exhibits strong and rapid ripening, resistant to lodging and shattering, easily hulled, and delivers a substantial great output.	Russia

varietal improvement through hybridization techniques. This pioneering work set the foundation of hybridization-based breeding, ultimately yielding 10 new varieties. Among these, 'Longshu 16' stood out as the first broomcorn millet variety to be bred through hybridization in China.

18.8.5 Kenya

The team at Kenya Agricultural Research Institute (KARI) initiated an initial enhancement initiative by making a limited number of choices from local and ICRISAT-associated lineages. One notable outcome of this effort is the 'KAT/PRO-1' cultivar, which was created through mass selection and is recommended for farming in Kenya. Developed by KARI, this cultivar exhibits a 50% higher yield compared to indigenous variants. Its origins trace back to 'N40101,' a plant introduced from the former Soviet Union via ICRISAT. The other institutes working on proso millet genetic resources are in brief reviewd by Santra et al. (2019).

18.9 Conclusion and Future Perspectives

Proso millet stands as a neglected crop, often overlooked. Its remarkable ability to thrive under varying climates due to its minimal water requirements sets it apart from other cereal crops. This hardy crop displays resilience in the face of challenging conditions, particularly in arid regions and nutrient-depleted soils. Despite its potential benefits, proso millet's significance has waned against more prominent cereals such as rice, wheat, and maize. Nevertheless, the tide is turning, as more people are expressing interest in exploring these traditional crops. Considering the existing scenario of climate change, constrained resources, and escalating food insecurity, there is an urgent requirement to revitalize resilient and nutritionally dense crops such as proso millet. This underscores the importance of enhancing proso millet's genetics to create improved cultivars that offer not only enhanced yields but also enriched protein and mineral content. As we look ahead, initiatives aimed at advancing proso millet should embrace technological progress. This involves the development of innovative varieties, novel cultivation techniques, effective agronomic approaches, plant protection strategies, and the mechanization of farming practices.

References

Anonymous (2014) Status paper on coarse cereals, directorate of millets development. Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India, Jaipur, Rajasthan

AICSMIP (2014) Annual Report 2013–2014 – All India Coordinated Small Millets Improvement Project (AICSMIP), Bengaluru, India

Araki M, Numaoka A, Kawase M, Fukunaga K (2012) Origin of waxy common millet, *Panicummiliaceum* L. in Japan. Genet Resources Crop Evol 59(7):1303–1308

Baltensperger DD (2002) Progress with proso, pearl and other millets. Trends New Crops New Uses:100–103

Baltensperger DD, Nelson LA, Frickel GE (1995a) Registration of 'Earlybird'proso millet. Crop Sci 35(4):1204–1205

Baltensperger DD, Nelson LA, Frickel GE, Anderson RL (1995b) Registration of 'Huntsman'proso millet. Crop Sci 35(3):941–941

Baltensperger DD, Nelson LA, Frickel GE, Heyduck RF, Yu TT (2004) Registration of NE-1 proso millet germplasm. Crop Sci 44(4):1493–1495

Changmei S, Dorothy J (2014) Millet-the frugal grain. Int J Sci Res Rev 3(4):75–90

Cho Y II, Chung JW, Lee GA et al (2010) Development and characterization of twenty-five new polymorphic microsatellite markers in proso millet (Panicum miliaceum L.). Genes Genom 32:267–273

Christenhusz MJ, Byng JW (2016) The number of known plants species in the world and its annual increase. Phytotaxa 261(3):201–217

Dikshit N, Sivaraj N (2013) Diversity for protein and morpho-agronomical characteristics in proso millet germplasm collections of Ratnagiri District, Maharashtra, India. Vegetos 26(2):164–170

Gomashe SS (2017) Proso millet, *Panicummiliaceum* (L.): genetic improvement and research needs. In: Millets Sorghum: biology and genetic improvement, pp 150–169. https://doi.org/10.1002/9781119130765.ch5

Goron TL, Raizada MN (2015) Genetic diversity and genomic resources available for the small millet crops to accelerate a new green revolution. Front Plant Sci 6:157

Graybosch RA, Baltensperger DD (2009) Evaluation of the waxy endosperm trait in proso millet (*Panicum miliaceum*). Plant Breed 128(1):70–73

Gupta A, Sood S, Agrawal PK, Bhatt JC (2011) Floral biology and pollination system in small millets. Eur J Plant Sci Biotechnol 6:81–86

Habiyaremye C, Barth V, Highet K, Coffey T, Murphy KM (2017a) Phenotypic responses of twenty diverse proso millet (*Panicummiliaceum* L.) accessions to irrigation. Sustainability 9(3): 389

Habiyaremye C, Matanguihan JB, D'Alpoim Guedes J, Ganjyal GM, Whiteman MR, Kidwell KK, Murphy KM (2017b) Proso millet (*Panicummiliaceum* L.) and its potential for cultivation in the Pacific northwest, US: a review. Front Plant Sci 7:1961

Harlan JR (1975) Crops and man. American Society of Agronomy, Madison, Wisconsin, p 295

Haussmann BIG, Hess DE, Omanya GO, Folkertsma RT, Reddy BVS, Kayentao M, Welz HG, Geiger HH (2004) Genomic regions influencing resistance to the parasitic weed Striga hermonthica in two recombinant inbred populations of sorghum. Theor Appl Genet 109(5): 1005–1016

Hawlader MSH (1991) Genetic variability and correlation study In proso millet (*Panicum miliaceum* L.). the annals of Bangladesh. Agriculture 1(2):61–64

Hou S, Sun Z, Li Y, Wang Y, Ling H, Xing G, Han Y, Li H (2017) Transcriptomic analysis, genic SSR development, and genetic diversity of proso millet (*Panicummiliaceum*; Poaceae). Appl Plant Sci 5(7):1600137

Hu YG, Zhu J, Liu F, Zhang Z, Chai Y, Weining S (2008) Genetic diversity among Chinese landraces and cultivars of broomcorn millet (*Panicummiliaceum*) revealed by the polymerase chain reaction. Ann Appl Biol 153(3):357–364

Hu X, Wang J, Lu P, Zhang H (2009) Assessment of genetic diversity in broomcorn millet (*Panicummiliaceum* L.) using SSR markers. J Genet Genomics 36(8):491–500

Hunt HV, Vander Linden M, Liu X, Motuzaite-Matuzeviciute G, Colledge S, Jones MK (2008) Millets across Eurasia: chronology and context of early records of the genera *Panicum* and Setaria from archaeological sites in the Old World. Veg Hist Archaeobotany 17(1):5–18

Hunt HV, Campana MG, Lawes MC, Park YJ, Bower MA, Howe CJ, Jones MK (2011) Genetic diversity and phylogeography of broomcorn millet (*Panicummiliaceum* L.) across Eurasia. Mol Ecol 20(22):4756–4771

Hunt HV, Moots HM, Graybosch RA, Jones H, Parker M, Romanova O, Jones MK, Howe CJ, Trafford K (2013) Waxy phenotype evolution in the allotetraploid cereal broomcorn millet: mutations at the GBSSI locus in their functional and phylogenetic context. Mol Biol Evol 30(1): 109–122

Hunt HV, Badakshi F, Romanova O, Howe CJ, Jones MK, Heslop-Harrison JP (2014) Reticulate evolution in *Panicum* (Poaceae): the origin of tetraploid broomcorn millet, *P. miliaceum*. J Exp Bot 65(12):3165–3175

Jones M (2004). Between fertile crescents: minor grain crops and agricultural origins (p. 127). Na

Karam D, Westra P, Nissen SJ, Ward SM, Figueiredo JEF (2004) Genetic diversity among Proso millet (Panicum miliaceum L.) biotypes assessed by AFLP technique. PlantaDaninha 22 (2):167–174

Kaume RN (2006) Panicum miliaceum L. In: Brink M, Belay G (eds) PROTA 1: cereals and pulses/ Céréales et légumes secs. CD-Rom. PROTA, Wageningen

Konstantinov SI, Grigorashchenko LV (1987) Inheritance of resistance to melanosis in proso millet hybrids of the first generation. TsitologiyaiGenetika 21(5):335–338

Konstantinov SI, Linnik VM, Ya SL (1989) Use of smut-resistant induced mutants i n breeding proso millet. SelektsiyaiSemenovodstvo (Kiev) 66:25–28

Konstantinov SI, Linnik VM, Shapina LY, Grigorashchenko LV (1991) Breeding proso millet for resistance to diseases. In: Breeding proso millet for resistance to diseases, pp 112–117

Kurzeva A, Romanova O, Krylov A (2012) Genetic resources of common millet (*Panicummiliaceum* L.) at the Vavilov research Institute of Plant Industry (VIR). In: Advances in broomcorn millet research, pp. 76–81.

Lágler R, Gyulai G, Humphreys M, Szabó Z, Horváth L, Bittsánszky A, Kiss J, Holly L, Heszky L (2005) Morphological and molecular analysis of common millet (P. *Miliaceum*) cultivars compared to an aDNA sample from the 15th century (Hungary). Euphytica 146(1):77–85

Liu M, Xu Y, He J, Zhang S, Wang Y, Lu P (2016) Genetic diversity and population structure of broomcorn millet (*Panicummiliaceum* L.) cultivars and landraces in China based on microsatellite markers. Int J Mol Sci 17(3):370

Lu H, Zhang J, Liu KB, Wu N, Li Y, Zhou K, Ye M, Zhang T, Zhang H, Yang X, Shen L (2009a) Earliest domestication of common millet (*Panicummiliaceum*) in East Asia extended to 10,000 years ago. Proc Natl Acad Sci 106(18):7367–7372

Lu H, Zhang J, Wu N, Liu KB, Xu D, Li Q (2009b) Phytoliths analysis for the discrimination of foxtail millet (*Setariaitalica*) and common millet (*Panicummiliaceum*). PLoS One 4(2):e4448

Lyon DJ, Burgener PA, DeBoer KL, Harveson RM, Hein GL, Hergert GW, Holman TL, Nelson LA, Johnson JJ, Nleya T, Krall JM (2008) Producing and marketing proso millet in the Great Plains. Univ. of Nebraska, Lincoln, Extension (EC137)

Lyssov BH (1975) Proso (Panicum L.). In: Krotov AS (ed) The USSR flora of cultivated plants. 3. Croat Crops. USSR, Kolos, Leningrad

Manoharan V, Sivasubramanian V (1982) Variability studies in proso millet (*Panicummiliaceum* L.). Madras Agric J

Maslenkova LI, Resh LP (1990) Sources of resistance to head smut in proso millet. Nauchno-TekhnicheskiĭByulleten', VASKhNIL, SibirskoeOtdelenie, SibirskiĭNauchno-Issledovatel'skiĭInstitutSel'skogoKhozyaistva 6:28–33

Miller NF, Spengler RN, Frachetti M (2016) Millet cultivation across Eurasia: origins, spread, and the influence of seasonal climate. The Holocene 26:1566–1575. https://doi.org/10.1177/0959683616641742

M'Ribu HK, Hilu KW (1996) Application of random amplified polymorphic DNA to study genetic diversity in Paspalum scrobiculatum L. (kodo millet, Poaceae). Genet Resour Crop Evol 43:203–210

Murthi TK, Harinarayana G (1989) Insect pests of small millets and their management in India. In: Small millets in global agriculture proceedings of the first international small millets workshop Bangalore, India, pp 255–270

Nanda JS, Agrawal PK (2008) Botany of field crops, vol I. Kalyani Publisher, India

Nandini C, Srinathareddy J (2019) Modified crossing (SMUASB) method for artificial hybridization in proso millet (*Panicummiliaceum* L.) and Littlemillet (*Panicumsumatrense*). Electr J Plant Breed 10(3):1161–1170

Natarajan US, Raveendran TS, Appadurai R (1978) A path coefficient analysis of yield and yield components in proso millet (Panicum miliaceam L.). Madras Agric J 65(7):430–434

Nelson LA (1984) Technique for crossing proso millet 1. Crop Sci 24(1):205–206

NBPGR (1986) National Bureau of Plant Genetic Resources, India, Annual Report, 1986, 131

NBPGR (1987) National Bureau of Plant Genetic Resources, India. Annual Report, 1987, 81

NBPGR (2011) National Bureau of Plant Genetic Resources, India. Annual Report, 2010–2011

NBPGR (2012) National Bureau of Plant Genetic Resources, India. India. Annual Report, 2012–2013.

Panwar KS, Kapila RK (1992) Variation and character association in proso millet. Crop Improvement (India) 19:130–133

Prasad GS, Nagaraja TE, Seetharam A, Gowda BTS (1995) Genetic variability and character association studies in proso millet. Crop Improvement (India) 22(2):225–227

Rajasekaran R, Francis N (2021) Genetic and genomic resources for improving proso millet (Panicum miliaceum L.): a potential crop for food and nutritional security. Nucleus 64(1):21–32

Rajput SG, Plyler-Harveson T, Santra DK (2014) Development and characterization of SSR markers in proso millet based on switchgrass genomics. Am J Plant Sci 2014:05

Rajput SG, Santra DK, Schnable J (2016) Mapping QTLs for morpho-agronomic traits in proso millet (*Panicummiliaceum* L.). Mol Breed 36(4):1–18

Reddy VG, Upadhyaya HD, Gowda CLL (2007) Morphological characterization of world's proso millet germplasm collection. J SAT Agric Res 3:4

Sabir P, Ashraf M, Akram NA (2011) Accession variation for salt tolerance in proso millet (*Panicummiliaceum* L.) using leaf proline content and activities of some key antioxidant enzymes. J Agron Crop Sci 197(5):340–347

Salini K, Nirmalakumari A, Muthiah AR, Senthil N (2010) Evaluation of proso millet (*Panicummiliaceum* L.) germplasm collections. Electr J Plant Breed 1(4):489–499

Santra DK, Khound R, Das S (2019) Proso millet (Panicum miliaceum L.) breeding: progress, challenges and opportunities. In: Advances in plant breeding strategies: cereals, pp 223–257. https://doi.org/10.1007/978-3-030-23108-8_6

Sasamala AC, Sahoo LP, Mahapatra KC, Pandey S (2011) Genetic variability and character association in common millet germplasm of Odisha. Indian J Plant Genet Resour 24 (1):82–86.

Seetharam A, Gowda J, Halaswamy JH (2003) Small millets. In: Chowdhury SK, Lal SK (eds) Nucleus and breeder seed production manual. Indian Agricultural Research Institute, New Delhi, India, pp 54–67

Shailaja S, Jagadish PS, Kumar CTA, Neelu N, Jayarame G, Nagaraja A (2009) Evaluation of pre-release and released varieties of proso millet (*Panicummiliaceum* L.) to Corcyra cephalonica Stainton (Lepidoptera: Pyralidae) infestation. Environ Ecol 27(1A):445–447

Soldatov AF, Agafonov NP (1980) Susceptibility of *Panicum* millet to melanosis in western Kazakhstan. Trudy po PrikladnoïBotanike, GenetikeiSelektsii 69(1):64–69

Upadhyaya HD, Sharma S, Gowda CLL, Reddy VG, Singh S (2011) Developing proso millet (*Panicum miliaceum* L.) core collection using geographic and morpho-agronomic data. Crop Pasture Sci 62(5):383–389

Upadhyaya HD, Dwivedi SL, Singh SK et al (2014) Forming core collections in barnyard, kodo, and little millets using morpho agronomic descriptors. Crop Sci 54(6):2673–2682

Upadhyaya HD, Vetriventhan M, Dwivedi SL, Pattanashetti SK, Singh SK (2016) Proso, barnyard, little, and kodo millets. In: Genetic and genomic resources for grain cereals improvement. Academic Press, pp 321–343

Vavilov NI (1926) Studies on the origin of cultivated plants. Institut de BotaniqueAppliquée et d'Amélioration des Plantes

Wang L, Wang XY, Wen QF, Cao LP (2007) Identification of protein and fat content for Chinese proso millet germplasm. J Plant Genet Res 8:165–169

Zhang J, Lu H, Liu M, Diao X, Shao K, Wu N (2018) Phytolith analysis for differentiating between broomcorn millet (*Panicum miliaceum*) and its weed/feral type (*Panicum ruderale*). Sci Rep 8(1):1–9

Zhao Z (2011) New archaeobotanic data for the study of the origins of agriculture in China. Curr Anthropol 52(S4):S295–S306

Zohary D, Hopf M (2000) Domestication of plants in the Old World: the origin and spread of cultivated plants in West Asia, Europe and the Nile Valley, 3rd edn. Oxford University Press

Recent Advancements in Proso Millet (*Panicum miliaceum* L.) Breeding for Quality and Yield Improvement

19

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Abstract

Proso millet (*Panicum miliaceum* L.) is one of the important and oldest crops domesticated by humankind in China. It is a climate-smart crop inherently resilient to biotic and abiotic stresses, which led to its widespread across Europe and Asia during the Neolithic era. Besides, the crop is important among growers due to its extremely short life span and high efficiency of water use. Proso millet has a low glycemic index, gluten-free, and is rich in protein content among other millets. The lack of availability of appreciable genomic resources has slowed the crop improvement process. The draft proso millet genome sequence allows large-scale re-sequencing to investigate genetic resources and genetic diversity, leading to sequence-based breeding for quality and yield traits. Genome-wide association studies combined high-throughput genotyping and phenotyping to allow marker-trait association analysis to identify quality and yield responsive genes. The introduction of genomic selection and genome editing technologies, combined with recent advances in bioinformatics tools, has enabled a breakthrough in genomics-assisted breeding for the enhancement of yield and quality. This chapter focuses on the available genomic information, status, progress, prospects, and challenges of genome-assisted breeding to develop a road map for genetic improvement for quality (grain and protein quality, nutrient-enriched, tolerant for lodging, and non-shattering types) and yield improvement in proso millet, which

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is likely to play a tremendous role in improving global food and nutrition security under climate change and exponential population growth scenarios.

Keywords

Proso millet · Yield · Quality traits · Genome sequencing · Genomics-assisted breeding

19.1 Introduction

Proso millet (*Panicum miliaceum* L., $2n = 4x = 36$) is one of the ancient crops known to mankind and belongs to the Panicoideae subfamily of the family *Poaceae*. It is known by various names in different regions in India like Baragu (Kannada), Barri (Hindi), Chena (Hindi), Cheena (Bengali), Cheno (Gujarati), *China Bacharibagmu* (Odia), *Panivaragu* (Tamil), *Vari* (Marathi), Variga (Telugu), and in the world it has different names like broom corn millet (China), common millet (Japan), common millet and hog millet (United States of America), Hersey millet (Germany), and White millet (France). Currently, it is cultivated in many parts of the world. In developed countries, it is used to feed poultry and livestock and in some parts of Asia, it is used as food (Rajput et al. 2014). It is grown in 0.82 million ha in Russia followed by 0.82 m ha in China (Diao 2017), 0.20 m ha in the United States (Habiyaremye et al. 2017), and 0.03 m ha in India (Bhat et al. 2018). In India, Madhya Pradesh, eastern Uttar Pradesh, Bihar, Tamil Nadu, Maharashtra, Andhra Pradesh, and Karnataka are the major producers presented in Fig. 19.1 (Rajasekaran and Francis 2021). Although it was grown as a major cereal in the past across Eurasia, its cultivation has rapidly declined since the eighteenth century due to the dominance of other major food crops like maize, wheat, etc., along with the change in food habits (Kalinova and Moudry 2006).

The world's population is expected to grow to 9.8 billion people by 2050 and the demand for food production is increased upto 60–70% over the current production, creating a substantial challenge to feed the expanding population (Cakmak and Kutman 2018; Vetriventhan and Upadhyaya 2019). About 2 billion people worldwide suffer from hidden hunger caused by a lack of micronutrients. The major staple cereal grains like rice, wheat, and maize have a lower concentration of minerals (micro and macro nutrients) than other grains like millets (Bekkering and Tian 2019). It is necessary to significantly transform the current agriculture and food systems toward greater diversity (crops and diets) to promote the cultivation and consumption of historically significant underutilized, climate-resilient, and nutrient-dense crops for sustainable agriculture and healthy diets.

Small millets are a class of small-seeded grain crops, commonly referred to as minor millets. This includes millet varieties such as barnyard millet, brown top millet, foxtail millet, guinea millet, kodo millet, little millet, and proso millet. Small millets are renowned for their climate-resilient characteristics, such as their increased ecological adaptability, reduced water use, decreased incidence of insect

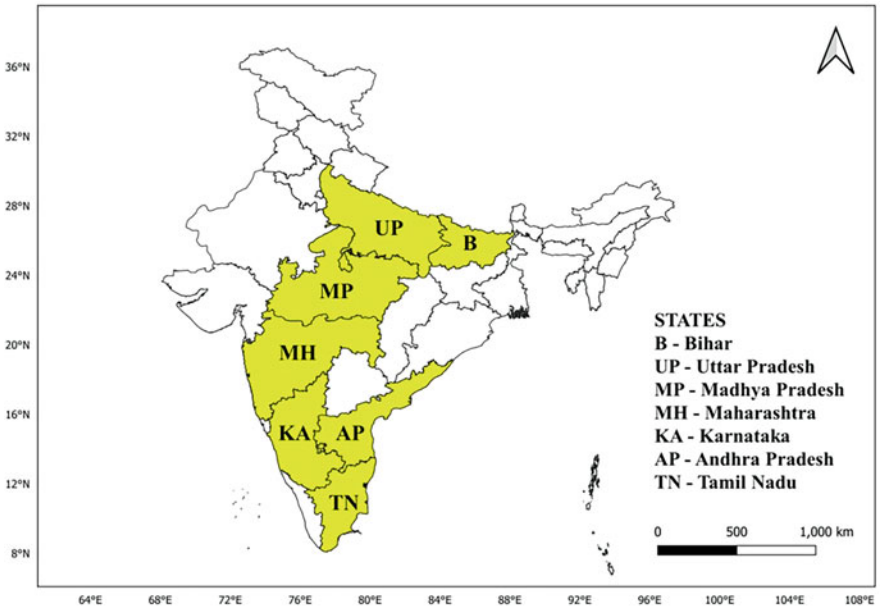

Fig. 19.1 The major proso millet-producing states in India

pests and diseases, and reduced susceptibility to environmental shocks. They are significant crops in semi-arid rainfed regions. Small millets are currently planted in a small area that only accounts for a small fraction of the world's millet production but they produce consistent yields on marginal soils and make a substantial contribution to food security. Among all the small millets, proso millet (13.21 g/100 g) had the highest mean protein content compared to other small millets (https://www.millets.res.in/ra19-20.php). The proso millet is a good source of energy, with 74 g of carbohydrates per 100 g of edible part. In addition, it has more crude fiber (5.5 g/100 g flour) than the majority of cereals and millets. The protein level (10–14 g/100 g) is higher than rice (8.5 g/100 g) and comparable to wheat (11.6 g/100 g), but with a higher proportion of important amino acids (leucine, phenylalanine, and methionine). According to reports, the essential amino acid index is 7% greater than wheat. Lysine was found to be limited, whereas leucine was responsible for the majority of the proso millet protein complex. Environmental factors are said to have a considerable impact on protein and amino acid composition (Kalinova and Moudry 2006; Devisetti et al. 2014). Proso is naturally a good source of antioxidants and other major nutrients with superior quality along with an optimal balance of essential amino acids among the small millets (Dikshit and Sivaraj 2013).

Proso millet is grown popularly as a minor millet in marginal lands with the provision of minimal resources. It has a very short growing duration, ranging from 60 to 100 days, and has very high water-use efficiency, resulting in the lowest water requirement among all cereal crops. Proso millet is a highly diversified crop with

excellent nutritional properties and suits for dryland agriculture in the current scenario of climate change. The creation of high-yielding, lodging, and seed-shattering resistant, proso millet cultivars would be beneficial.

Despite proso millet's unquestionable advantages for the environment and human health, it is still a crop that receives little attention and lags behind most cereals. There is a need to follow efficient breeding techniques (both traditional and modern breeding techniques) to create superior varieties. Proso millet germplasm with a diverse genetic makeup is preserved in gene banks run by various nations. Modern breeding techniques, such as genomics-assisted breeding (GAB), are essential for increasing crop productivity. Modern molecular tools like QTL (quantitative trait loci) mapping, fine mapping (dense genetic linkage map creation), association mapping, TILLING, Eco-TILLING techniques, genome-wide association studies (GWAS), and genomic selection (GS) can be used for the mining of mapping populations for its attributes. The development of molecular markers for the identification of candidate genes is made possible by the improvements in next-generation sequencing (NGS), which open a variety of omic platforms such as full genome sequencing, RNA-seq analysis, etc. Information on the genes involved in the trait of interest is also provided by the transcriptome analysis. The roles of genes can be discovered by analysis of genes that express differently under stress. Some millet's genomes have not yet been sequenced but for proso millet, there are only draft genome sequences available. In this review, we discuss the details of available genetic resources, breeding objectives, and modern advancements to accelerate genetic gains in proso millet.

19.2 Genetic Resources

Proso millet is domesticated nearly 10,000 years BP (Before Present) in Central and Eastern Asia (Hunt et al. 2014). According to Miller et al. (2016), it spread throughout West Asia and Europe at the end of the first millennium BC due to changes in farming methods in those regions. The crop cultivar diversity is crucial for sustainable agriculture and necessary variability for crop improvement is provided by germplasm. The low or limited genetic diversity increases the chance of crop failure because they are more susceptible to pest infestations, disease epidemics, and unpredictably changing climatic conditions. About 29300proso millet germplasm are preserved in various gene banks worldwide presented in Table 19.1 (https://www.fao.org/wiews-archive/germplasm_query.htm%3fi_l%3dEN). Most collections are kept in Russia (8778), China (8451), and Ukraine (1046 + 3976). In India, the AICRP (All India Coordinated Research Project) on Small Millets, International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), and National Bureau of Plant Genetic Resources (NBPGR) host around 3000 accessions.

The use of germplasm and management of germplasm is crucial for crop improvement in underutilized millets, as it is in major crops (Byrne et al. 2018). Small millets have low germplasm utilization, which has been severely constrained

Table 19.1 Proso millet germplasm maintained at various institutes in the world

Country	Institute	Accessions
Argentina	Banco Base de Germoplasma, Instituto de Recursos Biológicos, Instituto Nacional de Tecnología Agropecuaria	01
	Estación Experimental Agropecuaria 'Ing. Agr. GuillermosCovas'	01
Australia	Australia Australian Tropical Crops and Forages Genetic Resources Centre	228
Austria	AGES Linz—Austrian Agency for Health and Food Safety/Seed Collection	05
	Gene bank Tyrol/Tyrolean Government	08
	Office of the Styrian Regional Government, Department for Plant Health and Special Crops	01
Azerbaijan	Research Institute of Forage, Meadows and Pastures	10
Bangladesh	Plant Genetic Resources Centre, BARI	198
Bulgaria	Institute for Plant Genetic Resources 'K.Malkov'	489
Canada	Plant Gene Resources of Canada, Saskatoon Research Centre, Agriculture and Agri-Food Canada	23
China	Institute of Crop Science, Chinese Academy of Agricultural Sciences	8451
Czech Republic	Gene bank Department, Division of Genetics and Plant Breeding, Crop Research Institute	171
Georgia	Biological Farming Association (NGO) ELKANA	03
Germany	Genebank, Leibniz Institute of Plant Genetics and Crop Plant Research	166
Hungary	Institute for Agrobotany	244
	Station of Ujmajor, Research Institute for Vegetable Crops	50
India	AICRP (All India Coordinated Research Project)on Small Millets	920
	International Crop Research Institute for the Semi-Arid Tropics	842
	National Bureau of Plant Genetic Resources	998
Japan	Department of Genetic Resources I, National Institute of Agrobiological Sciences	516
	Plant Germplasm Institute, Faculty of Agriculture, Kyoto University	62
Kenya	National Genebank of Kenya, Crop Plant Genetic Resources Centre – Muguga	06
Mexico	Estación de Iguala, InstitutoNacional de InvestigacionesAgrícolas	400
Morocco	Centre Régional de la Recherche Agronomique de Settat	05
Nepal	Central Plant Breeding and Biotechnology Division, Nepal Agricultural Research Council	16
Pakistan	Pakistan Plant Genetic Resources Program	54
Philippines	Crop science cluster—Institute of Plant Breeding, College of Agriculture, University of the Philippines, Los Baños College	01
Poland	Botanical Garden of Plant Breeding and Acclimatization Institute	354
	Plant Breeding and Acclimatization Institute	359
Portugal	Portuguese Bank of Plant Germplasm	01
Romania	Research Institute for Cereals and Technical Plants Fundulea	65
	Suceava Genebank	32

(continued)

Table 19.1 (continued)

Country	Institute	Accessions
Russian Federation	N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry	8778
Slovakia	Slovakia Plant Production Research Center Piestany	57
Spain	Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria. Centro Nacional de Recursos Fitogenéticos	03
Sri Lanka	Dry Zone Agricultural Research Institute	04
	Plant Genetic Resources Centre (conservation, evaluation, data management)	27
Ukraine	Institute of Plant Production n.a. V.Y. Yurjev of UAAS	1046
	Ustymivka Experimental Station of Plant Production	3976
United Kingdom	Genetic Resources Unit, Institute of Biological, Environmental and RuralSciences, Aberystwyth University	03
	Millennium Seed Bank Project, Seed Conservation Department, Royal Botanic Gardens, Kew, Wakehurst Place	01
United States	National Center for Genetic Resources Preservation	01
	North Central Regional Plant Introduction Station, USDA-ARS, NCRPIS	721
Uruguay	INIA La Estanzuela	03
Viet Nam	Plant Resources Center	01
	Total collections	**29,301**

(https://www.fao.org/wiews-archive/germplasm_query.htm%3fi_l%3dEN)

by challenges with artificial hybridization. Reddy et al. (2007) described 842 germplasm accessions that came from 27 different nations. Since 1977, the characterization of proso millet has been done at ICRISAT in India and these studies revealed the significant diversity for several characteristics, pointing to superior country-specific accessions for those attributes. In 2011, Upadhyaya et al. evaluated around 833 accessions and developed a core collection of 106 proso millet accessions, which is based mostly on data from 20 qualitative and quantitative features and geographic data. Dikshit and Sivaraj (2013) discovered accessions with high protein content and great diversity for several morpho-agronomical features. Vetriventhan and Upadhyaya (2018) examined changes in morpho-agronomic characteristics and grain nutrients in the proso millet seed and discovered that geographic race, regions, and country of origin all have an impact on diversity patterns. The management of germplasm is crucial for the genetic advancement of this crop species. Utilizing the resources systematically for breeding can assist in increasing grain, fodder productivity, grain quality with better composition, and grain tolerance to biotic and abiotic stress.

Even though there are currently many accessions available globally, plant breeders face the daunting task of selecting the right parents for their incorporation into crop breeding projects (Jia et al. 2017). The availability of trait-specific germplasm and its effective application is essential for a breeding program to be successful (Upadhyaya et al. 2013). A group of genotypes representing the entire diversity

(core collections) must be found among the numerous germplasm accessions. Future breeding attempts to increase productivity will undoubtedly benefit from the creation of core collections and mini-cores. They can help with the right selection of accessions with desirable features in a resource-efficient manner (Upadhyaya et al. 2013, 2014). Furthermore, a core collection might aid in the wise management of a great collection and it can be used to eliminate duplication in several collections. To boost the productivity of proso millet, further expansion of core and micro core collections would strengthen plant breeding capacities.

19.3 Breeding Objectives

The researchers and farmers have to deal with variable global climatic fluctuations and a variety of pressures that have a negative impact on agricultural production and productivity. Climate change will have a great impact on crop yield in the coming decades, and it may lead to an impact on future food demand. The biotic and abiotic factors cause a hazard to millet production (Antony et al. 2018). Numerous genes in proso millet need to be found for tolerance to biotic and abiotic stress. The understanding of proso millet adaptation mechanisms and gene mining could be a useful resource for researching their climate-resilience traits.

The development of new cultivars in accordance with the region-specific climatic factors, such as soil, temperature, humidity, day length, rainfall, and cropping patterns, genotypes must be produced for various maturity duration groups. Some proso millet cultivars are particularly preferred by farmers because they work well in particular environments. Since variety innovations are primarily focused on dry land cultivation, all varieties might not offer yields that are noticeably higher across sites. Proso millet consumption by humans is high in Asia and growing in developed nations. Due to their sticky character and superior adhesion after cooking, the waxy grain proso millet genotypes are chosen in these regions. The germplasm of proso millet contains waxy varieties that can be exploited to create cultivars of proso millet that are waxy. Larger seed size has a significant impact on the variety of applications besides grain quality features. The endosperm's brightness, which contains carotenoids, is quite valuable and the amount of carotenoids in grains closely correlates with their yellowish, vibrant, and glossy characteristics. The red-coated cultivars are favored in field situations because they protect against light-induced carotenoid degradation.

It is becoming more popular as a healthy diet worldwide. It has the greatest protein concentration among the tiny millets. The quality and amount of protein in eight different types of proso millet were assessed. Leucine, isoleucine, and methionine are three necessary amino acids that are abundant in proso protein, making it a superior source of protein to wheat, according to Kalinova and Moudry (2006). Some innovative end-use properties, such as waxiness, grain size and shine, might attract more market interest when creating different food products with proso millet grains. Their usage in practical breeding would aid in improving the grain quality of

new-generation cultivars. The inheritance of proso millet's grain quality features is quite complicated. There are inverse correlations between the quality attributes.

The first 2 weeks after planting are among the most crucial times for growing proso millet. It is a poor competitor to weeds hindering potential yield when grown on weedy terrains due to small seed size and delayed growth that results during the first several weeks. Finding germplasm with early vigor and the best yield is necessary. Genotypes with short duration typically exhibit significant early vigor.

Shoot fly (*Atherigona* spp.), a significant pest, has been identified in proso millet. The significant diseases that have been identified so far on this crop include blast, sheath and leaf blights, and grain smut. One of the important approaches to reduce losses due to these biotic stresses is to use immune or resistant germplasm in breeding programs to enhance host-plant resistance in the high-yielding varieties.

Proso millet begins to ripen from the top of the panicle, and it typically is not uniform after reaching maturity. Seeds that ripen unevenly stay green in the part of the panicle below (Matz 1986). The grain shattering takes place if harvesting is delayed. Thus, there is a need to develop shattering-resistant cultivars. During the rainy season, crop cultivation under the Indian scenario is not a major concern, but, this could have a significant impact on overall yields for post-rain and summer crops. It can be threshed after the grain moisture is reduced to 13% (Baltensperger et al. 1995).

The distribution of photosynthates among the plant's grains and biomass is reflected in the harvest index. The upgradation of the harvest index of proso millet in the current cultivars is an important breeding objective. It is especially challenging to sustain farm production and profitability with rising cultivation costs because the labor pool for agriculture has significantly shrunk and is currently steadily shrinking. Mechanizing labor-intensive farm operations is one of the solutions. Therefore, it is imperative to develop cultivars that can be harvested mechanically. The benefits of creating plant ideotype that may be harvested mechanically are numerous. As a result of the improved plant architecture and greater photosynthetic activity, the yield would increase. For mechanical harvesting, the development of erect genotypes with ideal height, well-organized branching, and panicle morphology is highly helpful.

19.4 Proso Millet Crop Improvement

Proso millet is primarily a self-pollinated short-day plant. A drooping inflorescence is present with the panicle is broom-like appearance (Habiyaremye et al. 2017). A panicle may take 12–15 days to complete flowering. Though self-pollination predominates, there is also more than 10% of natural cross-pollination may occur (Popov 1970; Baltensperger 2002). Proso millet is difficult to use for crossing without damaging the stigma and without pollen leakage before emasculation (Gupta et al. 2011). The use of hand emasculation for crossing proso millet is reported by Nelson et al. (1984) while the use of cold water spray for both emasculation and crossing is documented by Nandini et al. (2019).

19.4.1 Conventional Breeding Approaches

Crop improvement programs mainly focused on improving the features including yield, non-lodging, non-shattering, early maturity, waxiness, etc. In conventional plant breeding, numerous self-pollinating breeding techniques, such as pure line selection, pedigree selection, mass selection, and mutant breeding are used in proso millet breeding program. Nations like China, India, Russia, etc., have considerable contributions to proso millet breeding (Gomashe 2017). In the 1940s, China started a Proso millet improvement program and pioneered its breeding later. Germplasm management, which includes collecting, evaluating, and breeding germplasm, is the main focus of proso millet research in China (Diao and Jia 2017). The reports on proso millet cultivars over time revealed that the majority of them were released after being selected from local cultivars and landraces followed by pedigree selection (hybridization and selection). Following the selection of landraces, 11 proso millet cultivars and by pedigree method 8 cultivars were released in the United States (Santra 2013; Santra et al. 2019). In India, Ram Cheena was the first proso millet cultivar bred in 1960 through pure-line selection with a better yield of 2000–2500 kg/ha, and later the non-lodging and non-shattering variety K2 was produced through pure-line selection by the Tamil Nadu Agricultural University (Rajasekaran and Francis 2021). An important breeding technique for small millets, especially finger millet, foxtail millet, and proso millet, involves hybridization to promote variety followed by selection in a segregating population. In India, 45% of the cultivars of finger millet, 22% of foxtail millet, and 29% of proso millet were bred using the hybridization and selective breeding process (Vetriventhan et al. 2020). In India, high-yielding cultivars (TNAU 202 and ATL 1) developed through a hybridization program (Rajasekaran and Francis 2021).

In self-pollinated crops, the creation of variation through hybridization is difficult where mutation breeding plays an important role in the generation of variation. Kate et al. (2018) exposed a local cultivar of proso millet to gamma radiation and found two early-maturing mutants from 40 krad and 50 krad doses, as well as two high-yielding mutants from 20 krad and 60 krad doses.

The cultivars Neimi 5, Ningmi 9, Longmi 4, Ningmi 10, Longshu 16, and Jinshu 4 are the most renowned cultivars in China and since the 1950s, around 162 cultivars have been released in China (Diao and Jia 2017). Early maturity cultivars Dawn and Early-bird were created in the United States. Alba, a Russian cultivar, is renowned for its non-shattering qualities. The Nebraska Agricultural Experiment Station in the United States created the cultivar "Plateau," which is waxy or devoid of amylose. Because of their sticky properties and their ability to ferment effectively, waxy forms are preferred by food and beverage industries (Santra et al. 2019). The University of Nebraska-Lincoln, Panhandle Research and Extension Center (UNL-PHREC), Scottsbluff, Nebraska, is home to the proso millet breeding program in the United States and released several cultivars in the United States.

19.4.2 Modern Advancements to Accelerate Genetic Gains in Proso Millet

19.4.2.1 Genomics

Plant genomics deals with sequencing, characterizing, and studying the genetic compositions, structures, organizations, functions, and interactions or networks of an entire plant genome (Abdurakhmonov 2016). Earlier genomics included studies on genomic size and physical and genetic mapping but later with the advent of NGS, the ability to sequence, assemble, and analyze genomes of plant species was made easier. Identification of genes behind agronomically and economically important traits is now possible with the accessibility to whole genome sequences in the majority of the crops.

Genome sequences as well as molecular markers are important in establishing linkage maps, which are considered the key initial step in molecular breeding and have been considerably used in studies related to the taxonomy, genetic diversity, and population structure studies in many major crops (Antony et al. 2018; Nadeem et al. 2018). Molecular markers like restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), and simple sequence repeats (SSR) markers are regularly used in genetic diversity and QTL mapping studies in finger millet (Antony et al. 2018). In foxtail millet, SSRs and single nucleotide polymorphism (SNP) markers are being used for genomic studies (Jaiswal et al. 2019). But in proso millet, limited molecular markers such as SSR, and GBS (genotyping-by-sequencing)-SNP are available (Habiyaremye et al. 2017).

SSR markers are often studied in proso millet by the use of genomic sequences from other related plant species due to limited genomic resource(s) availability. SSR markers from the rice, wheat, oats, and barley were used in genetic diversity studies of Chinese proso millet accessions by Hu et al. (2009). Cho et al. (2010) developed and characterized 25 polymorphic microsatellite markers through the construction of an SSR-enriched library from proso millet genomic DNA. The dissemination of proso millet from its center of domestication to other regions of the world using 16 proso millet-specific SSR markers was studied by Hunt et al. (2011). Switchgrass being genetically nearest to proso millet, its SSR markers were used for genetic diversity of proso millet genotypes (Rajput et al. 2014). Liu et al. (2016) used high-throughput sequencing to develop 500 proso millet-specific SSR-primer pairs to identify polymorphism and analyze the genetic diversity and structure of 88 accessions. Jiang et al. (2018) developed 8139 expressed sequence tags-simple sequence repeat (EST-SSR) markers from the transcript sequencing of proso millet cultivar Yanshu 5.

SNP markers often are considered reliable for molecular studies like high-density linkage mapping and genomic selection due to their plentitude in the genome (Xia et al. 2019; Santra et al. 2019) but limited studies on SNP markers are available. Rajput et al. (2016) constructed genetic linkage map for the first time in proso millet to identify the DNA markers linked to QTLs of nine morpho-agronomical traits using 93 recombinant inbred lines (RILs) derived from F_1 (Huntsman × Minsum). Rajput et al. (2016) identified 833 GBS-SNPs using 93 RILs and 18 major linkage

groups (LG), considered as 18 chromosomes of proso millet. They were able to identify QTLs for eight morpho-agronomic traits, viz., heading date, 100 grain weight, grains per panicle, lodging, peduncle length, plant height, grain shattering and panicle length out of nine traits (Rajput et al. 2016). Wang et al. (2018) identified six SNPs of the waxy locus (wx-L) using sequences of PCR amplicons of 132 proso millet accessions and reported allelic variation of the waxy gene. In genome-wide population genetic studies of three millets, viz., kodo millet, little millet, and proso millet, 1882 SNPs were identified for proso millet that can accurately capture the population structure (Johnson et al. 2019).

This species chromosomal assembly and sequence were recently reported (Shi et al. 2019; Zou et al. 2019), bringing it into the current genomics and revealing its immense potential for gene mining (Shan et al. 2020) and even large-scale genotyping applications like genome-wide association studies (GWAS). Indeed, GWAS research is based on the NGS data and offer the potential to speed up neglected and underutilized crop breeding by integrating previously untapped collections of allelic variation into mainstream research (Yabe and Iwata 2020). The large-scale GWAS studies have been used to study different millets in the past, advancing our knowledge of genetic variation and its relationships to phenotypes. Boukail et al. (2021) examined a global collection of 88 proso millet varieties and landraces for proso millet's seed properties and to find connections between markers and phenotypes. Restrictions-site-related DNA fragments were sequenced and the results included 494 million reads and 2412 high-quality SNPs. SNPs were employed in a GWAS and to analyze the collection's diversity. Boukail et al. (2021) identified 13 marker-trait associations for important agronomic and seed attributes indicating the presence of alleles with potential for use in proso millet breeding programs.

Whole Genome Sequencing in Proso Millet

The accessibility to whole genome sequence is considered a milestone in genome-wide molecular studies as it helps in understanding the genome's complex molecular structure of any organism and acts as a powerful resource for the genetic improvement of any crop species (Antony et al. 2018). Among the millets, whole genome sequences of pearl millet (Varshney et al. 2017), finger millet (Hittalmani et al. 2017), and foxtail millet (Zhang et al. 2012) are already available, and proso millet's whole genome sequence is made available recently (Zou et al. 2019). A high-quality, chromosome-scale genome assembly was developed and reported by the authors and identified 55,930 protein-coding genes and 339 microRNA genes (Zou et al. 2019). The whole genome sequence would serve as an invaluable resource for genomic selection (GS) and molecular breeding works in proso millet (Santra et al. 2019).

19.4.2.2 Transcriptomics

"Transcriptome" is the full range of messenger RNA (mRNA) molecules expressed by an organism. Term transcriptome can also be used to refer to the array of mRNA transcripts produced in a cell or tissue type in particular time. Transcriptomics refers to the study of transcriptomes which involves a comprehensive analysis and

quantification of the genome-wide changes of transcripts under study (Lata 2015). There are limited reports on proso millet transcriptomic research, whereas appreciable reports are available in other millets like pearl millet (Kulkarni et al. 2016; Varshney et al. 2017; Dudhate et al. 2018; Shinde et al. 2018; Ndiaye et al. 2022; Satyavathi et al. 2022), finger millet (Hittalmani et al. 2017; Parvathi et al. 2019; Pathak et al. 2022), foxtail millet (Shi et al. 2018; Xu et al. 2019; Guo et al. 2022; Yi et al. 2022), and barnyard millet (Jayakodi et al. 2019). Initially, in the year 2017, Hou et al. (2017) reported the transcriptome of proso millet to develop SSR markers.

Transcription factors (TFs) play a significant role in regulating a number of crucial growth and development processes during growth and development of plants, including signal transduction, cellular morphogenesis, and response to environmental stressors (Riaño-Pachón et al. 2007; Zhang et al. 2011). One of the biggest families of plant-specific TFs is the NAC family (Yao et al. 2012) and three proteins that give rise to the word NAC: No apical meristem (NAM), Arabidopsis transcription activation factor (ATAF)1/2, and cup-shaped cotyledon are the (CUC2). Numerous studies have demonstrated that certain NAC genes are essential for controlling plants resistance to drought. In proso millet, drought tolerant mechanisms are poorly understood. Shan et al. (2020) studied first time NAC transcription factors in broomcorn millet genome and 180 NAC (PmNAC) genes were discovered and equivalently named based on their chromosomal distribution. In this180 NAC genes in proso millet, Shan et al. (2020) investigated the evolutionary relationships, gene architectures, protein motifs, chromosomal distribution, duplication, expression patterns in various tissues, and responses to drought stress. These findings will be helpful for further research into the PmNAC genes' functional properties, particularly in relation to drought resistance.

Recently, a transcriptome study on proso millet was conducted by Liu et al. (2022) using two proso millet cultivars with contrasting nitrogen use efficiency (NUE). Low–N–tolerant cultivar (T184) and the low–N–sensitive cultivar (S111) were used to explore physiological and comparative transcriptome mechanisms for earning high NUE in the field as well as assign hydroponic experiments. Through this study, Liu et al. (2022) found that T184 had less differentially expressed genes (DEGs) than S11. Gene Ontology (GO) term analysis of leaf transcriptomes of two cultivars showed that "transporter activity", "transmembrane transporter activity", and "transmembrane transport" were the main GO terms in the upregulated DEGs of HN–T184 vs. LN–T184. A large number of downregulated DEGs of HN–S111 vs. LN–S111 are related to photosynthesis, such as the clusters of light reactions in photosystem I and photosystem II.

19.4.2.3 Proteomics

"Proteome" refers to a complete set of proteins expressed by an organism or specific organ or tissue or specific cell at a given time, while "proteomics" refers to the extensive study of proteomes to understand the underlying regulatory mechanism behind the expressed proteins. Proteomics plays a key role in understanding biological functions as proteins act directly on biochemical processes (Gygi et al. 1999). Two-dimensional electrophoresis (2-DE) and mass spectrometry (MS)-based

techniques such as MALDI-TOF are the most commonly used tools in structural proteomics. It is considered that most of the proteome is proportional to the transcriptome but levels and diversity of proteome are not in direct proportion to the transcriptome always which is due to post-translational modification (Khound and Santra 2020). Extensive proteomic studies are now available in the majority of crop plants but very little in proso and other millets.

In 2017, Roy et al. reported an initial proteomics report on seed protein analysis in proso millet using four Korean proso cultivars with the objective to map seed proteins and study their functional characteristics. 2-DE and mass fingerprinting were conducted for seed protein analysis. The results revealed that out of 1152 differentially expressed proteins on the 2-D gel, 26 were identified using MALDI-TOF/MS. Among these 26 identified proteins, two proteins were upregulated in all the cultivars; in two cultivars, 13 proteins were upregulated, and 11 proteins were downregulated. It is thus understood that differential expression of seed proteins would be cultivar specific and most of the identified upregulated proteins are related to transcription, metabolism of starch, and pathogenesis, whereas the downregulated proteins were found to be involved in glycolysis, stress response mechanisms, and transduction.

In a recent study conducted by Yuan et al. (2022), two proso millet cultivars (salt sensitive and salt tolerant) were used to assess their comparative phenotypes, physiological characteristics, microstructure, multi-omic profiles under salt stress, and subsequent re-watering to plants. The proteome investigations revealed that under salt stress and subsequent re-watering, up to 38.28% and 44.68% of proteins were individually expressed specifically at the protein level in salt-sensitive cultivars, respectively. In salt-tolerant cultivars, 43.56% of proteins expressed at the protein level under salt stress and 39.31% of proteins were expressed at the subsequent re-watering. These results indicated the involvement of post-transcriptional regulation under salt stress and subsequent re-watering.

19.4.2.4 Metabolomics

"Metabolome" refers to the available complete set of small molecules called metabolites in a biological sample, and "metabolomics" is the comprehensive analysis of metabolites in a biological organism, organ, tissue, or cell at a specific stage (Diola et al. 2014). A metabolome profile depicts the physiological state of a cell, tissue, or whole organism, which indirectly provides functional aspects of metabolites. Metabolomics becomes essential to better understand the functional aspects of the plant genome when the transcriptome or proteome alone cannot provide a biochemical basis for biological functions such as tolerance to stresses (Sumner et al. 2003).

Phytochemicals, particularly phenolic compounds are abundant in proso millet grains which act as reducing agents, free radical scavengers, hydrogen donors, and singlet oxygen quenchers, exhibiting high antioxidant properties (Shahidi and Chandrasekara 2013). The main classes of phenolic compounds identified in millets were phenolic acids and flavonoids. Although the composition and contents of phenolic acids and flavonoids vary from millet to millet (Chandrasekara et al.

2012), limited information is available on the quantification and characterization of these compounds in proso millet.

Initial work on metabolomics studies in proso millet was performed using gas chromatography-time-of-flight mass spectrometry (GCTOFMS) for assessing grain quality by Kim et al. (2013). Three Korean proso millet varieties were used in their study to investigate primary metabolites and phenolic compounds in matured seeds. Forty-eight metabolites were identified; out of them, 43 were primary metabolites, and five were phenolic compounds. By using the principal component analysis and partial least-squares discriminate analysis (PLS-DA), the seed metabolomes could be separated for the three varieties under this study by Kim et al. (2013).

Recently, ultra-high performance liquid chromatography coupled to triple quadrupole mass spectrometry (UHPLC-QqQ-MS) techniques are being used widely for metabolomic studies, which is a powerful approach that combines the advantages of non-targeted and targeted metabolomics. Due to advantages like high-throughput nature, high sensitivity, fast separation, and wide coverage, this method has been widely applied in a variety of crop and vegetable species for plant metabolite analysis (Cho et al. 2016; Zhu et al. 2018). Therefore, it can be effective in conducting qualitative and quantitative analyses to determine the nutrient composition in proso millet grains. Li et al. (2021a, b) conducted the study using a UHPLC-QqQ-MS/MS-based widely targeted metabolomics approach in proso millet varieties of different bran colors to evaluate the difference in metabolites quantitatively and qualitatively. A total of 672 metabolites in whole grains were identified, of which 121, 116, and 148 differential metabolites in comparison between colored proso millet grains (Black/Grey/Red) and white grains (White), respectively. A comparative study was also conducted to identify the functional components associated with antioxidant activities which substantially contribute to the knowledge of metabolite composition and functional compounds involved in antioxidant activities in whole grains of proso millet. This would help in future pros millet breeding programs to screen varieties with functional properties through comparative evaluation (Li et al. 2021a, b).

19.4.2.5 High-Throughput Phenotyping

High-throughput plant phenotyping (HTP) includes a comprehensive assessment of complex plant traits based on physiological parameters using mechanization, sensors, cameras, and robotics for genetic gains. Various available HTP platforms can determine plant traits non-destructively based on imaging technology at different stages of the plant cycle. It is also used to identify the QTLs, and aid in marker-assisted selection, association mapping, forward and reverse genetics, annotation of genes, and determination of gene function (Barbadikar et al. 2019). The inability and inefficient implementation of high-throughput field phenotyping is increasingly perceived as a key component that limits genetic gain in crop breeding programs (Araus et al. 2018).

Zhao et al. (2022) aimed to develop a method for automatic proso millet panicle detection and estimating heading percentage using regular red-blue-green images collected by an unmanned aerial vehicle (UAV). The authors can achieve a

coefficient of determination of 0.728 and an accuracy of 92.4% was achieved in determining whether a plot has reached 50% heading or not. The methods developed in this study can head toward developing HTP pipelines in proso millet breeding.

19.5 Future Prospects

Over the past few years, there has been an increasing preference for millets. Proso millet is a lesser-studied climate-smart crop that requires strong research intervention with the available resources. The availability of molecular markers in proso millet is increasing. With the discovery of SNPs by GBS, the identification of differentially expressed genes, and thousands of SSR and SNP loci by transcriptome analysis, a considerable number of molecular markers are now available for genomic research in proso millet. Research in proso millet oriented towards suitability for different agro-ecosystems, non-shattering types, lodging tolerant, suitability for mechanical harvesting, grain yield and quality, and protein quality is to be intensified in the climate change scenario. The use of double haploid technology and modern breeding tools like genome editing, and genomic selection will encourage faster genetic gains and encourage future research in proso millet.

References

Abdurakhmonov IY (2016) Genomics era for plants and crop species—advances made and needed tasks ahead. Plant Genomics. https://doi.org/10.5772/62083

Antony Ceasar, S., Maharajan, T., Ajeesh Krishna, T.P., Ramakrishnan, M., Victor Roch, G., Satish, L. and Ignacimuthu, S., 2018. Finger millet [Eleusine coracana (L.) Gaertn.] improvement: current status and future interventions of whole genome sequence. Frontiers in plant science, 9, p.1054. https://doi.org/10.3389/fpls.2018.01054

Araus JL, Kefauver SC, Zaman-Allah M, Olsen MS, Cairns JE (2018) Translating high-throughput phenotyping into genetic gain. Trends Plant Sci 23(5):451–466. https://doi.org/10.3389/fpls.2018.01054

Baltensperger DD (2002) Progress with proso, pearl and other millets. Trends in new crops and new uses, pp.100–103.

Baltensperger D, Lyon DJ, Anderson R, Holman T, Stymieste C, Shanahan J, Nelson LA, DeBoer KL, Hein GL, Krall J (1995) EC95-137 producing and marketing proso millet in the high plains. Historical materials from University of Nebraska-Lincoln Extension, p.709.

Barbadikar KM, Balakrishnan D, Gireesh C, Kardile H, Bosamia T, Mishra A (2019) High-throughput phenotyping: potential tool for genomics. OMICS Based Approach Plant Biotechnol:303–321

Bekkering CS, Tian L (2019) Thinking outside of the cereal box: breeding underutilized (pseudo) cereals for improved human nutrition. Front Genet 10:1289. https://doi.org/10.3389/fgene.2019.01289

Bhat BV, Tonapi VA, Rao BD, Singode, A, Santra D, Johnson J (2018) Production and utilization of millets in India. In: International millet symposium on 3rd international symposium on broomcorn millet (3rd ISBM), pp. 24–36.

Boukail S, Macharia M, Miculan M, Masoni A, Calamai A, Palchetti E, Dell'Acqua M (2021) Genome wide association study of agronomic and seed traits in a world collection of proso millet (*Panicum miliaceum* L.). BMC Plant Biol 21(1):1–12. https://doi.org/10.1186/s12870-021-03111-5

Byrne PF, Volk GM, Gardner C, Gore MA, Simon PW, Smith S (2018) Sustaining the future of plant breeding: the critical role of the USDA-ARS National Plant Germplasm System. Crop Sci 58(2):451–468. https://doi.org/10.2135/cropsci2017.05.0303

Cakmak I, Kutman UÁ (2018) Agronomic biofortification of cereals with zinc: a review. Eur J Soil Sci 69(1):172–180. https://doi.org/10.1111/ejss.12437

Chandrasekara A, Naczk M, Shahidi F (2012) Effect of processing on the antioxidant activity of millet grains. Food Chem 133(1):1–9. https://doi.org/10.1016/j.foodchem.2011.09.043

Cho YI, Chung JW, Lee GA, Ma KH, Dixit A, Gwag JG, Park YJ (2010) Development and characterization of twenty-five new polymorphic microsatellite markers in proso millet (*Panicum miliaceum* L.). Genes Genom 32(3):267–273. https://doi.org/10.1007/s13258-010-0007-8

Cho K, Cho KS, Sohn HB, Ha IJ, Hong SY, Lee H, Kim YM, Nam MH (2016) Network analysis of the metabolome and transcriptome reveals novel regulation of potato pigmentation. J Exp Bot 67(5):1519–1533. https://doi.org/10.1093/jxb/erv549

Devisetti R, Yadahally SN, Bhattacharya S (2014) Nutrients and antinutrients in foxtail and proso millet milled fractions: evaluation of their flour functionality. LWT-Food Sci Technol 59(2): 889–895. https://doi.org/10.1016/j.lwt.2014.07.003

Diao X (2017) Production and genetic improvement of minor cereals in China. Crop J 5(2): 103–114. https://doi.org/10.1016/j.cj.2016.06.004

Diao X, Jia G (2017) Origin and domestication of foxtail millet. In: Doust A, Diao X (eds) Genetics and genomics of Setaria. Plant genetics and genomics: crops and models, vol 19. Springer, Cham, pp 61–72. https://doi.org/10.1007/978-3-319-45105-3_4

Dikshit N, Sivaraj N (2013) Diversity for protein and morpho-agronomical characteristics in proso millet germplasm collections of Ratnagiri District, Maharashtra, India. Vegetos 26(2):164–170. https://doi.org/10.5958/j.2229-4473.26.2.070

Diola V, MenezesDaloso de D, Antunes WC (2014) Metabolomics. In: Omics plant breeding, pp 81–101. https://doi.org/10.1002/9781118820971.ch5

Dudhate A, Shinde H, Tsugama D, Liu S, Takano T (2018) Transcriptomic analysis reveals the differentially expressed genes and pathways involved in drought tolerance in pearl millet [*pennisetumglaucum* (l.) r. Br]. PLoS One 13:1–14. https://doi.org/10.1371/journal.pone.0195908

Gomashe SS (2017) Proso millet, Panicum miliaceum (L.): genetic improvement and research needs. In: Millets and sorghum: biology and genetic improvement, pp 150–169. https://doi.org/10.1002/9781119130765.ch5

Guo Y, Hao D, Wang X, Wang H, Wu Z, Yang P, Zhang B (2022) Comparative transcriptomics reveals key genes contributing to the differences in drought tolerance among three cultivars of Foxtail millet (*Setariaitalica*). Plant Growth Regul 99(1):45–64. https://doi.org/10.21203/rs.3.rs-1687090/v1

Gupta A, Sood S, Agrawal PK, Bhatt JC (2011) Floral biology and pollination system in small millets. Eur J Plant Sci Biotechnol 6:81–86

Gygi SP, Rochon Y, Franza BR, Aebersold R (1999) Correlation between protein and mRNA abundance in yeast. Mol Cell Biol 19(3):1720–1730. https://doi.org/10.1128/MCB.19.3.1720

Habiyaremye C, Matanguihan JB, D'AlpoimGuedes J, Ganjyal GM, Whiteman MR, Kidwell KK, Murphy KM (2017) Proso millet (Panicum miliaceum L.) and its potential for cultivation in the Pacific northwest, US: a review, vol 7. Front Plant Sci, p 1961. https://doi.org/10.3389/fpls.2016.01961

Hittalmani S, Mahesh HB, Shirke MD, Biradar H, Uday G, Aruna YR, Lohithaswa HC, Mohanrao A (2017) Genome and transcriptome sequence of finger millet (*Eleusinecoracana* (L.) Gaertn.) provides insights into drought tolerance and nutraceutical properties. BMC Genomics 18(1): 1–16. https://doi.org/10.1186/s12864-017-3850-z

Hou S, Sun Z, Li Y, Wang Y, Ling H, Xing G, Han Y, Li H (2017) Transcriptomic analysis, genic SSR development, and genetic diversity of proso millet (*Panicum miliaceum*; Poaceae). Appl Plant Sci 5(7):1600137. https://doi.org/10.3732/apps.1600137

Hu X, Wang J, Lu P, Zhang H (2009) Assessment of genetic diversity in broomcorn millet (*Panicum miliaceum* L.) using SSR markers. J Genet Genomics 36(8):491–500. https://doi.org/10.1016/S1673-8527(08)60139-3

Hunt HV, Campana MG, Lawes MC, Park YJ, Bower MA, Howe CJ, Jones MK (2011) Genetic diversity and phylogeography of broomcorn millet (*Panicum miliaceum* L.) across Eurasia. Mol Ecol 20(22):4756–4771. https://doi.org/10.1111/j.1365-294X.2011.05318.x

Hunt HV, Badakshi F, Romanova O, Howe CJ, Jones MK, Heslop-Harrison JP (2014) Reticulate evolution in Panicum (Poaceae): the origin of tetraploid broomcorn millet, *P. miliaceum*. J Exp Bot 65(12):3165–3175

Jaiswal V, Gupta S, Gahlaut V, Muthamilarasan M, Bandyopadhyay T, Ramchiary N, Prasad M (2019) Genome-wide association study of major agronomic traits in foxtail millet (*Setariaitalica* L.) using ddRAD sequencing. Sci Rep 9(1):1–11. https://doi.org/10.1038/s41598-019-41602-6

Jayakodi M, Madheswaran M, Adhimoolam K, Perumal S, Manickam D, Kandasamy T, Yang TJ, Natesan S (2019) Transcriptomes of Indian barnyard millet and barnyardgrass reveal putative genes involved in drought adaptation and micronutrient accumulation. Acta Physiol Plant 41(5): 1–11. https://doi.org/10.1007/s11738-019-2885-4

Jia J, Li H, Zhang X, Li Z, Qiu L (2017) Genomics-based plant germplasm research (GPGR). Crop J 5(2):166–174. https://doi.org/10.1016/j.cj.2016.10.006

Jiang Y, Li H, Zhang J, Xiang J, Cheng R, Liu G (2018) Whole genomic EST-SSR development based on high-throughput transcript sequencing in proso millet (*Panicummiliaceum*). Int J AgricBiol 20:617–620. https://doi.org/10.17957/IJAB/15.0531

Johnson M, Deshpande S, Vetriventhan M, Upadhyaya HD, Wallace JG (2019) Genome-wide population structure analyses of three minor millets: Kodo millet, little millet, and Proso millet. Plant Genome 12(3):1–9. https://doi.org/10.3835/plantgenome2019.03.0021

Kalinova J, Moudry J (2006) Content and quality of protein in proso millet (*Panicummiliaceum* L.) varieties. Plant Foods Hum Nutr 61(1):43–47. https://doi.org/10.1007/s11130-006-0013-9

Kate SM, Desai SS, Bhave SG, Thorat BS, Bal CP (2018) Mutagene induced variability in proso millet (*Panicummiliaceum* L.). IJCS 6(5):13–16

Khound R, Santra DK (2020) Omics for proso millet genetic improvement. Nucleus 63(3): 241–247. https://doi.org/10.1007/s13237-020-00339-8

Kim JK, Park SY, Yeo Y, Cho HS, Kim YB, Bae H, Park CH, Lee JH, Park SU (2013) Metabolic profiling of millet (*Panicummiliaceum*) using gas chromatography-time-of-flight mass spectrometry (GC-TOFMS) for quality assessment. Plant Omics 6(1):73–78. https://doi.org/10.3316/informit.226858981083461

Kulkarni KS, Zala HN, Bosamia TC, Shukla YM, Kumar S, Fougat RS, Patel MS, Narayanan S, Joshi CG (2016) De novo transcriptome sequencing to dissect candidate genes associated with pearl millet-downy mildew (*Sclerosporagraminicola*Sacc.) interaction. Frontiers. Plant Sci 7: 847. https://doi.org/10.3389/fpls.2016.00847

Lata C (2015) Advances in omics for enhancing abiotic stress tolerance in millets. Proc Indian Natl Sci Acad 81:397–417

Li J, Wang Y, Wang L, Zhu J, Deng J, Tang R, Chen G (2021a) Integration of transcriptomic and proteomic analyses for finger millet [*Eleusinecoracana* (L.)Gaertn.] in response to drought stress. PLoS One 16(2):e0247181. https://doi.org/10.1371/journal.pone.0247181

Li W, Wen L, Chen Z, Zhang Z, Pang X, Deng Z, Liu T, Guo Y (2021b) Study on metabolic variation in whole grains of four proso millet varieties reveals metabolites important for antioxidant properties and quality traits. Food Chem 357:129791. https://doi.org/10.1016/j.foodchem.2021.129791

Liu M, Xu Y, He J, Zhang S, Wang Y, Lu P (2016) Genetic diversity and population structure of broomcorn millet (*Panicummiliaceum* L.) cultivars and landraces in China based on microsatellite markers. Int J Mol Sci 17(3):370. https://doi.org/10.3390/ijms17030370

Liu C, Yuan Y, Liu J, Wang H, Ma Q, Zhou Y, Liu C, Gong X, Feng B (2022) Comparative transcriptome and physiological analysis unravel proso millet (*Panicummiliaceum* L.) source leaf adaptation to nitrogen deficiency with high nitrogen use efficiency. Environ Exp Bot 199: 104891. https://doi.org/10.1016/j.envexpbot.2022.104891

Matz SA (1986) Millet, wild rice, adlay, and rice grass. In: Cereal science. Avi Press, Westport, CT, pp 225–229

Miller NF, Spengler RN, Frachetti M (2016) Millet cultivation across Eurasia: origins, spread, and the influence of seasonal climate. Holocene 26(10):1566–1575. https://doi.org/10.1177/0959683616641742

Nadeem MA, Nawaz MA, Shahid MQ, Doğan Y, Comertpay G, Yıldız M, Hatipoğlu R, Ahmad F, Alsaleh A, Labhane N, Özkan H (2018) DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. Biotechnol Biotechnol Equip 32(2):261–285. https://doi.org/10.1080/13102818.2017.1400401

Nandini C, Bhat S (2019) Modified crossing (SMUASB) method for artificial hybridization in proso millet (*Panicummiliaceum* L.) and little millet (*Panicumsumatrense*). Electronic journal of. Plant Breed 10(3):1161–1170. https://doi.org/10.5958/0975-928X.2019.00147.9

Ndiaye A, Diallo AO, Fall NC, Diouf RD, Diouf D, Kane NA (2022) Transcriptomic analysis of methyl jasmonate treatment reveals gene networks involved in drought tolerance in pearl millet. Sci Rep 12(1):1–13. https://doi.org/10.1038/s41598-022-09152-6

Nelson LA (1984) Technique for crossing proso millet 1. Crop Sci 24(1):205–206. https://doi.org/10.2135/cropsci1984.0011183X002400010049x

Pathak RK, Singh DB, Pandey D, Gaur VS, Kumar A (2022) Finger millet transcriptome analysis using high throughput sequencing technologies. In: The finger millet genome. Springer, Cham, pp 123–134. https://doi.org/10.1007/978-3-031-00868-9_8

Parvathi, M.S., Nataraja, K.N., Reddy, Y.N., Naika, M.B. and Gowda, M.C., 2019. Transcriptome analysis of finger millet (Eleusine coracana (L.) Gaertn.) reveals unique drought responsive genes. Journal of Genetics, 98(2), p.46. https://doi.org/10.1007/s12041-019-1087-0

Popov G (1970) The importance of cross pollination in plant breeding. Lietzemdirb mohsltyrimo inst darbai 14:23–30

Rajasekaran R, Francis N (2021) Genetic and genomic resources for improving proso millet (*Panicummiliaceum* L.): a potential crop for food and nutritional security. Nucleus 64(1):21–32. https://doi.org/10.1007/s13237-020-00331-2

Rajput SG, Plyler-Harveson T, Santra DK (2014) Development and characterization of SSR markers in proso millet based on switchgrass genomics. Am J Plant Sci 5(1):175–186. https://doi.org/10.4236/ajps.2014.51023

Rajput SG, Santra DK, Schnable J (2016) Mapping QTLs for morpho-agronomic traits in prosomillet (*Panicummiliaceum* L.). Mol Breed 36(4):1–18. https://doi.org/10.1007/s11032-016-0460-4

Reddy VG, Upadhyaya HD, Gowda CLL (2007) Morphological characterization of world's proso millet germplasm collection. J SAT Agric Res 3:4

Riaño-Pachón DM, Ruzicic S, Dreyer I, Mueller-Roeber B (2007) PlnTFDB: an integrative plant transcription factor database. BMC Bioinform 8(1):1–10. https://doi.org/10.1186/1471-2105-8-42

Roy SK, Kwon SJ, Yu JH, Sarker K, Cho SW, Moon YJ, Jung TW, Park CH, Woo SH (2017) Comparison of protein profiles of Proso millet (*Panicummiliaceum*) seeds of various Korean cultivars. Korean J Crop Sci 62(1):40–50. https://doi.org/10.7740/kjcs.2016.62.1.040

Santra DK (2013) Proso millet varieties for western Nebraska western Nebraska. University of Nebraska-Lincoln NebGuide G, p 2219

Santra DK, Khound R, Das S (2019) Proso millet (*Panicummiliaceum* L.) breeding: progress, challenges and opportunities. In: Advances in plant breeding strategies: cereals, pp 223–257. https://doi.org/10.1007/978-3-030-23108-8_6

Satyavathi CT, Tomar RS, Ambawat S, Kheni J, Padhiyar SM, Desai H, Bhatt SB, Shitap MS, Meena RC, Singhal T, Sankar SM (2022) Stage specific comparative transcriptomic analysis to reveal gene networks regulating iron and zinc content in pearl millet [*Pennisetumglaucum* (L.) R. Br.]. Sci Rep 12(1):1–13. https://doi.org/10.1038/s41598-021-04388-0

Shahidi F, Chandrasekara A (2013) Millet grain phenolics and their role in disease risk reduction and health promotion: a review. J Funct Foods 5(2):570–581. https://doi.org/10.1016/j.jff.2013.02.004

Shan Z, Jiang Y, Li H, Guo J, Dong M, Zhang J, Liu G (2020) Genome-wide analysis of the NAC transcription factor family in broomcorn millet (*Panicummiliaceum* L.) and expression analysis under drought stress. BMC Genomics 21(1):1–13. https://doi.org/10.1186/s12864-020-6479-2

Shi W, Cheng J, Wen X, Wang J, Shi G, Yao J, Hou L, Sun Q, Xiang P, Yuan X, Dong S (2018) Transcriptomic studies reveal a key metabolic pathway contributing to a well-maintained photosynthetic system under drought stress in foxtail millet (*Setariaitalica* L.). PeerJ 6:e4752. https://doi.org/10.7717/peerj.4752

Shi J, Ma X, Zhang J, Zhou Y, Liu M, Huang L, Sun S, Zhang X, Gao X, Zhan W, Li P (2019) Chromosome conformation capture resolved near complete genome assembly of broomcorn millet. Nat Commun 10(1):1–9. https://doi.org/10.1038/s41467-018-07876-6

Shinde, H., Tanaka, K., Dudhate, A., Tsugama, D., Mine, Y., Kamiya, T., Gupta, S.K., Liu, S. and Takano, T., 2018. Comparative de novo transcriptomic profiling of the salinity stress responsiveness in contrasting pearl millet lines. Environmental and Experimental Botany, 155, pp.619-627. https://doi.org/10.1016/j.envexpbot.2018.07.008

Sumner LW, Mendes P, Dixon RA (2003) Plant metabolomics: large-scale phytochemistry in the functional genomics era. Phytochemistry 62(6):817–836. https://doi.org/10.1016/S0031-9422(02)00708-2

Upadhyaya HD, Sharma S, Gowda CLL, Reddy VG, Singh S (2011) Developing proso millet (*Panicummiliaceum* L.) core collection using geographic and morpho-agronomic data. Crop and pasture. Science 62(5):383–389. https://doi.org/10.1071/CP10294

Upadhyaya HD, Dronavalli N, Dwivedi SL, Kashiwagi J, Krishnamurthy L, Pande S, Sharma HC, Vadez V, Singh S, Varshney RK, Gowda CLL (2013) Mini core collection as a resource to identify new sources of variation. Crop Sci 53(6):2506–2517. https://doi.org/10.2135/cropsci2013.04.0259

Upadhyaya HD, Dwivedi SL, Singh SK, Singh S, Vetriventhan M, Sharma S (2014) Forming core collections in barnyard, kodo, and little millets using morphoagronomic descriptors. Crop Sci 54(6):2673–2682. https://doi.org/10.2135/cropsci2014.03.0221

Varshney RK, Shi C, Thudi M, Mariac C, Wallace J, Qi P, Zhang H, Zhao Y, Wang X, Rathore A, Srivastava RK (2017) Pearl millet genome sequence provides a resource to improve agronomic traits in arid environments. Nat Biotechnol 35(10):969–976. https://doi.org/10.1038/nbt.3943

Vetriventhan M, Upadhyaya HD (2018) Diversity and trait-specific sources for productivity and nutritional traits in the global proso millet (*Panicummiliaceum* L.) germplasm collection. Crop J 6(5):451–463. https://doi.org/10.1016/j.cj.2018.04.002

Vetriventhan M, Upadhyaya HD (2019) Variability for productivity and nutritional traits in germplasm of Kodo millet, an underutilized nutrient-rich climate smart crop. Crop Sci 59(3):1095–1106. https://doi.org/10.2135/cropsci2018.07.0450

Vetriventhan M, Azevedo VC, Upadhyaya HD, Nirmalakumari A, Kane-Potaka J, Anitha S, Ceasar SA, Muthamilarasan M, Bhat BV, Hariprasanna K, Bellundagi A (2020) Genetic and genomic resources, and breeding for accelerating improvement of small millets: current status and future interventions. Nucleus 63(3):217–239. https://doi.org/10.1007/s13237-020-00322-3

Wang R, Wang H, Liu X, Ji X, Chen L, Lu P, Liu M, Teng B, Qiao Z (2018) Waxy allelic diversity in common millet (*Panicummiliaceum* L.) in China. Crop J 6(4):377–385. https://doi.org/10.1016/j.cj.2018.02.004

Xia W, Luo T, Zhang W, Mason AS, Huang D, Huang X, Tang W, Dou Y, Zhang C, Xiao Y (2019) Development of high-density SNP markers and their application in evaluating genetic diversity and population structure in *Elaeisguineensis*. Front Plant Sci 10:130. https://doi.org/10.3389/fpls.2019.00130

Xu BQ, Gao XL, Gao JF, Jing LI, Pu Y, Feng BL (2019) Transcriptome profiling using RNA-seq to provide insights into foxtail millet seedling tolerance to short-term water deficit stress induced by PEG-6000. J Integr Agric 18(11):2457–2471

Yabe S, Iwata H (2020) Genomics-assisted breeding in minor and pseudo-cereals. Breed Sci 70(1):19–31. https://doi.org/10.1270/jsbbs.19100

Yao D, Wei Q, Xu W, Syrenne RD, Yuan JS, Su Z (2012, September) Comparative genomic analysis of NAC transcriptional factors to dissect the regulatory mechanisms for cell wall biosynthesis. BMC Bioinformatics 13(15):1–12. https://doi.org/10.1186/1471-2105-13-S15-S10

Yi F, Huo M, Li J, Yu J (2022) Time-series transcriptomics reveals a drought-responsive temporal network and crosstalk between drought stress and the circadian clock in foxtail millet. Plant J 110(4):1213–1228. https://doi.org/10.1111/tpj.15725

Yuan Y, Liu J, Ma Q, Gao Y, Yang Q, Gao X, Feng B (2022) Cleaner production of proso millet (*Panicummiliaceum* L.) in salt-stressed environment using re-watering: from leaf structural alleviations to multi-omics responses. J Clean Prod 334:130205. https://doi.org/10.1016/j.jclepro.2021.130205

Zhang H, Jin J, Tang L, Zhao Y, Gu X, Gao G, Luo J (2011) PlantTFDB 2.0: update and improvement of the comprehensive plant transcription factor database. Nucleic Acids Res 39 (Suppl_1):D1114–D1117. https://doi.org/10.1093/nar/gkq1141

Zhang G, Liu X, Quan Z, Cheng S, Xu X, Pan S, Xie M, Zeng P, Yue Z, Wang W, Tao Y (2012) Genome sequence of foxtail millet (*Setariaitalica*) provides insights into grass evolution and biofuel potential. Nat Biotechnol 30(6):549–554. https://doi.org/10.1038/nbt.2195

Zhao B, Khound R, Ghimire D, Zhou Y, Maharjan B, Santra DK, Shi Y (2022) Heading percentage estimation in proso millet (*Panicummiliaceum* L.) using aerial imagery and deep learning. Plant Phenome J 5(1):e20049. https://doi.org/10.1002/ppj2.20049

Zhu G, Wang S, Huang Z, Zhang S, Liao Q, Zhang C, Lin T, Qin M, Peng M, Yang C, Cao X (2018) Rewiring of the fruit metabolome in tomato breeding. Cell 172(1–2):249–261. https://doi.org/10.1016/j.cell.2017.12.019

Zou C, Li L, Miki D, Li D, Tang Q, Xiao L, Rajput S, Deng P, Peng L, Jia W, Huang R (2019) The genome of broomcorn millet. Nat Commun 10(1):1–11. https://doi.org/10.1038/s41467-019-08409-5

Breeding Proso Millet (*Panicum miliaceum* L.) for Abiotic Stress Resistance

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Abstract

Proso millet (*Panicum miliaceum* L.) is an annual cereal crop known for its historical significance. This millet is decent provenance of energy, providing edible share of carbohydrates, crude fibre and protein content along with enhanced amino acid composition. But proso millet is predominantly cultivated in marginal, arid, and semi-arid regions. It is one of the most imperative crops in today's climate-change scenario and has high weightage for sustaining production in dry-land agriculture. The mode to expand tolerance to abiotic stresses such as drought and salinity is crucial to expand yielding potentially. The prevalence of moisture stress or elevated temperature during the ear emergence stage noticeably lessens seed weight and seed number per panicle leading it as critical stage. The secondary stresses like oxidative and osmotic stress also cause early aging of plants. It is also a target for frost due to the detail that is a warm-season crop. Hence, concentrated efforts to appreciate and expand tolerance to abiotic burdens such as drought, heat, and salinity are crucial to improve yield. Identification of QTLs/genes, proteins conferring resistance to abiotic and biotic stress and manipulative and development of gene specific markers would further aid in

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understanding the molecular machinery of stress tolerance. Thus, this will make proso as a best climate smart crop.

Keywords

Proso millet · Drought · Salinity · Abiotic tolerance

20.1 Introduction

Proso millet or broomcorn millet was prevalent minor millet throughout prehistoric lifestyle. However, with the inclusion of grains like rice and wheat human diet, the practice of proso millet has been significantly condensed. Proso millet seems to be grown and consumed by an inconspicuous population. Notwithstanding its unpopularity, the crop is super prevalent for its nutritional qualities and features related to climatic adaptability. Protein generous (>12%) nature of proso millet infers its status in nutritional and health perspectives (Saleh et al. 2013). As a C_4 panicoid species, common millet promises to be a brilliant crop to breed in the event of universal climate change since it has superior nitrogen and water usage efficiency.

Proso millet crop enhancement agendas are primarily intensive on improving agro morphological traits involving yield, panicle type, early maturity, waxiness, etc. Improved varieties of proso millet have been created using traditional practices including backcrossing, pedigree, and pure-line selection. Since proso millet is predominantly cultured in marginal, arid, and semi-arid regions, it is exaggerated by biotic and abiotic aspects though they are habitually considered well-adapted to abiotic stresses (Dwivedi et al. 2012: Rajasekaran and Francis, 2021: Das et al. 2019).

Moisture stress, temperature stress, soil-related issues like salinity, alkalinity, acidity or elemental toxicity, lodging from wind, rain, snow, or hail are significant abiotic factors causing yield losses in farmers' fields (Lobell et al. 2009). Global food security is severely hampered by abiotic stresses, which not only diminish productivity but also lower the quality of harvested grains (Wang and Frei 2011). There are various morphological, genetical, and biochemical factors, which contribute to potential of millets for being abiotic stress tolerant. Therefore, in order to generate genotypes that are climate adaptable, it is vital to prioritize these qualities in addition to drought.

20.2 Abiotic Stress Prevailing in Proso Millet; Challenges and Mechanisms

20.2.1 Drought Tolerance Mechanisms and Accomplishments

Proso, the cereal with the shortest growth duration (60–90 days), which enables it survive drought (Goron and Raizada 2015; Hunt et al. 2014). Due to its rapid maturation, this minor millet either escapes aridness and excessive heat or is resilient

Table 20.1 Some of the molecular markers conveyed in proso millet (*Panicum miliaceum* L.) for abiotic stress

Molecular markers	Total number of molecular markers	Authors
Expressed sequence tags (ESTs)	211 ESTs, which are derivative from drought stress encouraged leaf tissues	Saha et al. (2016)
Differentially expressed genes (DEGs)	62,543 unigenes, functionally annotated from the de novo assembly and transcriptome description of the proso millet	Yue et al. (2016a)
	32 *PmWRKY* genes intricated in abiotic stress response	Yue et al. (2016b)
DEGs	For cell wall biosynthesis used by salt-tolerant proso millet under salt stress	Yuan et al. (2021)
Transcription factors	180 NAC (*PmNAC)* genes involved in drought response	Shan et al. (2020)
DEGs	1695 DEGs in response to salt stress	Zhang et al. (2019)
DEGs	42,240 unigenes, 2301 SSRs, 1,447,148 SNPs under moisture stress	Wang et al. (2017)
Expressed sequence tags (ESTs)	32 ESTs, homologous to recognized plant sequences articulated in response to abiotic or biotic stress factors	Lin et al. (2006b)

of these conditions, which makes it of curiosity to areas with small water accessibility and longer ages without shower (Henry et al. 2008). In order to better withstand drought conditions, proso millet halts growing vegetatively at temperatures above 30 °C, stops flowering, and retains its primary stem at a reduced height (Sateesh 2010; Changmei and Dorothy 2014). Accordingly, proso millet growing primarily falls within dryland farming which makes crucial to breed for drought tolerance (Upadhyaya et al. 2016).

Drought has a significant impact on agricultural production by reducing the available water for metabolic processes. The extent of this loss varies based on factors such as the crop's genotype, developmental stage during the drought, its duration, and severity (Ngara and Ndimba 2014). Under the influence of drought and other abiotic factors, plants can experience premature aging, often evidenced by leaf senescence. In comparison to *Sorghum bicolor*, *P. miliaceum* is more vulnerable to moisture stress. In fact, there was a considerable 77% decrease in yield, particularly among the middle and late-maturing variants (Emendack et al. 2011). The impact of drought during the ear emergence stage was noteworthy. This stage led to a significant decrease in grain production and weight across all five genotypes, though the panicle per plant remained unaffected (Seghatoleslami et al. 2008). Consequently, the ear emergence stage emerged as the most critical period during limited water availability for irrigation. In situations where water scarcity was induced both before and after heading stages, *P. miliaceum* encountered a 36% reduction in yield due to factors such as fewer grains per panicle, decreased panicle count, and an overall decline in dry weight (Matsuura et al., 2012).

Table 20.2 Recently released drought tolerant varieties of proso millet in India

Variety	Pedigree	Release centre	Year of release	Salient features
CO(PV)5 (TNAU143)	PV 1403 × GPUP 21	TNAU, Coimbatore	2007	Profuse tillering with high yielding ability and drought tolerance
TNPm 230	TNAU 164 × IPM-19	TNAU, Coimbatore	2017	Short duration, drought tolerant variety
CO-3	Pure-line selection from the local collection maintained at TNAU	TNAU, Coimbatore	1985	Grain with shining golden yellow colour, drought tolerant, and leaf pubescent
TNAU202	PV 1453 × GPUP 16	TNAU, Coimbatore	2011	Drought resistant

In a study conducted by Habiyaremye in 2017 (Habiyaremye et al. 2017a, b), significant crop yields were observed in situations without irrigation where water stress was present. However, the research did not find a significant correlation between the choice of crop variety and yield when comparing irrigated and non-irrigated land. This implies that a variety performing well under irrigation might not yield the same or higher results under non-irrigated conditions. Notably, the varieties 'GR658' and 'Minsum' exhibited substantial success in non-irrigated plots, while 'GR665' and 'Earlybird' thrived in irrigated plots. In a distinct study by Fanyun et al. (2008), a complete cDNA of the S-adenosylmethionine synthase (SAMS) gene was successfully amplified using polymerase chain reaction (PCR) from a complementary DNA (cDNA) library. The gene's expression pattern was evaluated using semi-quantitative reverse transcription polymerase chain reaction (RT-PCR). The results indicated that the expression of the *SAMS* gene lessened under drought conditions, amplified after rehydration and subsequently stabilized at normal levels 6 h after rehydration. The researchers put forth a hypothesis suggesting the crucial role of this gene in enhancing drought tolerance and water use efficiency based on these findings and there are some drought tolerant varieties (Table 20.2).

20.2.2 Salinity Stress

Salinity is one of the most damaging abiotic stresses on plant growth and development, including seed germination and reproductive development (Ismail et al. 2014; Rasheed et al. 2019). Only two studies have looked at the germination of broomcorn millet under salt stress, and investigations on how it responds to salt stress and re-watering have not been published (Caruso et al. 2018; Liu et al. 2015). Although a few publications on the potential of genus *Panicum* for salt tolerance are accessible, no data is available on the compatible solutes such as proline and antioxidant enzyme activities in *P. miliaceum*. Thus, the salt tolerance of broomcorn millet

remains unknown. Understanding the mechanisms of resistance to and adaptation to salt stress can reveal important knowledge for enhancing plant species' salt tolerance.

Sabir et al. (2011) observed that exposure to salt stress led to a significant drop in both relative water content (RWC) and yield of proso millet accessions that were investigated. Among the 18 accessions examined, three specific accessions (008211, 008214, and 008226) demonstrated a yield of over 50% of the seed weight compared to the control, marking them as salt-tolerant. In a separate study by Yue et al. (2016b), they identified 22 *PmWRKY* genes that displayed distinct responses to various abiotic stress treatments. Through transcriptome sequencing of two genotypes, namely Yumi No. 2 (which is sensitive to drought) and Yumi No. 3 (exhibiting tolerance to both drought and salt), the researchers pinpointed genes showing differential expression related to stress tolerance, with a notable focus on cold and salt stress. Among these genes, Unigene33484 exhibited variations in expression levels across different genotypes under diverse stress conditions, indicating its role in osmoregulation. Another gene, Unigene35973, showed a remarkable 100-fold increase in expression during cold and salt stress in the Yumi No. 3 genotype. These findings underscore the involvement of these unigenes in conferring tolerance to abiotic stresses, suggesting potential avenues for further investigation into unraveling the molecular mechanisms governing stress tolerance. These discoveries could be leveraged to develop functional markers for screening and identifying tolerance traits.

20.2.3 Secondary Stresses: Osmotic and Oxidative Stress

Secondary stresses which embrace osmotic and oxidative stress can emerge as a result of primary abiotic stresses like salinity or drought (Wang et al. 2003). A combination of physiological and biochemical defence mechanisms gets triggered in response to these secondary stresses. These responses can serve as indicators of drought resistance. Crops utilize antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) to shield themselves against drought-related challenges (Zhang et al. 2012a).

During the heading stage, proso millet accessions with heightened osmoregulatory capabilities demonstrated moderate drought resistance (Karyudi and Fletcher 2002). Under polyethylene glycol (PEG) treatment, the more vulnerable cultivar 'Yumi 1' exhibited increased electrolyte leakage, malondialdehyde content (MDA), proline, and soluble sugar content (Jia et al. 2008). Research has demonstrated that the activity of SOD and POD, along with chlorophyll content, can serve as reliable indicators of proso millet's capacity to endure drought during its seedling stage (Zhang et al. 2012b; Jia et al. 2008). Similarly, under challenging conditions, proso millet subjected to drought exhibited elevated levels of antioxidant enzymes and metabolites (Jeevanandhan et al. 2021). In the process of selecting drought-resistant genotypes, the prolonged functional leaf duration and improved SOD activity could be utilized as indicators of drought resilience (Dai et al. 2011; Zhang et al. 2012b).

20.2.4 Grain Shattering and Lodging

After hitting maturity, proso millet commences to ripen from the apex of the panicle progressing downward and it is typically non-uniform. Green immature seeds remain in the lowest part of the panicle due to this unsynchronized ripening (Matz 1986). There is a probability of massive losses of yield owing to seed breaking if harvesting is prolonged until these grains are completely dried (Gomashe 2017, Santra et al. 2019). Threshing can delay until the grain moisture seems to be under 13% (Baltensperger et al. 1995a, b).This is not a great concern in Indian circumstances for crops grown during the rainy season, but it might have a significant impact on overall yields for post-rain and summer crops.

Furthermore, because of its strong propensities for lodging and seed shatter, proso millet cannot be directly combined (Rajput et al. 2016). Once it reaches physiological maturity, proso millet is often wrapped to reduce yield loss from lodging and seed breaking of standing plants from strong wind, rain, and hail damage(Henry et al. 2008; Rajput et al. 2016). Nonetheless, this approach dramatically reduces yield and quality. Due to these challenges, proso millet cultivars that are ideal for direct combining and can withstand the worst effects of seed lodging and shattering on yield must be designed (Rajput et al. 2016). The major obstacles of mechanising the harvesting process in proso millet involve seed lodging, breaking, and high moisture content in the straw. The advance of direct harvesting skills would halt the yield loss that occurs when harvesting is done typically via windrows or swaths. Accordingly, cultivars with erect stature, ideal height, branching, and panicle morphology appropriate for combine harvest must be established (Gomashe 2017). Proso millet lines developed in the United States have had strong selection for lodging resistance (Baltensperger et al. 1995a, b, 2004).

The identification of cultivars possessing resistance to lodging and shattering, along with the underlying genes responsible for these traits, is of paramount importance. Rajput et al. (2016) successfully identified and located QTLs (quantitative trait loci) as well as the associated markers for seed shattering and lodging using the linkage map they developed. These molecular markers (Table 20.1) could prove invaluable in scientific endeavours aimed at comprehending the genetic basis of these valuable attributes and in the development of robust proso millet cultivars. Rajput et al. (2016) meticulously charted 18 quantitative QTLs corresponding to eight distinct traits, including lodging, heading date, plant height, peduncle length, panicle length, grain shattering, 100-grain weight, and grains per panicle. Particularly noteworthy are the flanking SNP markers, which hold the potential to be converted into PCR-based markers. These markers could find effective application in marker-assisted selection (MAS) once the QTLs have been thoroughly verified and validated. This is especially relevant for phenotypes displaying a considerable additive value (ranging from 22% to 35%), such as lodging and tolerance to grain shattering.

20.3 Abiotic Stress in Proso Millet; Breeding Opportunities

20.3.1 Wide Hybridization

The proso millet has excellent resilience to abiotic stress since it can endure more heat and salt than that of other cereal crops (Zou et al. 2019).The genetic base of the cultivated proso millet is quite limited. It is essential to incorporate novel genomic resources into the modified cultivars to maximize yield potential and resistance levels for numerous biotic and abiotic stimuli. The use of this species' wild progenitors and relatives can be crucial for introgression of novel genes. With the aid of potential ancestors of proso millet like *Panicum capillare* or *Panicum repens*, it is presumable to widen the genetic base and find unique, highly adapted genotype combinations (Hunt et al. 2014).

20.3.2 Genomics of Proso Millet

In regard to the area, production, and amount of investigation on its genetics, genomics, and breeding, proso millet is recognized as a minor/underutilized crop (Rajput et al. 2016). The proso millet species has experienced an upsurge in scientific interest since the genome was accessible. This will offer up a wide range of possibilities for launching innovative genomic resources for breeding proso millet that seems to be climate resilient. Notably, genome assembly of broomcorn millet serves as an important resource to breed for climate smart panicum cultivars (Shi et al. 2019).

20.3.3 miRNAs in Proso Millet

Proso millet microRNAs (miRNAs), crucial for post-transcriptional control, have been the subject of exploratory research. Wu et al. (2012) employed ESTs to predict miRNAs rather than genomic survey of sequence analysis as done in other crops, due to the scarcity of proso millet genome sequences. Forty-three putative miRNAs and the genes they target were discovered to be crucial in biological processes such as stress response, metabolism, and development. Their findings on the functioning of miRNAs may assist in comprehending the mechanisms that proso millet exploits to survive drought, specifically in dryland agricultural environments.

20.3.4 Whole Transcriptome Analysis for Gene Discovery During Abiotic Stress

Using the advanced RNA sequencing technology provided by Illumina, researchers led a comprehensive analysis of the proso millet transcriptome through de novo assembly and characterization (Yue et al. 2016a). The exploration specifically targeted two proso cultivars: Yumi 2, a waxy variety susceptible to drought, and Yumi 3, a non-waxy type capable of tolerating both salt and drought conditions. The

primary focus of the study was on categorizing genes that showed differential expression (DEGs) between these two cultivars when exposed to various stress factors like low temperatures, high heat, and elevated salt levels. To verify the verdicts, the researchers employed quantitative reverse transcription polymerase chain reaction (qRT-PCR) for validation purposes.

A specific gene, Unigene 34,608, was anticipated to encrypt the heat-shock factor-binding protein (HSBP1), that can interfere with the DNA-binding capability of HSF1, thus influencing cellular responses to heat stress. In 'Yumi 2', transcript echelons of Unigene 34,608 exhibited minimal changes under cold stress, a decrease of 0.26-fold in response heat stress, and a 0.16-fold increase in response to salt stress compared to control plants. In contrast, 'Yumi 3' demonstrated a transient elevation in the expression of Unigene 34,608 following heat and cold stress. Notably, this expression surged by a significant 400-fold during 6 hours of cold stress when compared to control plants.

Through further transcriptome analysis, Yue et al. (2016a) identified 32 *PmWRKY* genes associated while responding to abiotic stress aspects. These WRKY genes function as vital transcription factors in plants, overseeing several physiological processes encompassing plant growth, development, and stress responses. Beyond the documentation of differentially expressed genes, the study also unearthed 35,000 simple sequence repeat (SSR) loci and 406,000 single nucleotide polymorphism (SNP) loci that could serve as valuable molecular markers.

20.3.5 Conclusion and Future Perspectives

Proso millet stands out as an exceptional rotational crop owing to its numerous remarkable attributes. Its notably low water requirements set it apart, enabling it to effectively conserve moisture more competently than grains like wheat and longer-season crops such as maize, grain sorghum, or sunflower. The proso millet genome has opened up possibilities for genomic selections, facilitated by the discovery of single nucleotide polymorphisms (SNPs) through genotyping-by-sequencing (GBS). Additionally, differential gene expression, unigenes, as well as a substantial number of simple sequence repeat (SSR) and SNP hotspots identified via transcriptome analysis, have paved the way for this advancement. Molecular markers, constructed from coding and non-coding sequences of the proso millet genome, have been utilized to assess the genetic diversity within landraces, breeding lines, and cultivars. If SNPs around quantitative trait loci (QTLs) have been identified and confirmed, marker-assisted breeding can be integrated into proso millet breeding strategies. These molecular techniques can also be extended to explore the extensive evolutionary variation present in proso millet accessions held in gene banks globally, in addition to the landraces well-preserved by farmers. The genetic enhancement and breeding of proso millet stand to greatly benefit from the distinctive alleles harboured within these landraces. Although attempts are being made to uncover the genetic areas that impart protection to heat and salt stress, the

literature on millet crops is scant. Consequently, in order to formulate genotypes that are climate durable, it is crucial to prioritize these qualities in conjunction to drought.

References

Baltensperger D, Lyon D, Anderson R, Holman T, Stymiest C, Shanahan J, Nelson L, DeBoer K, Hein G, Krall J (1995a) Producing and marketing proso millet in the high plains. Coop. Ext. fact sheet EC95-137-C. Univ. of Nebraska, Lincoln.

Baltensperger DD, Nelson LA, Frickel GE (1995b) Registration of 'Earlybird' proso millet. Crop Sci 35(4):1204–1205

Baltensperger DD, Frickel GE, Nelson LA, Krall JM, Vigil M, Hain J, Johnson J, Stymiest C, Rickertsen JR (2004) Registration of Horizon' proso millet. Crop Sci 44(2):688–690

Caruso C, Maucieri C, Berruti A, Borin M, Barbera AC (2018) Responses of different *Panicum miliaceum* L. genotypes to saline and water stress in a marginal Mediterranean environment. Agronomy 8(1):8

Changmei S, Dorothy J (2014) Millet-the frugal grain. Int J Sci Res Rev 3(4):75–90

Dai HP, Zhang PP, Lu C, Jia GL, Song H, Ren XM, Chen J, Wei AZ, Feng BL, Zhang SQ (2011) Leaf senescence and reactive oxygen species metabolism of broomcorn millet ('*Panicum miliaceum*'L.) under drought condition. Aust J Crop Sci 5(12):1655–1660

Das S, Khound R, Santra M, Santra DK (2019) Beyond bird feed: proso millet for human health and environment. Agriculture 9(3):64

Dwivedi SL, Upadhyaya HD, Senthilvel S, Hash CT, Fukunaga K, Diao X, Santra D, Baltensperge D, Prasad M (2012) Millets: genetic and genomic resources, 247-375

Emendack Y, Herzog H, Gotz KP, Malinowski DP (2011) Mid-season water stress on yield and water use of millet ('*Panicum miliaceum*') and sorghum ('Sorghum bicolour'L. Moench). Aust J Crop Sci 5(11):1486–1492

Fanyun L, Shiqiang W, Yingang H (2008) Cloning of a S-adenosylmethionine synthetase gene from broomcorn millet (*Panicum miliaceum* L.) and its expression during drought and re-watering. Acta Agron Sin, 34(5): 777-782

Fletcher RJ (2002) Osmoregulative capacity in birdseed millet under conditions of water stress. I. Variation in *Setaria italica* and *Panicum miliaceum*. Euphytica 125(3):337–348

Gomashe SS (2017) Proso millet, *Panicum miliaceum* (L.): genetic improvement and research needs. In: Patil V (ed) Millets and sorghum: biology and genetic improvement. Wiley, New Jersey, pp 150–169

Goron TL, Raizada MN (2015) Genetic diversity and genomic resources available for the small millet crops to accelerate a new green revolution. Front Plant Sci 6:157

Habiyaremye C, Barth V, Highet K, Coffey T, Murphy KM (2017a) Phenotypic responses of twenty diverse proso millet (*Panicum miliaceum* L.) accessions to irrigation. Sustainability 9(3): 389

Habiyaremye C, Matanguihan JB, D'Alpoim Guedes J, Ganjyal GM, Whiteman MR, Kidwell KK, Murphy KM (2017b) Proso millet (*Panicum miliaceum* L.) and its potential for cultivation in the Pacific northwest, US: a review. Front Plant Sci 7:1961

Henry WB, Nielsen DC, Vigil MF, Calderón FJ, West MS (2008) Proso millet yield and residue mass following direct harvest with a stripper-header. Agron J 100(3):580–584

Hunt HV, Badakshi F, Romanova O, Howe CJ, Jones MK, Heslop-Harrison JP (2014) Reticulate evolution in *Panicum* (Poaceae): the origin of tetraploid broomcorn millet, *P. miliaceum*. J Exp Bot 65(12):3165–3175

Ismail A, Takeda S, Nick P (2014) Life and death under salt stress: same players, different timing? J Exp Bot 65(12):2963–2979

Jeevanandhan K, Rajesh S, Sujatha KB, Dhivya K, Uma D (2021) Physio-biochemical analysis reveals drought stress tolerance mechanism in Proso millet (*Panicum miliaceum* L.). Pharma Innov J (11):1455–1461

Jia GL, Dai HP, Feng BL, Zhang SQ, Zhang SW (2008) Biochemical characteristics in broomcorn millet (*Panicum miliaceum* L.) seedlings under PEG simulated drought stress. Acta Botan Boreali-Occiden Sin 28(10):2–073

Lin F, Hu Y, Song G, Zhang H, Liu T, He B (2006b). Isolation andanalysis of genes induced by rehydration after serious drought in broomcornmillet (Panicum miliaceum L.) by SSH. Chin J Agric Biotechnol 3:237–242. https://doi.org/10.1079/CJB2006119

Liu M, Qiao Z, Zhang S, Wang Y, Lu P (2015) Response of broomcorn millet (*Panicum miliaceum* L.) genotypes from semiarid regions of China to salt stress. Crop J 3(1):57–66

Lobell DB, Cassman KG, Field CB (2009) Crop yield gaps: their importance, magnitudes, and causes. Annu Rev Environ Resour 34:179–204

Matsuura A, Tsuji W, An P, Inanaga S, Murata K (2012) Effect of pre-and post-heading water deficit on growth and grain yield of four millets. Plant Prod Sci 15(4):323–331

Matz SA (1986) Millet, wild rice, adlay, and rice grass. In: Cereal science. Avi Press, Westport, CT, pp 225–229

Ngara R, Ndimba B (2014) Understanding the complex nature of salinity and drought-stress response in cereals using proteomics technologies. Proteomics 14:611–621

Rajasekaran R, Francis N (2021) Genetic and genomic resources for improving proso millet (*Panicum miliaceum* L.): a potential crop for food and nutritional security. Nucleus 64(1):21–32

Rajput SG, Santra DK, Schnable J (2016) Mapping QTLs for morpho-agronomic traits in proso millet (*Panicum miliaceum* L.). Mol Breed 36(4):1–18

Rasheed A, Hameed A, Gul B, Khan MA (2019) Perianth and abiotic factors regulate seed germination of Haloxylon stocksii—a cash crop candidate for degraded saline lands. Land Degrad Dev 30(12):1468–1478

Sabir P, Ashraf M, Akram NA (2011) Accession variation for salt tolerance in proso millet (*Panicum miliaceum* L.) using leaf proline content and activities of some key antioxidant enzymes. J Agron Crop Sci 197(5):340–347

Saha D, Gowda MV, Arya L et al (2016) Genetic and genomic resources of small millets. CRC Crit Rev Plant Sci 35:56–79

Saleh AS, Zhang Q, Chen J, Shen Q (2013) Millet grains: nutritional quality, processing, and potential health benefits. Compr Rev Food Sci Food Saf 12(3):281–295

Santra DK, Khound R, Das S (2019) Proso millet (*Panicum miliaceum* L.) breeding: progress, challenges and opportunities. Adv Plant Breed Strategies: Cereals 5:223–257

Sateesh PV (2010) Millets: future of food and farming. Millet Network of India Deccan Development Society FIAN, Hyderabad, pp 2–9

Seghatoleslami MJ, Kafi M, Majidi E (2008) Effect of drought stress at different growth stages on yield and water use efficiency of five proso millet (*Panicum miliaceum* L.) genotypes. Pak J Bot 40(4):1427–1432

Shan Z, Jiang Y, Li H, Guo J, Dong M, Zhang J, Liu G (2020) Genome-wide analysis of the NAC transcription factor family in broomcorn millet (Panicum miliaceum L.) and expression analysis under drought stress. BMC Genomics 21(1):1–13

Shi J, Ma X, Zhang J, Zhou Y, Liu M, Huang L, Sun S, Zhang X, Gao X, Zhan W, Li P (2019) Chromosome conformation capture resolved near complete genome assembly of broomcorn millet. Nat Commun 10(1):1–9

Upadhyaya HD, Vetriventhan M, Dwivedi SL, Pattanashetti SK, Singh SK (2016) Proso, barnyard, little, and kodo millets. In: Genetic and genomic resources for grain cereals improvement. Academic Press, pp 321–343

Wang Y, Frei M (2011) Stressed food–the impact of abiotic environmental stresses on crop quality. Agric Ecosyst Environ 141(3–4):271–286

Wang W, Vinocur B, Altman A (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218(1):1–14

Wang RY, Wang HG, Liu XY, Lian S, Chen L, Qiao ZJ, McInerney CE, Wang L (2017) Drought-induced transcription of resistant and sensitive common millet varieties. J Anim Plant Sci 27(4): 1303-1314

Wu Y, Du J, Wang X, Fang X, Shan W, Liang Z (2012) Computational prediction and experimental verification of miRNAs in *Panicum miliaceum* L. Sci China Life Sci 55(9):807–817

Yuan Y, Liu C, Li J, Ma Q, Yang Q, Feng B (2021) Unravelling the strategies for cell wall biosynthesis used by salt-tolerant Proso millet (*Panicum miliaceum* L.) under salt stress: from root structure to molecular mechanism.

Yue H, Wang M, Liu S, Du X, Song W, Nie X (2016a) Transcriptome-wide identification and expression profiles of the WRKY transcription factor family in broomcorn millet (*Panicum miliaceum* L.). BMC Genomics 17(1):1–11

Yue H, Wang L, Liu H, Yue W, Du X, Song W, Nie X (2016b) De novo assembly and characterization of the transcriptome of broomcorn millet (*Panicum miliaceum* L.) for gene discovery and marker development. Front Plant Sci 7:1083

Zhang P, Feng B, Wang P, Gao X, Gao J, Song H, Zhang X, Chai Y (2012a) Study on identification of drought-resistance indexes at seedling stage in broomcorn millet under PEG stress. J China Agric Univ 17(1):53–59

Zhang P, Feng B, Wang P, Song H, Gao X, Gao J, Chen J, Chai Y (2012b) Leaf senescence and activities of antioxidant enzymes in different broomcorn millet (*Panicum miliaceum* L.) cultivars under simulated drought condition. J Food Agric Environ 10(2 Part 1):438–444

Zhang Y, Gao X, Li J, Gong X, Yang P, Gao J, Wang P, Feng B (2019) Comparative analysis of proso millet (*Panicum miliaceum* L.) leaf transcriptomes for insight into drought tolerance mechanisms. BMC Plant Biol 19(1):1–17

Zou C, Li L, Miki D, Li D, Tang Q, Xiao L, Rajput S, Deng P, Peng L, Jia W, Huang R (2019) The genome of broomcorn millet. Nat Commun 10(1):1–11

Breeding Proso Millet for Biotic Stress Resistance

21

Rukoo Chawla, Uttej Karla, Sonal Chavan, Hemlata Sharma, Minakshi Jattan, and D. S. Phogat

Abstract

Proso millet (*Panicum miliaceum*), one of the nutria-cereals which is renowned for its hardiness, ability to withstand drought, and short growing season. It is a water efficient crop and is enriched with nutrients. Due to its relative aversion to pests and diseases, it has an immense potential to be an exemplary crop for global climate change. Despite this, there are few prevalent diseases of which head smut, bacterial stripe disease, kernel smut, and leaf spot are important, causing substantial reduction in grain yield. Among the pests, shoot fly is the most serious. Proso millet is similarly subjected to annual and perennial weed competition. Crop rotation and adjusting sowing times are often used techniques in millet to prevent disease and insect pressure. Different traditional and advanced breeding techniques are used by plant breeders to develop disease and pest tolerant varieties. However, in spite of the impeccable benefits, development of agricultural improvement in this millet remains under explored due to extreme scarcity of research fundings leading to lack of sufficient genomic resources. Although, there are few reports on molecular markers for identifying pathotypes by screening genotypes that were discovered to be possible differential hosts. Recent advancements in identification of novel molecular markers and development of

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first linkage map has paved the way for accelerating proso millet breeding for biotic stress resistance with further availability of next-generation sequencing, together with high-throughput phenotyping.

Keywords

Proso millet · Biotic stress · Resistance · Pests · Diseases · Weeds

21.1 Introduction

The FAO estimated that 86.3 million metric tons of millet was produced globally, from an area of 71.8 million hectares during 2019 (FAO stat 2021). With an annual yield of 15.53 million tons cultivated on approximately 12.45 million acres, India stands as a leading producer of millets, accounting for nearly 40% of global millet output (Report Millets 2022). Millets can be cultivated in condition of minimal agricultural inputs. This offers a chance for profitable farming in regions where cereal yield output is poor (Amadou et al. 2013). Millets stand out from other cereals in being highly nutritious (Bhat et al. 2018). Due to their adaptability, millets are suitable for reducing the agricultural consequences of drought and climate change as well as addressing nutritional shortages (Kumar et al. 2018). As compared to popular cereals like wheat, rice, and maize, millets are major source of both protein and calories (Amadou et al. 2013; Saleh et al. 2013).

Proso millet is a warm-season, transient crop (Baltensperger 2002). It prospers best in sandy soils with an acidic pH of (5.5–7) (Changmei and Dorothy 2014; Lyon et al. 2008). However, it performs poor in waterlogged conditions (Seghatoleslami et al. 2008). Proso millet thrives in desert semi-arid regions with annual precipitation levels as low as 200–500 mm (Ceccarelli and Grando 1996). The hatch and slack pathway could be partially responsible for extremely low transpiration ratio in proso millet. The millet was introduced to North America and is now primarily grown for fodder. It was a staple grain across many regions of Europe and Asia until recently. Although proso millet ($2n = 36$) is regarded as a self-pollinated crop, there may be up to 10% natural cross-pollination.

Proso millet has wide nutritional qualities and is beneficial for health aspects. Proso millet has also been found to have a variety of phytochemicals, antioxidant activity, and anti-proliferative characteristics (Zhang et al. 2014). Kalinova and Moudry (2006) reported that particularly in comparison to wheat, proso millet protein (11% dry basis) has higher amount of important amino acids (leucine, methionine, and isoleucine). The cholesterol metabolism is influenced favorably by millet protein (Nishizawa and Fudamo 1995). Consuming proso millet is coupled with a lower prevalence of type-2 diabetes because of its high magnesium content.

Crop plants are severely compromised by biotic stress and controlling this pressure raises the production costs. Additionally, it could have detrimental consequences on ecology and the environment (Bainsla and Meena 2016). Potential breeding objectives include ways to develop cultivars more tolerant to both single

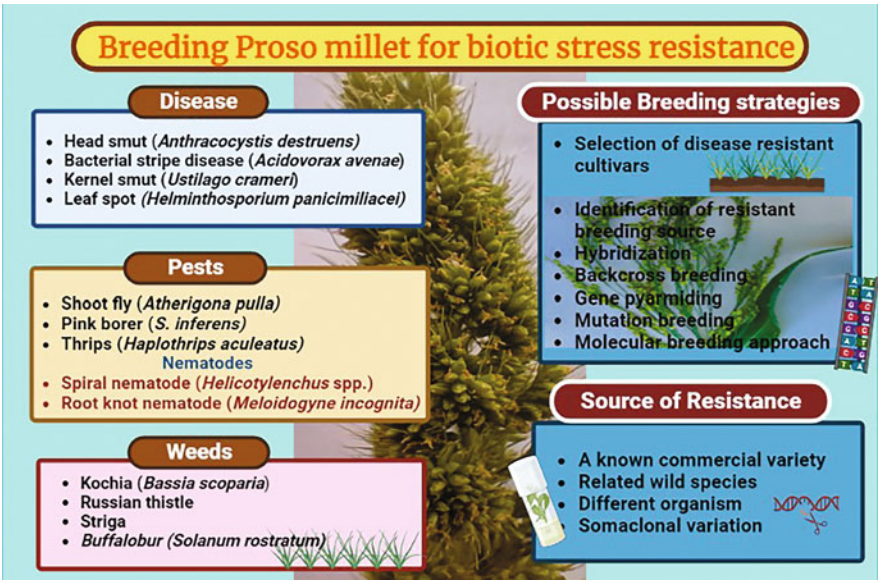

Fig. 21.1 Concise illustration of breeding prospective of proso millet for biotic stress resistance. (Created with BioRender.com)

and multiple stress and many techniques have been employed to achieve this (Calanca 2017). Although proso millet does not encounter a significant amount of biotic stress, this chapter discourses few prevalent diseases, insects, and weed invasions in the millet as well as breeding for resistant cultivars (Fig. 21.1). The biotic stress imposed on proso millet includes disease infestation such as head smut, bacterial stripe disease, kernel smut, and leaf spot (McDonald et al. 2003). The pathogens mostly *Helminothosporium* and *Fusarium* spp. are believed to be responsible for infecting early emerging seedlings in the soil. *Anthracocystis destruens* which causes head smut has also been documented in the crop. Furthermore, a few pest infestations were also identified in the crop. Proso millet suffers from a serious pest, i.e., shoot fly (*Atherigona pulla*). By itself, this pest may result in losses of up to 80% or perhaps even 100% (Sathish et al. 2017). *S. inferens*, a pink borer, also sporadically attacks common millet. Proso millet experiences several rather significant weed-related issues. Mostly in pacific northwest, interference from grassy weeds and broad-leaved weeds are potential issues for millet production (Herdrich 2001). Proso millet has low seed vigor and is particularly a weak weed competitor (Hinze 1977). This increases the scope of improving the seed vigor for its better persistence.

Competent exploitation of diverse germplasm, recognition, and characterization of core collections is required for successful crop improvement programs (Fig. 21.2) (Saha et al. 2016; Goron and Raizada 2015). This assists in emphasizing on employing direct selection for screening promising germplasm that can withstand different biotic stresses (Naylor et al. 2004). Among the different methods practiced

Fig. 21.2 Field view of proso millet cultivation. (Created with BioRender.com)

for breeding for disease and pest resistance (Table 21.1), the quickest way is to identify resistant breeding stocks that are somewhat less acceptable in terms of yield, still provide relevant disease resistance. Backcross provides an opportunity for crossing two plant varieties, one has resistant gene while the other, commercially desirable one is however prone. According to whether the gene for resistance is recessive or dominant to that for susceptibility, the backcross method would vary. This requires studying gene responsible for resistance. Introducing a pathogen artificially into the plant population will help in screening of resistant cultivars. Resistant varieties might also be introduced in a new location. This serves as quick and inexpensive way to control disease and pest. Pedigree method is mostly practiced in self-pollinating crops, individual F_2 plants are chosen using the pedigree approach based on their desirable characteristics, such as disease resistance.

Despite the socioeconomic significance of millet pests, knowledge on potential management techniques is meagre. This is partly because plant protection information is scarce owing the low crop value (Gahukar and Reddy 2019). This emphasizes the significance of developing proso millet varieties resistant to biotic stress and the same is discussed in this chapter.

21.2 Resistance to Disease Pressure

In the present context of climate change where small millets are considered to be the next-generation smart crops, disease susceptibility is of immediate concern to the scientific community. The changing climatic conditions such as increased

Table 21.1 List of resistant varieties developed through crop improvement in proso millet

Sr. no.	Resistant variety	Biotic stress	Particulars
1.	Co 4	Tolerant to shoot fly	Released in 1989 (India), TNAU, Tamil Nadu **Pureline selection**
2.	GPUP 8	Resistant to brown spot	Released in 2001 (India), (**S 7 × L 111**) Karnataka, 70–75 days
3.	GPUP 21	Moderately tolerant to shoot fly	Released in 2003 (India), (**GPUP 14 × K 1**) Karnataka and Tamil Nadu, 70–75 days
4.	CO (PV) 5	Resistant to brown spot as well as tolerant to rust and grain smut	Released in 2007 (India), (**PV 1403 × GPUP 21**), Tamil Nadu, 70–75 days
5.	PRC-1	Resistant to *Helminthosporium*	Released in 2008 (India), selection from **GPMS 519**, Uttarakhand, 90 days
6.	TNAU 145	Tolerant to rust and shoot fly	Released in 2009 (India), (**PV 1454 × TNAU 96),** Central, 88 days
7.	TNAU 151	Tolerant to rust and shoot fly	Released in 2009 (India), (**TNAU 96 × PV 1673**), Central, 72 days
8.	TNAU 164	Resistant to rust and grain smut disease incidence	Released in 2010 (India),(**TNAU 137 × CO 4**), Central, 72 days
9.	1. Slavjanskoe 2. Soyuz 3. Quartet 4. Sputnik	Resistant to head smut	Released in Russia
10.	Khar'kovskoe	Resistant to head smut	Released in Ukraine and Dagestan

temperature, relative humidity, soil moisture, sunlight intensity, and rainfall patterns contribute to the environmental conditions in disease triangle, this makes the environment suitable for infection leading to an increased chance of disease incidence in millets. This ultimately has a negative impact on the output of these crops, underscoring the need of developing proso millet for disease resistance.

Head smut is the utmost widespread disease in proso millet among other prevailing diseases, caused by the fungal pathogen *Anthracocystis destruens* (Schltdl.) Bref. 1912 (McTaggart et al. 2012), also commonly referred as *Sporisorium destruens* (Schltdl.) Vánky 1985 (Syn. *Sphacelotheca destruens*). The occurrence of this disease is about 5–10% and will reach about 40% in severe cases, affecting the production to extremities. The disease itself manifests lower yields due to destruction of the panicle, accounting for a total of 20–30% (Zhou et al. 2016; Dyussibayeva et al. 2020). The pathogen survives in soil and is transmitted as externally seed borne teliospores. High humidity and high temperature are conducive conditions for spread of this fungus (Wu et al. 2020), making it a major concern for climate change conditions. At the seedling stage, the hypha infects the host plant's coleoptile and most likely spreads throughout the entire host organism. The

pathogen produces teliospores only in inflorescence and symptoms become visible only at the time of panicle emergence with appearance of smut sori making the entire inflorescence turn into a sorus bounded by a greyish membrane and exposing dark-brown spores after rupture at maturity leading to contamination of straws and grains apart from yield loss (Fan et al. 2016). The other symptoms include cluster leaves, incomplete fruiting and hedgehog head. As the symptoms appear late, it becomes difficult to identify and control this disease (Wu et al. 2022).

Raising concerns of the cost of cultivation in control of this disease and negative impact of mandatory pre-sowing chemical seed treatment on environment necessitates the development of efficient and environment friendly new resistant varieties (Dyussibayeva et al. 2020). Proso millet has seen considerable progress attributable to conventional breeding techniques including selection from landraces, intra-specific hybridization followed by pedigree selection and mutation breeding (Numan et al. 2021). Availability of germplasm with resistance is the basic raw material for usage in resistant cultivar breeding programs, making it vital to collect germplasm, followed by subsequent preservation, assessment, and dissemination (Seetharam 1989). Information and knowledge of genetic diversity is pre-requisite for conservation and increased use of germplasm for proso millet breeding (Hu et al. 2008; Dvorakova et al. 2015). From 170 national and international varieties under examination, around 36 variants from the international collection and two varieties from the national collection were discovered to be resistant to artificial inoculation of seeds with spores (Dyussibayeva et al. 2020). While 11 and 574 landraces were identified to be highly resistant and resistant, respectively from a total of 6526 landraces under study collected from 14 provinces of China (Wang et al. 2008). Breeding for resistance to head smut and melanosis have been reported (Konstantinov and Grigorashchenko 1986, 1987; Maslenkova and Resh 1990; Konstantinov et al. 1989). These identified varieties and germplasms can be used directly for cultivation or involved as parents for genetic improvement. In India, 29% of the released cultivars of proso millet have been developed through hybridization followed by selection (AICSMIP Report 2014).

Construction of core collection and application of molecular markers are key factors in genetic diversity study (Wang et al. 2016). A core collection of 106 accessions from 833 proso millet genotypes from 30 countries was compiled by ICRISAT based on observations of 20 quantitative and qualitative attributes (Upadhyaya et al. 2011). Institute of Plant Production in Khar'kov has released smut resistant varieties in 1986 (Kh86) and 1989 (Kh22) and Slavjanskoe, Soyuz, Quartet, and Sputnik are the smut resistant cultivars available from Russia. Smut resistant mutants like 83-10146, 83-10170, Mutant 5, and Mutant 6 obtained from mutation breeding were employed in hybridization program for development of "Khar'kovskoe" mutant variety in Ukraine and Dagestan (Konstantinov et al. 1989).

Discovery and identification of stress resistant genes will offer crucial gene resources for this crop's enhancement in terms of tolerance. Scarcity of research fundings has led to lack of sufficient genomic resources. However, a few reports are available on using molecular markers for identifying pathotypes by screening genotypes to discover the possible differential hosts. Characterization in genetic

diversity for 51 *S. destruens* isolates were done with RAPD markers and the 16 isolates representing different geographic origins when tested for virulence have identified eight accessions to be immune to *S. destruens*, providing a base for head smut resistance breeding (Zhou et al. 2016). Following the advent of next-generation sequencing, genotyping-by-sequencing (GBS) was used by ICRISAT and Cornell University to genotype proso millet. This information of genome sequences aids in the creation of genome-scale markers for smut resistance and gives direct access to the coding and non-coding regions of the genome (Vetriventhan et al. 2020). In 2016, the first massive de novo assembly and examination of the transcriptome has functionally annotated 62,543 unigenes and has identified over than 35,000 SSRs and 406,000 SNP loci, providing a vital resource for marker expansion in proso millet (Yue et al. 2016). Using transgenic and genomics-assisted breeding, genes for smut resistance can be employed as donors for introgression in popular cultivars. This includes haplotype breeding, Marker Assisted Selection, Marker-Assisted Back Cross, Speed breeding, etc. (Rajasekaran and Francis 2021). The resistant and susceptible varieties of proso millet have 514 and 5452 differentially expressed genes (DEGs), respectively, according to a transcriptomics investigation on smut in the same. A foundation for further and additional research on the molecular mechanisms of interaction between proso millet and pathogen was laid by the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of DEGs, which revealed significant enrichment of pathways like phenylpropanoid biosynthesis, plant-pathogen interaction, and plant hormone signal transduction in case of resistance (Jin et al. 2021).

Bacterial stripe disease, also known as bacterial leaf blight, is an important disease in proso millet caused by the bacterium *Acidovorax avenae* subsp. *avenae* (Manns 1909; Willems et al. 1992) [Homotypic synonym: *Pseudomonas avenae* subsp. *avenae* Manns 1909]. The disease is more severe in the years following floods making it an important concern of climate change. In younger stages of plant, the pathogen results in stunting and death of seedlings (Liu et al. 2012). No reports are available on breeding for resistance against this disease. Kernel smut, a disease affecting the grains of proso millet, is caused by the fungus *Ustilago crameri* Korn. 1874. This disease is also known as grain or covered smut as the symptoms include transformation of grains into white grayish sacs (smut sori). The sori are filled with chlamydospores appearing as black powder responsible for spread of disease as externally seed borne pathogen. CO 5 (TNAU 143) variety developed in TNAU, Tamil Nadu, involving the pedigree PV 1403 × GPUP 21 was reported to be resistant for grain smut. No studies are available on genomic resources pertaining to this disease. Leaf spot is a seed transmitted disease in proso millet caused by *Bipolaris panici-miliacei* (Syn. *Helminthosporium panici-miliacei*) exhibiting brown rectangular spots over infected leaves. Seed rotting, coleoptile spot, and seedling blight are the common symptoms of seed infection (Lee 1997). Resistant variety RAUM 7 is recommended for cultivation.

In addition to these diseases, banded leaf and sheath blight, rust, melanosis, and blast are also observed in this crop which are of little importance. Based on the literature review for disease resistance breeding in proso millet, it is clearly reflected

that it lags much behind other cereals in terms of genetic, genomic, and breeding improvements. Keeping in mind the present context of changing climate and chances of increased disease incidence, there is an immense need for evaluation of germplasm for biotic stress tolerance to be used as donors in further breeding programs. In addition to phenotypic studies, involvement of markers for diversity studies, identification of genes, and novel molecular markers related to disease resistance with the use of available genome sequence will help in accelerating the breeding process together with omics study and high-throughput phenotyping.

21.3 Resistance to Pest Incidence

Inexorably, insects are major threat to plant kingdom causing food insecurity to the human kind and may also pose threat to survival of humanity. Insects have been attacking millets for a longtime, as millets are susceptible to a large number of insect pests. Millets are grown in resource-deprived areas, making it difficult to implement expensive pest control measures. Millet production is severely hampered by pests and diseases across the continents.

Seedling pest, shoot fly *Atherigona pulla* is found to be a substantial pest of proso millet in African countries and India. The larvae feeds on the central shoot of the plant and thereby causing dead hearts as a result of damage. An infestation of this pest can result in losses of up to 80% or even 100% (Jagadish et al. 1995). Incidence of other species of shoot fly such as *A. miliaceae*, *A. punctata* were also reported in proso millet. Insects such as wheat stem maggot *Meromyza americana*, which feeds on upper nodes of the stem and causing whiteheads, were reported in the United States. Thrips, *Haplothrips aculeatus*, can severely damage young plants. Mites and thrips are major seedling pests in the United States. Frequently, the damage caused by mites and thrips becomes excessive and uncontrollable. Nematode attack has also been documented in proso millet. Infestation of spiral nematode *Helicotylenchus* species was recorded on proso millet. The juvenile and adults of spiral nematode feed on epidermal cells of the roots and harbor endoparasitically in the roots, producing many root hairs at the site of the infection. *Meloidogyne incognita*, root knot nematode was found to be moderately to severely infectious in proso millet (Roy 1972).

Perhaps it is possible to exploit insect resistance mechanisms, such as non-preference/antixenosis, antibiosis, and tolerance, to minimize the damages caused by pest infestation. When insects try to exploit plants for food, oviposition, or habitat, these mechanisms might well be capable of mitigating damage. Traits like hairiness/smoothness of leaves, solid stem, waxiness etc. help in making the plant unacceptable for the insect thereby equipping the plant with protection against insects. As a consequence of its ability to prevent pest infestation, antixenosis is the predominant mechanism of resistance. Whereas, in antibiosis type of mechanism, the resistance results during the pest infestation, in which the metabolic activity of the insect is affected due to host plant interaction. The capacity of the plant to heal from the harm caused by the pest/insect is called as tolerance. Thus,

these three mechanisms combinedly confer the resistance to the plant from the pest. Therefore, it can be the nonacceptance mechanism observed during the initial stages of pest infestation, i.e., non-acceptance resistance mechanism is engaged before the pest attack, antibiosis resistance is the consequence of host plant interaction and this mechanism is engaged during the pest infestation. Whereas, tolerance mechanism is operated after the pest damage. Scientists were successful in developing sorghum genotypes which confer resistance to shoot fly, *Atherigona soccata* using non-acceptance/non-preference and antibiosis mechanism. Thus, this mechanism can be exploited. The traits such as leaf glossiness (Kamatar and Salimath 2003), morphology, and density of the trichomes (Gibson and Maiti 1983), seedling vigor (Dhillon 2004) were positively associated with the resistance against the shoot fly. The two main factors leading to resistance to shoot fly are non-acceptance and antibiosis (Raina et al. 1981). Thus, these strategies against sorghum shoot fly can also be incorporated in the breeding program to develop proso millet cultivars resistant against shoot fly *Atherigona pulla*.

21.4 Resistance to Weeds

Proso millet is susceptible to major weeds, few of which are Kochia, Russian thistle, Striga, Buffalobur, Pigweed spp., Wild buckwheat, etc. Striga is a significant biological restriction in subsistence agriculture that significantly damages millet crops in semi-arid tropical regions. Buffalobur is considered as a noxious weed in many regions of the world and is strictly regulated in quarantine.

Different integrated methods are practiced to maintain the productive yield and avoid the growth of weeds. Various cultural methods are employed to achieve optimum yield output in proso millet. Among the different cultural methods, two are most predominant. This involves tillage practices done shortly prior to planting. By delaying planting, significant weed outbreaks like kochia and Russian thistle might happen before sowing and be controlled with tillage. The other practice entails planting the seeds in a warm, moist environment to promote the plantlet's quick development. Herbicides also play a significant role in the management of weeds; 2,4-D is an effective herbicide for controlling most broad-leaved weeds in proso millet. To control emerging weeds, non-selective herbicides like glyphosate can also be used before sowing.

21.5 Molecular Interventions over Traditional Breeding

Diversity analysis provides an opportunity to screen diverse accessions for biotic resistance. The tetraploid nature ($4x = 36$) of millet and lack of sequence information makes it difficult to assess the genetic diversity of this plant. This further hinders with the creation of markers. To assess the genetic diversity of accessions of proso millet, coding and non-coding regions of the genome were used to develop molecular markers (Habiyaremye et al. 2017). In order to reduce time and money on

screening, phenotypic screening can be replaced by DNA markers in the early stages of breeding programs. They can also be used to identify resistant lines at the juvenile stage. Isozymes (Warwick 1987) and AFLPs (Karam et al. 2004) have been used in past studies of variation in proso millet. Following that, different analyses were conducted employing microsatellites (SSRs) obtained from different cereal species (Hu et al. 2009).

21.6 Conclusions

With changing climatic conditions, combating biotic stress has emerged as the top priority when concerned with increasing crop production. Proso millet has tremendous nutritional benefits and renowned for its hardiness. This emphasizes how important it is to scan the literature in order to develop resistant cultivars. Conventional and molecular breeding approaches are employed for resistance breeding. Growing resistant cultivars is the best option because proso millet is a low value crop with little scope for additional financial inputs like fungicides and chemical methods of management are typically not recommended.

21.7 Future Thrust

Focus should be on exploring the potential sources of resistance in the proso millet germplasm with better screening techniques that confer resistance against biotic stress and thereby decoding the resistance mechanism and inheritance pattern of the genes that confer resistance. Since many resistant parents/cultivars are low in yielding capacity, introgression of such resistance genes from donor to agronomically elite cultivars using conventional breeding methods or through biotechnological intervention with the aid of marker assisted selection and QTL mapping of our desired trait will result in development of resistant variety with good seed yield.

References

AICSMIP (2014) Report on compendium of released varieties in small millets, Bangalore, India. http://www.dhan.org/smallmillets/docs/report/CompendiumofReleasedVarietiesinSmallmillets.pdf. Accessed 13 Mar 2019

Amadou I, Gounga ME, Le GW (2013) Millets: nutritional composition, some health benefits and processing—a review. Emirates J Food Agric 25:501–508

Bainsla NK, Meena HP (2016) Breeding for resistance to biotic stresses in plants. In: Recent advances in plant stress physiology, pp 379–411

Baltensperger DD (2002) Progress with proso, pearl and other millets. In: Janick J, Whipkey A (eds) Trends in new crops and new uses. ASHS Press, Alexandria, pp 100–103

Bhat S, Nandini C, Tippeswamy V (2018) Significance of small millets in nutrition and health—a review. Asian J Dairy Food Res 37(1):35–40

Calanca PP (2017) Effects of abiotic stress in crop production. In: Ahmed M, Stockle C (eds) Quantification of climate variability, adaptation and mitigation for agricultural sustainability. Springer, Cham, pp 165–180

Ceccarelli S, Grando S (1996) Drought as a challenge for the plant breeder. Plant Growth Regul 20(2):149–155

Changmei S, Dorothy J (2014) Millet—the frugal grain. Int J Sci Res Rev 3:75–90

Dhillon MK (2004) Effects of cytoplasmic male-sterility on expression of resistance to sorghum shoot fly, *Atherigona soccata* (Rondani). PhD thesis, Department of Entomology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India, p 382

Dvorakova Z, Cepkova P, Janovska D, Viehmannova I, Svobodova E, Cusimamani E, Milella L (2015) Comparative analysis of genetic diversity of 8 millet genera revealed by ISSR markers. Emirates J Food Agric 27(8):617–628. https://doi.org/10.9755/ejfa.2015.04.077

Dyussibayeva E, Seitkhozhayev A, Rysbekova A, Tleppayeva A, Yessenbekova G, Zhirnova I (2020) Studying the world collection of millet with a view to select forms immune to lose smut. Bulgarian J Agr Sci 26(6):1203–1208

Fan J, Yang J, Wang YQ, Li GB, Li Y, Huang F, Wang WM (2016) Current understanding on *Villosiclava virens*, a unique flower-infecting fungus causing rice false smut disease. Mol Plant Pathol 17:1321–1330

FAO (2021) World food and agriculture—statistical yearbook 2021. FAO, Rome. https://doi.org/10.4060/cb4477e

Gahukar RT, Reddy GV (2019) Management of economically important insect pests of millet. J Integr Pest Manag 10(1):28

Gibson PT, Maiti RK (1983) Trichomes in segregating generations of sorghum matings. I. Inheritance of presence and density. Crop Sci 23:73–75

Goron TL, Raizada MN (2015) Genetic diversity and genomic resources available for the small millet crops to accelerate a New Green Revolution. Front Plant Sci 6:157. https://doi.org/10.3389/fpl.2015.00157

Habiyaremye C, Matanguihan JB, D'Alpoim Guedes J, Ganjyal GM, Whiteman MR, Kidwell KK, Murphy KM (2017) Proso millet (*Panicum miliaceum* L.) and its potential for cultivation in the Pacific Northwest, U.S.: a review. Front Plant Sci 7:1961. https://doi.org/10.3389/fpls.2016.01961. PMID: 28119699; PMCID: PMC5220228

Herdrich N (2001) Grower experiences with millet in Eastern Washington, 1997–1999. Cooperative Extension, Washington State University

HINZE G0 (1977) Millets in Colorado. Colorado State Univ Exp Station Bull 553S:1–12

Hu YG, Zhu J, Liu F, Zhang Z, Chai Y, Weining S (2008) Genetic diversity among Chinese landraces and cultivars of broomcorn millet (*Panicum miliaceum*) revealed by the polymerase chain reaction. Ann Appl Biol 153(3):357–364

Hu X, Wang J, Lu P, Zhang H (2009) Assessment of genetic diversity in broomcorn millet (*Panicum miliaceum* L.) using SSR markers. J Genet Genomics 36(8):491–500

Jagadish PS, Murali PR, Seetharam A (1995) Evaluation of germplasm lines and cultural practice on the incidence of little millet shoot fly, *Atherigona pulla* Wiede. Environ Educ Res 7:45–47

Jin F, Liu J, Wu E, Yang P, Gao J, Gao X, Feng B (2021) Leaf transcriptome analysis of broomcorn millet uncovers key genes and pathways in response to *Sporisorium destruens*. Int J Mol Sci 22(17):9542

Kalinova J, Moudry J (2006) Content and quality of protein in proso millet (*Panicum miliaceum* L.) varieties. Plant Foods Hum Nutr 61(1):43–47

Kamatar MY, Salimath PM (2003) Morphological traits of sorghum associated with resistance to shoot fly, *Atherigona soccata* Rondani. Indian J Plant Protect 31:73–77

Karam D, Westra P, Nissen SJ, Ward SM, Figueiredo JEF (2004) Genetic diversity among proso millet (*Panicum miliaceum*) biotypes assessed by AFLP technique. Planta Daninha 22:167–174

Konstantinov SI, Grigorashchenko LV (1986) Productivity of proso millet forms of different ecological and geographical groups and their susceptibility to melanosis infection. Selektsiyai Semenovodstvo (Kiev) 61:40–44

Konstantinov SI, Grigorashchenko LV (1987) Inheritance of resistance to melanosis in proso millet hybrids of the first generation. Tsitologiyai Genetika 21(5):335–338
Konstantinov SI, Linnik VM, Ya SL (1989) Use of smut-resistant induced mutants in breeding proso millet. Selektsiyai Semenovodstvo (Kiev) 66:25–28
Kumar A, Tomer V, Kaur A, Kumar V, Gupta K (2018) Millets: a solution to agrarian and nutritional challenges. Agric Food Secur 7:31
Lee DH (1997) Morphological characters and seed transmission of *Bipolaris panici-miliacei* causing leaf spot of common millet. Korean J Plant Pathol (Korea Republic) 13(1):18–21
Liu H, Qiu H, Zhao W, Cui Z, Ibrahim M, Jin G, Li B, Zhu B, Xie GL (2012) Genome sequence of the plant pathogen *Pseudomonas syringae pv. panici*. LMG 2367:5693–5694
Lyon DJ, Burgener PA, DeBoer K (2008) EC08-137 producing and marketing proso millet in the Great Plains. University of Nebraska-Lincoln, Lincoln, NB
Manns (1909) The blade blight of oats: a bacterial disease. Bull Ohio Agric Exp Station 210:91–167
Maslenkova LI, Resh LP (1990) Sources of resistance to head smut in proso millet. Nauchno-Tekhnicheskiĭ Byulleten', VASKhNIL, Sibirskoe Otdelenie, Sibirskiĭ Nauchno-Issledovatel' skiĭ Institut Sel' skogo Khozyastvaĭ 6:28–33
McDonald SK, Hofsteen L, Downey L (2003) Crop profile for proso millet in Colorado. USDA Crop Profiles, Regional IPM Centers
McTaggart AR, Shivas RG, Geering ADW, Vanky K, Scharaschkin T (2012) Taxonomic revision of *Ustilago*, *Sporisorium* and Macalpinomyces. Persoonia 29(1):116–132
Naylor RL, Falcon WP, Goodman RM, Jahn MM, Sengooba T, Tefera H, Nelson RJ (2004) Biotechnology in the developing world: a case for increased investments in orphan crops. Food Policy 29(1):15–44. https://doi.org/10.1016/j.foodpol.2004.01.002
Nishizawa N, Fudamo Y (1995) The elevation of plasma concentration of high-density lipoprotein choesterol in mice fed with protein from proso millet (*Panicum miliaceum*). Biosci Biotechnol Biochem 59:333–335. https://doi.org/10.1271/bbb.59.333
Numan M, Serba DD, Ligaba OA (2021) Alternative strategies for multi-stress tolerance and yield improvement in millets. Genes 12(5):739
Raina AK, Thindwa HK, Othieno SM, Cork-Hill RT (1981) Resistance in sorghum to the sorghum shoot fly: larval development and adult longevity and fecundity on selected cultivars. Int J Trop Insect Sci 2:99–103
Rajasekaran R, Francis N (2021) Genetic and genomic resources for improving proso millet (*Panicum miliaceum* L.): a potential crop for food and nutritional security. Nucleus 64(1):21–32
Reports millets (2022) MILLETS the future super food for India. The Associated Chambers of Commerce and Industry of India
Roy AK (1972) Reaction of some plants to the attack of Meloidogyne incognita in Assam. Indian J Nematol 2:86–89
Saha D, Channabyre Gowda MV, Arya L, Verma M, Bansal KC (2016) Genetic and genomic resources of small millets. Crit Rev Plant Sci 35:56–79. https://doi.org/10.1080/07352689.2016.1147907
Saleh AS, Zhang Q, Chen J, Shen Q (2013) Millet grains: nutritional quality, processing, and potential health benefits. Compr Rev Food Sci Food Saf 12(3):281–295
Sathish R, Manjunatha M, Rajashekarappa K (2017) Incidence of shoot fly, *Atherigona pulla* (Wiedemann) on proso millet at different dates of sowing. J Entomol Zool Stud 5:2000–2004
Seetharam A (1989) Genetic resources of small millets in India. In: Small millets in global agriculture. Oxford & IBH Publishing Co. Pvt. Ltd., p 45
Seghatoleslami MJ, Kafi M, Majidi E (2008) Effect of drought stress at different growth stages on yield and water use efficiency of five proso millet (*Panicum miliaceum* L.) genotypes. Pak J Bot 40:1427–1432
Upadhyaya HD, Sharma S, Gowda CLL, Reddy VG, Singh S (2011) Developing proso millet (*Panicum miliaceum* L.) core collection using geographic and morpho-agronomic data. Crop Pasture Sci 62(5):383–389

Vetriventhan M, Azevedo VC, Upadhyaya HD, Nirmalakumari A, Kane-Potaka J, Anitha S, Ceasar SA, Muthamilarasan M, Bhat BV, Hariprasanna K, Bellundagi A (2020) Genetic and genomic resources, and breeding for accelerating improvement of small millets: current status and future interventions. Nucleus 63(3):217–239

Wang L, Wang XY, Wen QF, Zhao WH, Liu JY (2008) Identification and evaluation of resistance to dustbrand in Chinese proso millet germplasm resources. J Plant Genet Resour 9(4):497–501

Wang R, Hunt HV, Qiao Z, Wang L, Han Y (2016) Diversity and cultivation of broomcorn millet (*Panicum miliaceum* L.) in China: a review. Econ Bot 70(3):332–342

Warwick SI (1987) Isozyme variation in proso millet. J Hered 78(3):210–212

Willems A, Goor M, Thielemans S, Gillis M, Kersters K, Ley J (1992) Transfer of several phytopathogenic Pseudomonas species to *Acidovorax* as *Acidovorax avenae subsp. avenae subsp. nov*., comb. nov., *Acidovorax avenae subsp. citrulli, Acidovorax avenae subsp.* cattleyae and *Acidovorax konjaci*. Int J Syst Bacteriol 42(1):107–119

Wu EG, Zhou Y, Zhu MQ, Liu JJ, Gao XL, Feng BL (2020) Identification and biological characteristics of *Sporisorium destruens* in broomcorn millet. J Plant Protect Res 47:101–109 (In Chinese)

Wu E, Liu L, Zhu M, Wu H, Yang Q, Li J, Han X, Feng B (2022) The life cycle and ultrastructure of the host response of the smut pathogen *Anthracocystis destruens* on broomcorn millet. Phytopathology 112(5):996–1002

Yue H, Wang L, Liu H, Yue W, Du X, Song W, Nie X (2016) De novo assembly and characterization of the transcriptome of broomcorn millet (*Panicum miliaceum* L.) for gene discovery and marker development. Front Plant Sci 7:1083

Zhang L, Liu R, Niu W (2014) Phytochemical and antiproliferative activity of proso millet. PLoS One 9:e104058. https://doi.org/10.1371/journal.pone.0104058

Zhou Y, Qu Y, Zhu M, Liu J, Wang Y, Song H, Feng B (2016) Genetic diversity and virulence variation of *Sporisorium destruens* isolates and evaluation of broomcorn millet for resistance to head smut. Euphytica 211(1):59–70

Genetic Improvement of Proso Millet Through Advanced Biotechnological Approaches

22

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Abstract

Proso millet (*Panicum miliaceum* L.) is a short duration climate resilient millet crop with high nutritional quality. It is one of the oldest domesticated cereal crop and is distributed throughout the world. This C_4 crop has very high resource and water use efficiency and grows very well in poor and marginal lands. Though the crop has inherent stress tolerance and rich nutritional profile, the genetics and mechanism behind are not well understood. The rekindled importance of millets worldwide has attracted research investments and kick started advanced research programs in millets recently. The modern biotechnological approaches are powerful in dissecting complex processes and developing deployable tools for crop improvement. The recently released genome sequence information of proso millet has generated considerable genomic information in the crop which has taken the crop a step forward. Comparative genomic and pangenomic approaches can provide an even concrete understanding of the genes that are conserved and amenable for cross-transfer from other related crops. Few transcriptomic studies have thrown light onto the differentially expressed genes and proteins in proso under various stresses. Proteomics and metabolomics approaches can also be employed to dissect the nutritional and nutraceutical profile of the crop. Apart from the omics tools, techniques like site-directed mutagenesis, RNA interference, CRISPR, DNA methylation etc. can also be exploited in the crop. This

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chapter discusses the progress made in genetic improvement of proso millet through modern biotechnological approaches and the way ahead.

Keywords

Proso millet · Genetic improvement · Biotechnology · Transcriptomics · Proteomics

22.1 Introduction

Proso millet (*Panicum miliaceum* L.) is a climate resilient millet crop with short duration which is used for food and fodder purposes. It is a C_4 crop with allotetraploid ($2n = 4x = 32$) genome. It is one of the oldest domesticated cereal crop and is distributed throughout the world and can grow well in poor and marginal lands. The crop has low water and nutrient requirement and is the most water use efficient cereal crop. The seeds are nutritionally rich in protein (10–14 g/100 g), carbohydrates (74 g/100 g), vitamins, and minerals (Rajasekaran and Francis 2020). The husk and bran layers are great sources of antioxidants and can be used in functional foods. Though the crop has inherent stress tolerance and rich nutritional profile, the genetic mechanisms behind them are not well understood. As the major food crops are underperforming under the climate constrained production systems, the importance of hardy millets is being recognized.

Conventional breeding and biotechnological approaches have been contributing to crop improvement of several crops. The biotechnological approaches have revolutionized the crop breeding scenario by reducing the time and effort required for identification, selection, and transfer of beneficial traits from one plant to another. Genomic and molecular tools and the bioinformatic analyses of the information generated have bettered the understanding of complex pathways and processes in common food crops like rice and wheat. However, in proso millet, research in these aspects is far behind and many of the modern approaches are not yet attempted. This chapter discusses the advancements made in proso millet using the various "omics" approaches like genomics, transcriptomics, proteomics, and metabolomics and also the possibility of utilizing other advanced approaches like New Plant Breeding Techniques (NBPTs).

22.2 Genomic Approaches in Proso Millet

Earliest attempts to develop genomic resources used Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), and Amplified Fragment Length Polymorphism (AFLP) markers, which was later replaced by Simple Sequence Repeats (SSRs). RAPD markers were used to differentiate and characterize the wild and cultivated proso (Colosi and Schaal 1997). Similarly, AFLP was used to assess the genetic polymorphism among the American

genotypes of proso (Karam et al. 2004, 2006). Inter Simple Sequence Repeats (ISSR) were also used to study the variability among germplasm accessions of Himalayan region (Trivedi et al. 2015). Another study used ISSRs to understand the relatedness of a medieval proso millet genotype to the present day cultivars (Lágler et al. 2005). Chung et al. (2010) developed an SSR enriched library using the genomic DNA of 50 proso millet accessions from various countries. Twenty five polymorphic SSRs were developed from the study. These markers with further validation could be utilized for molecular characterization, genetic diversity studies, and marker assisted selection in the crop. Genetic diversity among the Slovenian landraces of the crop was determined using these SSR markers (Flajšman et al. 2019). As there is dearth of genetic markers in the crop, markers developed in closely related crop species can also be utilized. In a similar attempt, 62% of switchgrass (*Panicum virgatum*) SSR makers (339 markers) were found transferable to proso millet (Rajput et al. 2014). Marker trait associations were studied under saline stressed conditions among the 143 proso millet genotypes using 514 AFLP markers. Markers M14/E10–45 and M14/E10–60 were associated with seed yield and M14/E10–45 and M14/E11–44 were related to forage yield (Yazdizadeh et al. 2020). Genome wide association study among 88 accessions of diverse geographical origin identified ten marker trait associations for seed characters and three for agronomic traits (Boukail et al. 2021).

Genome sequencing and assembly have become simpler with the introduction of next-generation sequencing (NGS) technology. Single nucleotide polymorphisms (SNPs), the most popular markers at the moment, were created in large quantities by sequencing genomes. Genotype-By-Sequencing (GBS) is a cost-effective NGS platform particularly for crops with large genomes. Genome-wide association study (GWAS), genetic diversity analysis, linkage map construction, molecular marker identification, and genomic selection can all be done using GBS which can accelerate the plant breeding programs (He et al. 2014). GBS was used to genotype the Recombinant Inbred Lines (RILs) and their parents to develop the first genetic linkage map in the crop. In all, 833 unique polymorphic SNPs were found, and they were used to build linkage maps. These SNPs formed 18 major and 84 minor linkage groups (LG). Eighteen QTLs and flanking markers associated with eight agronomic traits were also identified. The QTLs *QLh.unac-lg5*, *QGs.unac-lg5,* and *QGpp.unac-lg4* respectively for tolerance to lodging, grain shattering, and grains per panicle that exhibited high additive effect were suggested as the most suited candidates for marker assisted selection after validation (Rajput et al. 2016). GBS was used to generated 1882 genome wide SNPs for 190 proso millet accessions. The study also identified eight subpopulations after population structure analysis of the genetic information (Johnson et al. 2019). Bacterial artificial chromosome (BAC) libraries preserve DNA materials as inserts in the bacteria like *Escherichia coli*. BAC clones with an average insert size of 123.48 kb were made to develop a BAC library for proso millet. A BAC mapping pipeline depended on the cloned-array pooled shot-gun sequencing and Illumina sequencing strategy was also constructed. This resource can be utilized for simple DNA segment sequencing to functional genomics and genetic engineering approaches of the crop (Xu et al. 2021).

Recently, proso millet's entire genome was sequenced and assembled by Zou et al. (2019), producing a vast amount of molecular data. An F_6 RIL population derived from a proso millet landrace of Northern China was sequenced and assembled, estimating the genome size to be 923 Mb. A high density linkage map with 221,787 single SNPs was also generated. Contigs were assembled onto 18 pseudochromosomes. The average size of protein coding genes was found to be 3260 bp long. Genome sequence data when subjected to phylogenetic analysis verified close kinship with Foxtail millet (*Setaria italica*). Comparative genomics deals with comparison and transferability of genomic resources from one organism to another. As molecular markers, genomic and transcriptomic resources in the crop are scarce, the possibility of cross-transferability of resources can be explored. Currently, only the draft genome sequence is available in proso millet. Whereas in foxtail and finger millet, the genome sequences have been annotated. The genomic resources and functional information available in these millets can be used to improve and interpret complex mechanisms in proso to expedite the breeding programs (Maharajan et al. 2022).

22.3 Transcriptomic Approaches in Proso Millet

The entire collection of RNAs produced by the genome from a particular type of tissue or cell at a certain developmental stage or under a particular set of physiological conditions is known as the transcriptome. Transcriptomics is the study of the transcriptome which includes the transcription, expression levels, functions, locations, trafficking and degradation of RNAs (Milward et al. 2016). It has been majorly utilized in understanding the various stress tolerance mechanisms in the crop. The functional study of WRKY genes applied computational prediction using already existing Expressed sequence tags (ESTs) and transcriptome contigs of proso millet and identified 32 unique PmWRKY genes. Under abiotic stresses like drought, cold and salt stresses a total of 22 PmWRKY genes exhibited significant differential expression and was categorized as stress responsive genes (Yue et al. 2016b). The whole transcriptome of two genotypes of the crop, Yumi No. 2 and Yumi No. 3 have been sequenced and assembled. A total of 62,543 of the 113,643 unigenes identified were found, functionally annotated. The study also generated a large number of SSRs and SNPs which can be utilized in marker development in the crop (Yue et al. 2016a). RNA-Seq analysis of tolerant (Huangmizi) and susceptible (sensitive) genotypes in proso millet generated 2301 SSRs and 1,447,148 SNPs. Gene Ontology (GO) terms of the Unigenes were majorly classified as having biological functions (74.30%). Sixty three metabolic pathways were identified for more than 5000 Unigenes from the KEGG database analysis (Wang et al. 2017). RNA-seq analysis and transcriptome assembly of another proso millet cultivar Neimenggu-Y1 were carried out. Based on the 4724 genic SSR loci and sequences identified, 229 SSR primers were designed and validated among 56 accessions of various geographic locations in China. Fourteen primers showed polymorphism for the accessions which could be utilized for marker assisted selection, QTL mapping

etc. The results of the study also suggested that there is no distinct relation between genetic distance and geographic origin among these lines. Trinucleotide repeats were found to be the most common repeats in the crop (Hou et al. 2017). Transcriptomic analysis of a yellow leaved mutant and wild type proso millet identified nine genes involved in the formation of chloroplasts and chlorophyll production. This study integrated physiological, agronomic, conventional breeding and advanced biotechnological approaches to reveal functional information of genes (Wang et al. 2022). A similar attempt to delineate the physiological and molecular mechanisms involved in salt stressed genotypes of proso millet was done with the help of digital RNA sequencing. Fifteen genes involved in chlorophyll production were differentially expressed in the genotypes. The digital RNA-seq analysis revealed that the salt tolerant genotype ST 47 had better Na^+ and K^+ homeostasis, increased expression of genes involved in photosynthesis and chlorophyll metabolism and in-turn higher biomass accumulation under stress (Yuan et al. 2022). Contrasting genotypes for cadmium tolerance, SH (susceptible) and TL (tolerant) were analyzed using transcriptome and weighted gene co-expression network analysis. The genes controlling glutathione metabolism, cell wall metabolism, phenylpropanoid biosynthesis, ATP-binding cassette (ABC), and metal ion transporters were differentially expressed among the tolerant and susceptible genotypes. This suggests their critical role during Cd stress (Liu et al. 2022b). Transcriptomes of two cultivars (T184: low-N tolerant and S111: low-N sensitive) in proso millet were compared to understand the mechanisms controlling Nitrogen Use Efficiency. Findings showed that higher efficiency of N uptake and utilization boosted assimilation and translocation and increased the photosynthetic capacity of the crop. Nitrogen transporter genes and genes related to photosynthesis were upregulated in the tolerant cultivar T184 in the low N conditions (Liu et al. 2022a). Two contrasting cultivars for sodium ion (Na^+) toxicity resistance, i.e., ST 47 and SS 212, were analyzed using phenotypic, physiological, and RNA sequencing techniques to understand the resistance strategies in the crop. Comparative analysis of various growth stages and plant parts was carried out as part of the study. The digital RNA sequencing analysis of the genotypes revealed that transporter genes and genes involved in photosynthesis were critical players in maintain better homeostasis of Na^+ and K^+ ions in the saline tolerant genotype ST 47. This integrated approach into elucidating the salt stress mechanism in the crop suggested it as promising salt-tolerant bioenergy crop for the future agriculture (Yuan et al. 2022).

22.4 Proteomic Approaches in Proso Millet

Proteomic analysis identifies the key participants in controlling different biological activities. They give information on the genes that are expressed as proteins and degree of their expression. Information about the post transcription and translational changes and resulting proteins and their functions are obtained through proteomic studies. It complements genomics and transcriptomics and completes the information flow from genetic material to its expression. Similar to the concept of

Quantitative Trait Loci (QTL), Protein Quantity Loci (PQL) have been identified. These regions give information about the responsive or expressive genes (Damerval et al. 1994). This is a powerful tool that can be integrated with genomics and used in Marker Assisted Breeding programs for crop improvement (Eldakak et al. 2013). Proso millet seed proteins have been profiled using electrophoresis and mass spectrometry among Korean cultivars "Leebaekchal," "Manhongchal," "Miryang 7," and "Miyang 8." Proteins were differentially expressed between the cultivars and two protein showed upregulation in all the cultivars. Most proteins that upregulated were involved in starch metabolism, transcription, and pathogenesis. Proteins associated with glycolysis, stress responses, and transduction were majorly downregulated in the seeds (Roy et al. 2017). In the model millet crop, foxtail millet (*Setaria italica* L. P. Beauv.) comparatively more proteomic studies have been carried out. Proteomic analysis of foxtail millet seedlings exposed to saline stress identified 29 differentially expressed proteins. These proteins were involved in various biological processes like signal transduction, photosynthesis, cell wall biogenesis, stress responses and energy, lipid, nitrogen, carbohydrate, and nucleotide metabolisms (Veeranagamallaiah et al. 2008). A total of 2474 differentially expressed proteins and biological functions were detected in seedlings subjected to drought (Pan et al. 2018).

22.5 Metabolomic Approaches in Proso Millet

Metabolome of a biological sample is constituted by all the low molecular weight metabolites present in it. The study that deals with the identification and quantification of these metabolites comes under metabolomics. Metabolomics has become an important part of integrated omics approach of crop improvement. It can provide insights into the growth and development of plants particularly under different growing conditions. This omics approach has been used in abiotic and biotic stress resistance breeding, nutritional quality improvement, understanding complex physiological processes like photosynthesis. Advanced metabolomics techniques including gas chromatography-mass spectrometry (GC-MS), liquid chromatography mass spectroscopy (LC-MS), and non-destructive nuclear magnetic resonance spectroscopy (NMR) can identify, measure, and evaluate these compounds (Razzaq et al. 2019).

Metabolome profiling and quantification of four varieties of proso millet that differed for their grain bran color was carried out. The study established association between grain color and physiological and antioxidant activities. The ultra-high performance liquid chromatography coupled with triple quadrupole mass spectrometry (UHPLC-QqQ-MS) technique was used for the metabolomic study. A total of 672 metabolites were detected in the proso millet grains. White type in comparison with black, gray, and red types showed differential accumulation of metabolites. Tryptophan metabolism, flavonoid, isoflavonoid, flavone, and flavonol production were the primary pathways that were differently activated. Upregulation of metabolic pathways related to flavonoid biosynthesis, isoflavonoid biosynthesis, and

propanoate metabolism was seen in the red type over white type (Li et al. 2021). Integrated approach using transcriptomics and metabolomics revealed that energy metabolism, photosynthesis, anthocyanin metabolism, plant hormone signal transduction, and drought related transcription factors were involved in drought tolerance in proso millet. High resolution mass spectrometry (HRMS) technology was used to analyze the metabolome of two drought stressed varieties in proso millet. A total of 2082 metabolites were differentially expressed in these genotypes (Cao et al. 2022).

22.6 Scope of other Advanced Approaches in Proso Millet and the Way Forward

New plant breeding techniques (NPBTs) is another set of modern tools that apply the knowledge obtained through various omics approaches to crop improvement. They can make specific genomic changes in a much shorter time compared to conventional breeding methods and expedite the crop improvement programs. These techniques can modify the genome with no remnants of foreign genes or DNA segments (from other species) in the end products. Cisgenesis, Intragenesis, sequence-specific nuclease technology (SSN 1,2), Oligo-directed mutagenesis, RNA-dependent DNA methylation, Reverse breeding are a few of the major NBPTs used for crop improvement (Nair et al. 2022). RNA guided endonucleases like clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) and other CRISPR techniques are the latest NPBTs that are being used extensively in many crops. They are high precision genome scissors that can accomplish site specific mutagenesis and genome editing. These tools can speed track the crop improvement efforts for future food and nutritional security.

The major hurdle in exploiting these techniques in proso millet is that the genes responsible for most of the agronomic, physiological, and nutritional traits are not identified. Gene identification and sequence information is critical in making precise modifications to the genome. In proso millet, two major traits that make it suitable as a future food crop are climate resilience and nutritional quality. However, research into genetic improvement of these traits is in its infancy. The rekindled importance of millets has initiated several research programs and collaborations in the crop. Experimental and computational annotation of the released draft genome is necessary to explore many novel techniques in the crop. Establishing associations between genomics and phenomics of tolerance mechanisms and nutritional traits and understanding their genetics and inheritance can propel the genetic improvement efforts in the crop.

References

Boukail S et al (2021) Genome wide association study of agronomic and seed traits in a world collection of proso millet (Panicum miliaceum L.). BMC Plant Biol 21(1):1–12

Cao X et al (2022) Transcriptome sequencing and metabolome analysis reveals the molecular mechanism of drought stress in millet. Int J Mol Sci 23(18):10792. https://doi.org/10.3390/ijms231810792

Chung YCJ et al (2010) Development and characterization of twenty-five new polymorphic microsatellite markers in proso millet (Panicum miliaceum L.). Genes Genom 32:267–273. https://doi.org/10.1007/s13258-010-0007-8

Colosi JC, Schaal BA (1997) Wild proso millet (Panicum miliaceum) is genetically variable and distinct from crop varieties of proso millet. Weed Sci 45(4):509–518

Damerval C et al (1994) Quantitative trait loci underlying gene product variation: a novel perspective for analyzing regulation of genome expression. Genetics 137(1):289–301

Eldakak M et al (2013) Proteomics: a biotechnology tool for crop improvement. Front Plant Sci 4: 35

Flajšman M, Štajner N, Ačko DK (2019) Genetic diversity and agronomic performance of Slovenian landraces of proso millet (Panicum miliaceum L.). Doga Turk J Bot 43(2):185–195. https://doi.org/10.3906/bot-1807-83

He J et al (2014) Genotyping-by-sequencing (GBS), an ultimate marker-assisted selection (MAS) tool to accelerate plant breeding. Front Plant Sci 5:484

Hou S et al (2017) Transcriptomic analysis, genic SSR development, and genetic diversity of proso millet (Panicum miliaceum; Poaceae). Appl Plant Sci 5(7):1600137. https://doi.org/10.3732/apps.1600137

Johnson M et al (2019) Genome-wide population structure analyses of three minor millets: kodo millet, little millet, and proso millet. Plant Genome 12(3):190021. https://doi.org/10.3835/plantgenome2019.03.0021

Karam D et al (2004) Genetic diversity among proso millet (Panicum miliaceum) biotypes assessed by AFLP technique. Planta Daninha 22:167–174

Karam D et al (2006) Assessment of silver-stained AFLP markers for studying DNA polymorphism in proso millet (Panicum miliaceum L.). Rev Bras Bot 29:609–615

Lágler R et al (2005) Morphological and molecular analysis of common millet (P. miliaceum) cultivars compared to an aDNA sample from the 15th century (Hungary). Euphytica 146(1): 77–85

Li W et al (2021) Study on metabolic variation in whole grains of four proso millet varieties reveals metabolites important for antioxidant properties and quality traits. Food Chem 357:129791. https://doi.org/10.1016/j.foodchem.2021.129791

Liu C et al (2022a) Comparative transcriptome and physiological analysis unravel proso millet (Panicum miliaceum L.) source leaf adaptation to nitrogen deficiency with high nitrogen use efficiency. Environ Exp Bot 199:104891

Liu J et al (2022b) Transcriptome and co-expression network analyses reveal key factors responsible for cadmium tolerance and translocation in broomcorn millet. SSRN Electron J:1–51. https://doi.org/10.2139/ssrn.3983848

Maharajan T et al (2022) Mining genes and markers across minor millets using comparative genomics approaches. In: Omics of climate resilient small millets. Springer, pp 185–203

Milward EA et al (2016) Transcriptomics. In: Encyclopedia of cell biology, vol 4. Elsevier, pp 160–165. https://doi.org/10.1016/B978-0-12-394447-4.40029-5

Nair A et al (2022) 'European consumer and societal stakeholders' response to crop improvements and new plant breeding techniques. Food Energy Secur 12:e417

Pan J et al (2018) Comparative proteomic investigation of drought responses in foxtail millet. BMC Plant Biol 18(1):1–19

Rajasekaran R, Francis N (2020) Genetic and genomic resources for improving proso millet (Panicum miliaceum L.): a potential crop for food and nutritional security. Nucleus 64:21–32

Rajput SG, Plyler-Harveson T, Santra DK (2014) Development and characterization of SSR markers in proso millet based on switchgrass genomics. Am J Plant Sci 5(1):175–186. https://doi.org/10.4236/ajps.2014.51023

Rajput SG, Santra DK, Schnable J (2016) Mapping QTLs for morpho-agronomic traits in proso millet (Panicum miliaceum L.). Mol Breed 36(4):37

Razzaq A et al (2019) Metabolomics: a way forward for crop improvement. Metabolites 9(12):303. https://doi.org/10.3390/metabo9120303

Roy SK et al (2017) Comparison of protein profiles of proso millet (Panicum miliaceum) seeds of various korean cultivars. Kor J Crop Sci 62(1):40–50

Trivedi AK et al (2015) Genetic variability in proso millet [Panicum miliaceum] germplasm of Central Himalayan Region based on morpho-physiological traits and molecular markers. Acta Physiol Plant 37(2):1–16

Veeranagamallaiah G et al (2008) Proteomic analysis of salt stress responses in foxtail millet (Setaria italica L. cv. Prasad) seedlings. Plant Sci 175(5):631–641

Wang RY et al (2017) Drought-induced transcription of resistant and sensitive common millet varieties. J Anim Plant Sci 27(4):1303–1314

Wang Y et al (2022) Identification and function analysis of yellow—leaf mutant (YX—yl) of broomcorn millet. BMC Plant Biol 22:1–15. https://doi.org/10.1186/s12870-022-03843-y

Xu W et al (2021) Genomic resources of broomcorn millet: demonstration and application of a high-throughput BAC mapping pipeline. BMC Genomic Data 22(1):1–9. https://doi.org/10.1186/s12863-021-01003-z

Yazdizadeh M et al (2020) Association analysis between agronomic traits and AFLP markers in a wide germplasm of proso millet (Panicum miliaceum L.) under normal and salinity stress conditions. BMC Plant Biol 20(1):1–18

Yuan Y et al (2022) Unravelling the distinctive growth mechanism of proso millet (Panicum miliaceum L.) under salt stress: from root-to-leaf adaptations to molecular response. GCB Bioenergy 14(2):192–214

Yue H, Wang L et al (2016a) De novo assembly and characterization of the transcriptome of broomcorn millet (Panicum miliaceum L.) for gene discovery and marker development. Front Plant Sci 7:1083. https://doi.org/10.3389/fpls.2016.01083

Yue H, Wang M et al (2016b) Transcriptome-wide identification and expression profiles of the WRKY transcription factor family in Broomcorn millet (Panicum miliaceum L.). BMC Genomics 17:1–11. https://doi.org/10.1186/s12864-016-2677-3

Zou C et al (2019) The genome of broomcorn millet. Nat Commun 10:436. https://doi.org/10.1038/s41467-019-08409-5

Floral Biology, Pollination, Genetics, Origin, and Diversity in Barnyard Millet

23

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Abstract

Barnyard millet (*Echinochloa* spp.) has emerged as one of the earliest indigenous millets throughout the semi-arid tropical regions of Asia and Africa. Communities belonging to hilly and tribal regions in Asia, particularly in India, China, and Japan, cultivate two main species for food and fodder: *Echinochloa esculenta* (A. Braun) H. Scholz; syn. *E. utilis* Ohwi Yabuno (Japanese barnyard millet) and *Echinochloa frumentacea* Link; syn. *E. colona* var. *frumentacea* (Link) Ridl. Due to its short life cycle, the crop has an extensive range of adaptations and occupies a specialized niche in marginally humid regions. Despite the crop's endurance to withstand harsh conditions, its importance had been denied substantially over the last 50 years. It serves as a superior alternative during famine periods. In the Indian Himalayan region, the crop appeared to be utilized as a substitute for rice. It has been established to be a great choice for climate-resilient agriculture. It is a functional food crop because of its high nutrient content and antioxidant properties. Because the crop's grains are so incredibly nutritious, demand for it has recently surged. Therefore, it has the potential to ensure both nutritional as well as food security, particularly in high-terrain regions where deficiencies in nutrients are frequent. Despite its enormous potential, the crop has not achieved widespread acceptance and remains visible only as sustenance for the underprivileged. In order to make the crop competitive and modernize its production, this effort aims to consolidate the scant knowledge on agricultural history, domestication, phylogeny, plant architecture, and floral biology of the crop that will be of use to the readers and researchers working on this crop.

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Keywords

Barnyard millet · Domestication · History · Germplasm resources · Origin

23.1 Barnyard Millet

Barnyard millet, *Echinochloa* sp. ($2n = 36, 54$), grown as a cereal fodder crop, is one of the oldest domesticated millets in the semi-arid tropics of Asia and Africa. Barnyard millet is a self-pollinated crop which belongs to the family Poaceae, subfamily Panicoideae, and tribe Paniceae (Kellogg 2015). It is constituted as the fastest-growing millet crop and is typically grown on hill slopes and undulating fields in mountainous, tribal, or backward areas where crop choice and possibilities are constrained. The genus *Echinocholoa* includes 35 wild species spread over the world. Among these, *E. utilis* (A. Braun) H. Scholz; syn. *E. utilis* Ohwi et Yabuno also known as the Japanese barnyard millet and *E. frumentacae* Link; syn. *E. colona* var. *frumentacea* (Link) Ridl., also known as Indian barnyard millet or sawa millet, are the two domesticated species. *E. utilis* is a temperate annual hexaploidy ($2n = 6x = 54$). *E. frumentacae* ($2n = 36, 54$) are cultivated as minor cereals in Japan, Korea, the north-eastern parts of China and India, Pakistan, and Nepal, respectively (Yabuno 1987).

Barnyard millet is cultivated in India for human consumption as well as livestock feed. Barnyard millet production is often preferred in areas where climate and environmental factors are inappropriate for raising and cultivating of rice crop (Yabuno 1987). It is largely grown in India in two separate agroecologies, one in Tamil Nadu's Deccan plateau area and the other in Uttarakhand's mid-hills of the Himalayas in the north. The second-most significant small millet in India after finger millet, barnyard millet has production and productivity of 0.147 mt and 1034 kg/ha, respectively. It has received considerable interest as a fodder crop in the United States and Japan due to its early growth and maturity. Barnyard millet has drawn attention recently, largely due to its excellent nutritional content.

23.1.1 History, Origin, and Domestication

Echinochloa frumentacea, which likely evolved in both India and Africa, followed a comparable evolutionary path on both continents. It is cultivated annually in Malawi, Tanzania, the Central African Republic, and India (Doggett 1989). *E. colona* (L.) Link, also known as tropical grass, is the wild progenitor of *E. frumentacea*, sometimes referred to as jungle rice. It is raised for beer, fodder, and grain. It varies from *E. esculenta* due to the presence of white caryopses and correspondingly tiny embryos, while *E. esculenata* has brownish caryopses and longer pedicels.

Annual *Echinochloa esculenta* is mostly grown in temperate areas of Japan, Korea, China, Russia, and Germany (de Wet et al. 1983). Around 4000 years ago

in Japan, it was directly domesticated from barnyard grass (*Echinochloa crusgalli* (L.) Beauv) (Doggett 1989).

From a number of investigations, Yabuno (1962, 1984, 1966, 2001) provided a good grasp of this genus. Two domesticated species, *E. esculenta* (Japanese barnyard millet) and *E. frumentacea* (Indian barnyard millet), are closely related to their wild counterparts, *E. crus-galli* and *E. colona*. Both domesticated species and their predecessors are hexaploid ($2n = 6x = 54$, where $x = 9$) (Yabuno 1962, 1966).

Interspecific hybrids of *E. crusgalli* with *E. esculenta* and *E. colona* with *E. frumentacea* have been determined to have a typical meiotic division with 27 bivalents. Crosses between these two groups are sterile and generate micronuclei, univalents, and laggards throughout the meiotic process. These cytogenetic findings indicate that the cultivated species *E. utilis* and *E. frumentacea* are derived from two different hexaploid wild species, *E. colona* and *E. crusgalli* (Yabuno 1966).

Based on a comparison of their complete chloroplast (cp) genomes, both *E. crusgalli* and *E. colona* comprise 136 genes in their chloroplast genomes. However, phylogenetic studies revealed that *E. colona* diverged from *E. oryzicola* and *E. crusgalli* between 2.65 and 3.18 million years ago, respectively (Guo et al. 2017). Based on indication of awn or awnless trait, minute differences in the spikelets, and glume morphology differentiated Japanese and Indian barnyard millet (de Wet et al. 1983). In contrast to *E. esculenta's* big, typically awned spikelets with chartaceous upper glumes and lower lemma, *E. frumentacea* bears smaller, awnless spikelets with membranous glumes.

Echinochloa in temperate East Asia has been grouped into three classified polyploid categories:

Group	Species	Ploidy
E. oryzicola complex	Two forms of *E. oryzicola*, *E. persistentia* (non-shattering form of *oryzicola*), and cultivated forms of *E. oryzicola* and *E. phyllopogon*	Allotetraploid
E. crus-galli complex	*E. crus-galli* var. *crus-galli*, *E. crus-galli* var. *praticola*, *E. crus-galli* var. *formosensis*, *E. oryzoides*, *E. esculenta* (Japanese barnyard millet), and Lijiang millet (a cultivated form from China)	Allohexaploid
Echinochloa colonum complex	*E. colonum* and *E. frumentacea* (Indian barnyard millet)	Allohexaploid

23.1.2 Cytogenetics

The genus *Echinochloa* reflects an estimated 25 or more perennial or annual species, majority of those are weeds in paddy fields. In this species, the fundamental chromosomal number is $x = 9$. The diploid *E. obtusiflora* Stapf ($2n = 2x = 18$), the tetraploid *E. oryzicola* Vasing ($2n = 4x = 36$), and the hexaploid *E. untilis* Ohwi ($2n = 6x = 54$) are polyploid species in this genus. Yabuno (1962) documented inter-specific polyploidy in a number of cytotypes ($2n = 4x = 36$, $6x = 54$, $7x = 63$,

$12x = 108$, and $14x = 126$). The Japanese barnyard millet (*E. esculenta*) and Indian barnyard millet (*E. frumentacea*) are closely linked to the wild relatives of the barnyard millet species *E. crusgalli* and *E. colona*. Like their domesticated relatives, these species are hexaploids with the basic chromosomal number $x = 9$ ($2n = 6x = 54$).

With the formation of 27 bivalents, the interspecific crossings between the domesticated species (*E. crusgalli* × *E. esculenta* and *E. colona* × *E. frumentacea*) and their wild relatives displayed typical meiotic behavior. Crosses between cultivated ones and their progenitors, on the contrary, exhibited meiotic abnormalities (formation of univalents, laggards, and micronuclei). Based on cytological evidence, *E. crusgalli* and *E. colona* are presumably the progenitors of *E. esculenta* and *E. frumentacea*.

The wild species is allohexaploid and shares two of its three genomes with *E. crusgalli* due to the 18 bivalents and 9 univalents resulting from crossing *E. crusgalli* and *E. oryzoides*. This demonstrates that the tetraploid *E. oryzoides* and the *Echinochloa* species that have not yet been named spontaneously hybridized to produce *E. crusgalli* (Yabuno 1966, 1984; Sood et al. 2015). These results were also validated by using DNA sequences from the chloroplast and internal transcribed spacer (Ye et al. 2014).

23.2 Botanical Description

Barnyard millet is extensively distributed in the high-temperature regions around the world due to its profound adaptability. By the heat of summer, it can exceed an altitude of 2000 m above mean sea level (Gupta et al. 2009).

It is a hardy annual crop plant that can attain the heights of 220 cm. Compared to other small millets, it develops rapidly, has a relatively short growth period, and finishes the life cycle within 45–60 days (Sood et al. 2015). It could take a little longer to mature in the northern hill habitat. The leaf blades are flat, broad, and ligule-free. The inflorescence is a terminal panicle which is 10–25 cm long, is composed of dense racemes with 3–4-mm-long spikelets (Sood et al. 2015) and it can be compact, intermediate, or open in size, color (green, light purple, and dark purple), and shape (cylindrical, pyramidal, globose to elliptic) (Renganathan et al. 2017; Kuraloviya et al. 2019). The inflorescence seldom droops and is typically upright. Racemes differ in size from a few to many (22–64 per inflorescence), are full of numerous spikelets arranged in four arbitrary rows on the triquetrous rachis, and are freely or strongly attached to the rachis (Renganathan et al. 2017). The spikelets vary from green to brown to purple and are positioned on one side or all around the rachis of the raceme. Spikelets are two-flowered, awnless or awned, and consist of red or green awns on short, rough pedicels supported by two glumes (Sood et al. 2015).

The flower is having lower floret with five-veined sterile lemma and tiny palea whereas the upper floret is bisexual and consists of fertile lemma which is plano-convex, elliptic margins are enrolled below over palea with apex of palea not

enclosed (Gupta et al. 2010). The surface texture of palea is comparable to that of a fertile lemma (Napper 1965). The superior ovary features two distinct styles and a plumose stigma, whereas there are three stamens (Gupta et al. 2011). The grain is 1–2 mm broad and 2–3 mm long, and it is encased in a palea and lemma that are both hardened and white-shiny.

Flowering is in basipetal order, where the arrangement of flowers begins at the top of the inflorescence and progresses downward, takes approximately 10–15 days to complete. Anthesis takes place from 5 to 10 a.m., with the majority of the flowers open between 6 and 7 a.m. and close at 10 a.m. (Gupta et al. 2011). Flowering in an individual raceme originates from the periphery and progresses to the center. The flowers are hermaphrodite. Before the anthers dehisce, the stigmatic branches expand and the flower opens (Seetharam et al. 2003). Late-season florets are cleistogamous (not opening) (Sood et al. 2015). It is mainly self-pollinated and self-compatible, but few chances of outcrossing may occur owing to wind pollination (Sood et al. 2015) and the emergence of stigmatic branches prior to anther dehiscence allows for some cross-pollination (Seetharam et al. 2003). In both cultivated species, hot water treatment of inflorescence at 48 °C for 4–5 min was shown to be efficient to induce male sterility, particularly under hill conditions.

Compared to kodo millet and foxtail millet, barnyard millet seeds are less tough. A thorough investigation is needed to address the issue of seed dormancy, which is a key limiting factor in the production of tiny millets. According to Song et al. (2015), deep physiological dormancy in *E. crus-galli* grain is one of the most probable reasons for the long-term persistence of seed dormancy.

Furthermore, the innate dormancy of generated *Echinochloa* species hampers seed assessment and multiplication in germplasm conservation centers (Renganathan et al. 2020). Different dormancy-breaking techniques, including light, dark, cold, heat, or a mix of these, can be used (Renganathan et al. 2020). It has been demonstrated that application of 100 ppm of IAA (indoleacetic acid) to seedlings enhances the percentage of germination as well as germination speed and vigor.

Echinochloa species, classified as a short-day plant (Muldoon 1985), shows photoperiodism and the diverse photoperiod ranges from short days (8–13 h) to long days (16 h) generate various outcomes (Gupta et al. 2011). Such different performance with variable flowering time with hindered uniformity in grain yield has been observed in CO (Kv) 2 (Vanniarajan et al. 2018). To overcome this, MDU 1, a short-duration variety with stable yield performance, has been developed.

23.2.1 General Morphology of Barnyard Millet

Traits	Characteristics
Common name	Sawa millet/billion dollar grass/sava millet
Habitat	Annual crop, warmer region, Himalayan region in the North to Deccan plateau in the south

(continued)

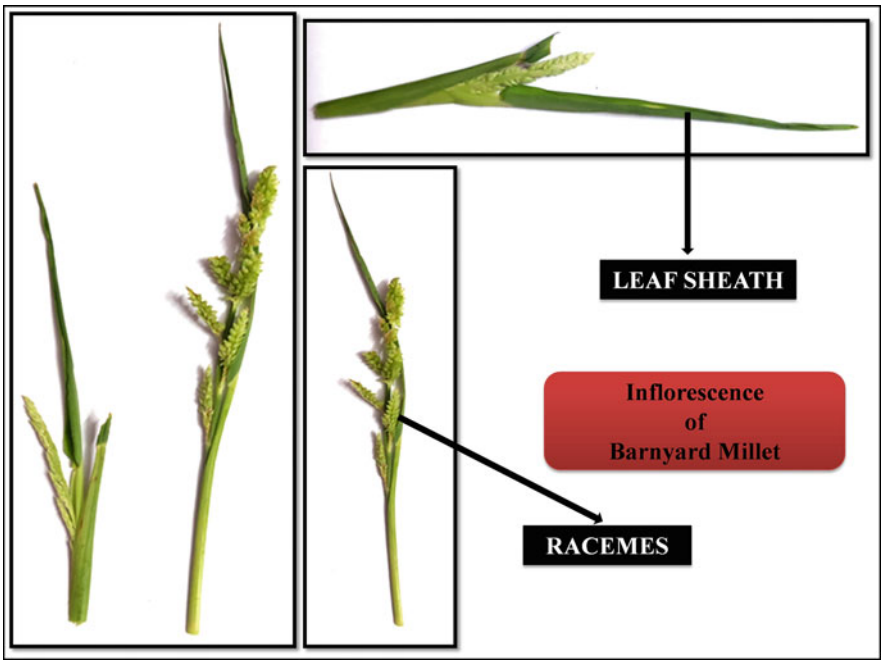

Fig. 23.1 Barnyard millet inflorescence

Traits	Characteristics
General morphology	Erect, 242 cm tall, leaf length 15–40 cm long and 1–2.5 cm wide, plants mostly green, moreover purple tings also present in both/either reproductive and vegetative parts. Culms being slender to robust, while leafblades are smooth and glaborous
Inflorescence morphology	Green to purple, erect and compact, 1–28 cm long inflorescence Racemes—numerous 20–70 in number, 1–3 cm long, rarely drooping and awnless
Spiklets on panicle	Spikelets on the rachis are compact and non-branched and are firmly grouped, 2–4 mm long, acute and awnless. It consists of 2 florets
Lemma and Palea	Lower floret: sterile lemma with tiny palea Upper floret: Bisexual, shiny lemma partially encloses with palea
Stamen and pistil	Stamen: 3 in number, fertile lemma and palea varying from white to dark purplish color Pistil: Bifid, plumose varying from white to dark purple
Caryopsis	Turgid and whitish
Seed dormancy	Physiological dormancy (seeds of both wild and cultivated species)
Seed shattering	High

See Figs. 23.1, 23.2, and 23.3.

Fig. 23.2 Different colors of Barnyard millet inflorescence

23.2.2 Anthesis and Pollination

The upper raceme of the flower opens first, and flowering occurs from the top of the inflorescence to the bottom. Under the conditions of the hills, the panicle emerges in 10–14 days and finishes flowering in 15–20 days. Six to eight days after flowering, the majority of the florets open (Bhinda et al. 2023). The majority of flower openings occur between 6 and 7 a.m., while flowers are open from 5 to 10 a.m. (Bhinda et al. 2023). Flowering within a single raceme first begins at both marginal ends, moves to the center, and finally finishes. Prior to the anthers dehiscing, the stigmatic branches extend and the flower develops (Seetharam et al. 2003). The blossom shuts in 30 min.

The total genome size is predicted to be 1.4 gigabases (Bennett et al. 1998, 2000). According to Schlegel (2010), the average diploid 2C DNA content of barnyard millet is 2.65–2.7 pg bot agr.

23.2.3 Germplasm Resources and Utilization

Genetic resources are the fundamental building blocks for genetic improvement in any crop. The preservation of the germplasm is utmost essential. Ex situ conservation is perhaps the most prevalent approach for conserving millet genetic resources.

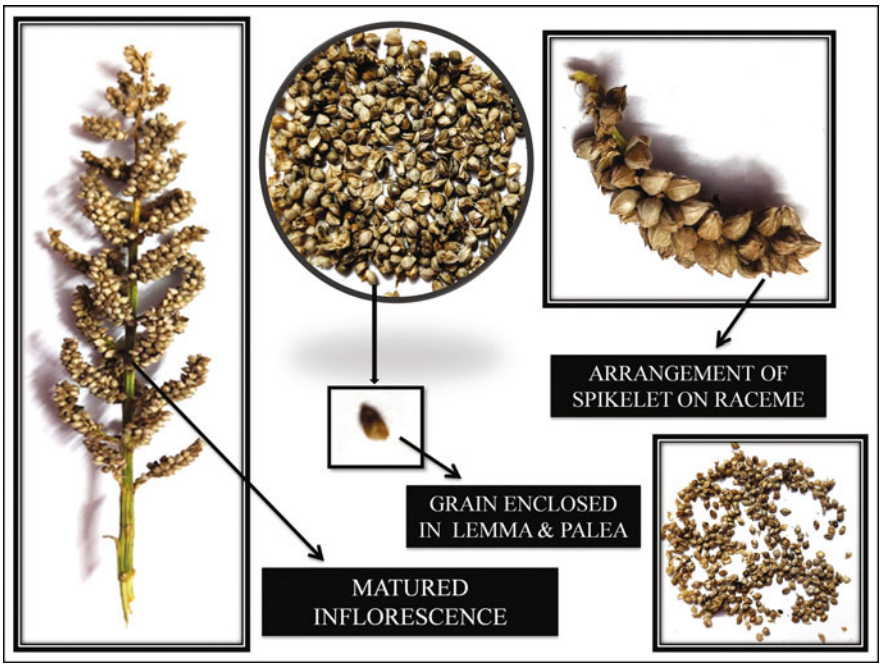

Fig. 23.3 Structure of matured barnyard millet inflorescence

Over 8000 barnyard millet accessions have been collected and conserved globally (Padulosi et al. 2009). The Consultative Group on International Agricultural Research (CGIAR) possesses the world's biggest ex-situ collection of barnyard millet, with 2365 accessions. The National Bureau of Plant Genetic Resources in India has the biggest barnyard millet collection, with 1677 accessions (Vetriventhan et al. 2020). The University of Agricultural Sciences in Bangalore, Karnataka, has 988 barnyard millet accessions as part of the All India Coordinated Small Millet Improvement Project. Barnyard millet has been represented by 749 active collections and 487 base collections at ICRISAT (Upadhyaya et al. 2008). Furthermore, the US Department of Agriculture lists 306 barnyard millet accessions in its National Plant Germplasm System (GRIN). Table 23.1 lists the major gene banks that are preserving barnyard millet.

For crop breeders to use germplasm, it must be characterized in addition to being collected, stored, and revived. It is crucial to collect barnyard millet before it goes extinct because accessions of the *laxa* race, which is unique to the Indian state of Sikkim, are underrepresented in ex-situ collections (Upadhyaya et al. 2014). The gathered materials' phenotypic analysis revealed that the barnyard millet germplasm is quite varied (Nirmalakumari and Vetriventhan 2009; Upadhyaya et al. 2016). Core collections encompassing 56 and 89 accessions, respectively, were produced by Gowda et al. (2009) and Upadhyaya et al. (2014) for the best and most accurate use of diversity for agronomic and nutritional development.

Table 23.1 Status of some significant germplasm collection of barnyard millet

Institute	Location	Number of accessions
		Barnyard millet
Department of Genetic Resources, National Institute of Agrobiological Sciences	Kannondai, Japan	3671 (3603 cultivated; 68 wild)
National Bureau of Plant Genetic Resources	Delhi, India	1888
Indian Institute of Millet Research	Hyderabad	1561
All India Coordinated Small Millets Improvement Project, University of Agricultural Sciences	Bengaluru, India	985
International Crops Research Institute for the Semi-Arid Tropics	Patencheru (Hyderabad), India	749
Institute of Crop Science, Chinese Academy of Agricultural Sciences	China	717
National Centre for Genetic Resources Conservation	Fort Collins, Colorado, USA	306
North Central Regional Plant Introduction Station, Ames	USA	304
Vivekananda Parvatiya Krishi Anusandhan Sansthan	Almora	300
USDA Agricultural Research Service, Washington	USA	232
National Gene Bank of Kenya, Crop Plant Genetic Resources Centre	Muguga, Kenya	208 (192 cultivated; 16 wild)
International Livestock Research Institute, Addis Ababa	Ethiopia	92
Tamil Nadu Agricultural University	Coimbatore, India	–
Australian Plant Genetic Resource Information Service	Biloela, Australia	66
Plant Germplasm Institute, Kyoto University	Japan	65
Plant Genetic Resources Program, Islamabad	Pakistan	50
Svalbard Gene Bank, Spitsbergen	Norway	44
Millennium Seed Bank Project, Seed Conservation Department, Royal Botanic Gardens, London	United Kingdom	44
Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben	Germany	36

Source: http://apps3.fao.org/wiews/germplasm_report.jsp?

Halaswamy et al. (2001) selected and characterized promising accessions for increased plant height (seven accessions), larger number of basal tillers (nine accessions), taller inflorescence (10 accessions), and early flowering (27 accessions). Nirmalakumari and Vetriventhan (2009) discovered two new lines, IEC 566 and IEC 566/2, that vary from cultivated varieties in various ways, including the generation of numerous pollen grains and flowers which remain open for a long period with the stigma projecting fully so as to allow emasculation and pollination. The

identification of Japanese barnyard millet cultivar PRJ 1 (2003) from Uttarakhand hills, a direct selection from ICRISAT germplasm, is believed to have the greatest influence on barnyard millet germplasm utilization in India (Gomashe 2017). PRJ 1 produced 40% more than the Indian barnyard millet control genotypes (Upadhyaya et al. 2008). In general, Japanese barnyard millet yields more than Indian barnyard millet in the hills; but, if sown late in the hills, the Japanese barnyard millet crop fails totally, whereas Indian barnyard millet yields some even under late-sown conditions. Similarly, an easy dehulling accession B29, found in Uttarakhand hills accessions and registered with NBPGR under the number INGR09023, had a 40% and 140% greater dehulled grain recovery than the control varieties VL 172 and PRJ 1, respectively (Gupta et al. 2009). GP 70 A, GP 90 A, GP 110, GP 117, and PRJ 1 are barnyard millet germplasm accessions exhibiting tolerance to grain smut. IIMR, Hyderabad (2016) analyzed 146 barnyard millet accessions and observed a greater variability in grain production and yield contributing factors, leading to the discovery of 18 potential barnyard millet breeding accessions.

Recently, MDU 1, a short-duration (<100 days), high-yielding barnyard millet with pyramidal inflorescence and incurved compact raceme has been developed at TNAU that yields on an average 1700 kg/ha and 2500 kg/ha grain under rain-fed and irrigated condition respectively (Nirmalakumari et al. 2009). Apart from that, the variety exhibits greater iron content (16 mg/100 g) in the grains with good milling and cooking quality.

There is a requirement for a detailed description and evaluation of the available germplasm preserved in national gene banks, particularly the wild weedy species, in barnyard millet. It aids in identifying trait-specific germplasm, especially for grain production, insect pest and disease resistance, and quality characteristics, which may then be utilized in broadening the crop's genetic basis.

Over several years, 729 accessions of *E. frumentacea* (Roxb.) Link. were examined for yield and yield characteristics in Barnyard millet, and a core set of 50 accessions was created (Gowda et al. 2009) (Table 23.2).

In barnyard millet, gene flow mainly occurs through intraspecific hybridization. Maun and Barrett (1986) reported a high degree of self-fertilization among barnyard millet population. The information on interspecific hybridization in case of barnyard millet is very meager. Inter-specific hybrids for early maturity and high yields were developed using *E. frumentacea* (early maturing) and *E. esculenta* (high yielding) but were found sterile both ways (Mehta et al. 2005). Similarly, sterile hybrids were obtained when inter-specific hybridization was carried out between Japanese and Indian Barnyard millet for grain smut resistance (Sood et al. 2014).

In India, Uttarakhand and Tamil Nadu are the major states involved in the breeding of barnyard millet, and most of the varieties have been developed through mass selection and pure-line selection followed by hybridization and pedigree selection. In *Echinocholoa*, gamma radiation has been used to develop mutants for various traits, including grain yield, plant height, tillers per plant, and head length and waxiness (Kumar et al. 2016).

Table 23.2 Trait-specific barnyard millet germplasm identified in the Indian program

Traits	Promising genotypes	Reference
Grain smut, head smut, sheath blight	HCBM 1016/HCBM 1018, HCBM1019, HCBM1020	NBPGR Annual Report (2012)
Grain smut	TNAU 25, TNAU 63, VL 10, PRJ1, PRB 903, DHBMV 56-6, DHBMV93-3, RBM 82, RBM 45, RBM 78, RBM 85	NBPGR Annual Report (2012; 2013)
Shoot fly	VL 207, VL 172, TNAU 151, TNAU 155, KOPBM 46	NBPGR Annual Report (2012)
Days to 50% flowering	IEC 336, IEC 343, IEC 344, IEC 330, IEC 217, JEC 340 and JEC 338, IC257799	NBPGR Annual Report (1986; 1987)
Days to maturity	IEC 157, IEC 159, IEC 71, IEC 78, IEC 257, IEC 271, IEC 158, IEC 156, IEC 282, IC426595, IC436926, IC426594, IC257799	NBPGR Annual Report (2011–2012)
Basal tillers	IC257799, IC24848, VL 29, IC52701, IC338896	NBPGR Annual Report (2011)
Length of inflorescence	IC426595, IC97034, VL. 29, IC97031	NBPGR Annual Report (2011)
Width of inflorescence	IC426594	NBPGR Annual Report (2011)
1000 seed weight	IC326725, KLI	NBPGR Annual Report (2011)
Yield per plant	IC97034, VL 29 IC338960, IC97031	NBPGR Annual Report (2011)
Ear head length	IC548696, IC548635, IC548697	NBPGR Annual Report (2011)
Plant height	IC548681, IC548696, IC548658	NBPGR Annual Report (2011)
100 seed weight	IC548641, IC538089, IC548697	NBPGR Annual Report (2011)

References

Bennett MD, Leitch IJ, Hanson L (1998) DNA amounts in two samples of angiosperm weeds. Ann Bot (Lond) 82:121–134. https://doi.org/10.1006/anbo.1998.0785

Bennett MD, Bhandol P, Leitch IJ (2000) Nuclear DNA amounts in angiosperms and their modern uses—807 new estimates. Ann Bot (Lond) 86:859–909. https://doi.org/10.1006/anbo.2000.1253

Bhinda MS, Joshi DC, Parihar M, Meena RP, Joshi P, Gupta A et al (2023) Genetics, breeding, and genomics of Indian barnyard millet (Echinochloa frumentacea): status and perspectives. In: Neglected and underutilized crops. Academic, pp 115–135

de Wet JMJ, Prasada Rao KE, Mengesha MH, Brink DE (1983) Domestication of mawa millet (Echinochloa colona). Econ Bot 37(3):283–291. https://doi.org/10.1007/BF02858883

Doggett H (1989) Small millets—a selective overview. In: Seetharam A, Riley KW, Harinarayana G (eds) Small millets in global agriculture. Oxford & IBH, New Delhi, pp 3–18

Gomashe SS (2017) Barnyard millet: present status and future thrust areas. In: Millets and sorghum. Wiley, pp 184–198. https://doi.org/10.1002/9781119130765.ch7

Gowda J, Bharathi S, Somu G, Krishnappa M, Rekha D (2009) Formation of core set in barnyard millet [Echinochloa frumentacea (Roxb.) Link] germplasm using data on twenty four morpho-agronomic traits. *Int J Plant Sci* **4**:1–5

Guo L, Qiu J, Ye C, Jin G, Mao L, Zhang H et al (2017) Echinochloa crus-galli genome analysis provides insight into its adaptation and invasiveness as a weed. Nat Commun 8(1):1031

Gupta A, Mahajan V, Kumar M, Gupta HS (2009) Biodiversity in the barnyard millet (Echinochloa frumentacea Link, Poaceae) germplasm in India. Genet Resour Crop Evol 56(6):883–889

Gupta A, Joshi D, Mahajan V, Gupta H (2010) Screening barnyard millet germplasm against grain smut (Ustilago panici-frumentacei Brefeld). *Plant Genet Resour* 8:52–54. https://doi.org/10.1017/S1479262109990141

Gupta A, Sood S, Agrawal PK, Bhatt JC (2011) Floral biology and pollination system in small millets. Eur J Plant Sci Biotechnol 6:81–86

Halaswamy BH, Srinivas GV, Ramakrishna BM, Magar VK, Krishnappa M, Gowda J (2001) Characterization and preliminary evaluation of national collections of Barnyard millet (*Echinochloa* spp.) germplasm. *Indian J Plant Genet Resour* **14**:213–216

Kellogg EA (2015) Flowering plants, Monocots: Poaceae, vol 13. Springer

Kumar S, Dikshit N, Singh M, Rana JC (2016) Foxtail and barnyard millets. In: Broadening the genetic base of grain cereals. Springer, pp 257–275

Kuraloviya M, Vanniarajan C, Vetriventhan M, Babu C, Kanchana S, Sudhagar R (2019) Qualitative characterization and clustering of early-maturing barnyard millet (*Echinochloa* spp.) germplasm. *Electron J Plant Breed* 10:535. https://doi.org/10.5958/0975-928x.2019.00067.x

Maun MA, Barrett SCH (1986) The biology of Canadian weeds. *Echinochloa crus-galli* (L.) Beauv. Can J Plant Sci 66:739–759

Mehta H, Tyagi PC, Mohapatra KP (2005) Genetic diversity in barnyard millet (Echinochloa frumentacea Roxb.). Indian J Genet Plant Breed 65(04):Article 04

Muldoon DK (1985) Summer forages under irrigation I. Growth and Developmet. Australlian Journal of Experimental Agriculture 25:402–410

Napper DM (1965) *Grasses of tanganyaika: with keys for identification*. In: *Bull. Min. Agric. For*. Ministry of Agriculture, Forest and Wildlife), (Tanzania, p 146

Nirmalakumari A, Vetriventhan M (2009) Phenotypic analysis of anther and pollen in diversified genotype of barnyard millet (*Echinochloa frumentacea*) floral characters. *ICFAI Univ J Genet Evol* **2**:12–16

Nirmalakumari A, Subramanian A, Sumathi P, Senthil N, Kumaravadivel N, Joel AJ et al (2009) A high yielding kudiraivali variety CO (KV) 2. Madras Agric J 96:319–321

Padulosi S, Mal B, Bala Ravi S, Gowda J, Gowda KTK, Shanthakumar G, Yenagi N, Dutta M (2009) Food security and climate change: role of plant genetic resources of minor millets. *Indian J Plant Genet Resour* **22**:1–16

Renganathan VG, Vanniarajan C, Nirmalakumari A, Raveendran M, Thiyageshwari S (2017) Cluster analyses for qualitative and quantitative traits in barnyard millet *Echinochloa frumentacea* (Roxb.Link) germplasm. *Bioscan* 12:1927–1931

Renganathan VG, Vanniarajan C, Karthikeyan A, Ramalingam J (2020) Barnyard millet for food and nutritional security: current status and future research direction. Front Genet 11. https://doi.org/10.3389/fgene.2020.00500

Schlegel RHJ (2010) *Dictionary of Plant Breeding*, 2nd edn, 422. CRC Press, Taylor and Francis Group, Boca Raton - London - New York

Seetharam A, Gowda J, Halaswamy JH (2003) Small millets. In: Chowdhury SK, Lal SK (eds) *Nucleus and Breeder Seed Production Manual*. Indian Agricultural Research Institute), (New Delhi, pp 54–67

Song BY, Shi JX, Song SQ (2015) Dormancy release and germination of *Echinochloa crus-galli* grains in relation to galactomannan-hydrolysing enzyme activity. *J Integr Agric* 14:1627–1636. https://doi.org/10.1016/S2095-3119(14)60940-0

Sood S, Khulbe RK, Gupta AK, Agrawal PK, Upadhyaya HD, Bhatt JC (2015) Barnyard millet—a potential food and feed crop of future. Plant Breed 134(2):135–147. https://doi.org/10.1111/pbr.12243

Upadhyaya HD, Gowda CLL, Sastry DVSSR (2008) Plant genetic resources management: collection, characterization, conservation and utilization. *J SAT Agric Res* **6**:1–15

Upadhyaya HD, Dwivedi SL, Singh SK, Singh S, Vetriventhan M, Sharma S (2014) Forming core collections in barnyard, kodo, and little millets using morphoagronomic descriptors. Crop Sci 54(6):2673–2682

Upadhyaya HD, Vetriventhan M, Dwivedi SL, Pattanashetti SK, Singh SK (2016) Proso, barnyard, little, and kodo millets. In: Singh M, Upadhyaya HD (eds) *Genetic and Genomic Resources for Grain Cereals Improvement*. Academic Press, (Cambridge, MA, pp 321–343

Vanniarajan C, Anand G, Kanchana S, Arun Giridhari V, Renganathan VG (2018) A short duration high yielding culture - Barnyard millet ACM 10145. *Agric Sci Dig A Res J* 8:123–126. https://doi.org/10.18805/ag.D-4574

Vetriventhan M, Azevedo VCR, Upadhyaya HD, Nirmalakumari A, Kane-Potaka J, Anitha S, Ceasar SA, Muthamilarasan M, Bhat BV, Hariprasanna K, Bellundagi A, Cheruku D, Backiyalakshmi C, Santra D, Vanniarajan C, Tonapi VA (2020) Genetic and genomic resources, and breeding for accelerating improvement of small millets: current status and future interventions. Nucleus 63(3):217–239. https://doi.org/10.1007/s13237-020-00322-3

Yabuno T (1962) Cytotaxonomic studies on the two cultivated species and the wild relatives in the genus *Echinochloa*. Cytologia 27(3):296–305. https://doi.org/10.1508/cytologia.27.296

Yabuno T (1966) Biosystematic study of the genus *Echinochloa*. *Jpn J Bot* **19**:277–323

Yabuno T (1984) A biosystematic study on Echinochloaoryzoides (Ard.) Fritsch. Cytologia 49(3):673–678. https://doi.org/10.1508/cytologia.49.673

Yabuno T (1987) Japanese barnyard millet (Echinochloa utilis, poaceae) in Japan. Econ Bot 41(4):484–493. https://doi.org/10.1007/BF02908141

Yabuno T. 2001. [Taxonomy and phylogeny of the genus *Echinochloa*.] In: *Natural History of Genus Echinochloa*, revised edn (ed. by T. Yabuno and H. Yamaguchi). Zennokyo Shuppan, Tokyo, 15–30 (in Japanese)

Ye CY, Lin Z, Li G, Wang YY, Qiu J, Fu F et al (2014) Echinochloa chloroplast genomes: insights into the evolution and taxonomic identification of two weedy species. PLoS One 9(11):e113657

Breeding Barnyard Millet for Abiotic Stress Tolerance

24

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Abstract

Barnyard millet (*Echinochloa* species) is a versatile crop with a rich nutritional profile known for its innate ability to tolerate most abiotic stresses, including drought and high temperatures. Although potential yield losses are inevitable, further improvement is needed to make them more resilient to unprecedented effects of climate change and associated environmental stresses. Hence, strenuous efforts are required to characterize the germplasm resources and identify the specific traits, QTLs, genes and pathways associated with stress tolerance. In addition, understanding the mechanism underlying stress response in *Echinochloa* species may not only be useful to develop superior cultivars but also serve as the reservoir of unique alleles which assist in improving tolerance in major cereal crops. The release of genome and transcriptome sequence of both wild and cultivated barnyard millets will undoubtedly facilitate the dissection of

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genetic architecture behind stress tolerance. However, a major downside is the lack of large-scale genomic resources, research efforts and funding compared to other major millets like sorghum, pearl millet, foxtail millet and finger millet. Thus, with available knowledge on genetic and genomic resources in other major millets and cereals, attempts should be made to improve the stress tolerance of barnyard millet.

Keywords

Barnyard millet · Abiotic stress · salinity · Drought · Mutation breeding

24.1 Introduction

Barnyard millet (*Echinochloa* species) is an age-old crop cultivated generally in both tropical and temperate regions around the globe. It is ranked fourth in small millet production and has been grown for its food and fodder value. The *Echinochloa* genus comprising of 35 species (Mabberley 1997), including weedy forms *E. crus-galli* (barnyard grass), *E. colona* (jungle rice), and cultivated forms such as *E. frumentacea* (Indian barnyard millet) and *E. esculenta* (Japanese barnyard millet), are predominantly grown in Asia, particularly in India, China and Japan (Sood et al. 2015). Owing to their fast growth, rapid maturation and high adaptability, barnyard millet is preferred as fodder in the United States and Japan and can produce as many as eight harvests per year (Sood et al. 2015). It is fermented to make beer in Africa. In India, the crop is grown on 0.146 m ha with a production of 0.151 mt, and Uttarakhand alone contributes 11.5% of the total fodder consumption (IIMR 2018). Barnyard millet matures fast, is able to withstand adverse environmental conditions and the cost of cultivation is low as compared to any major food crops. Despite its agronomic advantage, the *Echinochloa* species possesses a rich nutritional profile. The iron content in barnyard millet grain is about 15.6–18.6 mg/100 g, which is more than the daily recommended dietary allowance of men (8.7 mg) and women (14.8 mg). The protein content of Japanese barnyard millet is twice as high as that of rice (Sood et al. 2015). However, though the crop has a rich nutritional and agronomical value, lack of awareness has made this a neglected or underutilized crop.

The climate changes as a result of global warming, variation in the seasonal rainfall and unpredictable weather condition remind us of the need for the development of climate-resilient crops. The crops have undergone abiotic stresses, viz., drought, salinity, submergence, low and high temperature and metal toxicity, which lead to a yield loss up to 50%. Although the millets have an innate ability to cope with the stresses better than the cereals, additional efforts are required to make them more tolerant and increase their productivity in marginal lands (Muthamilarasan and Prasad 2021). Many breeding approaches are proposed to elucidate the tolerance mechanisms and evidently improve the stress tolerance of the crop species. The integration of the breeding programme with genomic tools will give new insights and hasten the development of crop species tolerant to abiotic stresses. Thus, with available knowledge on genetic and genomic resources in other major millets and

cereals, this chapter attempts to make a comprehensive review of the all-possible mechanisms behind their adaptability and various methods for the further improvement of abiotic stress tolerance in barnyard millet to serve as a better climate-resilient alternative for food and nutritional security.

24.2 Abiotic Stress Tolerance

Crops exposed to the abiotic stress will desiccate the cells, which leads to an array of changes, viz., reduced growth, sterility, poor grain filling and yield. However, millets have exceptional adaptation to stress by enunciating different adaptive strategies than the staple food crops (Numan et al. 2021). The tolerance to abiotic stresses is complex because the regulation happens at physiological, cellular and molecular levels which leads to the alteration in morphological structure, osmotic potential, differential growth rate and antioxidant defence. The mechanism of stress tolerance exhibited by the barnyard millet identified so far is reviewed as follows.

24.2.1 Drought Tolerance

Barnyard millet is one of the preferred choices for cultivation in unfortunate environmental conditions such as drought and flooding and shows better adaptation than other millets (Fig. 24.1). However, biomass accumulation gets reduced when grown under severe drought and compact soil conditions which limits root elongation and prevents water uptake. Zegada-Lizarazu and Iijima (2005) investigated both water stress extremes of barnyard millet along with some other millet species including pearl millet. The results suggest that pearl millet is tolerant to drought but not the waterlogging. In contrast, the barnyard millet shows stability in both conditions. The

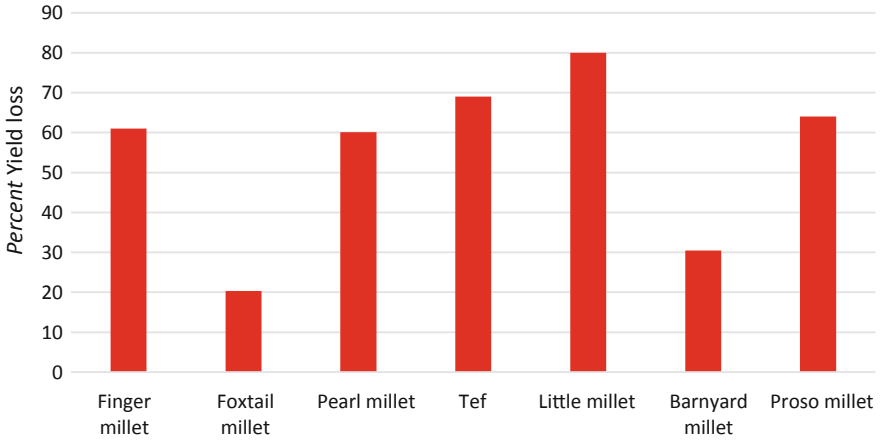

Fig. 24.1 Percent yield loss of various millets (Maqsood and Ali 2007; Matsuura et al. 2012; Numan et al. 2021; Subiramaniyan et al. 2018)

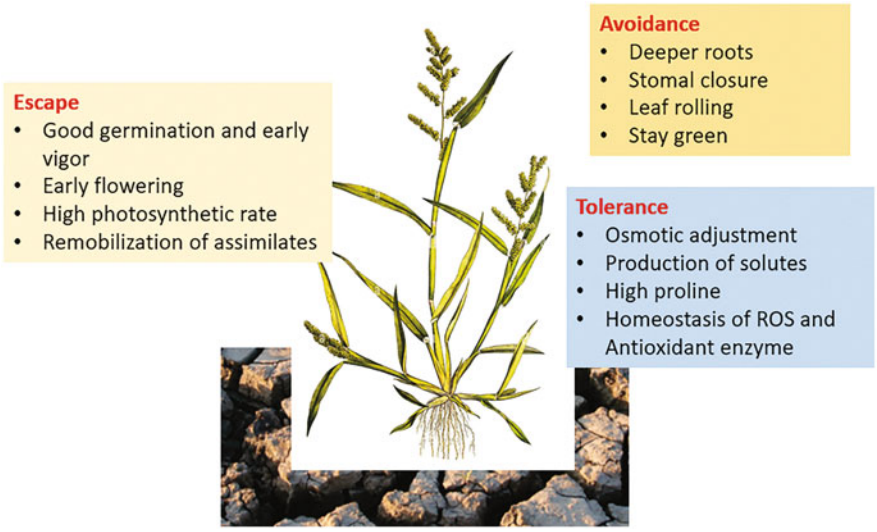

Fig. 24.2 Drought adaption strategy used by the barnyard millet

deuterium content in xylem sap, deep rooting, water use efficiency, leaf area index and dry matter production was maximised in barnyard millet in comparison to pearl millet and other species. The previous study of Zegada-Lizarazu and Iijima (2004) also shows that the finger millet, job tears and barnyard millet have increased their water extraction from deep soil layers when moisture deficit occurs in the topsoil. In addition, drought tolerance is characterized by the instinctive allocation of biomass to roots, resulting in high root/shoot ratio as observed in finger millet (Zhang et al. 2018) and wheat (Tavakol and Pakniyat 2007). This suggests that the tolerance exhibited by the barnyard millet might be due to the specialized rhizosphere organisation to cope up with the stress. Besides morphology, physiological and biochemical traits are considered inevitable to tolerate the adverse climatic conditions. The barnyard millet exhibits better physiological mechanisms like lesser reduction of leaf area, leaf area duration, increased net assimilation rate, nitrate reductase activity and soluble protein for finer adaptation during drought stress at all developmental stages, viz., vegetative, reproductive and maturity, and also have higher recovery percentage after rewatering than the finger millet and little millet (Senthil et al. 2018). This suggests that the tolerance might be due to the extended leaf area duration or slow rate of senescence which ultimately results in high photosynthetic ability and better yields (Fig. 24.2). At low concentration, reactive oxygen species serves as the secondary messengers in cell signalling, whereas their concentration increased during the stress which impairs damage to the cells and their metabolism (Bartoli et al. 2013). However, the damage depends on the equilibrium of reactive oxygen species and antioxidants/antioxidant enzymes at the time of the stress (Mirzai et al. 2013). The increase in MDA, catalase, phenol, flavonoids and proline concentration was observed in finger millet and barnyard millet during drought stress and those accumulate more in roots rather than the stem and leaves (Tiwari et al. 2020). This shows that barnyard millet shows better homeostasis

between the Ros production and antioxidants (Fig. 24.2). Moreover, molecular insights into the transcriptomes of *E. frumentacea* and *E. crus-galli* (weed) under drought stress condition suggests that the Indian barnyard millet is more drought-tolerant than the weedy species, even though both have well-adapted C4 machinery. The comparative transcriptomics make evident that the genes related to higher photosynthetic ability, such as chlorophyll-binding, light harvesting in PS I and PS II and electron transport chain were upregulated in Indian barnyard millet than the *E. crus-galli*. This reveals that barnyard millet uses drought escape strategies such as rapid growth and high photosynthetic ability (Jayakodi et al. 2019). In addition, other reports on major cereals such as wheat (Zivcak et al. 2013) and rice (Zhang et al. 2016) also support that high photosynthesis capacity might be the cause of drought tolerance.

24.2.2 Waterlogging or Submergence Tolerance

Various crops respond differently to the range of different water and temperature stress and a combination of these two stresses. Biomass accumulation decreases in all the crop species during stress conditions rather than the ideal growing environment. The barnyard millet was well adapted to waterlogging (Zegada-Lizarazu and Iijima 2005). But in combination with continuous waterlogging and gradual reduction of soil moisture with low temperatures respectively, the reduction of shoot biomass was observed. The stomatal conductance decreased earlier in low temperatures than high temperatures. This shows that the barnyard millet is temperature-dependant, the slow decrease of stomatal conductance leads to the maintenance of photosynthetic capacity which in turn affects the biomass accumulation (Khanthavong et al. 2022). Generally, the structural variants are associated with the functional variations. Waterlogging causes an anoxic condition which limits the root distribution, respiration and elongation due to the inadequate supply of O_2 to the submerged root. Other cereals like rice adapted to the anaerobic condition by the induction of aerenchyma cells and by forming the barriers to transport the O_2 to root tips without loss. A similar strategy was followed by barnyard millet by inducing the cortical sclerenchyma in their nodal roots. The functions of cortical sclerenchyma have been well understood viz., filtration of excess soil water, defence against microorganisms, prevents oxygen leak from roots and physical support to cortical aerenchyma (Galamay et al. 1991). Khanthavong et al. (2021) also reported that the root distribution pattern and elongation were not much affected under both drought and waterlogging when compared to the other family members of *Poaceae*. The *Echinochloa* spp. are able to germinate and grow under flooding associated with the Hexokinase (HXK) activity. The increase of HXK was hypothesised to maximize the entry of hexose into glycolysis to sustain anoxic condition by anaerobic ATP production (Fox et al. 1998). Besides, the species tolerant to submergence was characterized by the presence of transcription factors like ERF (ethylene response factor) which plays a vital role in coping with the stress. During flooding, the ethylene concentration in plants increases and either induces or prohibits the internode elongation by stimulating *SNORKEL1 (SK1)/SNORKEL2 (SK2)* and

Submergence1 (Sub1). Nah et al. (2015) investigated the differentially expressed genes of *Echinochloa crus-galli* species for submergence tolerance. The 60–70% transcripts of *E. crus-galli* was significantly matched with foxtail millet, indicating that some common genomic regions were conserved among the C4 crops. Unfortunately, the expression of *Sub1* or *Sk* genes was not identified in this study, instead, ERF homologs were found which might be a candidate for submergence tolerance. Moreover, RLk a large gene family involved in abiotic signal transduction was identified. The ERF and RLk are responsible for signal transduction not only in flooding but in numerous abiotic stresses, thus further investigation on these will help to unravel the multiple stress adaptation of barnyard millet.

24.2.3 Salinity Tolerance

Salinity tolerance in crops requires a cooperative act of numerous genes and gene families to administrate the array of physiological and biochemical processes lead to an adaptation. The mechanism of salt tolerance includes regulated plant growth (Passioura and Munns 2000), accumulating osmotic protectants (Munns 2005), scavenging ROS (Foyer and Noctor 2005), ion exclusion or sequestration (Munns and Tester 2008) and maintaining high cytosolic K+/Na^+ ratio (Yamamoto et al. 2003). Conventionally, the crops tolerant up to 1–10 ds/m have been classified as glycophytes and above the threshold of 10 ds/m are classified as halophytes. The majority of millets are classified as glycophytes, which can tolerate about the average thresholds of 6 ds/m; however, it varies from species to species. The *Echinochloa* species are well adapted to saline soils and produce quality biomass (Al Sherif 2007). In addition, the study shows that Indian barnyard millet can tolerate up to 200 mM sodium chloride solution, which is higher than the promising salt-tolerant cultivar FL 478 of rice, which tolerates up to 100 mM NaCl (Arthi et al. 2019). The further investigation of the mechanisms behind this helps to develop the saline-tolerant variety not only in the barnyard millet but also in other major cereals and related species. In addition, Subramanian et al. (2020) investigated the salt-tolerant ability of barnyard millet in both in vivo and in vitro condition suggests that the genotypes MDU 1 and BAR 119 show superior tolerance. However, sensitivity to salinity increases as the intensity of salt stress increases. The tolerant genotypes cope with the stress by scavenging the ROS by producing antioxidant enzymes like catalase and peroxidase (Williams et al. 2019). The Ec required to inhibit the shoot biomass of rice is lower than that of *Echinochloa* species (Chauhan et al. 2013). The *Echinochloa* sps. sustain the salinity stress through the production of more soluble sugars, high photosynthetic rates, high chlorophyll, rubisco activity and production of antioxidant enzymes (Abogadallah and Quick 2009; Abogadallah et al. 2010a, b). Furthermore, increase in proline and polyamines is also associated with the salt tolerance of *E. crus-galli* (Yamamoto et al. 2003). Another study demonstrates that *E. oryzicola* shows a higher saline tolerance than rice. It is capable of limiting the accumulating sodium ions (Na^+) and maintains a constant higher potassium ion (K^+) content than rice (Nguyen et al. 2005). Molecular investigations show that higher expression of GSTs (Glutothione *S*-transferase) is responsible for the saline-alkaline

tolerance of *Echinochloa* species (Wu et al. 2022). Apart from the *Echinochloa* species, a similar mechanism of ionic and redox homeostasis and osmotic adjustment was exhibited by the pearl millet during salt stress (Jha et al. 2022), whereas the foxtail millet was alleviated by decarboxylation mechanism (Shah et al. 2022) and increased energy production. The transcription factors such as bHLH, WRKY, AP2/ERF, and MYB-MYC are involved in the salinity tolerance of foxtail millet (Han et al. 2022). However, the mechanism behind the salt tolerance of barnyard millet was unclear due to the lack of scarce genomic information.

24.3 Metal Toxicity

The soil quality has significantly deteriorated as a consequence of anthropogenic climate change, pollution, and intensive farming, rendering sustainable land usage more difficult compared to earlier. There are several phyto-management techniques for lowering the ecological concerns in lands polluted with trace elements (TE). To reduce the biological impact of trace elements on land as well as to improve plant cover on contaminated soils, phytoextraction or in combination with the use of conditioners might be a solution (Kidd et al. 2015). In recent years, growing non-food crops for the phytoremediation of metal-toxic soils are increasing. However, using food crops like barnyard millet will be advantageous by providing food security as well as helping in purifying soils and also minimizing the acreage of uncultivable lands. Gorelova et al. (2022) studied the heavy metal accumulation capacity of *E. frumentacea* and revealed that they can accumulate Cu and Zn and are not suitable for the bioremediation of Mn, Co, As and Cd and accumulate some V (vanadium) mainly in the roots. Another study suggests that growing *E. frumentacea* with phosphorous solubilizing microorganism and EDTA increases the accumulation of As (Arsenic) in plants (Park et al. 2008). Besides, *E. polystachya* can tolerate up to 174 mg/L of Cd without any symptoms. The high concentration of Cd is accumulated more in root xylem cells than in the leaves however in the leaves, it accumulates in bulliform cells of the epidermis (Solís-Dominguez et al. 2007). The *E. stagnina* can accumulate Pb (lead) in the tissues (Eid et al. 2020). *E. crus-galli* is sensitive to Al (Aluminium) but is tolerant to Cd and Cu (Ezaki et al. 2008). It has been suggested that *Echniochloa* species have a better antioxidant defence system, viz., carotenoid, ascorbic acid and glutathione was increased during the heavy metal stress (Gorelova et al. 2022). The *E. crus-galli* uses phytochelation mechanism (Cd-Bl-cadmium binding ligand) to detoxify the heavy metals (Kim et al. 2009). However, the mechanism behind the heavy metal tolerance of *Echinochloa* sp. is still ambiguous. Furthermore, the desirable plant features required to withstand heavy metal stress was possessed by the *Echinochloa* species *Viz*., rapid growth and high biomass, extended root system, tolerance to high concentration of trace element, efficient translocation mechanism, adaptability to specific environments, easy agricultural management and good interaction with associated bacteria. Moreover, extensive efforts are required to investigate the molecular mechanism behind the *Echinochloa* sp. which uncovers the genes and pathways responsible for heavy metal tolerance and helps in achieving abiotic stress tolerance in plants.

24.4 Breeding for Abiotic Stress Tolerance

24.4.1 Germplasm Characterization and Utilization

Although barnyard millet has an inherent capacity to tolerate abiotic stresses such as drought, salinity and waterlogging, the spectrum of tolerance is genotype-specific. There are about 8000 accessions of barnyard millets conserved in the various centres around the world (Renganathan et al. 2020). The morpho-phenological characterization of the available germplasm resources is the preliminary step, however, it gives the idea of heritability of the traits, identification of the trait-specific donor is important to improve its further tolerance. Arthi et al. (2019) characterize 89 germplasm resources and grouped CO (KV) 2, MDU-1, PRJ1, TNEf 301, TNEf 204, TNEf 361, TNEf 364, VL 29 as saline-tolerant cultivars of barnyard millet. In addition, Williams et al. (2019) studied 32 accession and categorised ACM161, ACM295, ACM335, GECH10, IEc167 as tolerant at biochemical level. In another study, Trivedi et al. (2017) characterize the 178 accessions collected from the central Himalayan region to identify the trait-specific donors for crop improvement. Apart from the morpho-phenological traits, the accessions with a wide range of variation for 18 traits related to biochemical accumulation like lipid peroxidation, total glutathione and total ascorbate content will serve as the potential germplasm stocks for abiotic stress tolerance improvement in barnyard millet.

24.4.2 Mutational Breeding

Autogamy is the strict rule, thus the crop is on an evolutionary dead edge, which limits the development of natural variation. Nevertheless, the crop adapted to the different climatic zones and environments which helped them build better adaptive mechanisms by morphological, physiological, biochemical, genetic and epigenetic changes. However, the variation in the cultivated species is limited. Thus, mutational breeding is the inevitable choice to create novel variation and broadening genetic base which also supplements germplasm resources. Among the members of poaceae, rice, wheat, foxtail millet and finger millet induced mutagenesis is widely used as the pre-breeding technique, though the mutation breeding for improvement of abiotic stress tolerance is rather limited. The study attempts to demonstrate that mutation as a potential tool to improve the salt tolerance of barnyard millet. The wild type germinates up to 200 mM whereas the mutants have germination up to 300 mM NaCl, and it was also found that another mutant sustained its growth up to 25 days in salt stress then the wild type indicates that this mutant imparts vegetative stage salt stress tolerance apart from germination stress (Abogadallah and Quick 2009; Abogadallah et al. 2010a, b).

24.4.3 Crop Wild Relatives and Interspecific Hybridization

Both cultivated and wild forms of Echinochola species tolerant to abiotic stress are given in Table 24.1. The phylogenetic analysis reveals that *E. frumentacea*, *E. crus-galli* and *E. orizycola* were categorized into one cluster, suggesting that these species are closely related (Perumal et al. 2016). The hybridization between wild and its cultivated form *vViz.*, *E. crus-galli*/*E. esculenta* and *E. colona*/*E. frumentacea* shows normal meiotic behaviour (27 bivalents) and produces fertile plants. Besides interspecific hybrids of *E. esculenta*/*E. frumentacea* and *E. crus-galli*/*E. colona*, exhibit irregular meiotic behaviour resulting in sterility (Yabuno 1966, 1984). In addition, the cross-compatibility of these species was confirmed by Yamaguchi et al. (2005). However, hybridization is the preliminary step for any QTL or gene mapping studies and it is the inevitable step in marker-assisted back-cross breeding. For instance, *E. colona* exhibits higher salinity tolerance than the Indian barnyard millet, it belongs to the Gene pool 1 of the Indian barnyard millet (Table 24.1). Hence wide hybridization between them will help to map the genes or QTLs associated with salinity tolerance. Although both cultivated species share same morphology *E. esculenta* has high-yield potential, cold tolerance, and low seed shattering ability, whereas *E. frumentacea* possess wider adaptability, easy threshing and dehusking traits. Besides, it contains antifeedants, which are present at higher concentrations than rice, and it displays resistance against brown planthopper. Sood et al. (2014) attempt to make the interspecific hybrids of PRJ 1 (*E. esculenta*) and ER 72 (*E. frumentacea*). The hybrids are sterile, but they have superior agronomic performance and grain smut disease tolerance than the parents. Besides, they can

Table 24.1 Source of abiotic stress tolerance in barnyard millet

Species	Tolerance	Gene pool	References
E. frumentacea (Indian Barnyard millet)	Drought, salinity	GP1 of *E. colona*	Arthi et al. (2019), Jayakodi et al. (2019), Rocha et al. (2021)
E. crus-galli	Drought, salinity, Submergence/ flooding	GP1 of *E. colona* and Japanese barnyard millet GP3 of Indian barnyard millet	Jayakodi et al. (2019), Nah et al. (2015), Rocha et al. (2021), Chauhan et al. (2013)
E. colona	Salinity	GP1 of Indian barnyard millet	Al Sherif (2007), Rocha et al. (2021)
E. utlis/ *E. esculenta* (Japanese Barnyard millet)	Drought, water-logging	GP1 of *E. esculenta*	Galamay et al. (1991), Khanthavong et al. (2021), Rocha et al. (2021)
E. phyllopogon/ *E. orizycola*	Flooding, Salinity	NA	Fox et al. (1998)
E. polystachya	Cd tolerant	NA	Solís-Dominguez et al. (2007)

be utilized as fodder, but further genetic studies get hampered. The use of either colchicine or bridging species will help to restore its fertility. However, this opens up a new platform for the genetic enhancement and stress tolerance of both species. In another study, Samantaray et al. (2001) demonstrated the development of chromium- and nickel-tolerant cell lines derived from *E. colona*. Nevertheless, the wild relatives cannot be utilized directly in the breeding program, to minimize the undesirable linkages, proper pre-breeding is a pre-requisite to mine the potential of germplasms to improve the cultivated species in terms of yield and stress tolerance.

24.4.4 Genomic-Assisted Breeding

The stress tolerance relies on modulating the genes and genomic regions underlying key traits, either directly or indirectly. Direct approaches include overexpression, RNA interference, genome editing, etc., while breeding majorly constitutes the indirect approach. With the advent of the latest tools and technologies, these strategies could hasten the improvement of crop species. Next-generation sequencing, high-throughput genotyping, precision editing, use of space technology for accelerated growth, etc., had provided a new dimension to crop improvement and was a step forward towards developing better varieties to cope with the challenges. Among the millets, high-quality genome sequences are available for sorghum, pearl millet, foxtail millet and finger millet with well-documented linkage and physical maps, genetic stocks and large-insert libraries (Gomashe 2017; Varshney et al. 2006). Over the past decades, the research effort attempts to understand the features of this crop are rather limited than other major millets. However, in barnyard millet, the genetic information available is scanty. This is mainly due to the lack of reference genome for barnyard millet, whereas its wild relative *E. crus-galli* is only sequenced ($2n = 6x = 36$, 1.27 Gb). Besides, the chloroplast sequence of various *Echinochloa* sp. is available (Lee et al. 2015; Perumal et al. 2016; Sebastin et al. 2019). The core collection of 89 accession of barnyard millet from ICRISAT was characterized by genotype by sequencing. The total of 10,816 SNPs among 65 germplasm of *E. colona* and 8217 SNPs among 22 genotypes of *E. crus-galli* were identified and tended into presumably four clusters within *E. colona* and three within *E. crus-galli*. This reveals the phylogenetic relationship among the core collection and will enhance the utility of germplasm resources for crop improvement (Wallace et al. 2015). Recently, the release of transcriptome sequence of both wild and cultivated barnyard millets enrich the understanding of genetic mechanism behind drought tolerance and micronutrient accumulation (Jayakodi et al. 2019). The molecular markers from the other millet and non-millet species show more than 55% cross-genera transferability (Table 24.2) which emphasises their utility and enrichment of genomic information in barnyard millet. Additionally, the study shows that high synteny was found between rice and barnyard millet for traits like panicle length, spikelet and yield-associated traits, leaf senescence, BPH and blast tolerance and amylose content (Babu et al. 2018b). This provides the scope for the improvement of stress-related traits in barnyard millet with available knowledge on

Table 24.2 Cross-genera transferability of molecular markers from other species to barnyard millet

Crop	Type of marker	Cross-genera transferability percentage	Reference
Maize and finger millet	64 SSR	61	Babu et al. (2018a)
Foxtail millet	EST	90	Kumari et al. (2013)
Foxtail millet	SSR	91	Pandey et al. (2013)
Foxtail millet	microRNA based	89	Yadav et al. (2014)
Foxtail millet	SSR	65	Krishna et al. (2018)
Finger millet	SSR	55	Krishna et al. (2018)
Finger millet	SSR	100	Babu et al. (2018b)
Rice	SSR	71	Babu et al. (2018b)
Foxtail millet	ILP	94	Muthamilarasan et al. (2014)
Foxtail millet	SSR	74	Gupta et al. (2013)

SSR simple sequence repeats, *EST* expressed sequence Tags, *ILP* intron length polymorphic markers

related genera. It is shown that foxtail millet markers have more than 65% cross-genera transferability, thus there is a possibility of more synteny between foxtail millet and barnyard millet. The genetic information associated with the stress response in foxtail millet will aid in uncovering the adaptive strategy of barnyard millet. For instance, an SNP in the DREB (dehydration response element binding) gene was found to be associated with the stress response in foxtail millet (Lata et al. 2011). This SNP is validated and accounts for about 27% variance of stress-induced lipid-peroxidation and thus it can be potentially used in marker-assisted breeding to develop the stress-tolerant cultivar (Lata and Prasad 2014). However, extensive efforts should be needed to unravel the hidden potential of stress tolerance in barnyard millet.

24.4.5 Genome Editing

CRISPR/Cas9 and CRISPR/Cpf1 systems have been successfully adapted for genome editing in numerous crop plants (Osakabe et al. 2016) for climate-resilient traits like biotic and abiotic stress tolerance and yield improvement (Jaganathan et al. 2018). The proper selection of the target gene is the most decisive step to achieving the desirable trait. Targeted trait improvement for abiotic stress tolerance can be achieved by focusing on two groups of genes, viz., structural and functional genes (Table 24.3). Moreover, *Cis*-acting regulatory elements (Table 24.3) also play a vital role in abiotic stress tolerance (Zafar et al. 2020). Mostly, CRISPR application is focused mainly on insect and pest resistance whereas the abiotic stress tolerance is rather limited. However, CRISPR/Cas9 system employed for abiotic stress

Table 24.3 Candidate gene identified for stress tolerance in both millet and non-millet species

Crop	Gene	Trait	Reference
Pearl millet	VDAC (voltage-dependent anion channel)	Salt stress	Desai et al. (2006)
	Hsp 70 and HSP 90	Abiotic stress	Reddy et al. (2009)
	Glutathione reductase	Abiotic stress	Achary et al. (2015)
	Dehydroascorbate reductase	Abiotic stress	Pandey et al. (2014)
	LEA (late embryogenesis abundant)	Heat and salt stress	Reddy et al. (2012)
Finger millet	Ecdehydrin 7	Drought and heat	Singh et al. (2015)
Finger millet Sorghum	LEA14, rd29A, rd29B, SOD, APX, ADH1, HSP70 and PP2C	Multiple stress	Babitha et al. (2015)
Finger millet Rice	Vascular ATP synthase genes	Salinity	Rahman et al. (2014)
Finger millet Rice Maize	*BiP*, *PDIL*, and *CRT1*	Salinity	Ramakrishna et al. (2018), Liu and Howell (2010)
Finger millet Rice	*EcTAF6*	Drought and salinity	Parvathi et al. (2019)
Finger millet Maize	*EcGBF3*	Drought and salinity	Ramegowda et al. (2017)
Finger millet	*Threonine dehydratase mRNA*	Drought	Hittalmani et al. (2017)
Foxtail millet	Argonaute protein 1 encoding gene	Stress	Liu et al. (2016)
	Abscisic acid stress ripening gene (ASR)	Drought Oxidative stress	Feng et al. (2016)
	Autophagy-related gene (ATG)	Drought	Li et al. (2015)
	ABA-responsive DRE-binding protein (ARDP)	Salt and drought	Li et al. (2014)
	WD-40	Dehydration	Mishra et al. (2012)
	NAC transcription factor	Salinity tolerance	Puranik et al. (2011)
	Phospholipid hydroperoxide glutathione peroxidase (PHGPX)	Salinity tolerance	Sreenivasulu et al. (2004)
	Si69	Aluminium tolerance	Zhao et al. (2009)
Rice	OsDIS1, OsiSAP7, OMTN2, OMTN3, OMTN4, OMTN6	Drought	Sharma et al. (2015), Fang et al. (2014), Ning et al. (2011)

(continued)

Table 24.3 (continued)

Crop	Gene	Trait	Reference
	OsCDPK7, NF-Y18, Arginine decarboxylase (ADC), CIPK12	Drought	Soltani Najafabadi (2012), Capell et al. (1998)
Wheat	WRKY mRNA, TaWRKY146	Drought	Satapathy et al. (2018), Ma et al. (2017)

improvement is demonstrated in some major crops such as cotton (Chen et al. 2017), maize (Char et al. 2017; Svitashev et al. 2016), rice (Miao et al. 2013; Shan et al. 2014) and wheat (Shan et al. 2014). However, apart from other cereals like rice, wheat and maize, genetic transformation in millets falls behind. Few reports were available in millets species regarding genetic transformation. For instance, Hema et al. (2014) targeted the mtlD gene which imparts drought and salinity tolerance in finger millet. The transgenic foxtail millet, which possesses SiARDP (Li et al. 2014), SiASR4 (Li et al. 2017), SiLTP (Pan et al. 2016) and SiLEA14 (Wang et al. 2014) genes, imparts tolerance to drought, salinity and other multiple stresses. However, any successful transformation or genome editing requires an efficient transformation system and regeneration protocol. Only one in vitro regeneration (Rajak et al. 2018) and transformation protocol (Gupta et al. 2001) was reported for barnyard millet. Numerous genes have been identified in millets for stress tolerance, but they are not functionally annotated (Renganathan et al. 2020). Nevertheless, without the optimized protocols, direct manipulation of the gene, either by overexpression or knockdown/knockout is not feasible through the CRISPR/cas9 system. This bottleneck should be resolved to use the new biotechnological tools (NBTs) for the further improvement of abiotic stress tolerance in barnyard millet.

24.5 Conclusion

Barnyard millet is a climate-resilient crop with an excellent nutrition profile that has enormous potential to serve as the livelihood of the population of developing countries, particularly due to its enormous contribution to food security. Presently, widely cultivated staple food crops, which might be the dietary source for three-fourths of the population of the world, might not be grown extensively in the future due to their extensive exploitation for yield and their susceptibility to abiotic stresses due to the global climate change. However, during its evolution, barnyard millet grown as a weed or in cultivated forms in different ecosystems upgraded their abilities to respond to adverse climatic conditions. However, the crop has not been studied adequately, and both conventional and modern crop improvement techniques are also not well implemented. Thus, unrevealing the molecular and cellular mechanisms behind barnyard millet adaptation to abiotic stresses will help to improve not only the barnyard millet but will also serve as the reservoir of novel alleles of the climate-resilient traits which will help to improve other major cereals and related species.

References

Abogadallah GM, Quick WPJ (2009) Vegetative salt tolerance of barnyard grass mutants selected for salt tolerant germination. Acta Physiol Plant 31(4):815–824

Abogadallah GM, Serag MM, El-Katouny TM, Quick WP (2010a) Salt tolerance at germination and vegetative growth involves different mechanisms in barnyard grass (*Echinochloa crusgalli L.*) mutants. Plant Growth Regul 60(1):1–12

Abogadallah GM, Serag MM, Quick WPJ (2010b) Fine and coarse regulation of reactive oxygen species in the salt tolerant mutants of barnyard grass and their wild-type parents under salt stress. Physiol Plant 138(1):60–73

Achary V, Reddy CS, Pandey P, Islam T, Kaul T, Reddy MK (2015) Glutathione reductase a unique enzyme: molecular cloning, expression and biochemical characterization from the stress adapted C4 plant, *Pennisetum glaucum* (L.) R. Br. Mol Biol Rep 42(5):947–962

Al Sherif EA (2007) Echinochloa colona (L.) Link., a promising species to cultivate salt affected soils in arid lands. American-Euroasian J Agric Environ Sci 2(6):767–774

Arthi N, Rajagopal B, Geethanjali S, Nirmalakumari A, Senthil N (2019) Screening of barnyard millet (*Echinochloa frumentacea*) germplasm for salinity tolerance. Electron J Plant Breed 10(2):659–666

Babitha K, Vemanna RS, Nataraja KN, Udayakumar M (2015) Overexpression of EcbHLH57 transcription factor from Eleusine coracana L. in tobacco confers tolerance to salt, oxidative and drought stress. PLoS One 10(9):e0137098

Babu BK, Rashmi C, Sood S (2018a) Cross transferability of finger millet and maize genomic SSR markers for genetic diversity and population structure analysis of barnyard millet. Indian J Genet Plant Breed 78(03):364–372

Babu K, Sood S, Kumar D, Joshi A, Pattanayak A, Kant L, Upadhyaya H (2018b) Cross-genera transferability of rice and finger millet genomic SSRs to barnyard millet (*Echinochloa spp.*). 3 Biotech 8(2):1–10

Bartoli CG, Casalongué CA, Simontacchi M, Marquez-Garcia B, Foyer CH (2013) Interactions between hormone and redox signalling pathways in the control of growth and cross tolerance to stress. Environ Exp Bot 94:73–88

Capell T, Escobar C, Liu H, Burtin D, Lepri O, Christou PJ (1998) Over-expression of the oat arginine decarboxylase cDNA in transgenic rice (*Oryza sativa* L.) affects normal development patterns in vitro and results in putrescine accumulation in transgenic plants. Theor Appl Genet 97(1):246–254

Char SN, Neelakandan AK, Nahampun H, Frame B, Main M, Spalding MH, Becraft PW, Meyers BC, Walbot V, Wang K (2017) An Agrobacterium-delivered CRISPR/Cas9 system for high-frequency targeted mutagenesis in maize. Plant Biotechnol J 15(2):257–268

Charu, Lata Manoj, Prasad (2014) Association of an allele-specific marker with dehydration stress tolerance in foxtail millet suggests SiDREB2 to be an important QTL Journal of Plant Biochemistry and Biotechnology 23(1) 119–122 https://doi.org/10.1007/s13562-013-0193-y

Chauhan BS, Abugho SB, Amas JC, Gregorio GBJ (2013) Effect of salinity on growth of barnyardgrass (*Echinochloa crus-galli*), horse purslane (*Trianthema portulacastrum*), junglerice (*Echinochloa colona*), and rice. Weed Sci 61(2):244–248

Chen X, Lu X, Shu N, Wang S, Wang J, Wang D, Guo L, Ye W (2017) Targeted mutagenesis in cotton (*Gossypium hirsutum* L.) using the CRISPR/Cas9 system. Sci Rep 7(1):1–7

Desai M, Mishra R, Verma D, Nair S, Sopory S, Reddy MJ (2006) Structural and functional analysis of a salt stress inducible gene encoding voltage dependent anion channel (VDAC) from pearl millet (*Pennisetum glaucum*). Plant Physiol Biochem 44(7–9):483–493

Eid EM, Galal TM, Sewelam NA, Talha NI, Abdallah SM (2020) Phytoremediation of heavy metals by four aquatic macrophytes and their potential use as contamination indicators: a comparative assessment. Environ Sci Pollut Res 27(11):12138–12151. https://doi.org/10.1007/s11356-020-07839-9

Ezaki B, Nagao E, Yamamoto Y, Nakashima S, Enomoto T (2008) Wild plants, *Andropogon virginicus* L. and *Miscanthus sinensis* Anders, are tolerant to multiple stresses including aluminum, heavy metals and oxidative stresses. Plant Cell Rep 27(5):951–961. https://doi.org/10.1007/s00299-007-0503-8

Fang Y, Xie K, Xiong L (2014) Conserved miR164-targeted NAC genes negatively regulate drought resistance in rice. J Exp Bot 65(8):2119–2135

Feng Z-J, Xu Z-S, Sun J, Li L-C, Chen M, Yang G-X, He G-Y, Ma Y-Z (2016) Investigation of the ASR family in foxtail millet and the role of ASR1 in drought/oxidative stress tolerance. Plant Cell Rep 35(1):115–128

Fox TC, Green BJ, Kennedy RA, Rumpho ME (1998) Changes in hexokinase activity in Echinochloa phyllopogon and Echinochloa crus-pavonis in response to abiotic stress. Plant Physiol 118(4):1403–1409

Foyer CH, Noctor G (2005) Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. Plant Cell Environ Exp Bot 28(8): 1056–1071

Galamay TO, Kono Y, Yamauchi A, Shimizu M (1991) Comparative root anatomy of seminal and nodal root axes of summer cereals with special reference to the development of hypodermis and cortical sclerenchyma. Jpn J Crop Sci 60(1):184–190

Gomashe SS (2017) Barnyard millet: present status and future thrust areas. Millets Sorghum Biol Genet Improv 134:184–198

Gorelova SV, Muratova AY, Zinicovscaia I, Okina OI, Kolbas A (2022) Prospects for the use of Echinochloa frumentacea for phytoremediation of soils with multielement anomalies. Soil Syst 6(1):27

Gupta P, Raghuvanshi S, Tyagi AK (2001) Assessment of the efficiency of various gene promoters via biolistics in leaf and regenerating seed callus of millets, Eleusine coracana and Echinochloa crusgalli. Plant Biotechnol J 18(4):275–282

Gupta S, Kumari K, Muthamilarasan M, Subramanian A, Prasad M (2013) Development and utilization of novel SSR s in foxtail millet [*Setaria italica* (L.) P. Beauv.]. Plant Breed 132(4): 367–374

Han F, Sun M, He W, Guo S, Feng J, Wang H, Yang Q, Pan H, Lou Y, Zhuge Y (2022) Transcriptome analysis reveals molecular mechanisms under salt stress in leaves of foxtail millet (*Setaria italica* L.). Plants 11(14):1864

Hema R, Vemanna RS, Sreeramulu S, Reddy CP, Senthil-Kumar M, Udayakumar M (2014) Stable expression of mtlD gene imparts multiple stress tolerance in finger millet. PLoS One 9(6): e99110

Hittalmani S, Mahesh H, Shirke MD, Biradar H, Uday G, Aruna Y, Lohithaswa H, Mohanrao A (2017) Genome and transcriptome sequence of finger millet (*Eleusine coracana* (L.) Gaertn.) provides insights into drought tolerance and nutraceutical properties. BMC Genomics 18(1): 1–16

IIMR, (2018). Annual Report 2017-18. Hyderabad: Indian Institute of Millets Research.

Jaganathan D, Ramasamy K, Sellamuthu G, Jayabalan S, Venkataraman G (2018) CRISPR for crop improvement: an update review. Front Plant Sci 9:985

Jayakodi M, Madheswaran M, Adhimoolam K, Perumal S, Manickam D, Kandasamy T, Yang T-J, Natesan S (2019) Transcriptomes of Indian barnyard millet and barnyardgrass reveal putative genes involved in drought adaptation and micronutrient accumulation. Acta Physiol Plant 41(5): 1–11

Jha S, Singh J, Chouhan C, Singh O, Srivastava RK (2022) Evaluation of multiple salinity tolerance indices for screening and comparative biochemical and molecular analysis of pearl millet [*Pennisetum glaucum* (L.) R. Br.] genotypes. J Plant Growth Regul 41(4):1820–1834

Khanthavong P, Yabuta S, Asai H, Hossain M, Akagi I, Sakagami J-I (2021) Root response to soil water status via interaction of crop genotype and environment. Agronomy 11(4):708

Khanthavong P, Yabuta S, Malik AI, Hossain MA, Akagi I, Sakagami J-I (2022) Combinational variation temperature and soil water response of stomata and biomass production in maize, millet, sorghum and rice. Plants 11(8):1039

Kidd P, Mench M, Alvarez-Lopez V, Bert V, Dimitriou I, Friesl-Hanl W, Herzig R, Olga Janssen J, Kolbas A, Müller I (2015) Agronomic practices for improving gentle remediation of trace element-contaminated soils. Int J Phytoremediation 17(11):1005–1037

Kim S-H, Park J-S, Lee I-S (2009) Characterization of cadmium-binding ligands from roots of *Echinochloa crusgalli* var. *frumentacea*. J Plant Biol 52(2):167–170

Krishna TA, Maharajan T, David RHA, Ramakrishnan M, Ceasar SA, Duraipandiyan V, Roch GV, Ignacimuthu S (2018) Microsatellite markers of finger millet (*Eleusine coracana* (L.) Gaertn) and foxtail millet (*Setaria italica* (L.) Beauv) provide resources for cross-genome transferability and genetic diversity analyses in other millets. Biocatal Agric Biotechnol 16:493–501

Kumari K, Muthamilarasan M, Misra G, Gupta S, Subramanian A, Parida SK, Chattopadhyay D, Prasad M (2013) Development of eSSR-markers in Setaria italica and their applicability in studying genetic diversity, cross-transferability and comparative mapping in millet and non-millet species. PLoS One 8(6):e67742

Lata C, Bhutty S, Bahadur RP, Majee M, Prasad M (2011) Association of an SNP in a novel DREB2-like gene SiDREB2 with stress tolerance in foxtail millet [*Setaria italica* (L.)]. J Exp Bot 62(10):3387–3401

Lee J, Kim C-S, Lee I-Y (2015) Discrimination of *Echinochloa colona* (L.) Link from other Echinochloa species using DNA Barcode. Weed Turfgrass Sci 4(3):225–229

Li C, Yue J, Wu X, Xu C, Yu J (2014) An ABA-responsive DRE-binding protein gene from Setaria italica, SiARDP, the target gene of SiAREB, plays a critical role under drought stress. J Exp Bot 65(18):5415–5427

Li W-w, Chen M, Zhong L, Liu J-m, Xu Z-s, Li L-c, Zhou Y-B, Guo C-H, Ma Y-Z (2015) Overexpression of the autophagy-related gene SiATG8a from foxtail millet (*Setaria italica* L.) confers tolerance to both nitrogen starvation and drought stress in Arabidopsis. Biochem Biophys Res Commun 468(4):800–806

Li J, Dong Y, Li C, Pan Y, Yu J (2017) SiASR4, the target gene of SiARDP from Setaria italica, improves abiotic stress adaption in plants. Front Plant Sci 7:2053

Liu J-X, Howell SH (2010) Endoplasmic reticulum protein quality control and its relationship to environmental stress responses in plants. Plant Cell 22(9):2930–2942

Liu X, Tang S, Jia G, Schnable JC, Su H, Tang C, Zhi H, Diao X (2016) The C-terminal motif of SiAGO1b is required for the regulation of growth, development and stress responses in foxtail millet (*Setaria italica* (L.) P. Beauv). J Exp Bot 67(11):3237–3249

Ma J, Gao X, Liu Q, Shao Y, Zhang D, Jiang L, Li C (2017) Overexpression of TaWRKY146 increases drought tolerance through inducing stomatal closure in Arabidopsis thaliana. Front Plant Sci 8:2036

Mabberley DJ (1997) The plant-book: a portable dictionary of the vascular plants. Cambridge University Press

Maqsood M, Ali SA (2007) Effects of drought on growth, development, radiation use efficiency and yield of finger millet (*Eleucine coracana*). Pak J Bot 39:123

Matsuura A, Tsuji W, An P, Inanaga S, Murata K (2012) Effect of pre-and post-heading water deficit on growth and grain yield of four millets. Plant Product Sci 15(4):323–331

Miao J, Guo D, Zhang J, Huang Q, Qin G, Zhang X, Wan J, Gu H, Qu L-J (2013) Targeted mutagenesis in rice using CRISPR-Cas system. Cell Res 23(10):1233–1236

Mirzai M, Moeini A, Ghanati F (2013) Effects of drought stress on the lipid peroxidation and antioxidant enzyme activities in two canola (*Brassica napus* L.) cultivars. J Agric Sci Technol 15(3):593–602

Mishra AK, Puranik S, Bahadur RP, Prasad M (2012) The DNA-binding activity of an AP2 protein is involved in transcriptional regulation of a stress-responsive gene, SiWD40, in foxtail millet. Genomics 100(4):252–263

Munns R (2005) Genes and salt tolerance: bringing them together. New Phytol 167(3):645–663

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651

Muthamilarasan M, Prasad M (2021) Small millets for enduring food security amidst pandemics. Trends Plant Sci 26(1):33–40

Muthamilarasan M, Venkata Suresh B, Pandey G, Kumari K, Parida SK, Prasad M (2014) Development of 5123 intron-length polymorphic markers for large-scale genotyping applications in foxtail millet. DNA Res 21(1):41–52

Nah G, Im J-H, Kim J-W, Park H-R, Yook M-J, Yang T-J, Fischer AJ, Kim D-S (2015) Uncovering the differential molecular basis of adaptive diversity in three Echinochloa leaf transcriptomes. PLoS One 10(8):e0134419

Nguyen HTT, Shim IS, Kobayashi K, Usui K (2005) Effects of salt stress on ion accumulation and antioxidative enzyme activities of *Oryza sativa* L. and *Echinochloa oryzicola* Vasing. Weed Biol Manag 5(1):1–7

Ning Y, Jantasuriyarat C, Zhao Q, Zhang H, Chen S, Liu J, Liu L, Tang S, Park CH, Wang X (2011) The SINA E3 ligase OsDIS1 negatively regulates drought response in rice. Plant Physiol 157(1): 242–255

Numan M, Serba DD, Ligaba-Osena A (2021) Alternative strategies for multi-stress tolerance and yield improvement in millets. Genes 12(5):739

Osakabe Y, Watanabe T, Sugano SS, Ueta R, Ishihara R, Shinozaki K, Osakabe K (2016) Optimization of CRISPR/Cas9 genome editing to modify abiotic stress responses in plants. Sci Rep 6(1):26685. https://doi.org/10.1038/srep26685

Pan Y, Li J, Jiao L, Li C, Zhu D, Yu J (2016) A non-specific Setaria italica lipid transfer protein gene plays a critical role under abiotic stress. Front Plant Sci 7:1752

Pandey G, Misra G, Kumari K, Gupta S, Parida SK, Chattopadhyay D, Prasad M (2013) Genome-wide development and use of microsatellite markers for large-scale genotyping applications in foxtail millet [*Setaria italica* (L.)]. DNA Res 20(2):197–207

Pandey P, Achary V, Kalasamudramu V, Mahanty S, Reddy GM, Reddy MK (2014) Molecular and biochemical characterization of dehydroascorbate reductase from a stress adapted C4 plant, pearl millet [*Pennisetum glaucum* (L.) R. Br]. Plant Cell Rep 33(3):435–445

Park J-Y, Kim K-W, Kim J, Lee B-T, Lee J-S, Bae B-H (2008) Enhanced phytoremediation by Echinochloa frumentacea using PSM and EDTA in an arsenic contaminated soil. In: The international symposia on geoscience resources and environments of Asian terranes, 4th IGCP 516, and 5th APSEG

Parvathi M, Nataraja KN, Reddy Y, Naika MB, Gowda M (2019) Transcriptome analysis of finger millet (*Eleusine coracana* (L.) Gaertn.) reveals unique drought responsive genes. J Genet 98(2): 1–12

Passioura JB, Munns R (2000) Rapid environmental changes that affect leaf water status induce transient surges or pauses in leaf expansion rate. Funct Plant Biol 27(10):941–948

Perumal S, Jayakodi M, Kim D-S, Yang T-j, Natesan S (2016) The complete chloroplast genome sequence of Indian barnyard millet, *Echinochloa frumentacea* (Poaceae). Mitochondrial DNA B 1(1):79–80

Puranik S, Bahadur RP, Srivastava PS, Prasad M (2011) Molecular cloning and characterization of a membrane associated NAC family gene, SiNAC from foxtail millet [*Setaria italica* (L.) P. Beauv.]. Mol Biotechnol 49(2):138–150

Rahman H, Jagadeeshselvam N, Valarmathi R, Sachin B, Sasikala R, Senthil N, Sudhakar D, Robin S, Muthurajan R (2014) Transcriptome analysis of salinity responsiveness in contrasting genotypes of finger millet (*Eleusine coracana* L.) through RNA-sequencing. Plant Mol Biol 85(4):485–503

Rajak K, Sprae S, Rathour S, Kumari R, Nikeeta T (2018) To study the indirect plant regeneration of two cultivars in barnyard millet using different combination of plant growth regulators and compared between superior genotype for in-vitro culture. Int J Curr Microbiol Appl Sci 51: 2055–2061

Ramakrishna C, Singh S, Raghavendrarao S, Padaria JC, Mohanty S, Sharma TR, Solanke AU (2018) The membrane tethered transcription factor EcbZIP17 from finger millet promotes plant growth and enhances tolerance to abiotic stresses. Sci Rep 8(1):1–14

Ramegowda V, Gill US, Sivalingam PN, Gupta A, Gupta C, Govind G, Nataraja KN, Pereira A, Udayakumar M, Mysore KS (2017) GBF3 transcription factor imparts drought tolerance in Arabidopsis thaliana. Sci Rep 7(1):1–13

Reddy RA, Kumar B, Reddy PS, Mishra RN, Mahanty S, Kaul T, Nair S, Sopory SK, Reddy MK (2009) Molecular cloning and characterization of genes encoding *Pennisetum glaucum* ascorbate peroxidase and heat-shock factor: interlinking oxidative and heat-stress responses. J Plant Physiol 166(15):1646–1659

Reddy PS, Reddy GM, Pandey P, Chandrasekhar K, Reddy MK (2012) Cloning and molecular characterization of a gene encoding late embryogenesis abundant protein from *Pennisetum glaucum*: protection against abiotic stresses. Mol Biol Rep 39(6):7163–7174

Renganathan VG, Vanniarajan C, Karthikeyan A, Ramalingam J (2020) Barnyard millet for food and nutritional security: current status and future research direction. Front Genet 11:500

Rocha V, Duarte MC, Catarino S, Duarte I, Romeiras MM (2021) Cabo Verde's Poaceae flora: a reservoir of crop wild relatives diversity for crop improvement. Front Plant Sci 12:630217

Samantaray S, Rout GR, Das P (2001) Induction, selection and characterization of Cr and Ni-tolerant cell lines of *Echinochloa colona* (L.) Link in vitro. J Plant Physiol 158(10): 1281–1290

Satapathy L, Kumar D, Kumar M, Mukhopadhyay K (2018) Functional and DNA–protein binding studies of WRKY transcription factors and their expression analysis in response to biotic and abiotic stress in wheat (*Triticum aestivum* L.). 3 Biotech 8(1):1–18

Sebastin R, Lee KJ, Cho G-T, Lee J-R, Shin M-J, Kim S-H, Lee G-A, Chung J-W, Hyun DY (2019) The complete chloroplast genome sequence of Japanese millet *Echinochloa esculenta* (A. braun) H. scholz (Poaceae). Mitochondrial DNA B 4(1):1392–1393

Senthil A, Ashok S, Sritharan N, Punitha S, Divya K, Ravikesavan R (2018) Physiological efficiency of small millets under drought condition. Madras Agric J 105. https://doi.org/10.29321/MAJ.2018.000161

Shah WH, Rasool A, Padder SA, Singh RK, Prasad M, Tahir I, Hakeem KR (2022) Decarboxylation mechanisms of the C4 cycle in foxtail millet observed under salt and selenium treatments. Plant Growth Regul 99:65–83

Shan Q, Wang Y, Li J, Gao C (2014) Genome editing in rice and wheat using the CRISPR/Cas system. Nat Protoc 9(10):2395–2410

Sharma G, Giri J, Tyagi AK (2015) Rice OsiSAP7 negatively regulates ABA stress signalling and imparts sensitivity to water-deficit stress in Arabidopsis. Plant Sci 237:80–92

Singh RK, Singh VK, Raghavendrarao S, Phanindra MLV, Venkat Raman K, Solanke AU, Kumar PA, Sharma TRJ (2015) Expression of finger millet EcDehydrin7 in transgenic tobacco confers tolerance to drought stress. Appl Biochem Biotechnol Biofuels 177(1):207–216

Solís-Dominguez F, Gonzalez Chavez MC, Carrillo González R, Rodriguez Vazquez R (2007) Accumulation and localization of cadmium in *Echinochloa polystachya* grown within a hydroponic system. J Hazard Mater 141:630–636. https://doi.org/10.1016/j.jhazmat.2006.07.014

Soltani Najafabadi M (2012) Improving rice (*Oryza sativa* L.) drought tolerance by suppressing a NF-YA transcription factor. Iran J Biotechnol 10(1):40–48

Sood S, Khulbe R, Saini N, Gupta A, Agrawal P (2014) Interspecific hybrid between *Echinochloa esculenta* (Japanese barnyard millet) and *E. frumentacea* (Indian barnyard millet)—a new avenue for genetic enhancement of barnyard millet. Electron J Plant Breed 5(2):248–253

Sood S, Khulbe RK, Gupta AK, Agrawal PK, Upadhyaya HD, Bhatt JC (2015) Barnyard millet—a potential food and feed crop of future. Plant Breed 134(2):135–147

Sreenivasulu N, Miranda M, Prakash HS, Wobus U, Weschke W (2004) Transcriptome changes in foxtail millet genotypes at high salinity: identification and characterization of a PHGPX gene specifically up-regulated by NaCl in a salt-tolerant line. J Plant Physiol 161(4):467–477

Subiramaniyan A, Alagarswamy S, Sritharan N, Punitha S, Divya K, Ravikesavan R (2018) Yield potential of small millets under drought condition. Madras Agric J 105. https://doi.org/10.29321/MAJ.2018.000163

Subramanian A, Raj RN, Jeyaprakash P (2020) In vitro and in vivo screening of barnyard millet (Echinochloa frumentacea (roxb.) Link) germplasm for salinity tolerance. Plant Arch 20:7389–7397

Svitashev S, Schwartz C, Lenderts B, Young JK, Mark Cigan A (2016) Genome editing in maize directed by CRISPR–Cas9 ribonucleoprotein complexes. Nat Commun 7(1):1–7

Tavakol E, Pakniyat H (2007) Evaluation of some drought resistance criteria at seedling stage in wheat (*Triticum aestivum* L.) cultivars. Pak J Biol Sci 10(7):1113–1117

Tiwari A, Rastogi A, Singh V, Arunachalam A (2020) Effect of water stress on oxidative damage and antioxidant enzyme activity in finger millet and barnyard millet. Indian J Hill Farm 33(1): 36–45

Trivedi A, Arya L, Verma S, Tyagi R, Hemantaranjan A (2017) Evaluation of barnyard millet diversity in central Himalayan region for environmental stress tolerance. J Agric Sci 155(10): 1497–1507

Varshney RK, Hoisington DA, Tyagi AK (2006) Advances in cereal genomics and applications in crop breeding. Trends Biotechnol 24(11):490–499

Wallace J, Upadhyaya H, Vetriventhan M, Buckler E, Tom Hash C, Ramu P (2015) The genetic makeup of a global barnyard millet germplasm collection. Plant Genome 8:1–7

Wang M, Li P, Li C, Pan Y, Jiang X, Zhu D, Zhao Q, Yu J (2014) SiLEA14, a novel atypical LEA protein, confers abiotic stress resistance in foxtail millet. BMC Plant Biol 14(1):1–16

Williams G, Vanniarajan C, Vetriventhan M, Thiageshwari S, Anandhi K, Rajagopal B (2019) Genetic variability for seedling stage salinity tolerance in barnyard millet [*Echinochloa frumentaceae* (Roxb.) Link]. Electron J Plant Breed 10(2):552–558

Wu D, Shen E, Jiang B, Feng Y, Tang W, Lao S, Jia L, Lin H-Y, Xie L, Weng X (2022) Genomic insights into the evolution of *Echinochloa* species as weed and orphan crop. Nat Commun 13(1):1–16

Yabuno T (1966) Biosystematic study of the genus Echinochloa. Jpn J Bot 19:277–323

Yabuno T (1984) A biosystematic study on *Echinochloa oryzoides* (Ard.) Fritsch. Cytologia 49(3): 673–678

Yadav CB, Muthamilarasan M, Pandey G, Khan Y, Prasad M (2014) Development of novel microRNA-based genetic markers in foxtail millet for genotyping applications in related grass species. Mol Breed 34(4):2219–2224

Yamaguchi H, Utano A, Yasuda K, Yano A, Soejima AJ (2005) A molecular phylogeny of wild and cultivated *Echinochloa* in East Asia inferred from non-coding region sequences of trnT-L-F. Weed Biol Manag 5(4):210–218

Yamamoto A, Shim IS, Fujihara S, Yoneyama T, Usui K (2003) Physiochemical factors affecting the salt tolerance of *Echinochloa crus-galli* Beauv. var. *formosensis* Ohwi. Weed Biol Manag 3(2):98–104

Zafar SA, Zaidi SS-e-A, Gaba Y, Singla-Pareek SL, Dhankher OP, Li X, Mansoor S, Pareek A (2020) Engineering abiotic stress tolerance via CRISPR/Cas-mediated genome editing. J Exp Bot 71(2):470–479

Zegada-Lizarazu W, Iijima M (2004) Hydrogen stable isotope analysis of water acquisition ability of deep roots and hydraulic lift in sixteen food crop species. Plant Product Sci 7(4):427–434

Zegada-Lizarazu W, Iijima M (2005) Deep root water uptake ability and water use efficiency of pearl millet in comparison to other millet species. Plant Product Sci 8(4):454–460

Zhang Z-F, Li Y-Y, Xiao B-Z (2016) Comparative transcriptome analysis highlights the crucial roles of photosynthetic system in drought stress adaptation in upland rice. Sci Rep 6(1):1–13

Zhang H, Li Y, Zhu J (2018) Developing naturally stress-resistant crops for a sustainable agriculture. Nat Plants 4:989–996

Zhao L, Zhao Q, Ao G, Yu J (2009) The foxtail millet Si69 gene is a Wali7 (wheat aluminum-induced protein 7) homologue and may function in aluminum tolerance. Chin Sci Bull 54(10): 1697–1706

Zivcak M, Brestic M, Balatova Z, Drevenakova P, Olsovska K, Kalaji HM, Yang X, Allakhverdiev S (2013) Photosynthetic electron transport and specific photoprotective responses in wheat leaves under drought stress. Photosynth Res 117(1):529–546

Breeding Barnyard Millet for Biotic Stress Resistance

25

M. Rajesh, G. Shivaraj, V. Ambethgar, and C. Vanniarajan

Abstract

Barnyard Millet (*Echinochloa frumentacea* L.) has been an underutilized crop and has got a very scanty focus from agriculturists as well as researchers across the world. The resistance of this crop to biotic stress gives a significant guide to the challenges posed by pests and diseases on crucial fodder and food crops. Biotic factors such as weeds, insect pests, and disease are predicted to worsen as the climate change pattern continues; furthermore, this biotic stress induced pressure stands as one of the major limitations on the production of barnyard millet across the globe. Management of these biotic stresses is becoming increasingly essential. Present strategies primarily hinge on the development of resistant varieties since the use of chemicals is not economically feasible in developing countries where millets are of paramount importance as a source of food. Tackling the specific difficulties posed by weeds, insect pests, birds, and diseases—the most prevalent threats to the yield of barnyard millet. This chapter explores non-chemical-focused options for improving biotic stress resistance of barnyard and safeguarding the sustainability of yield. This solitary reference will be valuable for students, educators, and researchers in the field of plant breeding, plant pathology, and agricultural entomology.

Keywords

Barnyard millet · Biotic stress · Resistance · Breeding

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25.1 Introduction

Barnyard millet (*Echinochloa frumentacea* L.) is a prehistoric, fourth-most-produced crop among small millets. Generally, it is cultivated on the Asian continent, mainly in India, Japan, China, and Korea. It provides food security to many underprivileged people in warm and temperate regions of the planet. India is the leading producer of barnyard millet in the world and is mainly for consumption, though it is also used as a livestock feed. It is a rich source of carbohydrates, fiber, proteins, and micronutrients such as zinc (Zn) and iron (Fe) (Saleh et al. 2013; Chandel et al. 2014) that has more health benefits (Ugare et al. 2014). All these characteristics make barnyard millet highly suitable for contingency plan during the failure of monsoons for subsistence farmers (Gupta et al. 2009). Barnyard millet is an underutilized crop, as it has been neglected due to a lack of awareness, despite having excellent agronomic and nutritional value. Among the wild and cultivated species of barnyard millet, two of the most popular species are *Echinochloa esculenta* (Japanese barnyard millet) and *Echinochloa frumentacea* (Indian barnyard millet) (Sood et al. 2015). It is an early-maturing crop that can be grown in almost adverse and zero or no input conditions. In addition to these agronomic benefits, as compared to major cereals like maize, wheat, and rice, the barnyard millet grains are valued for their lower expenses and high nutrition.

Generally, biotic stresses cause considerable yield loss in all the crop plants and its management leads to direct impact on the increase of the cost of production and indirectly affects the environment and ecology. Now-a-days, the high usage of chemicals for biotic stress management is of utmost concern for humans and ultimately the overall environment. The best method to combat the biotic stress is to use resistant varieties. The resistant varieties are of less input cost and are environmentally safe when applied to manage the biotic stresses. Resistance breeding for pest and disease is one of the mandated objectives for crop breeding programs across the world. It requires the donor or resistance source. There are many breeding programmes to create resistance against biotic stress and it involves combinations of conventional and modern tools. The resistant variety development needs attention on the durability, expression, inheritance, and interaction with other genes and the environment. This chapter deals with the significance of biotic stresses on barnyard millet and its management through resistance breeding.

It is said that "Prevention is better than cure". Generally the curative approaches have successful and quickest control on the situation but costly to the pocket of farmers as well as a risk to the environment. The preventive methods other than resistance can be of mechanical, agronomical, and cultural; they are time-consuming but sustainable and eco-friendly and economic and the only solution in many cases. But resistant varieties are very effective in terms of cost and time basis compared to any other methods.

Jagadish et al. (2008) explained that the wild *Echinochloa* species has potential resistance to various biotic stresses. But the commercially grown *E. frumentacea* and *E. esculenta* are highly affected by pest, i.e. stem borer, shoot fly and the diseases like loose smut, grain smut at different stages of crop growth. The early state

infection of Aphid causes considerable yield reduction to barnyard millet. Rawat et al. (2019) described that so far, TNEF-204 and DHBM 996 were highly resistant source for stem borer and shoot fly. Kim et al. (2008) reported that some *E. frumentacea* entries have anti-feeding activity against brown plant hopper. Grain smut (*Ustilagopanici-frumentacea*) and Loose smut (*Ustilagotritici*) are the major constraints of grain formation in cultivated species of *Echinochloa* (Gupta et al. 2010). However, Nagaraja and Mantur (2008) showed that some of the *E. esculenta* lines had resistance against smut disease and can further provide the chance to breed the resistance cultivars.

25.2 Stress

Stress means whenever any combination of an individual with fellow individuals of the same or other species and surrounding environmental factors becomes detrimental to its survival, reproduction, for future. Generally, biotic stress per se requires many factors to play a role in its existence. The biotic factors are fungi, bacteria, viruses, mycoplasma, insects, nematodes, rodents, and even mammalian pests. But to cause a stress interaction of a susceptible host, aggressive or virulent pathogen or pest and favourable environment is must.

25.3 Importance of Resistant Varieties

The world population will increase continuously till2050, it is estimated that current global production of wheat must increase annually by about 2% (Bainslal and Meena 2016). But the demand for land for uses other than agriculture is also expanding. Because of biotic stress, up to 35% of food production is wasting, not reaching the poor people. The loss was worth Rs 140,000 crores in 2009 (Suresh and Malathi 2014). Overall, pests accounted for pre-harvest losses of 42% of the potential value of output were lost postharvest (Bainslal and Meena 2016). The biotic stresses by insects and diseases caused 31–42% loss, with an additional loss of 6–20% in post-harvest due to insects, bacterial, and fungal rots. The losses caused by pathogens are severe in developing nations (e.g. cereals, 22%) when compared to crop losses in developed nations (e.g. cereals, 6%). More than 42% of the potential world crop yield is lost due to biotic stresses (13% attributed to pathogens, 13% to weeds and 15% attributable to insects).

Darwin's theory of "survival of the fittest" highly relevant to biotic stress but here comes the factor of the degree of stress. Plants have naturally their own defence mechanism like pre-formed structures and compounds also inducible plant defences that are generated after infection. Plant immune systems exhibit many plant-specific characteristics and show some mechanistic similarities with the immune systems of insects and mammals. Plant breeding is a science that is an art of culturing plants with skills of selection, standing on the platform of the science of genetics at the meta as well as the micro/nano/pico or atomic level using statistics as its main tool,

which may be through simple chi squares, random tables, calculators or high throughput computational software. The genetic makeup of the plant makes it either resistant or susceptible.

25.3.1 General Procedure for Plant Resistance Breeding

- Classification of resistant sources—Ancient Land races and Wild relatives are highly valuable to enhance plant disease resistance, especially millets.
- Generate the segregation population by crossing the compatible accessions.
- Artificial screening against specific pathogens or diseases may result in the inheritance of reliable resistance sources.
- Selection of disease-resistant individuals.

25.3.2 Sources of Disease Resistance

- A familiar variety: Commercial varieties as a source of resistant plants.
- Germplasm collection: Resistant to pest and diseases.
- Related species: Prabhani Kranti, a variety resistant to YVM, is developed in which resistance is transferred from *Abelmoschas album*. Grassy stunt virus resistance in rice (*Oryza sativa* L.) is transferred from *Oryza nivara*.
- Somaclonal variation: Resistance to Fiji disease of sugarcane (*Saccharum officinarum* L.) was obtained from a variety named Ono (a somaclone of Pindar variety).
- Mutation: Spontaneous or induced mutations can be used to isolate some disease-resistant plants.
- Unrelated organism: e.g. genes for novel phytoalexins, coat protein genes of a pathogenic virus.

25.4 Breeding Methods for Biotic Stress Resistance

The pre-requisite for an advanced resistance breeding is the availability of reservoirs of resistance. In the initial phase of resistance breeding, the cultivated crops were used as a source of resistance. But, over time, the emphasis was also given to interspecific as well as inter-generic crosses, including wild species. Currently, alien species constitute an important component of genetic pool for disease resistance. The transfer of resistance to a commercial cultivar from a wild alien species has multiple steps, the initial step being the choice of a suitable source. The other traits are also considered while the resistance is being evaluated. Subsequently, obstacles of crossability and hybrid sterility have to be resolved when the source of resistance and the crop lack close genetic affinity. This constitutes a part of pre-breeding process that is designed to lead to a resistant and stable lines, though still lacking the necessary agronomic characteristics. Ultimately, the resistance or

biotic stress breeding is commonly achieved by conventional breeding methods when commercial cultivars are crossed with pre-bred resistant lines.

Commonly, methods of breeding for disease resistance are selection, introduction, mutation, hybridization (pedigree and backcross method), somaclonal variation, and genetic engineering.

25.4.1 Selection

Selection is an ancient and basic procedure in the methodology of plant breeding. Selection of plants with biotic resistance from among the commercially available variety is the cheapest and the expedient approach of developing a resistant variety. This method has been proved to be useful in the past, however, it has some limitations at the present level of crop improvement. Exploration of wild species, land races, farmers cultivating commercial varieties, etc., are used as main source for resistance breeding programmes.

Barnyard millet crop has encountered issues with infestation by sap feeders, defoliators, root feeders, and stem borers. Among the insect-pests affecting the barnyard millet crop, stem borer and shoot fly are the most yield-losing insect-pests in all around the Indian continent. Generally, shoot fly and stem borer have emerged as major constraints to barnyard millet cultivation, leading to reduction in productivity. The major focus is to identify resistant donors of barnyard millet against stem borer and shoot fly by screening the germplasm.

25.4.2 Introduction

Introductions has been proven as an effective and a very useful method for disease management in plants. Resistance cultivars may be introduced for cultivation in a novel region. This offers a relatively uncomplicated and quick means of acquiring resistant varieties. For example, the introduction of Ridley wheat from Australia had been useful as a rust-resistant variety. The wheat varieties Sonalika and Kalyan Sona originated from the segregating materials introduced form CIMMYT, Mexico, and exhibited rust resistant. Introductions also serve as reservoirs of resistance within breeding programmes. For example, introductions of African pearl millet have been used for developing male sterile lines (Tift23 cytoplasm) resistant to Downey mildew.

25.4.3 Mutation

The exposure of genotypes to mutagenic agents may convert a susceptible genotype into a resistant one against particular pests and diseases. In case of a point mutation, the resistant mutant will closely resemble to the original cultivar, except for its resistance. However, there are undesirable side effects linked to mutagenic

treatment. Several other genes may also have experienced alterations, or the resistance-associated mutation may lead to undesirable pleiotropic effects. As a consequence, the selection of a resistant mutant should be succeeded further by breeding efforts (i.e. backcrossing) to yield a commercially viable cultivar. The value of induced mutations is usually highlighted using the example of peppermint (*Mentha piperita*) cultivation in the USA with an annual value of 20 million U.-S. dollars. This monoclonal crop became susceptible to Verticillium wilt, and its commercial cultivation was in jeopardy. A large-scale mutation breeding project permitted the isolation of Verticillium wilt resistant mutant that had the same specific quality of peppermint oil as the parent variety; this saved the peppermint crop from being wiped out. Using γ-rays, amber-grained mutants of Lerma Rojo and Sonora-64 were produced and released as Pusa Lerma and Sharbati Sonora and respectively. In North America, one variety of common bean resistance to Anthracnose namely 'Samilac' was released by Down and Anderson in Michigan in 1956. Some other examples of disease resistance induced by mutagenic agents include resistance to mildew in barley, stripe rust in wheat, flax rust and leaf spot and stem rot resistance in peanuts, crown rust in oats.

25.4.4 Hybridization

The plant breeding technique that is most frequently used is hybridization. The main objective of hybridization is to amalgamate the desired characters found in different plant lines into a single plant line through cross-pollination. Hybridization stands as the prevailing approach for developing a disease resistant line. It fulfils the following two objectives:

1. Transfer of disease resistance to a susceptible but otherwise desirable variety from an agronomically undesirable variety (by back cross method).
2. Combining disease resistance and some other desirable characteristics of one variety with the superior characteristics of another variety (by pedigree method).

In all these cases, one parent is selected for disease resistance; it should have resistance that is governed by few oligogenes and have a high degree of resistance to as many races of the pathogen as possible. The backcross method is employed if the resistant variety is agronomically undesirable and unadapted. Otherwise, the pedigree method of breeding is employed if the resistant variety is well-adapted and has some other desirable characteristics as well. Kufri Jyoti a prominent variety of potato developed by hybridization. It is resistant to late blight of potato. Kufri Khyati produces white oval tubers with shallow eyes, is moderately resistant to late blight, and is suitable for cultivation in the plains of India.

25.4.5 Pedigree Method

Pedigree approach consists of selection of individual F2 plants on the basis of desirable traits, including disease resistance. Progenies from these selections are selected repeatedly in each succeeding generation until the homozygosity is claimed. This is most commonly used method for developing improved varieties of plants for disease resistance. This method is relevant for transferring polygenic or horizontal resistance, for which backcross method is of restricted value. The process of utilizing pedigree method to breed disease resistance is not substantially different from the approach used for other quantitative traits. However, in case of breeding for disease resistance, the artificial disease epidemics are generally created to enable an effective selection. Numerous disease resistant commercial varieties have been developed through this method e.g., Kalyan Sona, Malviya-12, Sonalika, Malviya-37, Malviya-206, Malviya-234 of wheat. *G. hirsutum* variety Laxmi resistant to red leaf blight was developed from a cross between the resistant parent Combodia-2 and the susceptible parent Gadag-1. Resistance to diseases like bean common mosaic necrosis virus (BCMNV) and anthracnose has been successful. Recently, the pedigree approach with marker-assisted selection is developed to pinpoint specific genes responsible of resistance to diseases. However, the time investment is the major drawback of the pedigree approach.

25.4.6 Backcross Method

This method is most effective in transferring resistant genes from an agronomically undesirable resistant variety to an agronomically superior but susceptible variety. Based on whether the allele being transferred for resistance is dominant or recessive, the backcross programme may differ. A strict selection is followed for disease resistance, and the plant type of recurrent parent is also selected. In general, 4–6 backcrosses are made, but with an effective use of marker-assisted selection three backcrosses may be adequate. At the end of backcross programme, the progeny are selfed and resistant plants are selected. The new disease-resistant variety is developed by bulking the progenies derived from different homozygous resistant plants that are similar in agronomic traits. The new variety developed would be almost similar to the recurrent parent, except for the disease resistance. Henceforth, its release doesn't require extensive yield trials. There are many examples of interspecific transfer of genes; "Transfer", the first commercial wheat variety for rust resistance was developed through backcross method. Rust resistance has been transferred from HS-19, Robin, K-1, Tobari, Bluebird, Frecor, etc., to Kalyan Sona via backcross method. Three multiline varieties, viz., KSML-3, KSML-11 and KSML-7406, have been released for cultivation and was developed using Kalyan Sona as recurrent parent and exotic lines resistant to leaf rust as non-recurrent parent.

The advantages of this method are:

1. Backcross method is an obvious choice, if resistant variety is agronomically undesirable and unadapted.
2. Possibility of multiple resistance breeding.
3. Usefulness in transferring one or few major genes (vertical resistance).
4. Non requirement of extensive yield trials, before its release for commercial cultivation.

The disadvantages of this method are:

1. No progress in yield capacity is attainable.
2. The Indian wheat varieties were incorporated with rust resistant genes using backcross method to develop several near isogenic lines such as HW 2044 (backcross line of PBW 226), HW 2004 (backcross line of C 306 carrying Lr24) for commercial cultivation.

25.4.7 Genetic Engineering

In this strategy, genes anticipated to confer resistance to disease are isolated, cloned and transferred into the relevant crop. For viral pathogens, numerous transgenes have been assessed, viz., DNA copy of viral satellite RNA, ribozymes, virus coat protein gene, defective viral genome and antisense constructs of critical viral genes. The technique involving the viral coat protein gene seems to be the most efficient (Singh 2011), and a virus-resistant transgenic squash variety is in commercial cultivation in the USA. Genes conferring insect resistance in plants have been transferred from *Bacillus thuringiensis* (cry gene) and from other plants through genetic engineering. The cry gene transfers are the most successful, and insect-resistant transgenic varieties of maize, soybean, cotton, etc., Expressing this gene is being cultivated in USA and other countries. In India, insect resistant Bt-cotton hybrids are in cultivation since 2002.

25.4.8 Barnyard Millet Breeding

Maun and Barrett (1986) explained that the *Echinochloa* species exhibits high degree of seif-pollination, although the level of cross-pollination is adequate to facilitate gene exchange within their population. Due to miniature flower size, early hours of blooming, brief pollen viability, limited availability of pollen grains and little opening of flowers that too for a transit period makes it difficult for carrying out emasculation and hand pollination (Nirmalakumari and Vetriventhan 2009). The crop is still regarded as a food and feed crop of poor and tribal people, yet limited work has been done by researchers and breeders for its improvement. The Indian states of Tamil Nadu and Uttarakhand are known for conducting breeding in barnyard millet. As of now, over 20 improved varieties of barnyard millet have been developed and released for different zones of the nation. Predominately, pure

line selection and mass selection have remained the principal breeding strategies for the crop improvement; however, varieties have been produced through hybridization, followed by pedigree approach.

Although inducing a mutant phenotype is difficult in a polyploid species such as *Echinochloa*, irradiation of low amylase landrace 'Nogehie' resulted in the development of a complete waxy stable mutant lines (Hoshino et al. 2010). An increased genetic variance for grain yield, plant height, tiller number and head length were noted when irradiated with gamma rays (Mehta et al. 2005) stressed the significance of inter-specific hybridization programme involving high-yielding *E. esulenta* and early maturing *E. frumentacea* to develop early maturing high-yielding segregants. However, hybrids formed from *E. esculenta* and *E. frumentacea* are sterile in both ways, unlike those with its wild ancestors are fertile. These wild ancestors can provide valuable genetic reservoirs for the improvement of cultivated species (Mandelbaum et al. 1995). Large variation in calcium and protein content observed within the accessions suggests selective breeding for nutritive attributes (Mandelbaum et al. 1995).

Barnyard millet has the potential to grow up to 2 m or even more under the high moisture conditions prevalent during monsoon season in India, rendering it susceptible to lodging. Curtailing the plant height to about 120–130 cm could mitigate the lodging problem. The decrease in the fodder yield due to this adjustment may be compensated by developing cultivars with large number of basal tillers, contributing to overall grain yield too. In case of barnyard millet, the number and length of spikes has positive correlation with grain yield. So the principal approach for enhancing the grain yield should be the development of cultivars with longer and more spikes. Another breeding objective is to develop a easy dehulling cultivars to reduce the drudgery involved in postharvest processing of barnyard millet. Within the local germplasm, the instances of easy dehulling cultivars have been documented (Anonymous 2010), enabling the transfer of this trait to a well-adapted and high-yielding cultivars. As barnyard millet is traditionally consumed just like rice, enlarging the grain size is likely to enhance its appeal among the consumers. Nonetheless, this aspiration poses a challenge due to the limited scope of variability in this attribute.

The grain smut of barnyard millet caused by *Ustilago* spp. is considered as the major constraint to yield as it contributes to the yield loss of up to 61% (Jain et al. 1997). The Japanese barnyard millet is reported to be resistant to the grain smut but the Indian barnyard millet is not reported with such a reservoir of resistance to this disease. The hybrid sterility between the two species hampers the transfer of resistance from Japanese to Indian barnyard millet (Sood et al. 2014). An alternative source resistance could potentially be the wild ancestor of Indian barnyard millet, *E. colona*, which exhibits both resistance to grain smut as well as compatibility for hybridization with cultivated barnyard millet. Simultaneously, the breeding efforts should also be targeted towards development of cultivars resistant to Helminthosporium leaf blight which poses serious problem in specific regions.

In barnyard millet, enhancing grain yield still remains the predominant breeding objective. A wider production gap can still be observed between the yields realized at farmer's fields due to the prevalence of localized farming methods of cultivation

(Gupta et al. 2006). Majority of areas cultivating barnyard millet is still dependent upon the indigenous and native cultivars with low grain yields of 1.0–1.5 t/ha, despite the crop's potential to yield >2.0 t/ha (Harinarayana 1989) and yield levels of 3.0 t/ha has been documented in the *E. esulenta* (Bandyopadhyay 2001). The significant improvement in the productivity of barnyard millet can be obtained by merging improved varieties with modern agronomic practices. When compared to rice straw, barnyard millet straw is preferred. Therefore, a variety with higher grain yield coupled with ample fodder is most favoured.

25.5 Fodder Quality

Barnyard millet has a faster growth rate and produces abundant forage. Its fodder is exceptionally palatable and can also be used for preparing silage or hay. Its straw is regarded as better in comparison to oat and rice straw due to the presence of high calcium and protein content (Yabuno 1987). The straw of barnyard millet contains up to 61% total digestible nutrients and a substantial quantity of protein and easily digestible fibre. Under Indian conditions, the barnyard yields an average fodder of 5 t/ha (AICSMIP 2014). In the Uttarakhand hills of India, barnyard millet fodder contributes to around 12% of the overall fodder consumption in the state, where even the Indian barnyard millet grains are used as animal feed (Singh and Singh 2005). Recent investigation has revealed that the fodder-yielding potential of Indian barnyard millet is lower than that of Japanese barnyard millet, and the RDF (recommended dose of fertilizers) application increases fodder yield in case of barnyard millet (Yadav and Yadav 2013). In a dual-purpose management approach involving double fodder cutting, Japanese barnyard millet outperformed the Indian barnyard millet (Bandyopadhyay 2009). However, non-significant differences were observed for dry matter digestibility between the two species.

The Japanese barnyard millet is being grown for grazing or hay in many parts of the world like the USA, Australia, and China. In the USA, it is documented to yield eight harvests per year (Kajuna 2001). It boasts the highest protein content among all the millet species (http://www.fao.org/docrep/008/y5831e/y5831e06.htm). In Australia, it has been acknowledged as a valuable crop for short-term rotations, particularly for spring to early summer grazing. The rapid growth rate of the crop can help to counter feed shortages in early summer, following drought or floods. In northern New South Wales, the crop offers two substantial grazings, while in cooler southern regions, repeated grazing is feasible. However, the crop's main drawback is its tendency to head faster under dry and hot conditions. When this fodder is fed to the animals at a young phase, it exhibits superior feed quality (Metabolizable energy 8.5–9.5 MJ/kg). Nonetheless, its protein content upon maturity declines from 25% to 6%. In Kyabram, Australia, *Echinochloa* millet cv. 'Shirohie' exhibited digestible dry matter (DDM) and dry matter (DM) (10.8 t/ha, 16.3 t/ha) equivalent to sorghum × sudan grass hybrid cv. 'Sudax' (17.1 t DM/ha and 10.7 t/ha). Furthermore, it showcased higher nitrogen concentration (1.9%) and digestibility (65.9%) compared

to sorghum × sudan hybrids (1.5% N, 63.3% dry matter digestibility). Similar investigations conducted in Bangladesh also demonstrated elevated productivity and improved nutritive value of *Echinochloa crus-galli* L. (Kanak et al. 2013).

25.6 Genomic Resources and Molecular Breeding Advancements

The application of molecular markers in barnyard millet closely mirrored their utilization in other minor millets such as finger millet and foxtail millet. In alignment with the extensive morphological variation noticed within the species, Hilu (1994) identified considerable diversity within Indian barnyard millet (*E. frumentacea*) using RAPD markers. In a similar vein, Danquah et al. (2002) devised five primer pairs for microsatellite loci, facilitating the assessment of genetic diversity and interspecific categorization across three vital *Echinochloa* spp.: *E. crus-galli* (L.) Link., *E. colona* (L.) Beauv., and *E. crus-pavonis* (Kunth.). Their findings underscored the utility of microsatellites in differentiating these three species and potentially aiding in the classification of species within this intricate genus. Nozawa et al. (2006) harnessed SSR markers to explore population structure and diversity among 155 barnyard millet accessions, encompassing 49 from var. esculenta, 94 from var. crus-galli, and 12 from var. formosensis. The SSR markers arranged esculenta accessions into two clusters, crus-galli accessions into 12 clusters, and formensis accessions into six clusters. The outcomes further illuminated that the diversity among var. esculenta accessions was less pronounced compared to those from var. crus-galli or var. formosensis.

The available genomic resources for barnyard millet are limited, but foxtail millet has become a prominent model crop for investigating the systems biology of other millets due to its comprehensive genomewide sequence reservoir. In the context of foxtail millet, substantial progress has been made in generating molecular markers like SSRs (Pandey et al. 2013), EST-derived SSRs (Kumari et al. 2013), and ILPs (Muthamilarasan et al. 2014). These markers have not only been employed in germplasm characterization, but also showcased their efficacy in transferability, phylogenetics, and comparative mapping studies across millets and bioenergy grasses (Muthamilarasan et al. 2014). Furthermore, a distinctive web-based database dedicated to Foxtail millet markers (FmMDb) has been constructed, providing breeders and researchers easy access to these marker resources (Venkata Suresh et al. 2013). The high transferability levels demonstrated in these inquiries underscore the practicality of sequence-based markers in comparative genome mapping and evolutionary investigations in other grass species. For instance, in barnyard millet, cross transferability exceeded 90% and was categorized within the same group as foxtail varieties and species (Yadav et al. 2014). This highlights the potential for the development and broad validation of genomic microsatellite markers on a genomewide scale for barnyard millet. This is particularly relevant as barnyard millet possesses limited or negligible genomic information (Lata et al. 2013).

Molecular breeding investigations such as scrutinizing genetic diversity, establishing linkage maps, and pinpointing QTLs necessitate a substantial quantity of molecular markers. Given the absence of genome sequence data in barnyard millet, comparative genomics assumes a pivotal role. In this context, the primary research previously conducted in foxtail millet (Yadav et al. 2014) holds potential in enhancing barnyard crop via molecular avenues. The discerned SSRs can be effectively harnessed for delving into genetic diversity, crafting linkage maps, and subsequently employed for detecting markers linked to vital agro-morphological QTLs within barnyard millet. These recognized QTLs can be adeptly integrated into locally adapted barnyard millet genotypes to enhance yield and counter stressors through the mechanism of marker-assisted selection.

In addition to the utilization of molecular markers, literature is replete with accounts of successful regeneration of callus and plants in *E. frumentacea* (Bobkov 2005), *E. crus-galli* (Gupta et al. 2001), and *E. colona* (Samantaray et al. 2001). Bobkov (2005) fine-tuned the culture medium to sustain viable *E. frumentacea* callus over several years. He propagated the seeds in 2KC medium comprising salts derived from the MS medium and vitamins sourced from the B5 medium. Additionally, the medium contained 100 mg/L myo-inositol, 4 g/L sucrose, 2 mg/L glycine, 6 g/L agar, and 2 mg/L 2,4-D. As for transformation, only one initial study in barnyard millet is available (Gupta et al. 2001), conducted alongside finger millet to assess the efficacy of five gene promoters (CaMV35, Actl, uql, rice ribulose 1,5-biphosphate carboxylase small subunit (RbcS), and Flaveriatrinervia (Ft)). In the context of barnyard millet, solely the uq1 promoter exhibited effectiveness in expressing the GUS gene. Conversely, based on histo-chemical analysis, the other four promoters (CaMV35S, Act1, RbcS, and Ft) proved ineffective.

25.7 Conclusion and Future Prospects

In spite of its nutritional significance and agricultural advantages, barnyard millet remains an underutilized crop, garnering scant attention from both researchers and farmers worldwide. The progression of barnyard millet breeding initiatives has become stagnant due to insufficient funding from diverse funding agencies and research institutions. Consequently, substantial endeavours are requisite to cultivate varieties or hybrids harbouring traits favoured by farmers and consumers alike. In the forthcoming period, an increased number of resistance breeding programs must be devised to exploit biotic resistance, primarily focused on pest and disease resistance, as well as high yield potential, yield stability, augmented salinity resilience, and improved nutritional quality, particularly in terms of micronutrient composition.

However, the advancement of barnyard millet breeding endeavours has been sluggish owing to the scarceness of genetic and genomic resources. Regarding genetic resources, the size of the core collection in barnyard millet is comparatively smaller compared to other minor millets like foxtail and finger millet. Additionally, breeding populations have yet to be formulated. Thus, it is crucial to establish and

assess core and minicore collections that encompass maximum diversity. Moreover, bi-parental and multi-parental populations need to be developed and appraised for diverse nutritional and agronomic traits. These resources will prove invaluable in tracing genomic regions linked to specific traits through linkage-based QTL mapping, genome-wide association studies (GWAS), and genomic selection (GS). Furthermore, they will aid in the identification of candidate genes associated with these traits.

Despite being in its early stages and lagging behind other minor millets, genome research in barnyard millet is progressing. Transcriptome sequencing has enabled the creation of various genomic resources such as EST-SSRs and SNPs, which hold potential for facilitating marker-assisted breeding. However, significant endeavours lie ahead to establish the reference genome, genome-wide SSRs and SNP markers, and to construct genetic linkage maps and physical maps. The recent unveiling of the genome sequence of a weedy ancestor (*E. crusgalli*), coupled with genomic resources from both major and minor millet crops, furnishes an initial framework to augment genomic exploration in barnyard millet through comparative genomic approaches. Utilizing the *E. crusgalli* genome as a reference for cultivated barnyard millet species, similar to the paradigm in bread wheat, is a productive strategy. This not only advances our comprehension of the genome composition of cultivated barnyard millet, enhancing mapping precision but also facilitates the elucidation of variant effects on protein function.

Barnyard millet exhibits adaptability to both warm and temperate regions, serving as a valuable reservoir of genes that confer stress tolerance. Consequently, comprehending the molecular mechanisms governing plant stress responses in inherently stress-tolerant crops like barnyard millet holds promise for cultivating highly stress-resistant cultivars. Although several stress tolerance genes have been pinpointed in barnyard millet, their functionality remains untested via overexpression investigations, largely due to the absence of a genetic transformation system. There have been limited instances of genetic transformation reported in barnyard millet to date. Thus, an imperative task for the future is the establishment of an efficient transformation system for barnyard millet. Such a system will facilitate functional genomics inquiries pertaining to biotic and abiotic stress tolerance, as well as micronutrient attributes.

In addition to these research gaps, the farming community lacks awareness regarding the full potential of barnyard millet cultivation concerning both nutritional value and productivity. Farmers generally engage in cultivating this crop in marginal areas, relying on low-yielding local landraces. Here, support from non-governmental organizations (NGOs) can play a pivotal role in heightening awareness among farmers, stakeholders, nutritionists, and consumers, thereby encouraging barnyard millet cultivation and consumption. Furthermore, given its polyploid nature, the establishment of standardized practices such as rationing or multi-cutting, similar to those in sorghum, would optimally utilize the growing season for both grain and green fodder production.

Addressing the pressing need for advancements in post-harvest technologies is crucial to enhance processing efficiency and value addition for barnyard millet and

other minor millets. Concurrently, a shift in consumer preferences towards small millets, alongside the concurrent development of suitable food products, and an escalation in market prices, would yield improved returns for farmers and offer healthier choices for consumers.

Ultimately, as these obstacles are surmounted, barnyard millet, characterized by its nutritional robustness and ecological resilience, is poised to emerge as a propitious crop in ensuring sustainable food and nutritional stability within forthcoming climatic conditions. Moreover, it is anticipated to exhibit resilience against biotic pressures.

References

AICSMIP (2014) Report on compendium of released varieties in small millets [Internet]. Bangalore, India

Anonymous (2010) Annual report 2009–2010. Vivekananda Parvatiya Krishi Anusandhan Sansthan (ICAR), Almora, Uttarakhand, p 23

Bainslal NK, Meena HP (2016) Breeding for resistance to biotic stresses in plants. In: Recent advances in plant stress physiology. Daya Publishing House, pp 379–411

Bandyopadhyay BB (2001) Climatic influence on grain yield of barnyard millet (*Echinochloa crus-galli* subsp *utilis*) at high hills in Garhwal Himalayas. Indian J Agric Sci 71:788–790

Bandyopadhyay BB (2009) Evaluation of barnyard millet cultivars for fodder yield under single and double cut treatments at higher elevations of hills. Agric Sci Digest 29:66–68

Bobkov SV (2005) Long-term regeneration in callus culture of paisa (*Echinochloa frumentacea* Link.). Int Sorghum Millets Newslett 46:120–122

Chandel G, Meena R, Dubey M, Kumar M (2014) Nutritional properties of minor millets: neglected cereals with potentials to combat malnutrition. Curr Sci 107:1109–1111

Danquah EY, Hanley SJ, Brookes RC, Aldam C, Karp A (2002) Isolation and characterisation of microsatellites in *Echinochloa* (L.) Beauv. spp. Mol Ecol Notes 2:54–56

Gupta P, Raghuvanshi S, Tyagi AK (2001) Assessment of the efficiency of various gene promoters via biolistics in leaf and regenerating seed callus of millets, *Eleusine coracana* and *Echinochloa crusgalli*. Plant Biotechnol 18:275–282

Gupta A, Mahajan V, Kumar M, Gupta HS (2006) Biodiversity in the barnyard millet (*Echinochloa frumentacea* Link, Poaceae). Genet Resour Crop Evol 56:883–889

Gupta A, Mahajan V, Kumar M, Gupta H (2009) Biodiversity in the barnyard millet (*Echinochloa frumentacea* Link. Poaceae) germplasm in India. Genet Resour Crop Evol 56:883–889. https://doi.org/10.1007/s10722-009-9462-y

Gupta A, Joshi D, Mahajan V, Gupta H (2010) Screening barnyard millet germplasm against grain smut (*Ustilagopanici-frumentacei* Brefeld). Plant Genet Resour 8:52–54. https://doi.org/10.1017/S1479262109990141

Harinarayana G (1989) Breeding and varietal improvement of small mil-lets in India. In: Seetharam A, Riley KW, Harinarayana G (eds) Small millets in global agriculture. Oxford & IBH Publishing Co, New Delhi, pp 59–70

Hilu KW (1994) Evidence from RAPD markers in the evolution of *Echinochloa* millets (Poaceae). Plant Syst Evol 189:247–257

Hoshino T, Nakamura T, Seimiya Y, Kamada T, Ishikawa G, Ogasawara A (2010) Production of a fully waxy line and analysis of waxy genes in the allohexaploid crop, Japanese barnyard millet. Plant Breed 129:349–355

Jagadish PS, Mohapatra HK, Chakravarthy MK, Srivastava N, Nangia N (2008) A compendium of insect pests of finger millet and other small millets

Jain AK, Jain SK, Yadava HS (1997) Assessment of yield losses due to grain smut in barnyard millet. Indian Phytopathol 50:49–52

Kajuna STAR (2001) MILLET: post-harvest operations. http://www.fao.org/fileadmin/user_upload/inpho/docs/Post_Harvest_Compendium_-_MILLET.pdf. Accessed 21 Jul 2014

Kanak AR, Khan MJ, Debi MR, Khandakar ZH, Pikar MK (2013) Comparison on biomass production of three fodder germplasms. Bang J Anim Sci 42:35–39

Kim CS, Alamgir KM, Matsumoto S, Tebayashi SI, Koh HS (2008) Antifeedants of Indian barnyard millet, *Echinochloa frumentacea* link, against brown planthopper, Nilaparvata lugens (Stål). Z Naturforsch C J Biosci 63:755–760. https://doi.org/10.1515/znc-2008-9-1022

Kumari K, Muthamilarasan M, Misra G, Gupta S, Subramanian A, Parida SK, Chattopadhyay D, Prasad M (2013) Development of eSSR-markers in *Setaria italica* and their applicability in studying genetic diversity, cross-transferability and comparative mapping in Millet and non-Millet species. PLoS One 8:e67742

Lata C, Gupta S, Prasad M (2013) Foxtail millet: a model crop for genetic and genomic studies in bioenergy grasses. Crit Rev Biotechnol 33(3):328–343

Mandelbaum CI, Barbeau WE, Hilu KW (1995) Protein, calcium, and iron content of wild and cultivated species of *Echinochloa*. Plant Foods Hum Nutr 47:101–108

Maun MA, Barrett SC (1986) The biology of Canadian weeds, *Echinochloa crus-galli* (L.) Beauv. Can J Plant Sci 66:739–759. https://doi.org/10.4141/cjps86-093

Mehta H, Tyagi PC, Mohapatra KP (2005) Genetic diversity in barnyard millet (*Echinochloa frumentacea* Roxb.). Indian J Genet Plant Breed 65:293–295

Muthamilarasan M, Venkata Suresh B, Pandey G, Kumari K, Parida SK, Prasad M (2014) Development of 5123 Intron-Length Polymorphic markers for large-scale genotyping applications in Foxtail millet. DNA Res 21:41–52

Nagaraja A, Mantur SG (2008) Evaluation of barnyard millet entries for grain smut resistance and yield. Mysore J Agric Sci 42:375–377

Nirmalakumari A, Vetriventhan M (2009) Phenotypic analysis of anther and pollen in diversified genotype of barnyard millet (*Echinochloa frumentaceae*) floral characters. ICFAI Univ J Genet Evol 2:12–16

Nozawa S, Takahashi M, Nakai H, Sato YI (2006) Differences in SSR variations between Japanese barnyard millet (*Echinochloa esculenta*) and its wild relative *E. crus-galli*. Breed Sci 56:335–340

Pandey G, Mishra G, Kumari K, Gupta S, Parida SK, Chattopadhyay D, Prasad M (2013) Genome-wide development and use of microsatellite markers for large-scale genotyping applications in Foxtail millet [*Setaria italica* (L.)]. DNA Res 20:197–207

Rawat L, Nautiyal A, Bisht TS, Prasad S, Naithani D, Makhloga K, Tewari A (2019) Screening of barnyard millet germplasm against shoot fly and stem borer damage under field conditions. Int J Curr Microbiol App Sci 8(02):1221–1226. https://doi.org/10.20546/ijcmas.2019.802.142

Saleh ASM, Zhang Q, Chen J, Shen Q (2013) Millet grains: nutritional quality, processing, and potential health benefits. Compr Rev Food Sci Food Saf 12(3):281–295

Samantaray S, Rout GR, Das P (2001) Induction, selection and characterization of Cr and Ni-tolerant cell lines of *Echinochloa colona* (L.) Link in vitro. J Plant Physiol 158:1281–1290

Singh BD (2011) Plant biotechnology, 2nd edn. Kalyani Publishers, New Delhi

Singh HS, Singh K (2005) Status and needs of pasture and fodder management in Uttaranchal. In: Bisht JK, Srivastava AK (eds) Road map for pasture and fodder development in NWHR for livestock sustenance. Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, pp 39–64

Sood S, Khulbe R, Saini N, Gupta A, Agrawal PK (2014) Research note interspecific hybrid between *Echinochloa esculenta* (Japanese barnyard millet) and *E. frumentacea* (Indian barnyard millet)—a new avenue for genetic enhancement of barnyard millet. Electron J Plant Breed 5:248–253

Sood S, Khulbe R, Kumar RA, Agrawal PK, Upadhyaya H (2015) Barnyard millet global core collection evaluation in the sub mountain Himalayan region of India using multivariate analysis. Crop J 3:517–525. https://doi.org/10.1016/j.cj.2015.07.005

Suresh S, Malathi D (2014) Gene pyramiding for biotic stress tolerance in crop plants. Weekly Sci Res J 1(23, 26):1–14

Ugare R, Chimmad B, Naik R, Bharati P, Itagi S (2014) Glycemic index and significance of barnyard millet (*Echinochloa frumentacae*) in type II diabetics. J Food Sci Technol 51:392–395. https://doi.org/10.1007/s13197-011-0516-8

Venkata Suresh B, Muthamilarasan M, Misra G, Prasad M (2013) FmMDb: a versatile database of foxtail millet markers for millets and bioenergy grasses research. PLoS One 8:e71418

Yabuno T (1987) Japanese barnyard millet (*Echinochloa utilis*, Poaceae) in Japan. Econ Bot 41: 484–493

Yadav R, Yadav V (2013) Comparative performance of Indian and Japanese barnyard millet cultivars under varied fertility conditions for dual use in Indian Central Himalaya. Range Manag Agrofor 34:175–178

Yadav CB, Muthamilarasan M, Pandey G, Khan Y, Prasad M (2014) Development of novel microRNA-based genetic markers in foxtail millet for genotyping applications in related grass species. Mol Breed 34:2219. https://doi.org/10.1007/s11032-014-0137-9

Genetic Improvement of Barnyard Millet Through Advanced Biotechnological Methods

26

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Abstract

In recent years, barnyard millet (*Echinochloa* species) has emerged as a prominent minor millet crop in Asia, witnessing a substantial boost in both productivity and production. The two primary species within the *Echinochloa* genus, namely *Echinochloa esculenta* and *Echinochloa frumentacea*, are predominantly cultivated for human and animal consumption. Barnyard millet exhibits remarkable resilience against both biotic and abiotic stressors, showcasing its ability to yield even in the face of the most challenging conditions. In contrast to other major grains, barnyard millet stands out as an exceptional source of essential minerals such as iron and zinc, as well as dietary fiber and carbohydrates. Despite its manifold agronomic and nutritional advantages, barnyard millet has unfortunately remained somewhat overlooked. Regrettably, only limited efforts have been directed towards investigating the unique characteristics of this crop in recent times. A concerted endeavor is warranted to delve into barnyard millet's germplasm resources, pinpoint donors with specific traits, establish mapping populations, and pinpoint key QTL (Quantitative Trait Loci) and genes associated with these traits. The imperative to undertake more rigorous research is evident. A deeper comprehension of the genetic framework and the intricate interplay between genotype and phenotype concerning micronutrients and agronomic traits can be facilitated by the recent unveiling of genome and transcriptome sequences of both wild and domesticated *Echinochloa* species. In the present review, we underscore the paramount importance of barnyard millet within the prevailing context. We delve into the current landscape of genetic and genomics research, while also identifying pivotal gaps that necessitate urgent attention. Bridging

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these lacunae is indispensable to transform barnyard millet into a viable and substantial crop capable of bolstering food and nutritional security on a broader scale.

Keywords

Barnyard millet · *Echinochloa* species · Nutritional · Genetic and genomic resources · Next-generation sequencing · Transcriptome sequencing

26.1 Introduction

The global population is anticipated to reach 9.8 billion by the year 2050, which necessitates a robust strategy to fulfill the escalating food demands. This surge in population is projected to be around 60–70% higher than current levels, posing a substantial challenge in providing nourishment for the expanding populace. Notably, more than 60% of human energy intake from plant sources worldwide is derived from three key cereal varieties: rice, wheat, and maize (Cakmak and Kutman 2018; Vetriventhan and Upadhyaya 2019). However, despite their widespread consumption, these major staple cereals have relatively lower concentrations of essential micro- and macronutrients. This deficiency in nutrition has led to hidden hunger, affecting over two billion individuals globally due to micronutrient scarcity (Bekkering and Tian 2019; Li and Siddique 2018; Ramegowda et al. 2013; Satyavathi et al. 2022). The primary cause is the heavy dependence on a limited variety of staple foods. As a result, addressing the challenge of sustaining agriculture and fostering healthy diets mandates a transformative shift towards greater diversity in both crops and diets. This entails cultivating and consuming underutilized, climate-resilient, and nutrient-dense crops, thereby establishing a foundation for sustainable agriculture.

In this context, small millets have gained traction as prudent choices for health-conscious individuals and are hailed as 'nutri-cereals' and 'Smart-Food Crops.' These millets, including barnyard millet, offer elevated levels of energy, protein, dietary fiber, and essential minerals, vitamins, antioxidants, and amino acids, alongside a low glycemic index (Dwivedi et al. 2012; Kam et al. 2016). Barnyard millet, specifically, emerges as a short-duration crop with remarkable adaptability to adverse environmental conditions. It exhibits resilience against various biotic and abiotic stresses and demands relatively lesser water input. Beyond their agronomic advantages, the grains hold value for their cost-effectiveness and high nutritional content in comparison to major cereals such as rice, wheat, and maize.

Barnyard millet, belonging to the *Echinochloa* species, holds a rich historical significance as one of the earliest millet crops. It boasts extensive cultivation across Asia, particularly in countries like India, China, Japan, and Korea, within warm and temperate regions. Notably, India stands as the leading producer of barnyard millet in terms of both production (0.147 million tons) and cultivated area (0.146 million hectares), with an average productivity of 1034 kg/ha in recent years (IIMR 2018).

Serving as the fourth most widely cultivated minor millet, barnyard millet plays a pivotal role in ensuring food security for numerous economically disadvantaged households. It serves a dual purpose by being used for human consumption as well as animal feed. The two primary species, *Echinochloa frumentacea* (Indian barnyard millet) and *Echinochloa esculenta* (Japanese barnyard millet), have gained prominence for their nutritional composition and adaptability.

Barnyard millet is characterized by its substantial protein, carbohydrate, fiber, and micronutrient content, including iron (Fe) and zinc (Zn). These attributes contribute to its extensive health benefits and potential as a supplementary crop for subsistence farmers, especially during monsoon failures in major crop-growing regions (Gupta et al. 2009). Despite its nutritional and agronomic prowess, barnyard millet has faced neglect and underutilization due to limited awareness. Research on this crop has lagged behind other minor millets in recent decades. While some studies have explored germplasm diversity through morphological and molecular markers, comprehensive research is required to fully understand germplasm accessions, identify trait-specific donors, establish mapping populations, and uncover quantitative trait loci (QTLs) and genes responsible for key attributes.

Advancements in genomic resources, particularly with the advent of second- and third-generation sequencing technologies, have the potential to accelerate research on barnyard millet. Genome and transcriptome sequences of cultivated and wild *Echinochloa* species have provided critical insights into the genetic makeup and potential avenues for crop enhancement. Genotyping investigations, including analyses of genetic diversity, linkage mapping, and QTL identification, have been empowered by leveraging these genomic resources. Notable studies have employed SNP markers to elucidate genetic diversity in barnyard millet's core collection and to identify genes associated with drought resistance and micronutrient content.

While progress has been made, the complexity of barnyard millet's hexaploid genome ($2n = 6x = 54$) has posed challenges, and research in this area remains relatively nascent compared to other minor millets. Recent years have witnessed an upsurge in interest and publications related to barnyard millet, indicative of the growing momentum in this field. This chapter delves into the taxonomy, nutritional value, and health benefits of barnyard millet while offering insights into the state of genetic and genomic research. The existing research gaps are emphasized, urging for increased efforts to harness the potential of barnyard millet as a valuable crop contributing to food and nutritional security (Table 26.1).

26.2 Nutritional Significance and Health Benefits

In contrast to major grains such as rice, wheat, and sorghum, barnyard millets emerge as a nutritional powerhouse, boasting a diverse array of essential components that contribute to human health and well-being. These millets stand out for their exceptional richness in dietary fiber, iron, zinc, calcium, protein, carbohydrates, magnesium, fat, vitamins, and a range of vital amino acids. Particularly noteworthy is their abundant concentration of micronutrients, including iron

Table 26.1 Differences in morphology between wild and cultivated *Echinochloa* species

Traits	*Echinochloa esculenta*	*Echinochloa crus-galli*	*Echinochloa colonaa*	*Echinochloa frumentacea*
Origin country	Eastern Asia, Japan, China, and Korea	China, Japan, and Korea	China and Japan	India, Pakistan, and Nepal
Common names of barnyard millet and synonyms	Japanese barnyard millet Japanese millet, marsh millet, Siberian millet, and white millet	Barnyard grass *Panicum crus-galli*, *Panicum hispidulum*, *Milium crus-galli,* and *Pennisetum crus-galli*	Jungle rice *Echinochloa colonum*, *Echinochloa crus-galli* subsp. *colona*, *Panicum colonum*, *Panicum cumingianum*, *Panicum zonale,* and *Milium colonum*, *Oplismenuscolonus*	Indian barnyard millet/sawa millet Billion Dollar grass, sawa millet, sama millet
Habitat	Annual, warm-season	Annual, temperate region	Annual, warmer region	Annual, warmer region
Distribution around world	Widely distributed in India, Temperate Asia, Australasia, and Pacific	Widely distributed in South and Southeast Asia, Africa, Europe, and America	Widely distributed in South and Southeast Asia, Australia, Africa, Europe, and America	Widely distributed in Central Africa, Africa, Temperate Asia, Tropical Asia, Australasia, and South America
Morphological parameters	Robust, 60–122 cm tall, leaf sheath smooth 10–50 cm long and 7–25 mm wide, plants green, however, light to dark purple pigmentation in various plant parts, thicker stem	Erect, 200 cm tall, leaf 10–40 cm long, leaf blade surface smooth, leaf blades 0.5–35 cm long and 6–20 mm wide	Erect to decumbent, 60 cm tall, short leaf length 10–15 cm, red tinges at the basal portion of leaf, leaf blade surface smooth, leaf blades 3–30 cm long and 2–8 mm wide	Erect, 242 cm tall, leaf length 15–40 cm long and 1–2.5 cm wide, plants mostly green, however, purple tinges also found in vegetative and reproductive parts, leaf blades are smooth and glabrous, culms slender to robust
Spikelets on panicle	Compact, branched spikelets, larger spikelets and longer	Open, branched spikelets on the rachis, setae on the primary branches	Spikelets arranged in 4 uniform rows on the primary rachis, spikelets 1–3 mm long	Compact, non-branched spikelets on the rachis, tightly clustered,

(continued)

Table 26.1 (continued)

Traits	*Echinochloa esculenta*	*Echinochloa crus-galli*	*Echinochloa colonaa*	*Echinochloa frumentacea*
	primary branches, dense clustered, 3–4 mm long, shortly cuspidate and rarely awned			2–4 mm long, acute and awnless
Physiology of inflorescence	Brown to purple, compact inflorescence 12–15 cm long, racemes 5–15 numbers, arcuate to flexuous, 0.5–3 cm long, rarely awned	Green to purple, inflorescence length 10–25 cm, compound ascending racemes 5–15 numbers, 2–10 cm long, slightly hairy, green to purplish awns 2–5 mm long	Green to purple, inflorescence length 5–15 cm, simple ascending racemes 5–15 numbers, 0.5–3 cm long raceme	Green to purple, usually erect and compact, inflorescence length 1–28 cm long, racemes numerous 20–70, 1–3 cm long, rarely drooping, awnless

Sources: De Wet et al. (1983); Yabuno (1987); Doggett (1989); Bandyopadhyay (1999); Yadav and Yadav (2013); Renganathan et al. (2015)

and zinc, a characteristic that sets them apart (Singh et al. 2010; Saleh et al. 2013; Chandel et al. 2014).

With a protein content of around 12%, barnyard millet presents a highly digestible protein source that is also relatively low in calories. What distinguishes barnyard millet further is its carbohydrate profile, with an average content ranging between 51.5 and 62.0 g/100 g (Saleh et al. 2013). Remarkably, these carbohydrates exhibit a slow digestibility, rendering barnyard millet a low-glycemic-index food. This feature proves particularly advantageous for modern sedentary lifestyles, offering sustained energy release (Veena et al. 2005). Moreover, barnyard millet's carbohydrates display a tendency toward high retrogradation of amylase, facilitating the formation of significant amounts of resistant starch.

Barnyard millet shines as an exceptional source of dietary fiber, encompassing both soluble and insoluble fractions. The fiber content, ranging from 8.1% to 16.3%, surpasses that of other millets. This robust fiber presence offers a plethora of health benefits, including the prevention of constipation, alleviation of excess gas and bloating, reduction of blood cholesterol levels, and management of conditions like diabetes mellitus and obesity (Kumari and Thayumanavan 1998; Ugare et al. 2014). Notably, barnyard millet's carbohydrate to crude fiber ratio plays a role in stabilizing blood glucose and lipid levels, making it a valuable dietary choice (Kumari and Thayumanavan 1998; Ugare et al. 2014).

Certain varieties of barnyard millet exhibit notably high iron content, ranging from 15.6 to 18.6 mg/100 g, making them a promising source of this essential mineral, especially for vegetarian diets (Renganathan et al. 2017; Vanniarajan et al. 2018). Additionally, the presence of phytates and phytic acid is relatively lower in barnyard millet grains, particularly after the dehulling process. This reduction in anti-nutritional factors enhances mineral bioavailability, further contributing to the overall nutritional value (Kulkarni et al. 1992).

Barnyard millet's nutritional profile aligns perfectly with the health-conscious inclinations of today's society, positioning it as an ideal dietary choice for individuals dealing with lifestyle-related diseases, anemia, and especially women in developing nations. The elevated levels of polyphenols and carotenoids found in barnyard millet, surpassing those in finger millet, further underscore its potential to confer various health advantages to consumers (Panwar et al. 2016).

Additionally, barnyard millet houses a treasure trove of bioactive compounds, including alkaloids, steroids, glycosides, tannins, phenols, and flavonoids, which have long been associated with traditional medicinal properties. These encompass a wide array of benefits, spanning antioxidant, anti-carcinogenic, anti-inflammatory, antimicrobial, and wound-healing capacities (Kim et al. 2011; Ajaib et al. 2013; Moreno et al. 2014; Borkar et al. 2016; Nguyen et al. 2016; Sharma et al. 2016; Sayani and Chatterjee 2017).

The collective attributes of barnyard millet culminate in an offering that not only satiates nutritional needs but also promotes overall physical well-being. Its intricate blend of essential nutrients, slow-releasing carbohydrates, and health-boosting bioactive compounds positions barnyard millet as an emblem of holistic nourishment for today's discerning consumers.

26.3 Utilization of Next-Generation Sequencing

The acquisition of a whole genome sequence (WGS) stands as a pivotal step in unraveling the gene repertoire and genome architecture of a crop. This endeavor holds the key to uncovering crucial genes and metabolic pathways that underlie economically significant traits in crops. The insights garnered from WGS are invaluable, offering a roadmap for identifying and understanding the genetic factors that govern various essential traits. This knowledge extends its benefits to the realm of crop improvement and breeding, fostering more targeted and effective strategies.

A complementary technique, RNA-sequencing (RNA-Seq), adds another layer of depth to our understanding. By capturing the transcriptional landscape of the crop, RNA-Seq provides a wealth of information about non-coding regions, gene expression patterns, and transcript structures. This technique affords the ability to measure gene expression at a granular level, shedding light on the dynamic interplay of genes within the organism.

The rapid progress achieved in large-scale genome and transcriptomic sequencing owes much to the advent of next-generation sequencing technologies, notably the second and third-generation platforms. These advancements have democratized

sequencing, making it both accessible and cost-effective. Researchers have harnessed these technologies to decode the complete genomes of various millets, including pearl millet, foxtail millet, finger millet, and proso millet (Mace et al. 2013; Hittalmani et al. 2017; Varshney et al. 2017; Zou et al. 2019). This expansion of genomic information has facilitated the development of comprehensive genomic resources for millets like pearl millet, foxtail millet, and finger millet, encompassing physical maps, genetic linkage maps, cytogenetic stocks, and large-insert libraries (Varshney et al. 2006; Gomashe 2017).

However, in the context of barnyard millet, challenges loom due to the intricacy of its genome and the limited funding dedicated to researching this orphan crop. The genomic structure and the downstream mechanisms associated with barnyard millet remain relatively unexplored, despite its well-documented nutritional significance. Surprisingly, research efforts aimed at harnessing the crop's potential to address protein-energy deficiency and nutritional gaps have remained marginalized. This disparity could stem from the dominance of research focused on staple crops like wheat, rice, and maize, particularly in the wake of the Green Revolution. Furthermore, the application of modern breeding and biotechnology approaches in barnyard millet improvement is hindered by the scarcity of genomic information. This is evident in the absence of large-scale expressed sequence tag (EST) sequencing, a powerful tool that has catalyzed research progress in numerous other crops.

To harness the full potential of barnyard millet, concerted research endeavors must be directed toward describing germplasm resources, identifying trait-specific donors, establishing mapping populations, and uncovering QTLs. The urgency to accelerate these efforts is underscored by the imperative to enhance genomic and transcriptomic information for this crop. Initiatives aimed at expediting the acquisition of such data are pivotal for unlocking barnyard millet's latent genetic treasures and ushering in a new era of research-driven advancements in crop enhancement. By embarking on this path without delay, we can catalyze research operations and expedite the realization of barnyard millet's untapped potential.

26.4 Advancement in Genome Sequencing of Barnyard Millet

The landscape of sequencing technology has evolved significantly, leading to cost reductions alongside amplified coverage, depth, and dependability. Illustratively, Wu and colleagues embarked on sequencing endeavors encompassing one domesticated millet accession (*E. Colona* var. frumentacea, PI463783) and two weedy accessions (*E. crus-galli* and *E. oryzicola*), culminating in the establishment of comprehensive genomes for *Echinochloa* species (Wu et al. 2022). The meticulous de novo assembly process yielded a genome measuring 1.13 Gb, featuring contigs spanning 24.8 Mb reads and an average read length of 15.1 kb, all executed through the PacBio HiFi sequencing method. A remarkable 97.1% of contig sequences were successfully attributed to the three sub-genomes: DH, EH, and FH, aptly denoted as D, E, and F in the context of hexaploidy *E. colona*, where 'H' denotes hexaploidy. This culminated in the realization of the *E. colona* genome,

comprising sub-genome sizes of 436.4 Mb (DH), 310.5 Mb (EH), and 347.1 Mb (FH). A stringent validation process ensued, wherein 90.4% of transcriptomic reads derived from RNA-Seq and a notable 97.9% of genomic short reads from Illumina sequencing were accurately mapped to the assembly. Additionally, the assembly successfully recovered 97.0% of the 4896 core conserved Poales genes (BUSCO), with the long terminal repeat (LTR) assembly index (LAI) score reaching an impressive 22.5, indicative of a gold quality standard. Noteworthy contributions from China have also been made, encompassing the genome sequencing of the allohexaploid *Echinochloa crus-galli* through PacBio technology (Chu-Yu et al. 2020). The culmination of this research yielded PacBio long reads that spanned an approximate 86× coverage of the genome, revealing a genome size of 1.4 Gb, assembly size of 1.34 Gb, and contig and scaffold N50 sizes of 1.57 Mb and 4.09 Mb, respectively. The annotation process uncovered a total of 103,853 protein-coding genes within the new *E. crus-galli* genome assembly. Another spotlight is cast on the whole genome sequence of weedy *E. crus-galli*, which exhibited successful annotation reflecting its unique invasiveness and adaptation attributes in crop plant domains of China. The genome, estimated at a depth of 171×, spanned a length of 1.27 Gb, encapsulating around 90.7% of the predicted genome size. Impressively, the genomic libraries displayed a range from 160 bp to 20 kb, yielding a total contig count of 4534, with contig sizes varying from 1 kb to 11.7 Mb. Further scrutiny of the gene annotation in *E. crus-galli* unveiled 108,771 protein-coding genes, 785 miRNAs, 514 Mb of repetitive elements, and non-coding RNAs.

In the expansive repository of the NCBI database, numerous *Echinochloa* species have been sequenced, notably *E. crus-galli* (669), *E. oryzicola* (126), *E. phyllopogon* (100), *E. colona* (89), *E. pyramidalis* (56), *E. frumentacea* (43), *E. esculenta* (42), and *E. oryzoides* (34), as exemplified in Fig. 26.1b. Strikingly, *E. crus-galli* claims the spotlight, with 50% of nucleotide sequences available to date. In contrast, the cultivated barnyard millet species, *E. frumentacea* and *E. esculenta*, remain relatively underrepresented, constituting a mere 3% of the available nucleotide sequences.

The rapid evolution of sequencing technologies has opened avenues to decode the genetic blueprints of various *Echinochloa* species, unveiling intricate genomic structures and lending insights into their unique attributes. These efforts stand poised to drive groundbreaking advancements in crop improvement and cultivation, promising a brighter future for agriculture and scientific exploration.

26.5 Chloroplast Genomes of *Echinochloa* sp.

The structural integrity and organizational coherence of the *Echinochloa* chloroplast genome remain remarkably conserved, exemplifying a testament to its evolutionary stability (Ye et al. 2014). This assertion finds substantiation in the comprehensive sequencing endeavors that have unveiled the chloroplast genomes of seven *Echinochloa* species, encompassing *E. crus-galli*, *E. ugandensis*, *E. stagnina*, *E. colona*, *E. esculenta*, *E. frumentacea*, *E. oryzicola*, and *E. haploclada* (Ye et al.

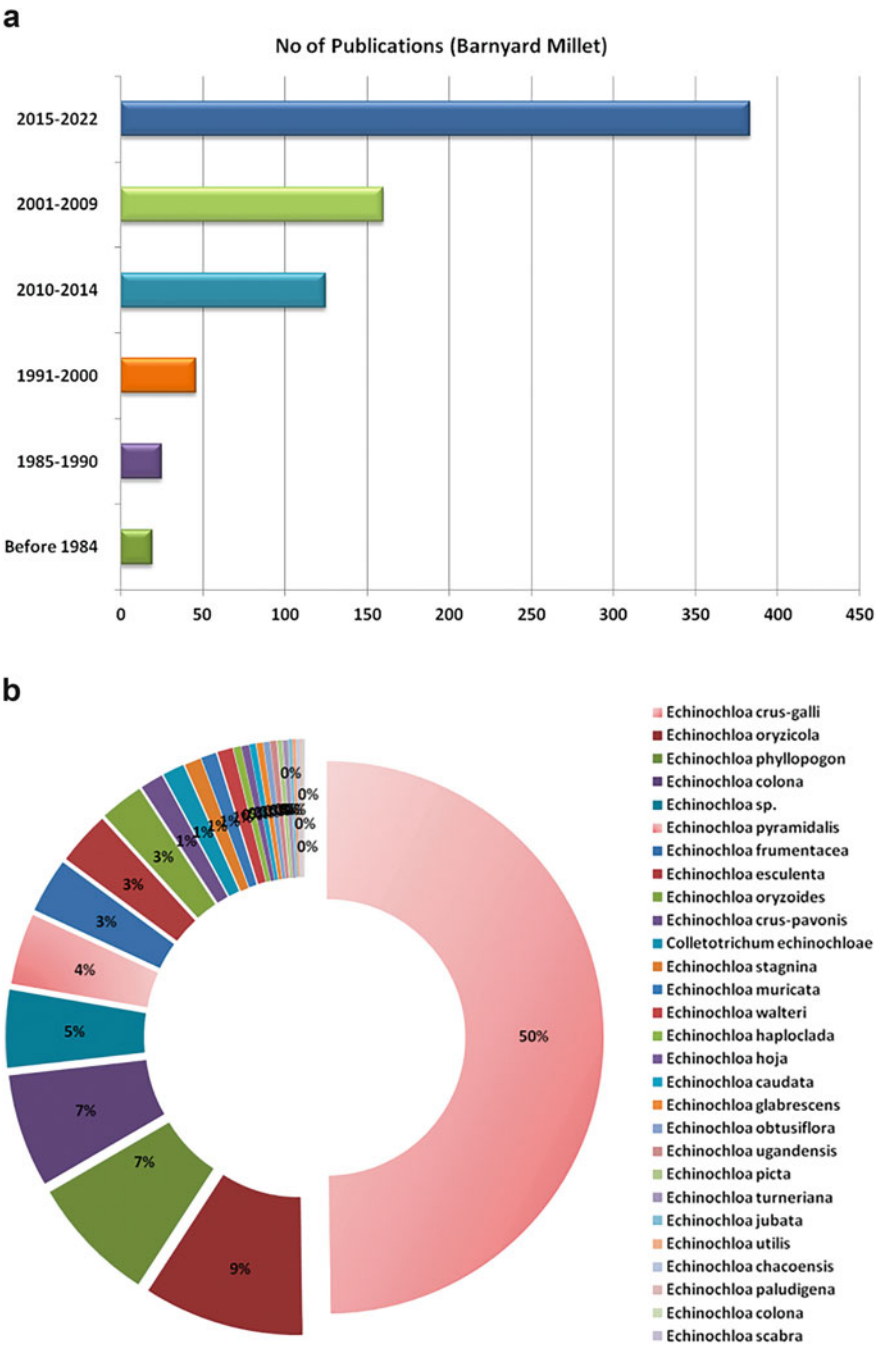

Fig. 26.1 Literature and nucleotide sequence availability. (Note: Data verified till Sep-2022). (**a**) PubMed (https://www.ncbi.nlm.nih.gov/pubmed). (**b**) Nucleotide sequence (https://www.ncbi.nlm.nih.gov/)

Table 26.2 Summary statistics of chloroplast genomes for wild and cultivated barnyard millet

	Echinochloa crus-galli	*Echinochloa frumentacea*	*Echinochloa esculenta*	*Echinochloa colonaa*
Genome size (bp)	139,851	139,593	139,851	139,592
Inverted Repeat size (bp)	22,640	22,618	22,748	22,618
Large-single copy region size (bp)	82,053	81,839	81,837	81,837
Small-single copy region size (bp)	12,518	12,518	12,518	12,519
Number of genes	131	112	111	131
Number of Protein coding genes	88	77	76	88
Number of tRNA	40	30	30	40
Number of rRNA	4	4	4	4
GC contents (%)	38.6	38.6	38.6	38.6

2014; Perumal et al. 2016; Lee et al. 2017; Sebastin et al. 2019; Jiang et al. 2021). These investigations collectively illuminated crucial aspects such as genome size, which ranges between 139,593 and 139,851 bp, and an astonishingly high degree of genomic identity, reaching an impressive 99.5% across the chloroplast genomes.

A pivotal determinant in the trajectory of plastome evolution is the intricate genome structure, typified by the presence of a pair of inverted repeats (IR) flanking a large single-copy region (LSC) and a small single-copy region (SSC) (Yang et al. 2013). A comprehensive overview of the chloroplast genomes of both wild and cultivated *Echinochloa* species is detailed in Table 26.2, outlining distinct characteristics and variations. Notably, the dimensions of the IR, LSC, and SSC regions range from 22,618 to 22,748 bp, 81,837 to 82,053 bp, and 12,518 to 12,519 bp, respectively, contributing to a genomic composition of 38.6% GC regions and 61% AT regions (Sebastin et al. 2019).

Further delving into the genetic makeup, the *Echinochloa* chloroplast genomes encompass a spectrum of 111–131 genes. This gene set encompasses 77–83 protein-coding genes, 30–32 tRNA genes, and 4 rRNA genes, with the cultivated species tending toward a lower count. The discernible variations in gene content are often attributed to the dynamic processes of gene copy number modulation and structural rearrangements that unfold throughout the course of evolution and speciation. The resultant genetic polymorphism between different *Echinochloa* species stems from the divergence in the copy number of individual genes, thus giving rise to substantial disparities in genome size and, consequently, phenotypic traits (Suryawanshi et al. 2016).

The manifestation of pronounced morphological diversity between wild and cultivated *Echinochloa* species can be traced back to the consequences of genome rearrangements that transpire during the intricate tapestry of angiosperm evolution (Wendel 2015). These revelations underscore the vital role played by chloroplast genomes in shaping the genetic underpinnings of *Echinochloa* species, elucidating

the mechanisms that steer their development, differentiation, and eventual adaptation to diverse ecological niches.

26.6 Nuclear and Organelle Genome Size Comparison of Barnyard Millet

Up to the present moment, a notable stride has been made in the sequencing of various millet genomes, encompassing foxtail millet, finger millet, proso millet, teff, and Japanese barnyard millet. Additionally, complete chloroplast genome sequences are now accessible for foxtail millet, proso millet, little millet, and barnyard millet, underscoring the comprehensive efforts invested in deciphering the genetic blueprints of these cereal crops (depicted in Fig. 26.2a, b). A spectrum of genome sizes has been unveiled, ranging from the smallest in foxtail millet (423–510 Mb) to the grandest in finger millet (1.5 Gb), with barnyard millet (*E. crus-galli*, 1.27 Gb) notably occupying an intermediary position (Perumal et al. 2016). Strikingly, the majority of millet species share similar chloroplast genome sizes, a phenomenon attributed to intrinsic characteristics such as their diminutive diploid genomes, compact growth durations, and self-pollinating behavior. Notably, foxtail millet emerged as the pioneering millet crop to have its genome entirely sequenced, further elevating its status as a model for C4 crop species, emblematic of its significance in agricultural research and advancement.

Despite the concerted efforts fueled by next-generation sequencing platforms, a substantial portion of millet genomes remains at a draft stage, signifying a phase of continuous refinement. This trajectory mandates resequencing and reannotation to rectify gaps, address mis-annotations, and optimize chromosomal assignments, an imperative step in ensuring the accuracy and comprehensiveness of the genomic data (Vetriventhan et al. 2020). However, it's important to note that while some millet genomes are still in draft form, the existing sequence information already holds substantial utility, particularly for large-scale genotyping applications and the invaluable pursuit of gene discovery and mining. This highlights the dual nature of ongoing genomics research, simultaneously catering to immediate practical applications while laying the foundation for deeper insights and breakthroughs in the future.

26.7 Transcriptomics of Barnyard Millet

The advent of advanced next-generation sequencing technologies (NGS) has heralded a prolific era of genomic resource generation, revolutionizing the landscape of genetic research. Particularly, RNA-seq (RNA-sequencing) technologies have emerged as a transformative tool, enabling cost-effective and time-efficient exploration of gene expression profiles, superseding the previous microarray approach (Weber 2015). This methodology not only facilitates the identification of differentially expressed genes but also serves as a conduit for the discovery of functional

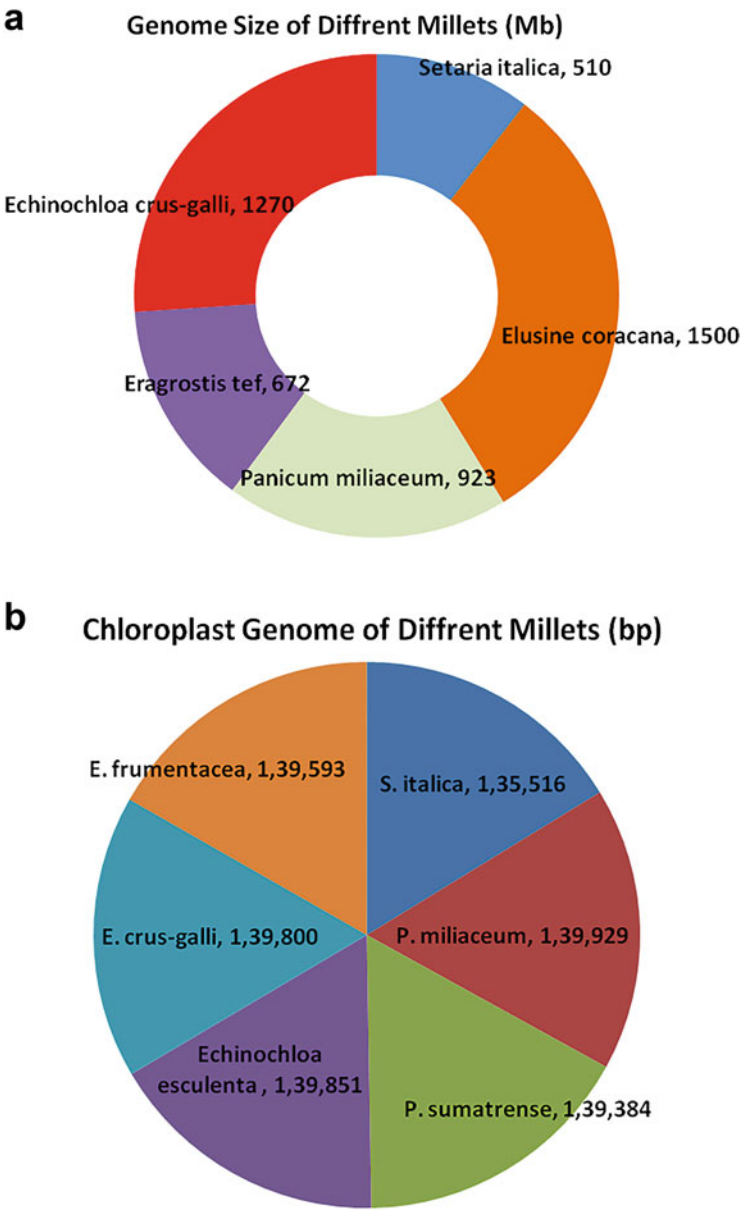

Fig. 26.2 Comparative genome size of Nuclear and chloroplast genome of different millets. (**a**) Comparative genome size of nuclear genome of different millets. (**b**) Comparative genome size of chloroplast genome of different millets

molecular markers, including simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs), within diverse minor millet species.

Remarkable strides have been made in elucidating intricate traits linked to invasiveness and adaptations, such as herbicide resistance, photosynthesis dynamics, flooding responses, and the involvement of homeobox genes. This pursuit has been championed through the application of RNA-seq in weedy *Echinochloa* species, yielding insights of profound significance (Yang et al. 2013; Nah et al. 2015; Xu et al. 2015; Guo et al. 2017; Gao et al. 2018; Pan et al. 2022; Rangani et al. 2022). Recently, Jayakodi et al. (2019) embarked on a comprehensive exploration, delving into the transcriptome sequences of cultivated *E. frumentacea* variety CO (Kv) 2. This endeavor bore fruit in the form of 97,065 transcripts, each characterized by an average length of 125 Mbp. The investigation also unveiled key genes intricately linked to crucial attributes like iron (Fe) and zinc (Zn) accumulation, as well as drought tolerance. The process encompassed multifaceted steps, including de novo assembly, functional annotation, and a meticulous comparison to *E. crus-galli* transcripts. Notably, this study further culminated in the development of 300 SSR primer pairs, meticulously designed from a repository of 10,881 SSR loci, with a predominant focus on trinucleotide (122) repeats, followed by dinucleotide (121), tetranucleotide (35), pentanucleotide (20), and hexanucleotide (2) repeats (Fig. 26.3).

The endeavor to unravel the genomic intricacies of *Echinochloa* species is underscored by an array of studies, several of which have been submitted as bioprojects and reside within the Sequence Read Archive (SRA) on the National Center for Biotechnology Information (NCBI). Table 26.3 provides an insightful glimpse into a selection of these studies, encapsulating a diverse array of *Echinochloa* species, ranging from *E. colona* to *E. oryzicola*.

Echinochloa species have yielded a plethora of gene sequences, encompassing a spectrum of pathways, abiotic and biotic stress resistance mechanisms, flavonoid biosynthesis pathways, and pivotal biological processes. The genetic repertoire is replete with genes intricately linked to photosynthesis components (e.g., PS I, PS II, NADH-plastoquinone oxidoreductase, ATP synthase), C4 pathways, micronutrient transportation (including genes such as Fe2C transport protein 2-like protein, nicotianamine synthase 1, and nicotianamine synthase 2), DNA/RNA polymerases, herbicide resistance factors, flooding tolerance determinants, waxy grain attributes, non-shattering grain traits (e.g., sh4), and even pivotal elements like ribosomal RNA and transfer RNAs.

However, while these endeavors have yielded substantial insights, it is important to acknowledge that the journey toward species-specific expression and a comprehensive understanding of the intricate genetic tapestry is an ongoing process, requiring a sustained commitment to research and data acquisition. As the field continues to evolve, each discovery contributes to the mosaic of knowledge, inching us closer to the grand tapestry of genetic comprehension that defines the captivating realm of *Echinochloa* species.

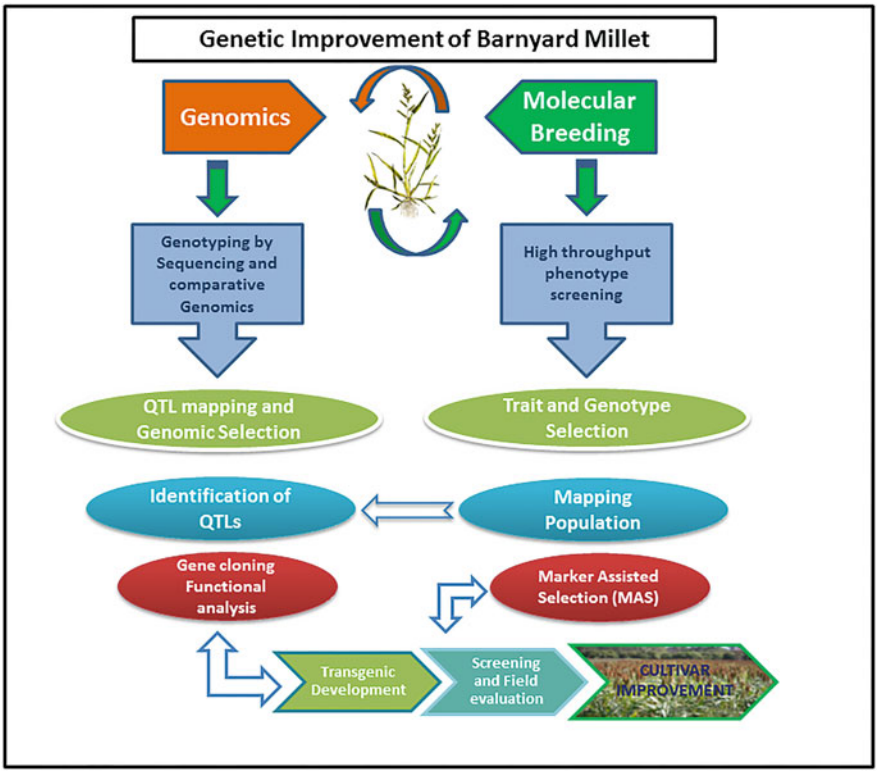

Fig. 26.3 Genetic Improvement of Barnyard Millet

26.8 Conventional Markers and Its Application in Barnyard Millet

Molecular markers stand as pivotal nucleotide sequences that play a crucial role in unraveling genetic diversity, constructing linkage maps, and facilitating marker-assisted crop selection strategies (Tomar 2010). In the nascent phases of molecular marker exploration, Random Amplified Polymorphic DNA (RAPD) markers emerged as pioneering tools to delve into the intricate genetic tapestry of *Echinochloa* species. These markers, as elucidated by Hilu (1994), exhibited an adept capacity to discriminate between the cultivated and wild progenitors within the *Echinochloa* genus. It was noteworthy that the genetic diversity among populations of *E. frumentacea* surpassed that of *E. utilis*. However, an intriguing revelation from an isozyme marker study between these species painted a more complex picture: accessions from distinct species coalesced into a single cluster, whereas those within the same species diverged into separate clusters, suggestive of intergrades and overlaps between these taxa (Prabha et al. 2010). The explorations of Rutledge

Table 26.3 Next generation sequencing experiments performed on different species of Barnyard Millet

No	Organism name	Experiment accession	Submitter	Study accession	Study title
1	*Echinochloa colona*	SRX14263425	Clemson University	SRP115957	*Echinochloa colona* Transcriptome or Gene expression
2	*Echinochloa crus-galli*	SRX3081138	College of Agriculture and Biotechnology, Zhejiang	SRP114887	*Echinochloa crus-galli* isolate: STB08 Genome sequencing
3		SRX5431761	Nanjing Agricultural University	SRP186893	*Echinochloa crus-galli* Transcriptome or Gene expression
4		SRX15039818	Auburn University	SRP372707	Evaluation of resistance mechanism of barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] to cyhalofop-butyl
5		SRX9782570	Hunan Agricultural University	SRP300396	*Oryza sativa* and *Echinochloa crus-galli* transcriptome
6	*Echinochloa crus-galli* var. *crus-galli*	SRX160526	Majorbio	SRP014452	*Echinochloa crus-galli* var. *crus-galli* Transcriptome or Gene expression
7	*Echinochloa crus-galli var. zelayensis*	SRX3574154	Nanjing Agricultural University	SRP130228	*Echinochloa crus-galli* var. *zelayensis* Transcriptome
8		SRX12027677	Nanjing Agricultural University	SRP335934	*Echinochloa crus-galli* var. *Zelayensis* Transcriptome or Gene expression
9	*Echinochloa crus-pavonis*	SRX7923697	Jiangsu Academy of Agricultural Sciences	SRP253049	*Echinochloa crus-pavonis* Transcriptome or Gene expression
10	*Echinochloa glabrescens*	ERX990973	University of Cambridge Department of Plant Science	ERP010718	A blueprint for C4 photosynthesis in the rice paddy: insights from the noxious weed *Echinochloa glabrescens*

(continued)

Table 26.3 (continued)

No	Organism name	Experiment accession	Submitter	Study accession	Study title
11	*Echinochloa haploclada*	SRX11675372	Zhejiang University	SRP331621	*Echinochloa haploclada* illumina sequencing
12	*Echinochloa oryzicola*	SRX542948	Seoul National University	SRP041999	Adaptive diversity in *Echinochloa* species

et al. (2000) and Ruiz-Santaella et al. (2006) further accentuated the scenario, unveiling 90 polymorphic bands from 21 primer pairs and 75 polymorphic bands from 13 primer pairs, respectively, with a modest average of 4.3 alleles per primer.

Yet, it becomes evident that the spectrum of polymorphism exhibited by RAPD markers within the germplasm remains relatively limited. This observation is congruent with findings in finger millet diversity studies (Muza et al. 1995), reflecting a constraint in capturing the full breadth of genetic variance. In this evolving landscape, the Amplified Fragment Length Polymorphism (AFLP) marker system emerged as a powerful contender, surpassing RAPD markers in its ability to unveil the intricate genetic tapestry of *Echinochloa* species (Danquah et al. 2002; Tabacchi et al. 2009). AFLP markers, through their ability to generate a higher number of alleles per primer, revealed a broader canvas of genetic diversity. Notably, the application of four primer pairs to 28 genotypes yielded a substantial 166 polymorphic bands, averaging an impressive 41.5 bands per primer pair. Moreover, the implementation of Insertion/Deletion (InDel) markers targeting crucial genes such as rbcL, matK, and ITS in *E. colona* not only enriched DNA barcoding initiatives encompassing *E. colona*, *E. oryzicola*, and *E. crus-galli* (Lee et al. 2014), but also underscored the inherent value of sequence-specific markers in delineating species boundaries and genetic relationships.

While these studies have illuminated the efficacy of RAPD, AFLP, and InDel markers, it is critical to acknowledge that these methodologies serve as a lifeline in situations where comprehensive sequence information is limited. As they stand as the vanguard of genetic differentiation within the enigmatic realm of *Echinochloa* species, these markers not only empower species discrimination but also lay the very foundation for a promising era of molecular breeding initiatives tailored to the context of barnyard millet. In an era marked by technological strides, these molecular beacons pave the way for a deeper understanding of genetic dynamics, driving the progress toward sustainable agricultural practices and enhanced crop resilience.

26.9 Microsatellite Markers and Their Application in Barnyard Millet

The landscape of genetic diversity exploration has been fundamentally transformed through the advent of sequence-based markers such as Simple Sequence Repeats (SSRs), Expressed Sequence Tag SSRs (EST-SSRs), and Single Nucleotide Polymorphisms (SNPs), propelled by the remarkable strides in sequencing technologies. This paradigm shift has effectively dismantled the constraints associated with earlier methodologies like RAPD, RFLP, and AFLP. The allure of these sequence-based markers lies in their co-dominance, repeatability, high polymorphism, and their immense utility in unraveling the intricate genetic tapestry of numerous crop plants, as eloquently highlighted by Lin et al. (2011). However, while microsatellite markers are now receiving a surge of attention in genetic diversity studies within germplasm collections, the deployment of sequence-based markers in the realm of barnyard millet remains in its nascent stages.

Consider, for instance, a pioneering study by Nozawa et al. (2006), wherein a cohort of 155 *Echinochloa* accessions, including *E. esculenta* (49), *E. crus-galli* (94), and *E. esculenta* var. formosensis (12), was meticulously examined using five SSR markers. This insightful investigation unraveled a distinct triad of clusters, underscoring the genetic divergence among these species. Interestingly, the diversity within *E. esculenta* accessions was found to be more restrained compared to its counterparts, *E. crus-galli* and *E. esculenta* var. formosensis.

Embracing the power of sequence-based markers, researchers have ventured into the terrain of EST markers, with promising outcomes for genetic diversity analyses within the realm of tiny millets. A landmark study by Li et al. (2013) and Yang et al. (2013) illuminated the potency of EST markers by generating 74 ESTs through comprehensive transcriptomics and annotation inquiries, specifically focusing on herbicide-resistant variants of *E. crus-galli*. Although their utilization in marker development and diversity exploration in barnyard millet has been limited due to their weedy nature, the in-silico mining of *E. crus-galli* ESTs bore fruit, unearthing 22 pairs of EST-SSR primers, as elegantly highlighted by Babu et al. (2017).

Furthermore, the pursuit of genetic diversity within Indian barnyard millet germplasm has been invigorated by recent studies. Manimekalai et al. (2018) and Murukarthick et al. (2019) harnessed the potential of EST-SSR markers sourced from the cultivated *E. frumentacea* transcriptome sequence to dissect the genetic tapestry of 61 barnyard millet germplasms. The former, probing 51 EST-SSR markers, discerned 14 polymorphic markers yielding 29 alleles with PIC values spanning from 0.276 to 0.652. Likewise, the latter identified 10 polymorphic markers from 30 EST-SSRs, unveiling significant polymorphism across 30 Indian barnyard millet germplasm samples.

Beyond the realm of SSRs, a panoramic view was unveiled through the genotyping-by-sequencing (GBS) method, spotlighting an astounding 21,000 SNPs across 95 barnyard millet accessions. The magnificence of these SNPs manifested in their discrimination prowess: approximately 10,816 SNPs spanning 65 biotypes of *E. colona* and 8217 SNPs spanning 22 biotypes of *E. crus-galli*. Of

paramount significance, 1299 SNPs distinguished *E. colona* biotypes, while 1444 SNPs achieved the same for *E. crus-galli*. The resounding clarity of SNP-based population structure analysis elegantly partitioned these two species, sculpting four clusters within *E. colona* and three within *E. crus-galli*, as gracefully unveiled by Wallace et al. (2015).

These captivating investigations collectively illuminate the burgeoning potential of sequence-based markers in unraveling the cryptic genetic makeup of barnyard millet, opening vistas of understanding that hold promise for molecular breeding and enhanced crop management practices.

26.10 Molecular Breeding and QTL Mapping

The advancement of various molecular marker technologies and their application in linkage mapping has enabled the examination of the impacts of specific genetic loci associated with quantitatively inherited traits, commonly referred to as quantitative trait loci (QTLs). These QTLs have the potential to be harnessed for enhancing resistance against biological stresses in crops. The identification of significant genes and QTLs responsible for crucial agricultural characteristics across diverse crops through genetic linkage mapping has streamlined the traditional breeding timeline and has facilitated the integration of biotechnology with conventional breeding methods (Tomar et al. 2017). The realm of barnyard millet breeding stands at an intriguing juncture, poised to harness the potential of microsatellite markers such as SSRs and SNPs to unravel critical micronutrient and agronomic traits. The arsenal of SSRs and SNPs has been deftly wielded to expedite linkage map construction and QTL mapping in barnyard millet, as illuminated by the insightful work of Murukarthick et al. (2019). Yet, amidst this fervor, a notable gap emerges: while an array of markers has been crafted, a genetic linkage map or QTL repository analogous to its millet counterparts, such as foxtail millet and finger millet, remains a conspicuous absence. Notably, Ishikawa et al. (2013) and Renganathan et al. (2019) emerged as trailblazers, each contributing a unique perspective to the barnyard millet mapping saga. The former delved into the intricate world of waxy traits, unraveling functional SNP markers and unraveling the triumvirate locus control of EeWx1, EeWx2, and EeWx3. Meanwhile, Renganathan and colleagues embarked on a journey into the pigmented realms, harnessing bulk segregant analysis (BSA) and 51 EST-SSR markers to shed light on the linkage between anthocyanin pigments and the SSR marker BMESSR 39 in barnyard millet. Yet, these forays, while tantalizing, beckon further exploration before they find their definitive stride in the realm of marker-assisted selection.

In this pursuit, the landscape unfurls with tantalizing prospects. The avenues to enhance barnyard millet's grain qualities and nutritional profiles, enriched with a treasure trove of essential amino acids, minerals, and vitamins, tantalizingly beckon. A pivotal stride in this direction is the optimization of nutrient bioavailability, which dances in tandem with the reduction of anti-nutrients or the ingenious utilization of novel promoters. Molecular markers, with their keen discriminatory power, hold the

key to unearthing novel millet germplasm graced with the desired nutrient bounty. Of singular significance is the quest for gene-based molecular markers tethered to specific nutritional traits, heralding a profound influence on their modulation (Tomar et al. 2016).

The transformative dawn of next-generation sequencing platforms casts a benevolent radiance upon the trajectory of barnyard millet molecular breeding. Its rapid sequencing prowess, synergistically coupled with trait-specific characterization, unfurls new vistas for advancement. These technological marvels harbor the potential to unravel the intricate genomic tapestry of barnyard millet with a precision hitherto unattained. As the curtain rises on this genomics-driven epoch, the promise of refined molecular breeding emerges, poised to bestow barnyard millet with enhanced nutritional vigor and resilience, charting a course toward a more nourished and sustainable future.

26.11 Conclusion

In spite of its remarkable nutritional and agronomic virtues, barnyard millet stands as an underexplored gem, languishing in relative obscurity on the global agricultural stage, largely overlooked by both farmers and researchers alike. The promising trajectory of barnyard millet breeding programs has been stifled, ensnared by the constraints of limited funding from diverse research bodies and funding agencies. Thus, a resolute commitment is imperative to fashion varieties or hybrids imbued with coveted attributes, poised to serve the interests of farmers and consumers alike. The forging of a dynamic landscape demands the birth of an array of future breeding initiatives, the vanguard of a concerted effort aimed at unlocking the crop's latent potential. Central to this mission is the orchestration of breeding programs meticulously architected to realize an amalgamation of lofty goals: soaring yield potential, unwavering yield stability, heightened salinity endurance, fortification against pestilence and disease, and a transformative elevation of nutritional quality, particularly in micronutrient content.

The pace of progress, however, is hampered by the scarcity of genetic and genomic resources intrinsic to barnyard millet. In stark contrast to its minor millet counterparts such as foxtail and finger millet, barnyard millet languishes with a diminished core collection of genetic reservoirs, while the crucial breeding populations remain conspicuously absent. Thus, a clarion call beckons for the creation and meticulous curation of core and mini-core collections that mirror the zenith of diversity inherent in this unassuming grain. Simultaneously, the orchestration of biparental and multiparental populations emerges as a pivotal prelude, an indispensable canvas upon which a spectrum of nutritional and agronomic virtues shall be etched.

Amidst this canvas, an array of indispensable tools awaits deployment, poised to illuminate the path to progress. These tools span a range of strategic fronts, encompassing the identification of seminal candidate genes, the meticulous tracing of genomic domains intricately interwoven with specific traits through the art of

linkage-based QTL mapping, the artful dance of genome-wide association analysis, and the judicious invocation of genomic selection. As the dawn of a genomic era unfurls, these tools, harnessed in unison, promise to be the sentinel companions propelling barnyard millet's ascent from the shadows, steering it toward a luminous future teeming with abundance, nourishment, and agricultural renaissance.

26.12 Future Prospects

In the realm of molecular exploration, the advent of transcriptome sequencing has unfurled a treasure trove of genomic resources, birthing entities like EST-SSRs and SNPs that shine as beacons of promise for the realm of marker-assisted breeding. Nonetheless, the canvas of barnyard millet genome research remains in its embryonic stage, casting a shadow of relative immaturity when juxtaposed with its minor millet counterparts. The path ahead is unequivocally marked by an imperious demand for robust initiatives that traverse the gamut of genetic exploration. Foremost among these endeavors is the imperative to forge a reference genome, an edifice of foundational significance. Hand in hand, the arsenal must include the construction of genome-wide SSR and SNP markers, an indispensable cartography that shall illuminate the tapestry of barnyard millet's genetic tapestry. The tapestry extends to the crafting of genetic linkage maps and cartographic guides unveiling the intricate choreography of genes as they intertwine and coalesce. Complementary to this pursuit, the endeavor to forge physical maps adds a tactile dimension, granting us tactile insights into the very topography of barnyard millet's genetic blueprint.

The dawn of comparative genomics lends its brilliance to this odyssey, as the publication of the weedy progenitor's (*E. crus-galli*) genome sequence and the wealth of genomic reservoirs from major and minor millets serve as stepping stones toward a fuller understanding of barnyard millet's genetic landscape. The crystalline clarity of a reference genome cultivates precision in mapping while simultaneously unraveling the structural tapestry of its domesticated kin, offering a vantage point to discern the cascading ripples of variations on the canvas of protein functionality.

Notably, barnyard millet emerges as a quintessential candidate for biofortification, a canvas upon which the art of imbuing micronutrients finds a fertile tableau. The grains of this unassuming crop abound in micronutrients, beckoning us to delve into the genetic lexicon, unearth the potential genes that orchestrate this symphony of abundance, and thereby foster their migration to high-yield cultivars or even other agricultural stalwarts like rice, wheat, and maize.

In the grand tapestry of plant adaptation, barnyard millet emerges as a resilient protagonist, with its genetic quiver adorned with the arrows of stress tolerance, a formidable armor that bolsters its ability to flourish amidst the rigors of both temperate and warmer climes. As the sun of progress continues to ascend, it becomes incumbent upon us to engineer an ecosystem that nurtures barnyard millet's trajectory into the realms of functional genomics. This alchemy shall unveil the hidden facets of traits entwined with resistance against biotic and abiotic adversities, as well as the intricate nuances of micronutrient endowments. Yet, amidst these scientific

endeavors, an inescapable truth lingers—the full scope of barnyard millet's nutritional and productive prowess remains veiled to many, especially the farming populace. Within the orbit of marginal landscapes, barnyard millet often languishes, woven into local landraces, yielding modest harvests. To pave the path toward maximal value and utility, innovations in post-harvest technologies beckon, heralding the promise of refined processing and value augmentation.

In the crucible of these challenges lies the metamorphosis of barnyard millet from an unsung hero to a resounding champion. Armed with resilience and imbued with the richness of nutrients, it emerges as a vanguard of sustainable nourishment and food security, poised to thrive amidst the ever-evolving tapestry of climatic exigencies. A symbol of environmental adaptability and nutritional potency, barnyard millet is poised to unfurl its banner as a bastion of future sustenance.

References

Ajaib M, Khan KM, Perveen S, Shah S (2013) Antimicrobial and antioxidant activities of *Echinochloa colona* (Linn.) link and *Sporobolus coromandelianus* (Retz.) kunth. J Chem Soc Pak 35:1384–1398

Babu BK, Joshi A, Sood S, Agrawal PK (2017) Identification of microsatellite markers for finger millet genomics application through cross transferability of rice genomic SSR markers. Indian J Genet 77:92–98

Bandyopadhyay BB (1999) Genotypic differences in relation to climatic adaptation of two cultivated barnyard millet at Garhwal hills. Indian J Genet 59:105–108

Bekkering CS, Tian L (2019) Thinking outside of the cereal box: breeding underutilized (pseudo) cereals for improved human nutrition. Front Genet 10:1289

Borkar VS, Senthil Kumaran K, Senthil Kumar KL et al (2016) Ethno medical properties of *Echinochloa colona* and *Hydrolea zeylanica*: a review. World J Pharmaceut Res 5:354–360

Cakmak I, Kutman UB (2018) Agronomic biofortification of cereals with zinc: a review. Eur J Soil Sci 69:172–180

Chandel G, Meena RK, Dubey M, Kumar M (2014) Nutritional properties of minor millets: neglected cereals with potentials to combat malnutrition. Curr Sci 107:1109–1111

Chu-Yu Y, Dongya W, Lingfeng M (2020) The genomes of the allohexaploid *Echinochloa crus-galli* and its progenitors provide insights into polyploidization-driven adaptation. Mol Plant 13(9):1298–1310

Danquah EY, Hanley SJ, Brookes RC, Aldam C, Karp A (2002) Isolation and characterization of microsatellites in *Echinochloa* (L.) Beauv. spp. Mol Ecol Notes 2:54–56

De Wet J, Prasada Rao K, Mengesha M, Brink D (1983) Domestication of mawa millet (Echinochloa colona). Econ Bot 37:283–291

Doggett H (1989) Small millets-a selective overview. In: Seetharam A, Riley KW, Harinarayana G (eds) Small millets in global agriculture. Oxford, Oxford, pp 3–18

Dwivedi S, Upadhyaya HD, Senthilvel et al (2012) Millets: genetic and genomic resources. Plant Breed Rev 35:247–375

Gao Y, Li J, Pan X, Liu D, Napier R, Dong L (2018) Quinclorac resistance induced by the suppression of the expression of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase genes in *Echinochloa crus-galli* var. *zelayensis*. Pestic Biochem Physiol 146:25–32

Gomashe SS (2017) Barnyard millet: present status and future thrust areas. Millets Sorghum Biol Genet Improv 134:184–198

Guo L, Qiu J, Ye C-Y, Jin G, Lingfeng M, Zhang H et al (2017) *Echinochloa crus-galli* genome analysis provides insight into its adaptation and invasiveness as a weed. Nat Commun 8:1031

Gupta A, Mahajan V, Kumar M, Gupta H (2009) Biodiversity in the barnyard millet (*Echinochloa frumentacea* Link. Poaceae) germplasm in India. Genet Resour Crop Evol 56:883–889

Hilu K (1994) Evidence from RAPD markers in the evolution of *Echinochloa* millets (Poaceae). Plant Syst Evol 189:247–257

Hittalmani S, Mahesh HB, Shirke MD, Biradar H et al (2017) Genome and transcriptome sequence of finger millet (*Eleusine coracana* (L.) Gaertn.) provides insights into drought tolerance and nutraceutical properties. BMC Genomics 18:465

IIMR (2018) Annual report 2017–18. Indian Institute of Millets Research, Hyderabad

Ishikawa G, Seimiya Y, Saito M, Nakamura T, Hoshino T (2013) Molecular characterization of spontaneous and induced mutations in the three homoeologous waxy genes of Japanese barnyard millet [*Echinochloa esculenta* (A. Braun) H. Scholz]. Mol Breed 31:69–78

Jayakodi M, Madheswaran M, Adhimoolam K et al (2019) Transcriptomes of Indian barnyard millet and barnyard grass reveal putative genes involved in drought adaptation and micronutrient accumulation. Acta Physiol Plant 41:66

Jiang B, Lao S, Wu D et al (2021) The complete chloroplast genome of *Echinochloa haploclada*. Mitochondrial DNA B Resour 6(11):3105–3106

Kam J, Puranik S, Yadav R, Manwaring HR et al (2016) Dietary interventions for type 2 diabetes: how millet comes to help. Front Plant Sci 7:1–14

Kim JY, Chang JK, Park B-R, Han S-I, Choi K-J, Kim S-Y et al (2011) Physicochemical and antioxidative properties of selected barnyard millet (*Echinochloa utilis*) species in Korea. Food Sci Biotechnol 20:461–469

Kulkarni LR, Naik RK, Katarki PA (1992) Chemical composition of minor millets. Karnataka J Agric Sci 5:255–258

Kumari KS, Thayumanavan B (1998) Characterization of starches of proso, foxtail, barnyard, kodo, and little millets. Plant Foods Hum Nutr 53:47

Lee J, Park K, Lee I, Kim C, Kown O, Park T (2014) Simple sequence repeat analysis of genetic diversity among Acetyl-CoA carboxylase inhibit or resistant and susceptible Echinochloa crus-galli and E. oryzicola populations in Korea. Weed Res 55:90–100

Lee J, Kim CS, Lee IY (2017) Discrimination of *Echinochloa colona* (L.) Link from other *Echinochloa* species using DNA barcode. Weed Turfgrass Sci 4:225–229

Li X, Siddique KHM (2018) Future smart food: rediscovering hidden treasures of neglected and underutilized species for zero hunger in Asia. Food and Agriculture Organisation of the United Nations, Bangkok

Li G, Wu SG, Yu RX, Cang T, Chen LP, Zhao XP et al (2013) Identification and expression pattern of a glutathione S-transferase in *Echinochloa crus-galli*. Weed Res 53:314–321

Lin H-S, Chiang CY, Chang S-B, Kuoh C-S (2011) Development of simple sequence repeats (SSR) Markers in *Setaria italica* (Poaceae) and cross amplification in related species. Int J Mol Sci 12: 7835–7845

Mace ES, Tai S, Gilding EK, Li Y, Prentis PJ, Bian L et al (2013) Whole genome sequencing reveals untapped genetic potential in Africa's indigenous cereal crop sorghum. Nat Commun 4: 2320

Manimekalai M, Dhasarathan M, Karthikeyan A et al (2018) Genetic diversity in the barnyard millet (*Echinochola frumentacea*) germplasms revealed by morphological traits and simple sequence repeat markers. Curr Plant Biol 14:71–78

Moreno AM, de Comino I, Sousa C (2014) Alternative grains as potential raw material for gluten-free food development in the diet of celiac and gluten-sensitive patients. Austin J Nutr Food Sci 2:9

Murukarthick J, Manimekalai M, Karthikeyan A, Perumal S et al (2019) Transcriptomes of Indian barnyard millet and barnyard grass reveal putative genes involved in drought adaptation and micronutrient accumulation. Acta Physiol Plant 41:66

Muza FR, Lee DJ, Andrews DJ, Gupta SC (1995) Mitochondrial DNA variation in finger millet [*Eleusine coracana* (L.) Gaertn.]. Euphytica 81:199–205

Nah G, Im J-H, Kim J-W, Park H-R, Yook M-J, Yang TJ et al (2015) Uncovering the differential molecular basis of adaptive diversity in three *Echinochloa* leaf transcriptomes. PLoS One 10: e0134419

Nguyen HD, Bingtian Z, Le DAT, Yoon YH et al (2016) Isolation of lignan and fatty acid derivatives from the grains of *Echinochloa utilis* and their inhibition of lipopolysaccharide-induced nitric oxide production in RAW 264.7 cells. J Agric Food Chem 64:425–432

Nozawa S, Takahashi M, Nakai H, Sato Y-I (2006) Difference in SSR variations between Japanese barnyard millet (*Echinochloa esculenta*) and its wild relative *E. crus-galli*. Breed Sci 56:335–340

Pan L, Guo Q, Wang J et al (2022) CYP81A68 confers metabolic resistance to ALS and ACCase-inhibiting herbicides and its epigenetic regulation in *Echinochloa crus-galli*. J Hazard Mater 428:128225

Panwar P, Dubey A, Verma AK (2016) Evaluation of nutraceutical and antinutritional properties in barnyard and finger millet varieties grown in Himalayan region. J Food Sci Technol 53:2779–2787

Perumal S, Jayakodi M, Kim DS et al (2016) The complete chloroplast genome sequence of Indian barnyard millet, *Echinochloa frumentacea* (Poaceae). Mitochondrial DNA B Resour 1(1):79–80

Prabha D, Negi YK, Khanna VK (2010) Morphological and isozyme diversity in the accessions of two cultivated species of barnyard millet. Nat Sci 8:71–76

Ramegowda Y, Venkategowda R, Jagadish P, Govind G et al (2013) Expression of a rice Zn transporter, OsZIP1, increases Zn concentration in tobacco and finger millet transgenic plants. Plant Biotechnol Rep 7:309–319

Rangani G, Rouse CE, Saski C et al (2022) High resistance to quinclorac in multiple-resistant *Echinochloa colona* associated with elevated stress tolerance gene expression and enriched xenobiotic detoxification pathway. Genes 13(3):515

Renganathan VG, Ibrahim SM, Vanniarajan C (2015) Combining ability for quantitative traits in Barnyard millet (Echinochloa frumentaceae L.). Electron J Plant Breed 6:389–394

Renganathan VG, Vanniarajan C, Nirmalakumari A et al (2017) Cluster analyses for qualitative and quantitative traits in barnyard millet *Echinochloa frumentacea* (Roxb. Link) germplasm. Bioscan 12:1927–1931

Renganathan VG, Vanniarajan C, Ramalingam J (2019) Genetic analysis and identification of molecular markers linked to the anthocyanin pigmentation in barnyard millet [*Echinochloa frumentacea* Roxb (Link)]. In: Proceedings of the neglected and underutilized crop species for food, nutrition, energy and environment. NIPGR, New Delhi, p 43

Ruiz-Santaella PJ, Bastida F, Franco A, Prado R (2006) Morphological and molecular characterization of different *Echinochloa* spp. and *Oryza sativa* populations. J Agric Food Chem 54: 1166–1172

Rutledge J, Talbert RE, Sneller CH (2000) RAPD analysis of genetic variation among propanil-resistant and -susceptible *Echinochloa crus-galli* populations in Arkansas. Weed Sci 48:669–674

Saleh ASM, Zhang Q, Chen J, Shen Q (2013) Millet grains: nutritional quality, processing, and potential health benefits. Compr Rev Food Sci Food Saf 12:281–295

Satyavathi CT, Tomar RS, Ambawat S et al (2022) Stage specific comparative transcriptomic analysis to reveal gene networks regulating iron and zinc content in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Sci Rep 12:276. https://doi.org/10.1038/s41598-021-04388-0

Sayani R, Chatterjee A (2017) Nutritional and biological importance of the weed *Echinochloa colona*: a review. Int J Food Sci Biotechnol 2:31–37

Sebastin R, Lee KJ, Cho G, Lee J, Kim S, Lee G et al (2019) The complete chloroplast genome sequence of Japanese millet *Echinochloa esculenta* (A. braun) *H. scholz* (Poaceae). Mitochondrial DNA B 4:1392–1393

Sharma A, Sood S, Agrawal PK, Kant L, Bhatt JC, Pattanayak A (2016) Detection and assessment of nutraceuticals in methanolic extract of finger (*Eleusine coracana*) and barnyard millet (*Echinochloa frumentacea*). Asian J Chem 28:1633–1637

Singh KP, Mishra HN, Saha S (2010) Moisture-dependent properties of barnyard millet grain and kernel. J Food Eng 96:598–606

Suryawanshi V, Talke IN, Weber M, Eils R, Brors B, Clemens S et al (2016) Between-species differences in gene copy number are enriched among functions critical for adaptive evolution in *Arabidopsis halleri*. BMC Genomics 17:1034

Tabacchi M, Mantegazza R, Spada A, Ferrero A (2009) Morphological traits and molecular markers for classification of *Echinochloa* species from Italian rice fields. Weed Sci 54:1086–1093

Tomar RS (2010) Molecular markers and plant biotechnology. New India Publishing

Tomar RS, Parakhia MV, Thakkar JR, Rathod VM, Padhiyar SM, Thummar VD, Dalal H, Kothari VV, Kheni JV, Dhingani RM, Golakiya BA (2016) Development of linkage map and identification of QTLs responsible for fusarium wilt resistance in castor (*Ricinus communis* L.). Res J Biotechnol 11:67–73

Tomar RS, Parakhia MV, Rathod VM et al (2017) Molecular mapping and identification of QTLs responsible for charcoal rot resistance in Castor (*Ricinus communis* L.). Ind Crop Prod 95:184–190

Ugare R, Chimmad B, Naik R, Bharati P, Itagi S (2014) Glycemic index and significance of barnyard millet (*Echinochloa frumentacae*) in type II diabetics. J Food Sci Technol 51:392–395

Vanniarajan C, Anand G, Kanchana S, Arun Giridhari V, Renganathan VG (2018) A short duration high yielding culture—barnyard millet ACM 10145. Agric Sci Dig A Res J 8:123–126

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

Varshney RK, Shi C, Thudi M, Mariac C, Wallace J, Qi P et al (2017) Pearl millet genome sequence provides a resource to improve agronomic traits in arid environments. Nat Biotechnol 35:969–976

Veena S, Chimmad BV, Naik RK, Shanthakumar G (2005) Physicochemical and nutritional studies in barnyard millet. Karnataka J Agric Sci 18:101e105

Vetriventhan M, Upadhyaya HD (2019) Variability for productivity and nutritional traits in germplasm of kodo millet, an underutilized nutrient-rich climate smart crop. Crop Sci 59: 1095–1106

Vetriventhan M, Azevedo VCR, Upadhyaya HD et al (2020) Genetic and genomic resources, and breeding for accelerating improvement of small millets: current status and future interventions. Nucleus 63:217–239

Wallace GJ, Upadhyaya H, Vetriventhan M, Buckler E et al (2015) The genetic makeup of a global barnyard millet germplasm collection. Plant Genome 08:01–07

Weber A (2015) Discovering new biology through RNA-Seq. Plant Physiol 169:01081

Wendel JF (2015) The wondrous cycles of polyploidy in plants. Am J Bot 102:1753–1756

Wu D, Shen E, Jiang B et al (2022) Genomic insights into the evolution of *Echinochloa* species as weed and orphan crop. Nat Commun 13:689

Xu W, Di C, Zhou S, Liu J, Li L, Liu F et al (2015) Rice transcriptome analysis to identify possible herbicide quinclorac detoxification genes. Front Genet 6:306

Yabuno T (1987) Japanese barnyard millet (Echinochloa utilis. Poaceae) in Japan. Econ Bot 41:484–493

Yadav R, Yadav V (2013) Comparative performance of Indian and Japanese barnyard millet cultivars under varied fertility conditions for dual use in Indian Central Himalaya. Range Manag Agrofor 34:175–178

Yang X, Yu X-Y, Li YF (2013) De novo assembly and characterization of the barnyard grass (*Echinochloa crus-galli*) transcriptome using next-generation pyrosequencing. PLoS One 8: e69168

Ye CY, Lin Z, Li G, Wang YY et al (2014) *Echinochloa* chloroplast genomes: insights into the evolution and taxonomic identification of two weedy species. PLoS One 9(11):e113657

Zou C, Li L, Miki D, Li D, Tang Q, Xiao L et al (2019) The genome of broomcorn millet. Nat Commun 10:436

27 Floral Biology, Pollination, Genetics, Origin and Diversity in Little Millet (*Panicum sumatrense* L. Roth ex. Roem. and Schultz)

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Abstract

Little millet was domesticated in India 5000 years ago. Little millet is a domesticated variety of the weed *Panicum silopodium*. Little millet is a tetraploid ($2n = 4x = 36$) plant in the Poaceae family. The chromosomes of hybrids of *Panicum sumatrense* and *Psilopodium* pair fairly completely with only one quadrivalent, demonstrating the two species divergence. Little millet is divided into two races based on panicle morphology, nana and robusta, each with two subraces (laxa and erecta for nana and laxa and compacta for robusta). This crop's flowering is of the chasmogamous variety, in which pollination occurs earlier than flower opening. Each spikelet consists of two-minute flowers. The lower is sterile, while the top is fertile or bisexual but lacks rachilla extension. Due to this, the self-pollination has a significant advantage. Hybridization is thus a requirement for the creation of variety. Emasculation is required for crossing due to self-

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pollination and the lack of male sterility. In little millet crop, numerous emasculation and crossing procedures are used, including the touch method, hot water treatment, hand emasculation and the USSR method. However, the problem with these procedures is that it causes stigma harm, which diminishes the success rate of obtaining actual F1s. To alleviate all of the shortcomings of previous methods, the modified crossing 'SMUASB' method was recently employed. Cold water (5–8 °C) is sprayed on the panicle as a mechanical stimulator for the opening of florets in male and female panicles in this approach. Female panicle is gently rinsed in cold water for emasculation. This has no effect on stigma or its sensitivity. Before pollination, all fertilised florets and unopened immature florets are removed. As a result, the success rate in little millet using the SMUASB approach was increased, producing actual F1 with more space and fewer resources for F1 evaluation.

Keywords

Little millet · Floral biology · Origin · Diversity · Hybridization

27.1 Introduction

Little millet, also known as *sama*, is grown in India, Sri Lanka, Pakistan, Myanmar, and other Southeast Asian nations (Hiremath et al. 1990). It is significant to tribes in India's Eastern Ghat Mountains and is planted with other millets (Hiremath et al. 1990). Little millet is a coarse cereal that is consumed in the form of rice. It is a member of the Poaceae family and the Panicoideae subfamily. It is a self-pollinated crop with $2n = 4x = 36$ chromosomes. Little millet is a food and feed crop grown in India's tribal belts of Madhya Pradesh, Chhattisgarh, Gujarat, Maharashtra, Odisha, and Andhra Pradesh. It is classified as a fast-growing grain with a short (60-day) to long (160-day) growing period that can survive both drought and water loading (Doggett 1989). It is also known as nutricereals or nutrimillets due to its nutritional excellence. The protein has a well-balanced amino acid profile and is high in methionine, cystine and lysine. Little millet can be produced in tropical and subtropical regions and is widely recognised for its drought tolerance. It is one of the least water-demanding crops and is suitable for delayed planting, rainfed conditions, drought tolerance, multiple and contingent cropping systems. Little millet contains a good level of iron and calcium when compared to other small millets and staple food crops like rice and wheat. Little Millet grains are as nutritious as or perhaps more so than some of the main cereals. Little millet is typically a disease-free crop; however, the incidence of grain smut (*Macalpinomyces sharmae*) can cause financial losses. Among insect pests, shoot flies are a common occurrence and are known to inflict financial losses; however, after receiving rainfall, shooflies become less common. Millets are typically renowned for their high nutritional value. The highest amount of crude fibre has been found in little millet. As a high source of minerals, vitamins, fat (4.79 g/100 g), protein (7.7%), and other nutrients, it must be taken into account as a necessary meal for dietary security (Hulse et al. 1980).

A domesticated variety of the weedy plant *Panicum psilopodium* is known as little millet (De Wet et al. 1983). *Panicum sumatrense* and *P. psilopodium* hybrids' nearly perfect chromosomal pairing and one quadrivalent suggest that the two species' original divergence may have been the result of a single reciprocal translocation (Hiremath et al. 1990). Due to the fertile, robust, and non-shattering spikelets of hybrid plants, gene introgression between the two species is frequently observed (Hiremath et al. 1990). Although specific dates are unknown, this capacity to hybridise and the variety of small millet crops grown across India suggest that little millet was independently domesticated multiple times (De Wet et al. 1983). In terms of fibre, fat, carbs, and protein, little millet is equivalent to other cereals. It is also high in phytochemicals such as phenolic acids, flavonoids, tannins, and phytate (Pradeep and Guha 2011). It can withstand dryness, pests, and salt, like many other little millets (Sivakumar et al. 2006; Bhaskaran and Panneerselvam 2013; Ajithkumar and Panneerselvam 2014).

27.2 Origin and History

Little millet (Panicum sumatrense Roth. Ex. Roem and Schultz) is one of the important small millets indigenous toIndian subcontinent and also has the presence of its wild ancestor Panicum psilopodium throughout India. Little millet was domesticated in the Eastern Ghats of India and became a staple food for tribal people there before spreading to Sri Lanka, Nepal, and Myanmar (Hiremath et al. 1990). There is no diversity and comparable wild species are not found outside of India, suggesting an Indian origin for this millet, which was also farmed or naturalised in nearby countries such as Sri Lanka and India. Little millet cultivation peaked at the Indus Valley Civilization of Harappa and Farman around 2600 BC, making up around 5% of the overall cereal assemblage. Earlier small millet was predominated grown in the Dang and Saurashtra region of Gujarat. In the Oriyo Timbo excavations in the Bhavnagar region of Gujarat state, ranging from 2000 to 1500 BC, 77% of the seeds were of millets, including little millet (https://milletmarvels.in).

A domesticated variety of the weedy plant *Panicum psilopodium* is known as little millet (De Wet et al. 1983). *Panicum sumatrense* and *P. psilopodium* hybrids' nearly perfect chromosomal pairing and one quadrivalent suggest that the two species' original divergence may have been the result of a single reciprocal translocation (Hiremath et al. 1990). Because hybrid plants are prolific, strong, and have non-shattering spikelets, it is typical for genes from the two species to interbreed (Hiremath et al. 1990). Although specific dates are unknown, its capacity to hybridise and the variety of small millet crops seen across India indicate that this plant was domesticated independently multiple times (De Wet et al. 1983).

Based on the morphology of the panicle, little millet is split into the nana and robusta races (Figs. 27.1 and 27.2). Compared to robusta, race nana grows earlier and produces less biomass (De Wet et al. 1983). Despite a short sampling area, diversity among locally cultivated landraces of small millet in a tribal region of the Indian Kolli hills was found to be high for all morphological features examined both within and between landraces (Arunachalam et al. 2005). A NBPGR collection of

Fig. 27.1 'Robusta' type Panicle of Little millet

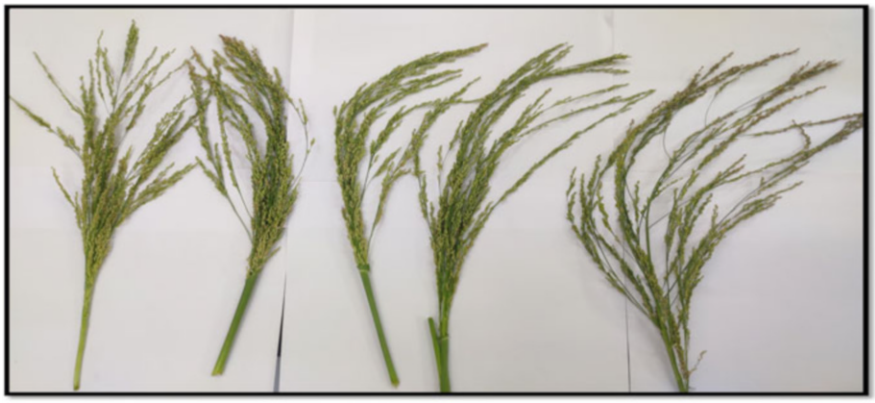
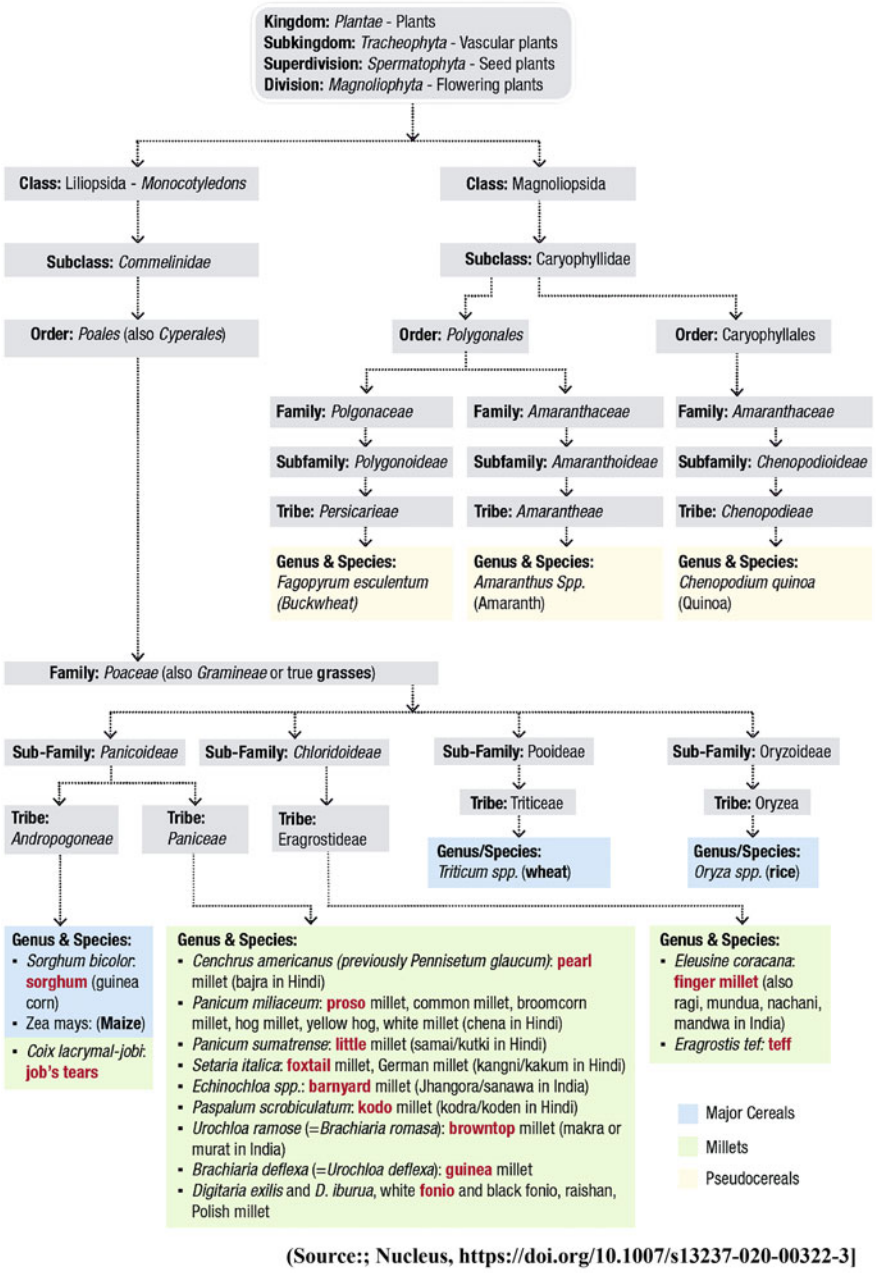


Fig. 27.2 'Nana' type Panicles of Little millet

10,409 landraces showed high diversity, heritability and genetic advancement in terms of yield and productive tillers, suggesting that the crop would be a strong candidate for varietal development (Nirmalakumari et al. 2010). For the majority of the variables analysed, a distinct collection of 460 accessions of small millet housed by ICRISAT showed genetic variation (Upadhyaya et al. 2014). It was determined that a core collection of 56 genotypes served as the seed bank's overall representation. With the help of mutational breeding, small millet populations have gained more heritable lodging resistance (Nirmalakumari et al. 2007).

Vetriventhan et al. (2020) provided the following taxonomical classification of tiny millets and other important cereals and millets, as well as pseudo-cereals. "Millet" is a frequent word for small-seeded grasses, sometimes known as dryland cereals. The grasses most commonly referred to as millets are: Major millet (pearl millet) and Minor/Small millets (finger millet, foxtail millet, proso millet, small millet, barnyard millet, kodo millet, browntop millet, fonio, teff and job's tears, and guinea millet) are the grasses that are most frequently referred to as millets.

Additionally, sorghum is occasionally referred to as major millet; in India, this is usually included in the classification of millets but is less common elsewhere.



Source: Nucleus, https://doi.org/10.1007/s13237-020-00322-3

27.3 Morphological, Cytogenetical and Genetic Diversity

Other names for the Indian cereal *P. sumatrense* include *miliare* and *attenuatum*. According to de Wet et al. (1983), *P. sumatrense* is the proper name for this native Indian cereal. The name was derived from a Sumatra specimen that was found. This plant is thought to have been brought to Indonesia by Indian immigrants and is thought to be grown on Tanimbar Island. It is difficult to understand the cytogenetics of panicum millets and their wild relatives. The basic chromosome number is $x = 9$, and evidence has been provided to support the theory that $x = 10$ is where this number first appeared. This genus frequently exhibits polyploidy, which can range from tetraploid to 12 ploidy levels (Chennaveeraiah and Hiremath 1990). The relationships between the genomes of these species are unknown. *P. psilopodium*, which is found all throughout India, is thought to be the ancestor of *Panicum sumatrense*. It thrives as a weed in the small millet farming in the Eastern Ghats of north Andhra Pradesh and creates fertile hybrids. *P. sumatrense* and *P. psilopodium*, two millet species that are grown for their morphology, can be identified from one another using a number of diagnostic traits. The hybrids had a strong reproductive capacity. In terms of non-shattering spikelets, the hybrids between *P. sumatrense* and *P. psilopodium* resembled *P. sumatrense* morphologically, and quantitative traits showed intermediate expression between the two parents. The purple glumes and stigma of the hybrids were similar to those of the male parent plant, *P. psilopodium*. The physical similarities, sympatric distribution, and creation of fertile hybrids are arguments in favour of *P. psilopodium* as the possible progenitor of *P. sumatrense*. For these two species, the genome designation is AABB. The meiotic behaviour seen in both taxa was completely normal. The parents showed regular 18 bivalents, and they are allo-tetraploid. The existence of regular 18 bivalents in the hybrids, along with the aforementioned characteristics, provide strong evidence that *P. sumatrense* may have evolved from the wild taxon *P. psilopodium* through selection and additional cultivation. The hybrid's presence of a single quadrivalent demonstrated the two species' genetic differentiation and divergence by a single reciprocal translocation (Gupta et al. 2010).

27.4 Floral Biology

The crop's floral biology is explained in detail (Fig. 27.3) by Clayton et al. in 2006. A panicle is the inflorescence. The panicle is rectangular, nodding, and about 5–40 cm long and 1–5 cm wide. The major panicle branches are 3–15 cm long and appressed. Scabrous panicle branches Spikelets are solitary, with fertile spikelets pedicelled. Fertile spikelets with one basal sterile floret and one fertile floret; no rachilla extension. Lemma II and its palea enclose the fertile flower, while lemma I and its palea enclose the staminate or sterile blossom (Sundararaj and Thulasidas 1976). Elliptical, dorsally compressed, and sharp spikelets characterise them. Spikelets are elliptic, dorsally compressed, and acute, measuring 2.5–3.5 mm in

Fig. 27.3 Little millet inflorescence and its parts. (**a**) Inflorescence; (**b**) Spikelet; (**c**) Side view of spikelet; (**d**) Opened spikelet; (**e**) Outer glume; (**f**) First lemma; (**g**) Sterile floret; (**h**) Fertile floret; (**i**) Upper glume; (**j**) Grain enclosed in lemma and palea; (**k**) Completely Open Flower

length and remaining on the plant. Glumes reach the apex of the florets and are narrower than the fertile lemma. Lower glume oval, 0.7–1.2 mm long and 0.25–0.33 mm long of spikelet, membranous with 1–3 veined keels. Lower glume lateral veins are either absent or obscured. Acute lower glume apex Upper glume is oval, membranous, and lacks keels; it has 11–15 veins. The upper glume apex is sharp. Palea barrens the basal sterile florets. Lower sterile floret lemma similar to upper glume, oval, 1 spikelet length, membranous; 9–13 veined; acute. Lower sterile floret palea 0.9 length of lemma, fertile lemma is oval, compressed dorsally, 2.2–2.5 mm long, indurate, dark brown, glossy, and lacks a keel. The margins of the lemma are involute, and the apex of the lemma is acute. Palea is involute and indurate. Three 1.5 mm long anthers 1.8–1.9 mm long caryopsis with attached pericarp.

27.5 Anthesis and Pollination

The second or third day after the panicle first appears the spikelets start to unfold. The panicle's blossoming develops from the top to the bottom. The majority of flowers bloom on the sixth or seventh day. A panicle takes around a fortnight to fully blossom (Sundararaj and Thulasidas 1976). Between 9.30 and 10.30 a.m., the anthesis takes place (Jayaraman et al. 1997). Self-pollination is the norm, and the

glumes only open for a brief period of time (Seetharam et al. 2003). The entire anthesis procedure takes around 2–5 min to complete.

27.6 Emasculation and Hybridization

Knowledge of the proper procedure for crossing and selfing in diverse crops allows the breeder to acquire the appropriate combination of traits. This requires ability and practice before the worker may expect the greatest outcomes. By recombining the alleles that contribute to yield components such as tiller number, primary branch number, secondary branch number, number of grains per panicle, and thousand-grain weight. Emasculation is the removal of stamens or anthers from a flower or the destruction of pollen grains without impacting the female reproductive organ in any manner. The goal of emasculation is to avoid self-fertilization in the female parent's flowers. Male plants in dioecious plants are removed, whereas male flowers in monoecious species are removed to avoid self-pollination. However, emasculation is required in bisexual blooms. Crossing is a procedure in which pollen from the desirable parent is sprinkled on the stigma of the seed parent because naturally self-pollinated crops are timid pollinators with very low movability to produce allogamy.

Crop enhancement effort in these crops has had some success so far. Some better cultivars have recently been created, although their yield potential is limited. Although there is substantial heterogeneity in the current germplasm collections, it has not been completely utilised. Hybridization and selection in the segregating population allow for the use of available variability to generate new improved cultivars. Hybridization is the interaction of people from different populations who differ in one or more heritable features (Harrison 1990). Hybridization can have immediate phenotypic repercussions due to hybrid vigour expression (Goulet et al. 2017). Hybridization is required for the efficient use of available germplasm, the development of breeding material, the introduction of novel genes, and the expansion of the genetic base. The generation of diversity in little millet is difficult due to challenges in artificial hybridization.

Understanding the characteristics that determine the duration of the flowering phase, pollination behaviour, and seed set is required for a successful hybridization programme in order to increase productivity and yield stability. The fundamental issue with all little millets is the difficulty in emasculation caused by the small size of the florets. The key factors linked to floral structure, diverse emasculation and crossing procedures, their downsides, and how to solve problems associated with old ways of crossing are summarised below.

27.7 Different Crossing Methods Used in Little Millet

1. Contact method/Approach method
2. Hand emasculation followed by pollination
3. Hot water treatment

4. USSR method
5. Modified crossing method (SMUASB method)

27.7.1 Contact Method/Approach Method

In this technique, the female parent that has been readied for pollination is planted next to the suitable male parent. The panicles of the sexes are loosely connected. The male parent should contain morphological markers so that it is easy to recognise real F1 after pollination and fertilisation have been finished and both have been separated (http://agritech.tnau.ac.in). Most self-pollinated crops use this form of crossing since it is the simplest. The likelihood of attaining real F1 is quite low. Only 2–3% of genuine F1 can be seen. To select real F1, a large plant population must be increased. For evaluation, more space and resources are required.

27.7.2 Hand Emasculation Followed by Pollination

This technique involved choosing flowers that will bloom the following day. The female reproductive organs were not in any way harmed by the removal of stamens or anthers. Pollination occurs the following morning after hand emasculation in the evening. Male flowers that would bloom that day are delivered to the emasculated female flower for pollination. Once tied, a butter paper bag is placed over them. Cross-pollination occurs naturally within 2–5 days. To determine the genuine hybrid, marker genes are used (http://agritech.tnau.ac.in). This is how self-pollinated crops have traditionally been produced. The main issue with this procedure was that the lemma and palea are extremely tight, making it impossible to open the flower before it goes through its regular anthesis without damaging it and preventing the development of seeds. The amount of time between the anthesis of the first flower on a panicle and the anthesis of the last floret on a panicle is another issue. Determining the appropriate time to emasculate before anthesis is therefore difficult (Jasovskij 1960). Nelson (1984), used a different method for selecting flowers for emasculation to address this issue. Using this technique, panicles were chosen around the place where the first florets open. The florets in the panicle started to open as the panicle was rubbed between the palms of the hands. To prevent the anthers from dehiscing before all of them were plucked, florets were sprayed with room-temperature water from an atomizer to keep them moist. The florets that hadn't been emasculated were removed once the florets finished opening and all of the opened florets had been closed. That comprised the top and bottom fertilised florets of the panicle as well as the immature florets at the bottom. The best time to emasculate was between 8 and 9 in the morning. At this time, the florets expanded at a rate that allowed effective emasculation conceivable. Emasculation was followed by fertilisation for 15 min. Male parents were rubbed and allowed to open for pollination. A glassine bag containing opened male florets was placed on top of the emasculated panicle. Five days were given to allow for crossing and moisture preservation. The benefit of this

approach is that the lemma and palea are permitted to open naturally, rather than being forced to do so. The drawback of this procedure is that, while emasculation, injury to the stigma prevents seed germination.

27.7.3 Hot Water Treatment

Many researchers have attempted to circumvent the issue of physically extracting the anthers from the florets by using the hot water treatment for emasculation (Keller 1952). This procedure involves choosing panicles that are expected to flower in the following 2–3 days and submerging them in hot water at 52 °C for 2 min. According to the percentage of hybrid seed set, this was the ideal temperature and timing (http://agritech.tnau.ac.in). According to Srivastava and Yadav (1972), emasculating little millet in hot water at 49 °C for 8–10 min or 50 °C for 5 min was successful. Similar to this, the male father that would open the next day is connected to the emasculated female parent and covered by a butter paper following emasculation using hot water for pollination. This method's limitations include the need for the appropriate equipment to maintain long time constant temperature. The stigma will be impacted by temperature, which could lead to a tiny amount of seeds being set (Primak and Jakovlev 1964)

27.7.4 USSR Method

This improved method of crossing was presented to alleviate the difficulty discovered in hand emasculation and hot water treatment in removing pollens (Seetharam et al. 2003). The induced opening of the flower (USSR method) has been effectively used in the creation and development of novel cultivars, as detailed below:

1. By gently stroking the panicle with the palm, florets are mechanically activated.
2. Florets open within 2–3 min, far earlier than usual flowering.
3. Dip in water that is room temperature to prevent another explosion.
4. With your forefingers, thrash the anthers from the opening florets.
5. Remove the unopened florets with scissors and keep the opened blossoms.

27.7.4.1 Pollination

The emasculated female spike was placed just below the male spike that was exuding pollen, and both spikes were then covered with a glassine bag to complete the pollination process. The female spike receives pollen from the male spike, which provides an excellent possibility for fertilisation. Throughout the daily anthesis periods, the spikes were jostled against one another for 2 days. The male spike was carefully removed on the third day of pollination, and the female spike was examined for any potential later-forming florets. Such florets frequently developed and produced seed when they weren't entirely removed, which could be mistaken for cross-fertilised seeds. The female spike was rebagged and kept until it was mature

enough to be harvested. The stigma may be damaged when the panicles are massaged to mechanically stimulate the florets to open, as well as when the anthers are removed from the florets with the forefingers in this way.

27.7.5 Modified Crossing Method (SMUASB Method)

This altered method of crossing was first used in little millet during kharif season at the Project Coordinating Unit on Small Millets at the University of Agricultural Sciences, GKVK, Bengaluru; it was given the designation SMUASB (Small millets, University of Agricultural Sciences, Bengaluru). Little millet has an extremely tight lemma and palea, making it impossible to attempt to open the floret before the typical anthesis without damaging the flower and preventing seed germination. Cold water is employed in the SMUASB method as a mechanical stimulator to encourage floret opening. Emasculation The Little millet flowers open from the top of the panicle to the bottom. For crossing, male and female parents are placed in different rows. The 8 to 9 a.m. is the ideal time to cross.

Details of emasculation are mentioned below

- The female plant's panicle must be chosen for emasculation so that the panicle's first floret has opened.
- Spraying cold water between 5 and 8 °C on the panicle. The florets are encouraged to open spontaneously 1 h earlier by this cold water spray than they would have otherwise.
- Emasculation is performed by dipping the panicle in cold water after all the florets have been opened, and all the anthers have been removed by washing the panicle in cold water.
- Unopened florets were eliminated. It has unfertilised florets at the top of the panicle and immature florets at the bottom.

Pollination

- The male parent is chosen in such a way that the first floret has opened.
- To open the florets and keep the anthers wet, male panicle is sprayed with cold water at 5–8 °C, the same as female panicle.
- It is knotted loosely around the female parent panicle immediately after the male florets open to facilitate proper oxygenation and pollination.
- The knotted panicles are then sprayed with water to keep the stigma and anthers moist.
- To avoid cross-pollination, male and female panicles are tied together and covered with butter cover.
- To prevent cross-pollination, male and female panicles are tied together and covered with butter cover. Because these crops are self-pollinated, only 3–5% cross-pollination may occur.
- Label the crossing panicle in the female parent for identification and seed collecting. The tag should include the cross combination and the date of crossing.

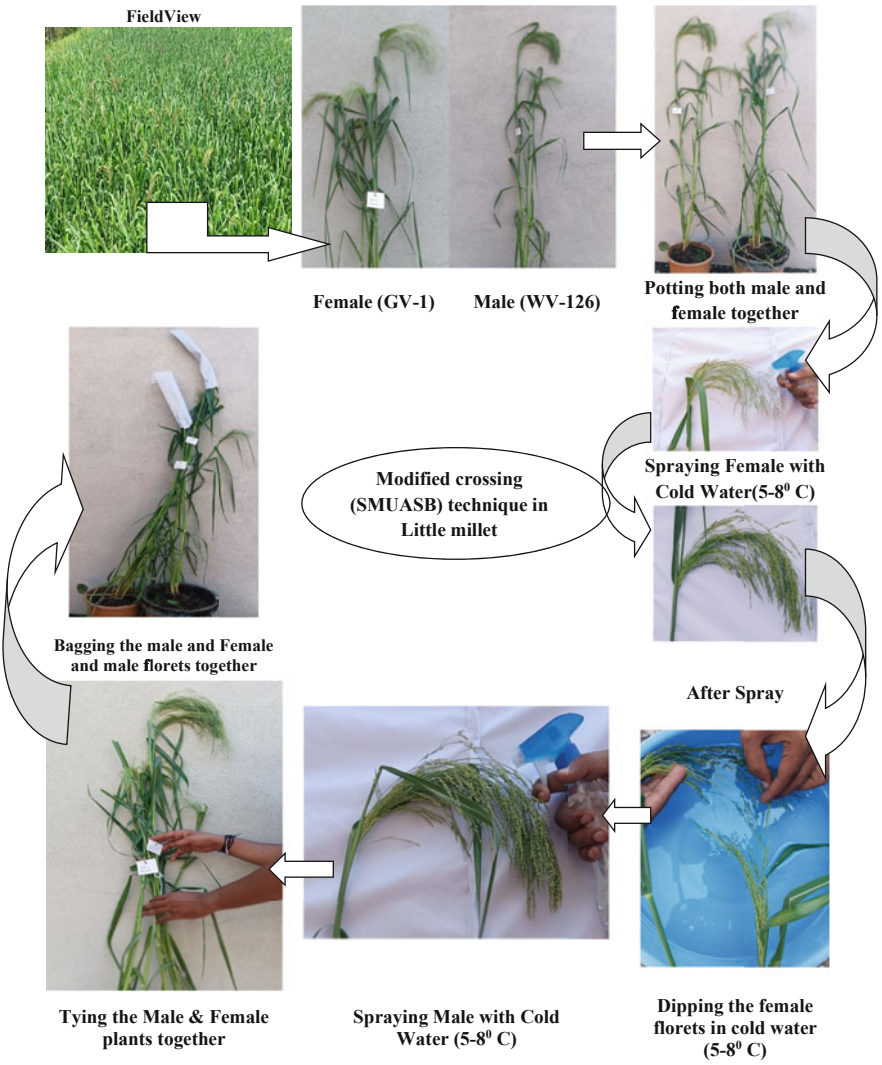


Fig. 27.4 Modified crossing (SMUASB) technique in little millet

In little millet, the SMUASB method is widely used for crossing Few crosses were attempted in Little millet using the SMUASB approach given in Fig. 27.4.

Female Parent: GV-1 (Nana flower type)
Male Parent: WV-126 (Robusta Flower type with Purple plant stem pigmentation-Gujarat local) and high-yielding genotype.

The primary limitation in this genotype is its long duration, which is incompatible with the cropping system. GV-1 is an early, tillering cultivar with high iron content. We crossed GV-1 with WV-126 to create a genotype that is short in duration, high in yield, and nutrient dense. WV-126 is utilised as a male parent and is purple in colour, whereas GV-1 is green in colour and is employed as a female parent. True F1s are distinguished by their purple coloration.

Success rate For crossing, three to four panicles were employed. Using the SMUASB procedure, panicles were emasculated and pollinated. Planting seed from the female parent allows for the identification of real F1s. Each panicle's seeds were collected and sown individually. Out of the 40 F1 plants that were planted in panicle 1, 20 plants were confirmed to be real F1s, indicating a 50% success rate. Similar results were obtained in panicles 2 and 3, with success rates of 55% and 56%, respectively. Using the SMUASB approach, small millet crossings experienced an average success rate of 40–50%.

Successful crosses in little millet are as below:

Sr. No.	Crosses	Objectives of the crossing programme
1.	GV-1 × WV 126	High yield and early to medium maturing, bold grain and lodging tolerant as well as shoot fly resistance
2.	GV-2 × WV 126	
3.	GNV-3 × WV 126	

Modified crossing (SMUASB) approach has the following advantages over other little millet crossing methods

The benefit of this SMUASB is that emasculation never results in stigma damage. In comparison to the other conventional approaches, this produces a larger seed set and a higher frequency of true F1's. It requires less effort and takes less time than manual emasculation. The removal of pollen from each and every floret during hand emasculation is time-consuming and technical skill is needed. Because the blossoms of these two crops are tiny, emasculation requires technical skill. In the modified crossing procedure, flowers open simply by being sprayed with cold water. It is a less difficult procedure because anthers are also easily removed by washing or dipping in water.

Emasculation and identifying real F1 requires less space and resources. For the contact approach, a significant number of seeds harvested from female plants must be examined for the presence of real F1s. The likelihood of acquiring actual F1s is lower. All unopened, immature, and previously pollinated florets are eliminated when using the modified approach. Only the florets with emasculated anthers are retained for pollination. As a result, the F1 plants that were collected from the female plant had less seeds. Therefore, fewer space and resources are

required for the examination and identification of actual F1s. In the modified crossing method, cold-water spray is utilised as a mechanical stimulator for opening the florets in place of hand messaging, as was done in the USSR approach, and hot water treatment for emasculation. The stigma is not harmed by this method. As a result, there is a higher success rate in getting actual F1s.

In little millet, the modified crossing (SMUASB) method of emasculation and crossing is a very beneficial method. This technique fixes the issues with the touch method, hand emasculation method, hot water emasculation method, and USSR way of crossing. Comparing this procedure to earlier ones, the success rate for obtaining actual F1s is higher. With this approach, less space and resources are needed to evaluate F1s. We attempted crosses in other small millets using the SMUASB approach. For little millets, the success rate was between 40% and 50%.

Constraints in Little millet hybridization and crossing techniques

- Small florets in this crop make it difficult to easily emasculate and hybridise it by hand.
- Knowledge of floral biology, a straightforward, practical hybridization technique, and an appropriate gene marker for identifying true F1 are necessary for artificial hybridization.
- The difficulty facing the breeders is to look for straightforward and efficient emasculation and pollination strategies in this crop, given the difficulties in creating artificial crosses.
- Despite the abundance of variability, artificial hybridization must be restored in order to combine the desirable traits from various accessions in genotypes, which necessitates the development of a quick and efficient process of pollination and emasculation. The best emasculation and pollination strategies can be planned with the aid of floral biology research.

27.7.5.1 Objectives for Little Millet Breeding and Improvement

When choosing the ideal donor parent, consideration should be given to the following targeted features in small millet: shoot fly resistance, non-lodging, days to maturity, and bold grain size (Nandini et al. 2019) in small millets improvement, yield and factors affecting yield are typically the most addressed features. As a result, selection for yield in and of itself has been the main driver of productivity improvement, but genotype-environment interactions greatly affect these features. To enhance yield, it is therefore crucial to evaluate yield stability across various conditions and look at physiological variables (such as harvest index, water use efficiency, etc.) linked to yield and adaptation. Depending on the location-specific requirements for soil, rainfall, temperature, humidity, day length, and cropping patterns, custom-made cultivars that fit into the different maturity groups—early, mid-late, and late—can be bred using the significant variation in maturity duration that germplasm collections exhibit. Medium- to long-duration types would be appropriate for places with a single cropping season and short-duration variations for double/intensive cropping regions (Haider 1997). The fundamental goal of

crossing is to broaden the genetic basis of the population for most efficient selection while also introducing variety and incorporating desired traits, such as high yield, pest and disease resistance and significant quality features, etc. into a single genotype.

References

Ajithkumar IP, Panneerselvam R (2014) ROS scavenging system, osmotic maintenance, pigment and growth status of *Panicum sumatrense* roth. under drought stress. Cell Biochem Biophys 68: 587–595. https://doi.org/10.1007/s12013-013-9746-x

Arunachalam V, Rengalakshmi R, Raj MSK (2005) Ecological stability of genetic diversity among landraces of little millet (Panicum sumatrense) in South India. Genet Res Crop Evol 52:15–19. https://doi.org/10.1007/s10722-005-6693-4

Bhaskaran J, Panneerselvam R (2013) Accelerated reactive oxygen scavenging system and membrane integrity of two Panicum species varying in salt tolerance. Cell Biochem Biophys 67:885–892. https://doi.org/10.1007/s12013-013-9576-x

Chennaveeraiah MS, Hiremath, SC (1990) Cytogenetics of minor millets. In Chromosome Engineering in PlantsGenetics, Breeding and Evolution. Vol. II. Chapter 32 Tsuchiya T, Gupta, PK (eds.) Elsevier Sci. Publ Amsterdam,Netherlands.

Clayton WD, Vorontsova MS, Harman KT, Williamson H (2006) GrassBase—The Online World Grass Flora. http://www.kew.org/data/grasses-db.html

De Wet JMJ, Prasada Rao KE, Brink DE (1983) Systematics and domestication of *Panicum sumatrense* (Graminae). J D'agriculture Tradit Bot Appliquée 30:159–168

Doggett H (1989) Small millet—a selective overview. In: Seetharam A, Riley K, Harinaryana G (eds) Small millets in global agriculture. New Delhi, India, Oxford and IBH Publ, pp 3–18

Goulet BE, Roda F, Hopkins R (2017) Hybridization in plants: old ideas, new techniques. Plant Physiol 173:65–78

Gupta A, Maharaj V, Gupta HS (2010) Genetic resources and varietal improvement of small millets for Indian Himalaya. In: Tewari LM, Pangtey YP, Tewari G (eds) Biodiversity potentials of Himalaya. Gyanodayaprakashan, Nainital, India, pp 305–316

Haider ZA (1997) Little millet in Indian agriculture: progress and perspectives. In: National seminar on small millets, 23–24, Coimbatore, India, pp 5–6

Harrison RG (1990) Hybrid zones: windows on evolutionary process. Oxford Surv Evol Biol 7:69–128

Hiremath SC, Patil GNV, Salimath SS (1990) Genome homology and origin of *Panicum sumatrense* (Gramineae). Cytologia (Tokyo) 55:315–319. https://doi.org/10.1508/cytologia.55.315

Hulse JH, Laing EM, Pearson OE (1980) Sorghum and the millets. Their composition and nutritional value. Academic, New York, p 997

Jasovskij IV (1960) An effective method of hybridization for millet (In Russian.). Sel Semenovod (3):69–70. (Plant Breeding abstr. 31: 4712)

Jayaraman N, Suresh S, Nirmala A, Ganeshan NM (1997) Genetic enhancement and breeding strategies in small millets. In: National seminar on small millets, 23–24, April, Coimbatore, India, pp 19–21. (Extended summaries)

Keller W (1952) Emasculation and pollination techniques. Int Grassland Congr Proc 6:1613–1619

Nandini C, Bhat S, Srinathareddy, Jayramegowda, Prabhakar (2019) Modified crossing (SMUASB) method for artificial hybridization in proso millet (Panicum miliaceum L.) and Little millet (Panicum sumatrense). Electron J Plant Breed 10(3):1161–1170., ISSN 0975-928X-1161. https://doi.org/10.5958/0975-928X.2019.00147.9

Nelson LA (1984) Technique for crossing proso millet. Crop Sci 21:205–206

Nirmalakumari A, Arulselvi S, Ganapathy S, Souframanian J, Senthil N, Devan P (2007) Gamma ray inducedvariation for lodging resistance and its associated characters in little millet (Panicum sumatrense Roth ex-Roem.and schult). Madras Agric J 94:151–155.

Nirmalakumari A, Salini K, Veerabadhiran P (2010) Morphological characterization and evaluation of little millet (*Panicum sumatrense* Roth ex. Roem. and Schultz) germplasm. Electron J Plant Breed 1:148–155

Pradeep SR, Guha M (2011) Effect of processing methods on the nutraceutical and antioxidant properties of little millet (*Panicum sumatrense*) extracts. Food Chem 126:1643–1647. https://doi.org/10.1016/j.foodchem.2010.12.047

Primak NN, Jakovlev AG (1964) Thermal emasculation of millet. Agro-biologija (Agrobiology):613–614

Seetharam A, Gowda J, Halaswamy JH (2003) Small millets. In: Chowdhury SK, Lal SK (eds) Nucleus and breeder seed production manual. Indian Agriculture Research Institute, New Delhi, India, pp 54–67

Sivakumar S, Mohan M, Franco OL, Thayumanavan B (2006) Inhibition of insect pest α-amylases by little and finger millet inhibitors. Pestic Biochem Physiol 85:155–160. https://doi.org/10.1016/j.pestbp.2005.11.008

Srivastava DP, Yadav A (1972) Emasculation of flowers of *Panicum miliare* Lam. by hot water treatment. Sci Cuk 38:450

Sundararaj DP, Thulasidas G (1976) Botany of field crops. Macmillan Publisher, India, p 509

Upadhyaya HD, Dwivedi SL, Singh SK, Singh S, Vetriventhan M, Sharma S (2014) Forming core collections in barnyard, kodo, and little millets using morphoagronomic descriptors. Crop Sci 54:1–10. https://doi.org/10.2135/crop-sci2014.03.0221

Vetriventhan M, Azevedo VCR, Upadhyaya HD, Nirmalakumari A, Potaka JK, Anitha S, Antony Ceasar S, Muthamilarasan M, Venkatesh Bhat B, Hariprasanna K, Bellundagi A, Cheruku D, Backiyalakshmi C, Santra D, Vanniarajan C, Tonapi VA (2020) Genetic and genomic resources, and breeding for accelerating improvement of small millets: current status and future interventions. Nucleus 63:217. https://doi.org/10.1007/s13237-020-00322-3

28 Genetic Improvement for Yield, Quality, Biotic, and Abiotic Stresses in Little Millet (*Panicum sumatrense* Roth. ex Roem. and Schult.)

Abhinav Sao, Vikas Pali, and H. E. Patil

Abstract

Small millet, a set of small-seeded crops that includes Finger millet, Little millet, Kodo millet, Foxtail millet, Barnyard millet, and Proso millet, was previously known as "orphan crops" but is now known as "nutri-cereals" due to its high nutritional value. *Panicum sumatrense* Roth. ex Roem. & Schult., often known as Little millet, is a native of India and is widely grown there as well as in Nepal, Thailand, China, Indonesia, and western Myanmar. It is grown as a key part of tribal agriculture in eastern India. Little millet has a diploid chromosomal number of $2n = 4x = 36$, an AABB genome, and is known in Hindi as *Samai/kutki*. On the basis of plant stature and inflorescence, there are two varieties of Little millet: *nana* and *robusta*. Plants in race *nana* range in height from 60 to 170 cm, and the inflorescence is small (14–15 cm), upright, open, and heavily branched, with branches that droop at maturity. Plants in the race *robusta* are tall (120–190 cm) with long (20–45 cm) inflorescences that open compactly and are extremely branching. It's mostly a self-pollinated crop. Little millet can provide significantly better grain yields even under moisture stress and on marginal fields. It is capable of a wide range of adaptations, including excellent efficiency in using water, tolerance for salt and waterlogging, and a low incidence of insect pests and diseases. For small millet, the most significant breeding goals are bold seed, non-lodging, and shoot fly resistance. Because of the small size of the florets and

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due to Little millet's high level of self-pollination, hybridization is very challenging. After multi-location evaluation for yield and other critical features, pureline and mass selection from high-yielding germplasm accessions may be a superior choice for varietal development. Most of the varieties of small millet in India are developed from existing germplasm. Extensive testing of preserved germplasm for grain production, nutritional content (Zinc and Iron), and biotic and abiotic stressors has the potential to improve Little millet crop yield.

Little millet genetic enhancement is also achievable by recombination breeding with superior accessions for yield, other quality, and stress tolerance, particularly shoot fly resistance. Little millet hybridization procedures include the contact method of crossing and artificial hybridization via hand emasculation or hot water emasculation. Mutation breeding can also play an important role, particularly in selfed crops where regular crossing is difficult to achieve genetic variety. Modern breeding strategies such as MAS and genomic selection are currently being used to improve the genetics of small millet. Little millet is also being produced in high Fe and high Zn variants. In India, about 20 varieties of small millet are generated through conventional selection, pedigree breeding, and mutation breeding. Chhattisgarh Kutki-2, a high iron-containing (approx. 28 ppm) tiny millet variety, was developed and distributed in 2018–2019 by IGKV, Raipur, India, due to the excellent nutritional status of millets. The metal transporter gene has also been cloned in tiny millet, which can be used to generate crops with high Zn and Fe levels. Traditional breeding techniques have led to the creation and dissemination of numerous cultivars, including those with resilience to biotic and abiotic stressors. The incorporation of genomic-assisted improvement through the use of diverse omics techniques has the potential to boost genetic gains in Little millet crop development. Although gene mapping, transcriptomics, proteomics, and genetic transformation using CRISPR/Cas9 have been accomplished in other millets, a holistic strategy for crop enhancement is still sought in this crop.

28.1 Introduction

The Poaceae grass family includes small millets, commonly referred to as minor millets, which are a type of cereal crop with small seeds. This group includes finger millet (*Eleusine coracana* L.), foxtail millet (*Setaria italica* L.), proso millet (*Panicum miliaceum* L.), barnyard millet (*Echinochloa crusgalli* L. and *Echinochloa colona* L.), kodo millet (*Paspalum scrobiculatum* L.), and Little millet (*Panicum sumatrense* Roth. ex. Roem. &Schult.). Small millets are well known for their climate-resilient characteristics, such as their greater adaptability to a variety of ecological situations and lower water requirements, lower incidence of insect pests and diseases, and low susceptibility to environmental stresses (Bandyopadhyay et al. 2017; Goron and Raizada 2015; Saxena et al. 2018). In semi-arid, rainfed locations, small millets are important crops. Due to the popularity of cash crops over traditional

crops, they are currently only grown in a small area, accounting for a small portion of the world's millet production. Farmers who previously practiced conventional agriculture have been forced to switch back because of the rising trend of erratic rainfall, changing weather patterns, and crop losses as a result of climate change. Millets, Little millet, and foxtail millet cultivation provide an assured crop yield under both less and excess rainfall situations while requiring little in the way of inputs. Millets are grasses that can be produced in dry places with poor soil fertility and moisture levels as rainfed crops. Sorghum, pearl millet, finger millet, barnyard millet, foxtail millet, kodo millet, proso millet, and Little millet are the millets that are most commonly grown. It is considered that they were likely the first cereal grains to be domesticated and cultivated. The four subraces of the cultivated small millet include laxa, erecta, robusta, and nana. These subraces are distinguished by the characteristics of their plants and panicles.

Millets are superior to other plants in their ability to absorb and utilize carbon dioxide. The majority of millets are known for their hardiness with the ability to endure extended droughts and hot heat while still producing grains and feed. Compared to other major grains, millets have a number of morphological, physiological, molecular, and biochemical traits that increase their ability to withstand environmental challenges. Millets are particularly helpful in relieving stress since their short life cycles (from seed to seed) take only 12–14 weeks, compared to 20–24 weeks for rice and wheat. However, characteristics including short stature, tiny leaf area, thickened cell walls, and the ability to establish deep root systems mitigate the occurrence of stress conditions and their effects (Li and Brutnell 2011). One of the small millet group's members, Little millet is eaten in the form of rice. It belongs to the Poaceae family's Panicoideae subfamily. The crop has a chromosomal number of $2n = 4x = 36$ and is self-pollinated. Little millet is a significant crop farmed in the tribal areas of eastern Indian states Madhya Pradesh, Chattisgarh, and Andhra Pradesh, as well as Nepal, Thailand, China, Indonesia, and western Myanmar for food and feed (Haider 1997). It is recognized as a cereal that grows quickly and only lasts a limited time and can endure both drought and waterlogging conditions (Doggett 1989). It is rich in minerals, vitamins, proteins, and carbs. It is also known as nutri cereals or nutri millet because of its excellent nutritional quality. The protein contains a balanced amino acid profile and is a strong source of methionine, cystine, and lysine. There is a significant amount of crude fiber in grains. One of the least water-demanding crops, Little millet can be grown in tropical and subtropical climates. It is suitable for mid-season seeding in a system of numerous dependent crops that are fed by rain. Little millet is widely renowned for its ability to withstand drought. Comparatively speaking to other small millet and common meals like rice and wheat, Little millet contains a decent amount of iron in the grains. It is also a substantial catch crop in India's tribal and mountainous regions.

Crop growth and productivity have historically been highly susceptible to the different biotic and abiotic pressures brought on by anthropogenic climate change. The creation of enhanced crop varieties and the formulation of fresher strategies for crop development against stress tolerance have taken precedence in the modern

period. However, the majority of crop breeding initiatives lean more toward major cereals like rice, wheat, and maize than they do toward minor cereals like small millet. It has great nutritional qualities and is a staple food in many semi-arid and tropical regions of the world, ensuring food security even in extreme environmental conditions. Therefore, Little millet improvement will greatly benefit from improved genetic manipulation for resistance/tolerance to both abiotic and biotic stresses, as well as for boosting nutritional quality.

28.1.1 Production Constraints in Little Millet

Breeding nutrient-rich varieties with higher-yielding backgrounds can be assisted by utilizing the genetic variability already present in the germplasm and hybridization-derived variations:

- Small millets are more resilient to severe biotic and abiotic challenges and are well-adapted to a variety of environmental situations. However, a few ailments and insect pests, such as the shoofly and grain smut, are significantly reducing yields; for this reason, it is crucial to breed for disease- and pest-resistant types of plants.
- Infections with grain smut, head smut, and leaf spots severely affect Little millet. The most harmful insect to small millet is the shoot fly. Small millets are frequently grown as rainfed crops, which makes them particularly vulnerable to drought because of less rainfall. Due to their fragile stalks, crop management practices, and climatic factors, lodging is a severe problem in all small millets in addition to moisture stress (drought).
- Although lodging has not been directly estimated to cause a production loss in small millets, losses in big grains like rice and wheat have reached 50%. Due to heavy panicles, a brittle stem, and poor root anchorage, complete plants bow when they reach maturity.
- To reduce yield and quality losses, it is crucial to design cultivars with higher lodge resistance because lodging is truly controlled by both the genotype and the environment.
- Little millet suffers from grain shattering during maturity. Grain shattering causes significant yield losses; consequently, shattering-resistant/tolerant Little millet will be crucial to preventing shattering-induced yield losses.
- Special breeding traits to enhance the cultivation and consumption of small millets include the development of machine-harvestable cultivars, improving the nutritional value of grain and fodder to fetch high market prices, developing cultivars suitable for post-harvest value-added products like rice, flour, vermicelli, flakes, hot and cold extruded snacks, noodles, and ready-to-cook mixtures, and low light-requiring genotypes for orchards and agroforestry.
- Little millet has a high nutritional status, but its usage is limited due to the presence of antinutritional substances such as phytate, phenols, tannins, and enzyme inhibitors, as well as a high concentration of amylase and protease

inhibitors, which reduce the digestibility of millet grains. Most of the nutrients in small millets are higher as compared to major cereals, however, there is a lot of variation in germplasm for grain nutrients and antinutrient characteristics.

28.1.1.1 Little Millets Breeding Objectives and Improvement

Improvements in yield, fodder, and quality attributes are of importance in tiny millet. When choosing the best donor lines, emphasis should be placed on shoot fly resistance, non-lodging, days to maturity, and bold grain size (Nandini et al. 2019). The main focused qualities in tiny millets improvement are often yield and its contributing factors. As a result, selection for yield in and of itself has been the main driver of productivity improvement, but genotype-environment interactions greatly affect these features. Therefore, it is necessary to assess yield stability across a range of conditions and include physiological factors (such as harvest index, water use efficiency, etc.) connected to yield and adaptation in order to increase production. The significant variation in maturity duration that germplasm collections exhibit can be used to breed cultivars that fit well into the different maturity groups—early, mid-late, and late—depending on the location-specific requirements for soil, rainfall, temperature, humidity, day length, and cropping systems. Medium- to long-duration types would be appropriate for places with a single cropping season and short-duration variations for double/intensive cropping regions. The primary objective of the crossing is to broaden the crop's genetic diversity in order to enable the most effective selection under a variety of environmental circumstances. This entails combining desired features, such as high yield, resistance to insects and diseases, the inclusion of important quality traits, etc., in a single genotype.

28.1.2 Little Millet Genetic Improvement Targets

Germplasm with high genetic diversity is required for each crop breeding operation. According to soil types, rainfall, temperature, humidity, day length, and cropping patterns, there is significant variation in the genetic resources for a variety of traits, including maturity duration, which can be used to breed tailored cultivars that can fit into the different maturity groups: early, mid-late, and late. While medium- to long-duration varieties would be suitable for sites with a single cropping season, short-duration varieties would be suitable for double/intensive cropping regions. There are just a few thousand germplasm varieties of Little millet, most of which are conserved by India (approx. 3000).

Little millet contains a high level of nutritional factors, but their utilization is limited due to the presence of anti-nutrients such as phytate, phenols, tannins, and enzyme inhibitors, as well as a high level of protease and amylase inhibitors, which affect the digestibility of Little millet grains (Vinoth and Ravindhran 2017). Most grain nutrients in millets are higher than in major cereals, but there is a lot of variation in gene banks for grain nutrients (Upadhyaya et al. 2011a, b; Vetriventhan and Upadhyaya 2018, 2019) and anti-nutritional factors (Panwar et al. 2016). Exploiting existing varieties in germplasm and hybridization variants can aid in

the creation of nutrient-rich varieties with high-yielding cultivars. Little millet is able to thrive in a variety of climates and is less vulnerable to significant biotic and abiotic challenges. However, a few ailments and insect pests are significantly reducing yields, thus it is crucial to breed cultivars that are disease- and pest-resistant. Little millet is frequently affected by the diseases grain and head smut and leaf spot. Shoot flies are the most significant pest of small millet. Little millet is primarily farmed as a rainfed crop, and as a result of India's failed monsoon season, it is severely impacted by drought. Due to their thin stems, poor crop management, and environmental conditions, lodging is one of the key issues facing all Little millets in addition to drought (Santra et al. 2019). The present breeding techniques integrate two or more goals, such as boosting grain output, improving nutritional quality, and resistance to various biotic and abiotic challenges. Therefore, under a climate change situation, new small millet cultivars that have all these characteristics are particularly desirable.

28.1.3 Hybridization Techniques in Little Millet

These crops have chasmogamous flower openings, in which pollination occurs prior to the flower opening. As a result, it encourages Little millet to self-pollinate. Therefore, hybridization is primarily necessary for the formation of variety. Emasculation is necessary in order to try crosses due to self-pollination and a lack of male sterility. The agricultural improvement effort done on these crops so far has had some success. Recently, some improved cultivars were created, but their potential for production is low. Although there is a lot of heterogeneity in the current germplasm collections, it has not been used to its full potential. By utilizing hybridization and selection in the segregating population, the existing variability can be used to create new, improved cultivars. Crossing from different groups that differ in one or more heritable features is known as hybridization (Harrison 1990). The expression of hybrid vigor causes phenotypic changes to occur immediately after hybridization (Goulet et al. 2017). To effectively use the present germplasm, provide breeding material, introduce novel genes, and expand the genetic basis of Little millet and other crops, hybridization is required. The fundamental issue with all small millets is the difficulty in emasculation due to the tiny floret size. The following are the primary factors relating to floral morphology, various emasculation and methods of crossing, their shortcomings, and ways to solve issues with conventional crossing methods using a new modified method (SMUASB), as well as their success rates and benefits in Little millet.

28.1.3.1 Constraints in Hybridization and Crossing Techniques in Little Millet

These plants have very tiny flowers that make hand emasculation and hybridization difficult. The floral biology knowledge, a straightforward, practical hybridization procedure, and appropriate molecular markers for identifying true F_1 are necessary for artificial hybridization planning. The problem faced by breeders is to look for easy and efficient emasculation and pollination strategies in these crops, given the

difficulties in creating artificial hybrids. Although there are a lot of variations available to combine the desired traits from germplasm resources, artificial hybridization must be done continuously, which necessitates the development of a quick and efficient method of crossing. The study of floral biology will aid in developing the best emasculation and pollination strategies.

28.1.3.2 Floral Morphology of Little Millet

Little millet's inflorescence is a panicle that is usually 15–45 cm long and 1–5 cm broad. It can be constricted or thyrsiform (Seetharam et al. 2003). The persistent spikelet is 2–3.5 mm in length (Bor 1960). At maturity, panicle branches are scabrous and drooping. At the ends of the branches, spikelets develop on uneven pedicels, but they are solitary. A spikelet has two tiny blooms on it. The upper one is sterile, whereas the bottom one is fertile or bisexual without rachilla growth. The lemma II and its palea envelope the fertile flower, whereas the staminate/sterile blossom is enclosed by the lemma I and its palea (Sundararaj and Thulasidas 1976). The spikelets are sharp, compressed dorsally, and elliptical. It has three anthers that are roughly 1.5 mm long. The lower glume is oblong, 0.7–1.2 mm long, membranous, without keels, and 1–3 veined, reaching the apex of the florets (Fig. 28.1). Lower glume lacks the lateral vein and its sharp apex. The top glume is also ovate

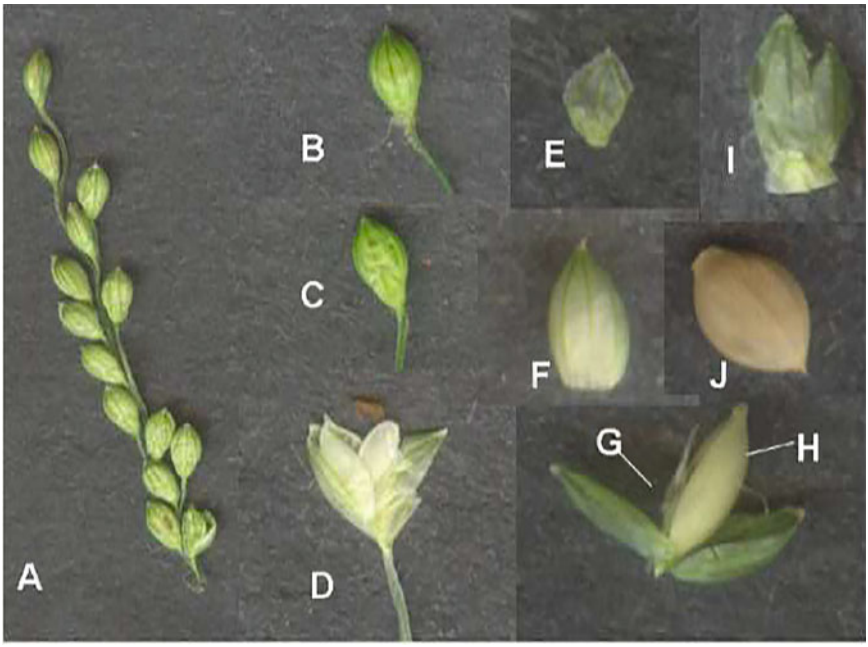

Fig. 28.1 Floral morphology of Little millet (**a**) Inflorescence; (**b**) Spikelet; (**c**) Lateral view of spikelet; (**d**) Open spikelet; (**e**) Outer glume; (**f**) Lemma I; (**g**) Sterile floret; (**h**) Fertile floret; (**i**) Top glume; (**j**) Seed covered in lemma and palea. (Source: Nandini et al., Electronic Journal of Plant Breeding, 10(3): 1161–1170)

and lacks a keel, but it is larger than the lower glume. It is veined 11–15 times (Nanda and Agarwal 2008).

28.1.3.3 Anthesis and Pollination

On the second or third day after the panicle emerges from the leaf blade, the spikelets start to unfold. The panicle's blossoming develops from top to bottom. On the sixth or seventh day, the majority of the florets begin to open. A panicle takes about a fortnight to completely flower (Sundararaj and Thulasidas 1976). The anther dehiscence happens between 9.30 and 10.30 a.m. (Jayaraman et al. 1997). The glumes open for a short while and selfing take place (Seetharam et al. 2003). The entire anthesis procedure is quite quick, lasting about 2–5 min.

28.1.4 Emasculation and Hybridization Techniques

The plant breeder can produce the required blend of traits by using the correct techniques for selfing and crossing various crops. Before the worker can hope to produce the finest outcomes, this requires skill and practice. by recombining the alleles that affect yield elements such as the number of tillers, primary branches, secondary branches, grains per panicle, and grain weight per thousand. Emasculation is the process of removing stamens or anthers or sterilizing pollen grains without harming a flower's female reproductive system. The objective of emasculation is to prevent self-pollination in the female parent's flowers. Male plants in dioecious plants are removed, whereas male flowers in monoecious species are removed to avoid self-pollination. However, emasculation is required in bisexual flowers. In order to maintain allogamy in naturally self-pollinated crops, pollen from the desired parent is purposefully sprinkled on the stigma of the female parent during the crossing process. This is because naturally self-pollinated crops have limited pollinators with very little mobility. The major goal of crossing is to increase variability and include desired qualities such as high yield, pest and disease resistance, critical quality features, and so on, into a genotype, as well as to extend the population's genetic basis.

28.1.4.1 Various Emasculation and Hybridization Methods Used in Little Millet

1. The Contact method
2. Manual emasculation followed by pollination
3. Treatment with hot water
4. USSR method

The Contact Method/Approach Method

This method involves planting the female parent near to the male parent that is suited for pollination. Male and female panicles are just hazily related. After pollination and fertilization are complete and both have been separated, the male father should have morphological markers to make it simple to identify actual F_1 (http://agritech.

tnau.ac.in). Due to its simplicity, this type of crossing is used by the majority of self-pollinated plants. Realizing F_1 status is quite unlikely. Only a small percentage of the real formula may be obtained and seen. A substantial plant population must be raised in order to find genuine F_1s. Greater resources are required for evaluation.

Manual Emasculation Followed by Pollination

Using this technique, flowers that are likely to bloom the following day are chosen. Removing stamens or anthers has no discernible impact on the female reproductive system. Hand emasculation occurs in the evening, followed by pollination the next morning. Delivered to the emasculated female flower for pollination are male flowers that are expected to bloom that day. They are knotted, then covered with a butter paper bag. Within 2–5 days, cross-pollination spontaneously takes place. The actual F_1s may be recognized using molecular markers (http://agritech.tnau.ac.in). This is how self-pollinated crops have traditionally been produced. The primary drawback of this approach is that the lemma and palea are so tightly packed that it is impossible to open the flower before anthesis without doing damage to it and erasing any traces of seed set. Another concern is the interval between the first flower on a panicle and the last floret on a panicle, which is known as the anthesis interval. This makes it challenging to choose the ideal moment for emasculation before anthesis. Panicle flowers with the first florets opened are chosen to address the issue. The florets in the panicle start to open as a result of being rubbed between the palms of the hands. The florets in the panicle start to open as a result of being rubbed between the palms of the hands. Florets were sprayed with room-temperature water from an atomizer to maintain their moisture, which helped to prevent the anthers from dehiscing before all of them were harvested. When all of the opened florets were closed and the florets had finished opening, the florets that hadn't been emasculated were removed. That included the panicle's top and bottom fertilized florets as well as the bottom immature florets. Between 8 and 9 a.m. is considered the best period for emasculation. During this time, the florets opened at a rate that allowed for proper emasculation. Following emasculation by 15 min, pollination is carried out. For pollination, male parents are stroked and let to open. An inverted glassine bag with opened male florets is placed above the emasculated panicle. For the purpose of crossing and moisture preservation, this will be left for 5 days. The advantage of this strategy is that lemma and palea open willingly rather than under duress. The procedure's disadvantage is that the stigma is damaged during emasculation, which results in sterile florets and prevents seed germination.

Treatment with Hot Water

Many researchers employed the hot water emasculation technique to alleviate the problem of manual emasculation in small florets (Keller 1952). In this process, panicles that are predicted to flower in the next 2–3 days are chosen and submerged in hot water at 52 °C for 2 min. The ideal temperature and time were determined based on the proportion of the crossing seed set (http://agritech.tnau.ac.in). Hot water emasculation of Little millet was successful at 49 °C for 8–10 min or 50 °C for 5 min, according to Srivastava and Yadav (1972). The male father is attached to

the emasculated female parent and covered with butter paper after being emasculated with hot water for pollination. Because of the method's limitations, essential equipment such as a temperature recorder (thermometer) is required to maintain proper temperature. High temperatures reduce stigma survival, which may result in a limited seed set.

USSR Method

The improved crossing method was developed by Seetharam et al. in 1986 to address the problems associated with manual emasculation and treatment with hot water to eliminate pollens.

Induced Opening of the Flower (USSR Method)

The following are some examples of how the USSR approach is being effectively used to create variations and develop new cultivars:

1. Florets are stimulated mechanically by gentle stroking of the panicle with the palm of the hand.
2. Florets open in 2–3 min, much faster than usual flowering.
3. Dip an anther in room-temperature water to prevent it from exploding.
4. Remove anthers from opening florets with your forefingers.
5. Remove the unopened florets while keeping the opened flowers.

Pollination Method

In order to finish the pollination process, the emasculated female spike was positioned directly beneath the male spike that was oozing pollen. Pollen from the male spike is transferred to the female spike, which offers a fantastic opportunity for fertilization. The spikes were jostled against one another for 2 days during the daily anthesis periods. On the third day of pollination, the female spike was carefully removed and checked for any potential later-forming florets. When these florets weren't completely eliminated, they frequently grew and produced seeds that looked like cross-fertilized seeds.

Modified Crossing Method (SMUASB Method)

The Project Coordinating Unit, All India Coordinated Project on Small Millets, UAS, GKVK, Bengaluru (India) introduced this enhanced method of crossing Little millet in 2014, and it was given the designation SMUASB (Small millets, University of Agricultural Sciences, Bengaluru). Little millet's lemma and palea are so tightly packed that opening the floret before the regular anthesis would result in damage to the flower and would hinder seed germination. The SMUASB method uses cold water as a mechanical stimulator to promote floret opening.

Emasculation

The opening of the Little millet flower occurs from the top to the bottom of the panicle. In order to facilitate crossover, male and female parents are planted in

different rows. Eight to Nine a.m. is the best time to cross. Following are the details of emasculation:

- The first floret of the female plant's panicle must be open before it may be sprayed with cold water that is between 5 and 8 °C.
- This cold water spray causes the florets to spontaneously open 1 h earlier than they would have otherwise.
- After all the florets have been opened and all the anthers have been removed by washing the panicle in cold water, the panicle is emasculated by being submerged in cold water.
- At the top of the panicle, it has unfertilized florets, and at the bottom, it has immature florets.

Pollination

- The male parent is chosen in such a way that the first floret has opened.
- Male panicle, like female panicle, is sprayed with cold water to open the florets and keep the anthers moist.
- As soon as the male florets open, it is loosely knotted around the female parent panicle to aid in adequate oxygenation and pollination.
- The knotted panicles are then sprayed with water to keep the stigma and anthers moist.
- To prevent cross-pollination, male and female panicles are tied together and covered with butter cover. Because these crops are self-pollinated, only 3–5% cross-pollination may occur.

28.1.5 Application of SMUASB Method for Crossing in Little Millet

Crosses were attempted in Little millet using the SMUASB method at the Project Coordinating Unit, Small Millets, UAS, Bengaluru.

Success Rate Three panicles were employed to cross; success rate: Using the SMUASB procedure, panicles were emasculated and pollinated. The female parent's seed is collected and planted to identify authentic F_1s. Each panicle's seeds were collected and sown individually. Out of the 30 F_1 plants that were planted in panicle 1, 18 plants were confirmed to be real F_1s, indicating a 56% success rate. Similar results were obtained in panicles 2 and 3, with success rates of 53% and 55%, respectively. SMUASB technique was used to cross Little millet, with an average success rate of 56%.

28.1.6 The Modified Crossing (SMUASB) Approach Benefits Over Other Crossing Methods in Little Millet

The benefit of this SMUASB is that the stigma is never harmed during emasculation. In comparison to the other conventional approaches, this produces a larger seed set and a higher frequency of true F_1's. It requires less effort and takes less time than manual emasculation. The removal of pollen from each and every floret during hand emasculation is time-consuming and technical skill is needed. Because the blossoms of these two crops are small, emasculation requires technical skill. In the modified crossing procedure, flowers open simply by being sprayed with cold water. It is a less strenuous procedure because anthers are also easily removed by washing or dipping in water. Emasculation and identifying real F_1 requires less space and resources. For the contact approach, a significant number of seeds harvested from female plants must be examined for the presence of real F_1s. In the modified crossing method, cold water spray is utilized as a mechanical stimulator for opening the florets in place of hand messaging, as was done in the USSR approach, and hot water treatment for emasculation. The stigma is not harmed by this. Therefore, there is a higher success rate in getting actual F_1s. In Little millet, the modified crossing (SMUASB) method of emasculation and crossing is a very beneficial strategy. This technique fixes the issues with the touch method, hand emasculation method, hot water emasculation method, and USSR way of crossing. Comparing this procedure to earlier ones, the success rate for obtaining actual F_1 is higher.

28.1.7 Breeding for Biotic Stresses in Little Millet

Biotic stress tolerance in millets is a crucial step in addressing disease and pest issues on essential food and feed crops such as sorghum, pearl millet, finger millet, foxtail millet, and other minor millets. Biotic pressures are one of the key limits to millet production, and the problems of disease, insect pests, and weeds are projected to worsen in the future as the climate changes. As a result, management of these threats is becoming increasingly critical in the current environment. Current initiatives concentrate mostly on the creation of resistant crop types. Due to rising input prices in developing nations where millets are a major food source, the use of pesticides is constrained. Compared to the use of chemical pesticides or other techniques of pest control, genetic resistance has a number of advantages. The difference between oligogenic and polygenic resistance is often defined by the mode of inheritance. Grain smut and sheath blight are the principal diseases that afflict Little millet, and donors for resistance sources have been found. The production of Little millet is sometimes hampered by pests like shoot flies, which cause significant crop loss. It has been discovered that morphological traits are connected to small millet's resistance to shoot flies. Higher trichome length and density were seen in tolerant genotypes, which mechanically hinder immature larvae from reaching their feeding locations (Gowda et al. 2003). Highly resistant accessions were found after testing small millet germplasm accessions in the field for shoot-fly resistance (Table 28.1).

Table 28.1 Germplasm/genotypes identified as resistant/tolerant sources for diseases in Little millet

Biotic stresses	Resistant/tolerant sources	References
Grain smut	IPmr 841, 1061	http://www.dhan.org/smallmillets/docs/report/1_Advances_in_Crop_Improvement_of_Small_Millets.pdf
Head smut	GPMR# 65, 82, 67, 105, 70, 73, 80, 83, 92; OLM 36, 40, 203, TNAU# 89, 98, RLM# 13, 14, VMLC# 281, 296	Jain and Tripathi (2007)
Shoot fly	GPMR# 164, 274, 236, 243, 110, 213, 584, 66, 683, 569, 189, 241, 98, 163, 324, 670, 598, 192, 96, 583, 161, 596, 95, 190, GPMR# 7, 17, 18, 20, 22, 26, 46, 53, 78, 84, 92, 98, 101, 104, 106, 107, 112, 114, 115, 116, 117, 124, 132, 134, 136, 141, 148, 149, 163, 169, 170, 171, 172, 175; PRC# 2, 3, 7, 8, 9, 10, 11, 12, RPM# 1-1, 8-1, 12-1, 41-1, RAU# 1, 2, K1, Co 2, Dindori 2-1	Gowda et al. (2003); Murthy and Harinarayana (1986)

28.1.7.1 Breeding Strategies

It is a common observation that when a new character is brought into a breeding program, either gains in existing characters (e.g., yield potential) are affected or the program must be expanded by another feature that is dependent on the rate of selection. The breeder must therefore consider whether it is profitable to breed for disease or pest resistance. The frequency and severity of disease in the region where the crop will be grown, as well as the financial losses brought on by the pest, all play a major role in this decision. Furthermore, the breeder must find appropriate defensive mechanisms for crop introduction. He must select a major gene resistance with dominant expression. The following are the benefits of this form of resistance: (1) their straightforward inheritance, which is obviously highly desirable in a breeding program; (2) the crop's generally perfect pest protection. The risk of selecting total major-gene resistance is that it will be broken down over time. However, there have been reports of major-gene resistance being discovered to be long-lasting. Following that, in a resistant breeding program, identifying an appropriate donor for resistance is critical. It is important to look into the genotypic variation in crop types and related species. Taxonomic categories associated with the crop, such as the cultivar itself, commercial cultivars, landraces, wild progenitors, allied species, and genera, can be used to identify sources of resistance. The list of potential sources of resistance is organized by increasing difficulty for the breeder. The key issues are (1) crop and donor species cross compatibility, (2) hybrid sterility of wide or remote crosses, and (3) inadequate intrachromosomal recombination (Harlan and de Wet 1971). In general, repeated backcrossing is required for resistance with desired features to remove unwanted effects introduced along with the resistance. Insects and pests are less prevalent in the Little millet crop. However,

one of the most significant is the shoot fly (*Atherigona miliaceae*), which causes damage to plants in the seedling stage in India. *Atherigona falcata* and *Atherigona lineata* are two other species of shootfly. Dead-heart generation is a common sign of shoot fly damage. During the seedling stage, armyworm (*M. separata*) and *S. furgiperda* occasionally caused harm under dry conditions.

28.1.8 Conventional Methods

A single R gene can completely protect against one or more disease strains when transferred into a plant of the same species that was previously susceptible. As a result, R genes have long been exploited in traditional resistance breeding projects (Austin et al. 2002). R gene-mediated resistance offers several appealing characteristics for disease control. When triggered in a timely manner, the coordinated responses can effectively inhibit pathogen growth while causing little collateral damage to the plant. Pesticides have no negative environmental consequences and require no chemical input from the farmer. Unfortunately, co-evolving diseases frequently beat R genes. In this context, persistent resistance is defined as "resistance that stays effective when a cultivar is widely grown in disease-prone conditions" (reviewed by Michelmore 2003). In disease resistance breeding, the idea of persistent resistance—which makes no assumptions about resistance mechanisms or genetic control—has shown to be extremely helpful. Multiple R genes ('pyramids') can be introduced into individual plant lines as an alternative to single-gene deployment (Pink 2002). If the mutations have a substantial cumulative impact on virulence, this is improbable. Another tactic is to grow a variety of lines in the same plot, each of which expresses a different R gene.

To minimize the selection pressure for mutations in *Avr* genes, a susceptible line can be introduced in the combination (Mundt 2002). A multiline technique was evaluated in a study and found to be highly effective (Zhu et al. 2000). Pyramiding and multiline deployment haven't been widely utilized since it takes time to develop R genes into elite cultivars. However, when the ideas mentioned earlier mature, these strategies will become far more practicable. Furthermore, many R genes detect only a few pathogen strains and hence do not confer broad-spectrum resistance. Furthermore, the introduction of R genes into top cultivars requires a time-consuming process known as traditional breeding. On the other hand, recent molecular-level insights into the role of R proteins and downstream signal transduction pathways may suggest remedies for these deficiencies.

28.1.9 Applications of Marker-Assisted Breeding

Though polygenically regulated hereditary types of resistance are always enduring (Parlevliet 1979), they are more difficult to manage in breeding programs. Backcross initiatives to transfer polygenes from wild relatives of the crop, in particular, are heavy or difficult to handle. In the case of disease resistance, marker-assisted

breeding may play a unique role. In this regard, the application of marker-based selection simplifies the pyramiding of many important resistance genes into a viable genetic background (Song et al. 1995). According to research, pyramiding resistance QTLs can attain the same or even a higher level of resistance than a R gene (Castro et al. 2003; Richardson et al. 2006). When screening for one resistance gene interacts with screening for another, as happens frequently in disease resistance breeding, this should be extremely helpful. Instead of screening individuals sequentially for the inheritance of a single resistance (or simultaneously through progeny screens), individuals who have retained all of the genes of interest might be chosen based on DNA markers. Similarly, using marker-assisted breeding can speed up gene distribution. By cultivating cultivars with complementary sets of resistance genes that are race-specific, farmers can achieve long-term disease protection. Big, single-locus resistance genes are not, in theory, the only genes that can be pyramided or deployed. Partial resistance loci can be viewed as Mendelian factors and controlled much like any other main gene using QTL mapping. This includes resistance alleles derived from otherwise vulnerable parents (Wang et al. 2006), allowing for the discovery of transgressive resistant genotypes. QTLs from different donors can thus be quickly incorporated into a desired genetic background or used in a number of cultivars. This approach can clearly benefit from information on the racial (or racial-nonracial) specificity of partial resistance loci.

28.1.10 Alternative Approaches for Resistance Breeding

It is important to note that many ways to acquire resistance have been used. These include transgenic technologies and mutation breeding.

28.1.10.1 Mutation Breeding

A mutagenic therapy has the potential to transform a susceptible genotype into a resistant genotype. If the mutation is a point mutation, the resistant mutant will be identical to the original cultivar, save for its resistance. However, the mutagenic treatment usually has unfavorable side effects. Several other genes may have changed as well, or the resistant mutation may have unfavorable pleiotropic implications. As a result, after selecting a resistant mutant, more breeding efforts (i.e., backcrossing) need to be undertaken to develop a commercially acceptable cultivar.

28.1.10.2 Germplasm Resources of Little Millet

Diversity in crop cultivars is essential for sustainable agriculture. The variety required for agricultural development is provided by germplasm. The risk of crop failure due to insect pests, disease epidemics, unexpected climatic impacts, and inadequate genetic foundation of cultivars is increased. The largest germplasm bank for Little millet is in India. Most landraces have already been lost as a result of the switch from traditional crops and landraces to commercial crops, improved varieties, and hybrids, which is the cause of the inadequate germplasm collection. As

Table 28.2 Status of Little millet genetic resources

S. No.	Institutes	Number of accessions
	India	
1.	AICRP on Small Millets (AICRP Small Millets)	928
2.	International Crop Research Institute for the Semi-Arid Tropics (ICRISAT)	473
3.	National Bureau of Plant Genetic Resources (NBPGR)	1253
4.	The Ramiah Gene Bank, Tamil Nadu Agricultural University, India	108
5.	Regional Station Akola, NBPGR (NBPGR) India	165
	USA	
6.	North Central Regional Plant Introduction Station, USDA-ARS, NCRPIS (NC7)	226

Source: www.fao.org/wiews.archieves/germplasm

a result, collecting and conserving the diversity of the remaining Little millets is critical before we lose them forever. Small millet germplasm exhibits a high variety of morpho-agronomic, quality, and stress tolerance traits, and promising germplasm sources have been identified in the majority of crops. In Little millet, 10 accessions for higher seed weight, 15 for higher grain yield and biomass yield, and 30 for higher grain nutrients have been identified, including 3 accessions for two or more nutrients (IPmr 449 for Fe, Zn, Ca, and protein; IPmr 981 for Zn and protein; and IPmr 977 for Ca, and protein); and 5 accessions (IPmr 855, IPmr 974, IPmr 877, IPmr 897, and IPmr 767) that produced grain yield over 1500 kg/ha were identified at ICRISAT. Grain smut and sheath blight are significant diseases in small millet. There are just a few resistance sources for major diseases and, to a lesser extent, pests in Little millet. Table 28.2 shows the amount of genetic resources available for small millet.

28.1.10.3 Conventional Breeding Approaches

Small millets are bred using a variety of breeding techniques, including pure line selection, mass selection, pedigree selection, and mutation breeding, which are suitable for self-pollinated crops. According to reports on small millet cultivars issued over time, the majority of them were released through pedigree selection, then selection from local landraces and cultivars (hybridization and selection). For instance, in India, out of the 248 varieties of the 6 small millets (Little millet, 20; finger millet, 121, foxtail millet 16, proso millet 14, proso millet 24, kodo millet 33; barnyard millet, 18), about 65% were introduced after selection from landraces, about 30% through pedigree selection, and 5% by mutation breeding. An important breeding technique for small millet, especially finger millet, foxtail millet, and proso millet, involves hybridization to promote variety followed by selection in a segregating population. In India, 45% of the cultivars of finger millet, 22% of foxtail millet, and 29% of proso millet were bred using the hybridization and selective breeding approach. However, due to their floral architecture and anthesis behavior, Little millets are not readily receptive to hybridization.

28.1.10.4 Mutation Breeding for Little Millet Improvement

It is one of the small millets for which a novel variety has been produced through mutation breeding. The majority of the newly released varieties were created using mass selection or pure line selection techniques. Due to challenges in crossing brought on by small spikelets on brittle pedicels, hybridization procedures for breeding new types are limited (Nirmalakumari et al. 2007). Therefore, mutation breeding was utilized to improve the genetics of small millet in addition to traditional breeding techniques. Gamma rays were used by Nirmalakumari et al. in 2007 to induce mutagenesis in CO_3 and CO_4 crops. In both cultivars, they noted the greatest heterogeneity in plant height and overall tiller count. For the total number of tillers, lodging, and grain production in the M_2 generation, high heritability and high genetic advance were observed. For plant height, tillers, nodes, internodal length, stem thickness, and grain yield, mutants in 500 and 600 Gray showed the highest variability, heritability, and genetic progress, demonstrating the effectiveness of selection for these traits for the generation of superior genotypes. Little millet has a wide range of variability, heritability, and genetic advance for grain yield, productive tillers, no. of tillers, flag leaf length, and flag leaf sheath length, indicating additive gene effects for these characters. Nirmalakumari et al. (2010), evaluated 109 germplasm of Little millet. The majority of the component characters and grain yield showed a substantial link in the study, suggesting that these characters may be simultaneously improved by selection. The usefulness and efficiency of two tiny millet cultivars, CO_3 and CO_4, as mutagens were investigated by Ganapathy et al. in 2008 utilizing gamma rays. In all kinds, the mutagenic dose of 500 Gray resulted in the highest rate of mutation on the basis of the M_1 panicle family and the M_2 seedlings. Additionally, they noticed that in both genotypes, efficiency declined as the mutagen dose increased.

In self-pollinated crops, where hybridization is an uncommon occurrence, mutation breeding has been crucial in generating variety in small-sized floret crops. Thirteen small millet cultivars (Little millet 2; finger millet 8; kodo millet 3) have been released in India as a result of mutation breeding. In order to find a high frequency and diversity of desired mutations, a mutagen's utility depends on its efficacy (mutations produced per dosage of the mutagen) and efficiency (the relationship between mutation and harmful alterations/damage, such as sterility, death, injury, etc.).The characterization of small millet germplasm and its application in crop development and the release of numerous variants for high yield with resistance/tolerance to biotic and abiotic challenges have both benefited from conventional breeding methods. Enhancing genetic improvements in tiny millet will be made possible by genomics-assisted improvement using a variety of omics techniques. Table 28.3 provides a list of the variations created using both traditional breeding techniques and mutation breeding.

28.1.10.5 Genomic-Assisted Breeding in Little Millet

Any crop's genome can be sequenced to learn more about the coding and non-coding portions of the genome that control the plant's growth, development, and reaction to environmental factors. Furthermore, sequencing data aids in the

Table 28.3 Salient features of released varieties of Little millet crops in India (1989–2016) (Source: AICSMIP, UAS, Bangaluru, India)

S. No.	Name of variety	Pedigree	Institute where developed	Year of release	Maturity (days)	Av. yield (Q/ha)	Area of adaptation	Special features
1.	Chhattisgarh Kutki-1 (BL6)	Paiyur 1 × OLM 29	Jagdalpur IGKV, Raipur	2016	90–95	12–14	All India	Suitable for upland and rich in zinc and calcium
2.	DHLM36-3	Co-4x Paiyur-2	ARS, Hanumanmatti, UAS, Dharwad	2016	95–100	14–16	All India	Late duration variety
3.	Chhattisgarh Kutki-2 (BL-4)	CO-2xTNAU97	Jagdalpur IGKV, Raipur	2016	90–95	10–12	Chhattisgarh	High iron content (28.3 mg/100 g grain). Tolerant to major pests of Little millet
4.	GV-2	Derivative from a mutant of released variety Gujarat Vari-1′	Waghai, NAU, Navsari	2016	115–125	26–28	Gujarat	Clean white colour and bold seeded, resistant to pest and diseases
5.	Phule Ekadashi (KOPLM83)	Selection from local germplasm	ZARS, Kolhapur, MPKV, Rahuri	2016	120–130	12–14	Sub-montane and Ghat Zone of Maharashtra	Non-lodging
6.	Jawahar Kutki 4 (JK4)	DLM42 × Kutki1	Rewa JNKVV, Jabalpur	2016	75–80	13–15	Rainfed areas of Madhya Pradesh	Suitable for sole as well as inter/mixed cropping, resistant to moisture stress, lodging, and key pest shootfly, and moderately resistant to head smut
7.	JK 36	Selection from local Shahdol germplasm	Rewa, JNKVV, Jabalpur	2009	75–80	10–12	M.P. state	Tolerant to shoot fly
8.	OLM208			2009	100–105	12–15	National	Shoot fly resistant

		Selection from Lajigada local	OUAT, Berhampur					
9.	OLM217	Selection from Udayagiri local	OUAT, Berhampur	2009	105–110	15–16	National	Rust and grain smut resistant to but moderately resistant to sheath blight, tolerant to shootfly
10.	Co4	Co 2 × MS1684	TNAU, Coimbatore	2005	75–80	16–20	Tamilnadu	Lodging resistant, suitable for double cropping
11.	OLM20	Mutant of SS-81-1	OUAT, Odisha	2003	75–80	11–12	Odisha, Madhya Pradesh, Chattisgarh	Tolerant to drought
12.	Tarini (OLM203)	Selection from local cultivar (KL 2) of Koraput Dist.	OUAT, Berhampur, Odisha	2001	105–110	10–11	Karnataka, Andhra Pradesh, Odisha, Bihar, Tamil Nadu	Blast and grain smut resistant
13.	Kolab (OLM36)	Mutant of SS-81-1	OUAT, Odisha	2001	95–100	10–11	MP, Odisha, Chattisgarh, Bihar, Karnataka, Gujarat	Brown spot and sheath blight resistant
14.	Paiyur 2	Pureline selection from Accession PM295	TNAU, Coimbatore	2000	95–100	7.5–8.5	Tamil Nadu	Late duration variety
15.	TNAU 63	Selection from Germplasm MS2369	TNAU, Coimbatore	1997	90–100	11–12	Tamil Nadu, Karnataka, Gujarat	High grain and fodder yield
16.	Birsa Gundli 1	Selection from Local	BAU, Ranchi	1993	55–60	7–8	Bihar Plateau, Jharkhand	Early duration
17.	Paiyur1	Pureline selection	RRS, Paiyur, TNAU	1989	90–95	8–10	Tamil Nadu	Late maturity

development of genetic markers that provide insight into diversity, population structure, and crop evolution. Mapping drawn variation associated with desirable traits and developing molecular tools for use in genomic-assisted crop breeding is useful for future breeding programmes in Little millet. High throughput flexible genotyping technologies are available that not only cut costs but also give greater coverage, depth, and dependability. The full chloroplast genome sequences of foxtail millet, proso millet, tiny millet, and barnyard millet are also available. So far, the genomes of foxtail millet, finger millet, proso millet, and Japanese barnyard millet have been sequenced. In collaboration with Cornell University, the ICRISAT genotyped a diverse set of six small millets (Proso millet, kodo millet, finger millet, foxtail millet, barnyard millet, and Little millet) using a genotyping-by-sequencing (GBS) approach, identifying genome-wide single nucleotide polymorphisms (SNPs) and assessing population structure and diversity (Johnson et al. 2019).

28.1.10.6 Gene Mapping

Genes and genomic areas for interesting features were mapped in millets using low-density markers such as RAPD, RFLP, AFLP, and SSRs before genome sequencing techniques were developed. The first RFLP-based map of foxtail millet was created by Wang et al. in 2006, and they discovered that chromosome 8 contains a gene that has a significant impact on gamete fertility. Dida et al. (2007) created the first genetic map, which measured 721 cM on the A genome and 787 cM on the B genome. They did this by combining RFLP, AFLP, and SSR markers. The RAPD- and ISSR-based germplasm characterization in finger millet was described by Gupta et al. (2013). Additionally, DNA markers with higher (>85%) cross-genera transferability among other millets, such as proso millet, barnyard millet, Little millet, and kodo millet, as well as non-millet species, were developed using information from the foxtail millet genome. SSR, EST-SSR, ILP, and microRNA-based molecular markers were among them. These higher cross-transferability levels demonstrate the usefulness of these markers for the characterization of the germplasm, marker, and trait association, and marker-assisted breeding in other millets for which no genomic data are available.

28.1.11 Other Omics Approaches for Gene Discovery and Crop Improvement

28.1.11.1 Transcriptomics

Even while omics approaches to these crops are still relatively new and there aren't many studies on the use of other omics methods to research millets, genetics and genomics have already made significant strides in the field. According to Joshi et al. (2019), transcriptome-based gene expression profiling is a functional genomic strategy for describing the candidate genes in charge of governing various biological processes. It offers detailed data on the expression pattern of genes and functional polymorphism. For nutritional characteristics like Zn and Fe, Little millet's transcriptome-based information has been established.

28.1.11.2 Proteomics and Metabolomics

Because transcript abundance and expression do not directly correlate with protein or metabolome levels, proteomics and metabolomics studies become more crucial. Additionally, it is crucial to comprehend the post-translational modifications that proteins and metabolomes undergo to perform any given function. It takes a lot of work to examine gene expression by proteomics in Little millet.

28.1.12 Genetic Transformation of Small Millets

The transformation of small millets has not frequently used an agrobacterium-mediated approach. However, genetic transformation research in millets is lagging behind that of other important cereals like rice. Recent breakthroughs include reports on the improvement of transformation procedures and transgenic plants that produce functionally active transgenes in millets. There aren't many findings on genetic alteration in finger millet and foxtail millet. While small millet, teff, and fonio have no studies on the genetic change, barnyard millet, teff, and fonio do. Little millet has been the subject of an in vitro regeneration process that, according to Mishra (2017), may make it possible to investigate transgenic work and introduce important genes that provide tolerance to various challenges. The use of a transgene-based method in Little millet has been made possible by the availability of enhanced genetic transformation techniques. However, this has not yet been standardized in millets, thus it will be useless to directly manipulate genes to overexpress, knock out/down, or use the CRISPR/Cas9 system. For functional genomics and crop development studies, key cereals like rice have routinely used genome editing techniques like CRISPR/Cas9. Numerous genes with their functions in improving disease resistance, stress tolerance, and other desirable traits have been found in small millets, but they still need to be thoroughly functionally characterized utilizing genomics methods. The lack of an efficient transformation system, which would enable functional study on certain genes in small millets and the analysis of their activities in related crops like rice, wheat, and sorghum, is a limitation that can be solved.

28.1.13 Breeding for Abiotic Stresses Resistance/Tolerance in Little Millet

World food security is a concern in a climate change scenario due to the ever-increasing population, the limited amount of arable land available for food production, and other factors. The management of these food security issues can be greatly aided by the varietal development of crops that can produce more effectively in challenging climatic conditions. In comparison to large field crops, tiny millet crops are more adaptable to climate change conditions like inconsistent rainfall and moisture stress (drought). Although it is extremely rarely researched in these crops, the use of contemporary genetic and genomic technologies in the

development of these crops can guarantee global food security. According to statistics, losses from crop production are caused by drought-15%, storms-23%, and floods-60%. The productivity of millet crops is substantially impacted by drought stress, particularly in African countries. Little millet is a crop that grows quickly and is immune to moisture stress-related losses. Due to a lack of precipitation, Africa's semi-arid and arid regions are unable to support a healthy ecology. Little millet crops frequently fail due to dry spells that occur during crucial growth stages of the crop, particularly during flowering, rather than a lack of precipitation. At the time of blossoming, small millet crops exhibit considerable output decreases. The majority of millet crops are C_4 plants, which in hot, dry regions absorb moisture more effectively than C_3 crops. Salinity is another issue in hot climates with high rates of evapotranspiration. For the creation of cultivars suited for salinity and drought, current genomic tools must be linked with breeding strategies.

28.1.14 Nutritional Quality of Little Millet and Their Improvement

Little millet is a nutritious grain that contains macro- and micronutrients as well as antioxidants, including phenols, tannins, and phytates. It has a good amount of both soluble (3.15–5.70%) and insoluble (10.20–14.95%) dietary fiber (15.90–18.10%). It is a good source of fat (2.45–9.04%), carbohydrates (62.50–76.30%), and protein (7.70–16.50%). Additionally, according to some experts in the field of nutrition, it also contains a respectable number of minerals, including iron (9.30–20.00 mg/100 g), magnesium (133 mg/100 g), and zinc (3.70 mg/100 g) (Hadimani and Malleshi 1993; Ramulu and Rao 1997; Itagi 2003). Additionally, it had a low glycemic index, making it good for diabetics, and hypolipidemic effects, and a high fiber content (Ravindran 1991; Krishna Kumari and Thayumanavan 1997; Itagi 2003). Little millet has better nutritional value than staple cereals, but its use is constrained because of rising cereal output and cheaper, more readily available cereals (like rice and wheat). Small grain sizes compared to other crops and unintentional mixing of inert particles and stones during field harvesting make it challenging for processing and consumption. Additionally, the absence of processing facilities in millet-growing regions reduces millet use. As a result, millets are less readily available as convenience foods than traditional cereals. Dehulled millet has a short shelf life in part because the high-fat content causes early autoxidation and pest infestation from storage insects. Several processing techniques, such as blanching, malting, dry heating, acid treatment, popping, etc., reduce the amount of antinutrients, boost digestibility, and lengthen the shelf life of food. Utilizing millet grains to make new products will assist to broaden their uses, which will ultimately be good for human health (Table 28.4).

Nambi et al. (2012) from MSSRF examined the mineral composition of 18 genotypes of small millet that were gathered from millet-growing areas in Tamil Nadu (Megamalai Hills, Yelagiri Hills, Javadu Hills, Pachamalai Hills, and Dharmapuri dist.). The accessions' seed colors varied. Advanced quantification methods, like the spectroscopic method using inductively coupled plasma (ICP),

Table 28.4 Nutrient composition of Little millet

Nutrients	Little millet	SD value
Moisture (%)	10.00	0.06
Protein (%)	7.45	0.01
Fat (%)	1.49	0.01
Total carbohydrates (%)	64.87	0.01
Total minerals (%)	0.39	0.01
Total dietary fiber (%)	15.80	0.1
Soluble dietary fiber (%)	5.30	0.1
Insoluble dietary fiber (%)	10.50	0.2
Energy (kcal)	303	1.0
Calcium (mg)	22.02	0.01
Iron (mg)	8.18	0.01
Zinc (mg)	3.40	0.1
Manganese (mg)	0.17	0.01

were used to conduct the analysis. The content of proximate components varied from 6.36 to 9.15 g/100 g for protein, 61.48 to 68.57 g/100 g for carbohydrate, 3.15 to 9.07 g/100 g for ash, 5.54 to 7.84 g/100 g for fiber, and 3.61 to 5.56 g/100 g for fat. Mineral content varied from 199.2 to 303.4 mg/100 g for potassium, 184.3 to 308.9 mg/100 g for phosphorous, 131.3 to 186.6 mg/100 g for magnesium, 7.84 to 90.8 mg/100 g for iron, 22.6 to 56.4 mg/100 g for calcium, and 1.52 to 2.14 mg/100 g for zinc. An overall examination of the mineral composition revealed the following order for the minerals: Potassium > Phosphorous > Magnesium > Iron > Calcium > Zinc. All of the proximate and mineral contents studied showed significant overall variability, indicating their use in breeding efforts. Usha et al. (2011) also looked at variations in the proximal elements and mineral compositions. The protein content ranged from 9.8 to 12.49. Crude fiber, fat, and carbohydrates ranged from 2.87 to 5.09, 0.98 to 4.78, 0.49 to 8.72, and 62.2 to 76.59 g per 100 g, respectively. In that order, the following micronutrients are listed: calcium (18–24), phosphorus (215–232), iron (3–10.5), potassium (125–131), sodium (6.3–7.8), zinc (2.63–4.20), copper (0.6–1.0), and silicon (0.07–1.97).

28.1.15 Characterization of Millet Germplasm for Grain Nutrients

The preservation of plant genetic resources (PGRs) guarantees a consistent supply of raw materials for agricultural innovation. The success of the biofortification endeavor depends on the continued usage of PGRs for nutritional enhancement (Muthamilarasan and Prasad 2015). The International Crop Research Institute for Semi-Arid Tropics (ICRISAT) is home to the largest collection of millet genetic resources available in gene banks. India is the largest source of tiny millet genetic material, with 473 accessions.

28.1.15.1 Characterization of Gene for Fe Content in Little Millet

For the purpose of finding and mining the genes/alleles that regulate nutritional characteristics, including micronutrients like Fe, Zn, and Vitamin A, small millets are an important resource. This is because they are very nutrient-dense. Despite earlier studies on cereal crops emphasizing the significance of genes for grain Fe and Zn homeostasis that involves absorption, transport, and loading, there are currently no publications on potential genes affecting these aspects in tiny millet crops. Finding the genes for the Fe and Zn concentration in millets grain became essential as a result. The tiny millet RLM-37 genotype had an ortholog of the rice OsFRO2 gene, which was extensively characterized in this study utilizing computational genomics. Cloning and characterization efforts for this gene are still continuing. OsFRO2 and the Little millet gene discovered in rice showed good consistency in terms of sequence, structure, and functional similarity, showing that the two genes are orthologous to one another. This study generated preliminary information for the discovery of millet crop metal homeostasis genes. The recently found beneficial genes or their orthologs will serve as fresh sources and targets for genetic engineering to raise the micronutrient content of cereals. This could potentially have an impact on the evolution of crop plants with better nutritional qualities and a higher propensity to flourish in nutrient-deficient soils. Initial research results in the discovery of millet crop metal homeostasis genes. Table 28.5 provides information on the genotype's nutritional components (Chandel et al. 2017).

28.2 Strategies for Genetic Improvement

1. The main goal of genetic improvement should be to evaluate the genetic diversity in the germplasm systematically and to harness its genetic potential.
2. Grain yield has always been a crucial feature for genetic advancement. A minor improvement has been seen as a result of improvement efforts for yield enhancement. Selection for component qualities like high seed size and compact panicle types should be done in addition to yield per se.
3. There is a lack of understanding regarding floral structure and hybridization methods. Additionally, the modest floret size has prevented this crop from being genetically improved through hybridization methods. Significant steps must be taken in this regard to improve this crop using genetic methods.

Table 28.5 Profiling of nutritional status of Little millet genotype selected for the study

Genotype	Collection	Nutritional profile full grain				
		Fe	Zn	Protein	Tryptophan	Lysin
RLM 37	Jagdalpur Chhattisgarh (India)	32.00 µg/g	32.40 µg/g	10.30%	0.09 g/16 g N	1.92 g/16 g N

Source: Chandel et al. (2017)

4. Millets are typically grown in dry, poor soils, however, it's important to identify genotypes and cultivars that are tolerant of high-input environments.
5. Little millet is renowned for its ability to withstand droughts and is regarded as one of the crops with the lowest water requirements. Only a few research have shown that *Panicum miliaceum*, *Setaria italica*, and *Setaria glauca* suffer from less loss in grain production when subjected to water stress. However, the findings have not been supported by any formal studies. The focus should be placed on cultivating varieties that can withstand drought and have superior regenerative ability when dry spells are reversed in difficult climates.
6. Because Little millet is grown on drylands with low fertility, conditions that differ from those found at the study station are encountered. This would cause farmers to reject new types, resulting in minimal or no cultivation in farmers' fields. As a result, farmers continue to grow traditional local cultivars with reduced genetic potential. As a result, grain yield productivity is low. As a result, when undergoing selection, a farmer-participatory technique should be used to derive improved varieties.
7. Little millet consumption is quite low when compared to other millets. However, there are several better nutritional features, such as high fiber and mineral content, which have numerous health benefits. Improved cultivars with excellent nutritional content would go a long way toward improving agricultural demand.
8. Following crop harvest, the stover is a source of animal feed. The study of genetic improvement has gotten less attention. The heterogeneity in the stover's quality might be used to create superior genotypes or varieties with a high nutritional value.
9. Little millet has few pests and diseases. However, past research suggested that shoot fly and grain smut were responsible for economic losses. To develop disease/insect resistance, host plant resistance should be tested.
10. The other significant agronomic features that should be given appropriate consideration when enhancing grain output are stem lodging and grain shattering.
11. Priority should be given to breeding for specified end applications because doing so encourages private-sector businesses to invest in millets.
12. Little millet can reach maturity in between 60 and 80 days. However, photo-insensitive genotypes suitable for various cropping schemes as well as early-maturing genotypes with significantly high yield should be produced.

28.3 Conclusion

The characteristics of Little millets that make them climate resilient include their greater flexibility to a variety of ecological settings and less water requirement, as well as their reduced vulnerability to environmental stresses and lower incidence of insect pests and illnesses. Small millets are significant crops in semi-arid, rainfed regions. Due to the popularity of cash crops over traditional crops, they are currently only grown in a small area, accounting for a small portion of the world's millet

Fig. 28.2 Inflorescence of Little millet

production. Even in marginal areas and under moisture stress, Little millet can yield a noticeably higher amount of grain. With high water-use efficiency, salt and waterlogging resistance, and a very low incidence of insect-pests and illnesses, it has the capacity for diversified adaptation. These crops are referred to as "orphan cereals" since very little scientific research has been conducted on them. Apart from these difficulties, a benefit of small millets is that they are still cultivated in isolated and tribal areas of the world, preserving biodiversity and giving breeders special alleles for crop improvement.

Conventional breeding techniques have proven effective in creating and releasing a number of cultivars, including those with biotic and abiotic stress resistance and improved nutrition. However, the majority of genomic technologies must be used in tiny millet, and cultivar development should be prioritized. The incorporation of genomic-assisted improvement through the use of diverse omics techniques has the potential to promote genetic improvements in tiny millet crops. While other millets have achieved gene mapping, transcriptomics, proteomics, and genetic transformation by CRISPR/Cas9, a comprehensive strategy is still anticipated in this crop for genetic improvement. Local farmers prize Little millets for their health and nutritional benefits, ability to withstand great stress, including drought, and ability to grow in unfavorable settings, making them the most suitable crop in an era of climate change and continuously dwindling natural resources (Fig. 28.2).

References

Austin MJ, Muskett P, Kahn K, Jones JDG, Parker JE (2002) Regulatory role of SGT1 in early *R* gene mediated plant defenses. Science 295:2077–2080

Bandyopadhyay T, Muthamilarasan M, Prasad M (2017) Millets for next-generation climate-smart agriculture. Front Plant Sci 8:1266

Bor NL (1960) The grasses of Burma, Ceylon, India and Pakistan (excluding Bambusease), vol I. Pergamon Press, London, UK. 767 pp

Castro AJ, Capettini F, Corey AE, Filichkina T, Hayes PM, Kleinhofs A, Kudrna D, Richardson K, Sandoval-Islas S, Rossi C, Vivar H (2003) Mapping and pyramiding of qualitative and quantitative resistance to stripe rust in barley. Theor Appl Genet 107:922–930

Chandel G, Dubey M, Gupta S, Patil AH, Rao AR (2017) Identification and characterization of a grain micronutrient related OsFRO2 rice gene ortholog from micronutrient-rich little millet (*Panicum sumatrense*). 3 Biotech 7:80

Dida MM, Srinivasachary, Ramakrishnan S, Bennetzen JL, Gale MD, Devos KM (2007) The genetic map of finger millet, *Eleusine coracana*. Theor Appl Genet 114:321–332

Doggett H (1989) Small millet—a selective overview. In: Seetharam A, Riley KW, Harinarayana G (eds) Small millets in global agriculture, 1st edn. Oxford and IBH Publishing company, Delhi, India, pp 59–70

Ganapathy S, Nirmalakumari A, Senthil N, Souframanien J, Raveendran TS (2008) Isolation of macromutations and mutagenic effectiveness and efficiency in Little millet varieties. World J Agric Sci 4:483–486

Goron TL, Raizada MN (2015) Genetic diversity and genomic resources available for the small millet crops to accelerate a New Green Revolution. Front Plant Sci 6:157

Goulet BE, Roda F, Hopkins R (2017) Hybridization in plants: old ideas, new techniques. Plant Physiol 173:65–78

Gowda J, Halswamy BH, Somu G, Krishnappa M, Vasanth KR, Sennappa K, Seetharam A (2003) Evaluation of proso millet (*Panicum miliaceum* L.) germplasm. Project coordination cell, All India Coordinated Small Millets Improvement Project, University of Agriculture Sciences, Bengaluru. 39 pp

Gupta S, Kumari K, Muthamilarasan M, Subramanian A, Prasad M (2013) Development and utilization of novel SSRs in foxtail millet [*Setaria italica* (L.) P. Beauv.]. Plant Breed 132: 367–374

Hadimani NA, Malleshi NG (1993) Studies on milling, physico-chemical properties, nutrient composition and dietary fibre content of millets. J Food Sci Technol (India) 30(1):17–20

Haider ZA (1997) Little millet in Indian agriculture: progress and perspectives. In: National seminar on small millets, 23–24 April 1997, Coimbatore, India, pp 5–6. (Extended summaries)

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

Harrison RG (1990) Hybrid zones: windows on evolutionary process. Oxford survey on evolutionary biology. 1990

Itagi S (2003) Development and evaluation of millet-based composite food for diabetes. M.H.Sc. Thesis, Univ. Agric. Sci, Dharwad, Karnataka

Jain AK, Tripathi SK (2007) Management of grain smut (*Macalpino mycessharmae*) in little millet. Indian Phytopathol 60:467–471

Jayaraman N, Suresh S, Nirmala A, Ganeshan NM (1997) Genetic enhancement and breeding strategies in small millets. In: National seminar on small millets, 23–24 April

Johnson M, Deshpande S, Vetriventhan M, Upadhyaya HD, Wallace JG (2019) Genome-wide population structure analyses of three minor millets: kodo millet, little millet, and proso millet. Plant Genome 12:190021

Joshi DC, Chaudhari GV, Sood S, Kant L, Pattanayak A, Zhang K (2019) Revisiting the versatile buckwheat: reinvigorating genetic gains through integrated breeding and genomics approach. Planta 250:783–801

Keller W (1952) Emasculation and pollination techniques. Int Grassland Congr Proc 6:1613–1619

Krishna Kumari S, Thayumanavan B (1997) Characterization of starches of proso, foxtail, barnyard, kodo, and little millets. Plant Foods Hum Nutr 53(1):47–56

Li P, Brutnell TP (2011) *Setaria viridis* and *Setaria italica*, model genetic systems for the Panicoid grasses. J Exp Bot 62(9):3031–3037
Michelmore RW (2003) The impact zone: genomics and breeding for durable disease resistance. Curr Opin Plant Biol 6:397–404
Mishra M (2017) Studies on in vitro regeneration of *Panicum sumatrense* using mature seed and leaf base explant. Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur
Mundt CC (2002) Use of multiline cultivars and cultivar mixtures for disease management. Annu Rev Phytopathol 40:381–410
Murthy TK, Harinarayana G (1986) Insect-pests of small millets and their management in India, in Small millets in global agriculture. Seetharam A, Riley KW, Harinarayana G (eds) Proceedings of the First International small millets workshop, October-29–November-2, 1986, Bangalore, Oxford and IBH Publishing, New Delhi, 1989; pp. 255–270
Muthamilarasan M, Prasad M (2015) Advances in *Setaria* genomics for genetic improvement of cereals and bioenergy grasses. Theor Appl Genet 128(1):1–14
Nambi VA, Eganathan P, Maria Philip PI (2012) Proximate and mineral composition analysis of little millet collected from some millet growing areas in Tamil Nadu. J Plant Genet Resour 25 (2):189–191
Nanda JS, Agarwal PK (2008) Botany of field crops, vol I. Kalyani Publisher, India. 381 pp
Nandini C, Bhat S, Srinathareddy, Jayramegowda, Prabhakar (2019) Modified crossing (SMUASB) method for artificial hybridization in proso millet (*Panicum miliaceum* L.) and Little millet (*Panicum sumatrense*). Electron J Plant Breed 10(3):1161–1170
Nirmalakumari A, Arulselvi S, Ganapathy S, Souframanian J, Senthil N, Devan P (2007) Gamma ray induced variation for lodging resistance and its associated characters in little millet (*Panicum sumatrense* Roth Ex-roem and schult). Madras Agric J 94(7–12):151–155
Nirmalakumari A, Salini K, Veerabadhiran P (2010) Morphological characterization and evaluation of little millet (*Panicum sumatrense* Roth. ex. Roem. and Schultz.) germplasm. Electron J Plant Breed 1(2):148–155
Panwar P, Dubey A, Verma AK (2016) Evaluation of nutraceutical and antinutritional properties in barnyard and finger millet varieties grown in Himalayan region. J Food Sci Technol 53:2779–2787
Parlevliet JE (1979) Components of resistance that reduce the rate of epidemic development. Annu Rev Phytopathol 17:203–222
Pink DAC (2002) Strategies using genes for non-durable resistance. Euphytica 1:227–236
Ramulu P, Rao PU (1997) Effect of processing on dietary fiber content of cereals and pulses. Plant Foods Hum Nutr 50:249–257
Ravindran G (1991) Studies on millets; proximate composition, mineral composition, phytate and oxalate content. Food Chem 39:99
Richardson KL, Vales MI, Kling JG, Mundt CC, Hayes PM (2006) Pyramiding and dissecting disease resistance QTL to barley stripe rust. Theor Appl Genet 113:485–495
Santra DK, Khound R, Das S (2019) Proso millet (*Panicum miliaceum* L.) breeding: progress, challenges and opportunities. In: Al-Khayri J, Jain SM, Johnson DV (eds) Advances in plant breeding strategies: cereals. Springer, Cham, pp 223–257
Saxena R, Vanga SK, Wang J, Orsat V, Raghavan V (2018) Millets for food security in the context of climate change: a review. Sustainability 10:2228
Seetharam A, Riley KW, Harinarayana G (1986) Small millets in global agriculture, Chapter 11. In: Proceedings of the first international conference on small millets
Seetharam A, Gowda J, Halaswamy JH (2003) Small millets. In: Chowdhury SK, Lal SK (eds) Nucleus and breeder seed production manual. Indian Agriculture Research Institute, New Delhi, India, pp 54–67
Song WY, Wang GL, Zhu LH, Fauquet C, Ronald P (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. Science 270:1804–1806
Srivastava DP, Yadav A (1972) Emasculation of flowers of *Panicum miliare* L. by hot water treatment. Sci Cuk 38:450

Sundararaj DD, Thulasidas G (1976) Botany of field crops. MacMilan Publishing, New Delhi
Upadhyaya HD, Ramesh S, Sharma S, Singh SK, Varshney SK (2011a) Genetic diversity for grain nutrients contents in a core collection of finger millet (*Eleusine coracana* (L.) Gaertn.) germplasm. Field Crop Res 121:42–52
Upadhyaya HD, Ravishankar CR, Narasimhudu Y, Sarma NDRK, Singh SK, Varshney SK (2011b) Identification of trait specific germplasm and developing a mini core collection for efficient use of foxtail millet genetic resources in crop improvement. Field Crop Res 124:459–467
Usha R, Madhuprasad VL, Suresha SV, Jagadeeshwara K (2011) Little millet (*Panicum miliare* L.)—a source of functional food for sustainable nutrition security. In: 7th Asia Pacific conference on clinical nutrition, 5–8 June 2011, Bangkok, Thailand, p 449
Vetriventhan M, Upadhyaya HD (2018) Diversity and trait-specific sources for productivity and nutritional traits in the global proso millet (*Panicum miliaceum* L.) germplasm collection. Crop J 6:451–463
Vetriventhan M, Upadhyaya HD (2019) Variability for productivity and nutritional traits in germplasm of kodo millet, an underutilized nutrient-rich climate smart crop. Crop Sci 59: 1095–1106
Vinoth A, Ravindhran R (2017) Biofortification in millets: a sustainable approach for nutritional security. Front Plant Sci 8:29
Wang G, Ding X, Yan M, Qiu D, Li X, Xu C, Wang S (2006) Dual function of rice OsDR8 gene in disease resistance and thiamine accumulation. Plant Mol Biol 60:437–449
Zhu Y, Chen H, Fan JH, Wang Y, Li Y, Chen J, Fan JX, Yang S, Hu L, Leung H, Mew TW, Teng AS, Wang Z, Mundt CC (2000) Genetic diversity and disease control in rice. Nature 406:718–722

An Upliftment Strategy for Little Millet Improvement by Unravelling the Hidden Molecular Network Behind Its Miracle Properties

29

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Abstract

Nature has provided us with plenty of plant species which serve as the reservoir of highly essential nutrients. The dietary benefits of small millets have been forcefully forgotten by the dominance of major cereals. Nowadays, such orphan cereals are again blooming since people have begun realizing the importance of nutraceutical and its beneficial health properties. Each of the small millets has its own unique nutritional benefits and climate-resilient properties. Little millet is one among them. Little millet, a nutricereal, is known for its unique nutritional traits because of the presence of GABA, carotenoids, tocopherols and phenolic compounds. Besides this, little millet is fairly rich in Fe and Ca. It also has antidiabetic and antioxidant properties. Phenotypically, little millet has good drought tolerance ability with wider adaptability. Little is known about the crop and subjecting the crop to crop improvement programme needs more understanding of the crop. Hence, the genetic and molecular basis of its adaptation traits and nature of climate resilience need to be explored further to utilise the maximum potential of this crop. It is also imperative to utilise the advanced biotechnological tools available to make crop improvement programmes smart and efficient.

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Keywords

Little millet · Molecular networks · Genomics · Gene editing

29.1 Introduction

Global crop improvement programme revolves around plant breeding as a core tool to improve a crop in terms of yield, biotic and abiotic stress tolerance and enhanced nutritional traits. However, conventional approaches to crop improvement is a time-consuming process. Also, the environment plays a major role in depicting a genotype. In such a case, increasing the understanding of molecular biology of the crop and using biotechnological tools will play a major role. Enhancing the crop improvement through molecular breeding, genomic approaches and through gene editing will decipher useful results. Present day sedentary lifestyle of people and over-dependence on major cereals alone have proliferated many health disorders in humans viz., diabetes, coronary diseases, obesity and gastro-intestinal diseases (Paschapur et al. 2021). Besides this, environmental degradation and recent global climate change has paved the way for greater use of arid and semi-arid tropic crops for food and fodder. Small millets being climate resilient crop was grown in India's arid regions and suitable for rainfed conditions in Asia and Africa's semi-arid tropical regions (Rai et al. 2008). Small millets are C_4 crop having excellent photosynthetic efficiency and active oxygen species activity which serves as protective machinery against various abiotic and biotic stresses. They are a nutritious food crop that is high in proteins, fibre and minerals. As a result, they are an essential component of dietary food (Amadou et al. 2013). Indian little millet (*Panicum sumatrense* Roth.) is one of the millets cultivated across India, Nepal and western Myanmar. It has its importance mainly among the tribes of Eastern Ghats of India. Among small millets, little millet is the crop which is least explored at a molecular level in spite of its beneficial properties. Hence, this chapter reviews on exploration of little millet for its beneficial properties and molecular players behind its beneficial properties along with future thrust.

29.2 Exploration of Little Millet for Its Beneficial Properties

Small millets are a class of small-seeded crops in the Poaceae family. It includes finger millet (*Eleusine coracana* (L.) Gaertn.), barnyard millet (*Echinochloa* spp.), kodo millet (*Paspalum scrobiculatum* L.), foxtail millet (*Setaria italica* (L.) P. Beauv.), little millet (*Panicum sumatrense* Roth. ex. Roem. & Schult.), proso millet (*Panicum miliaceum L.*), teff ((*Eragrostis tef* (Zucc.) Trotter), job's tears (*Coix lacryma-jobi* L.), fonio (*Digitaria exilis* Stapf and *D. iburua* Stapf.), guinea millet (*Brachiaria deflexa* (Schumach.)) and brown top millet (*Brachiaria ramosa* (L.).

In India, little millet mainly grown in parts of Karnataka and Tamil Nadu. Little millet is an early maturing crop with abiotic stress tolerance and it can grow well under poor agronomic conditions. It can withstand drought, waterlogging and salinity (Ganapathy 2017). Little millet is an excellent source of carbohydrates (67%). It has proteins (7.75%), amino acid lysine (0.11 g/gN), minerals (1.5%), low fat (4.7%), crude fibre (7.7 g), high phosphorous (220 mg/100 g), high iron (9.3 mg/100 g) and zinc (3.7 mg/100 g) (Divya et al. 2021; Dhawale et al. 2022). Little millet due to its good source of dietary fibre exhibits hypo-glycemic index. It is also called as cool food due to its cooling nature when consumed in summer time. Neutraceuticals including resistant starch, lignans, sterols, phenolics, phytates and gamma-amino-butyric acid are also prominent in little millet (Guha et al. 2015). Phytate retention aids in anti-diabetic and anti-cancerous properties and also acts as an antioxidants. Significant fibre content resolves stomach issues, constipation and also regulates movement of food and waste through the digestive system (Kushwaha et al. 2019).

29.3 Genomics of Climate Resilient Properties in Little Millet

Understanding the genetic architecture of any crop requires knowledge of genomics and transcriptomics. But they are not enough to completely elucidate the molecular mechanism, cellular signalling and pathway behind stress response and nutritional response. Hence, the so-called term "multi-omics" came into existence to unwind the complexity behind complex traits. Integrative omics with genomics, phenomics, transcriptomics, proteomics, metabalomics and bioinformatics provide greater insight into understanding the trait of interest. To date, limitted approaches in this direction were observed in little millet. Only few reports are available in little millet in terms of transcriptomics, proteomics and metabolomics. For orphan crops like small millets, where research fundings are limited, exploring the genetic and molecular mechanism needs in depth studies. Application of omics and next generation sequencing studies can generate significant information about the global pattern of gene expression under different environmental conditions. Little millet is a crop with wider adaptation and has the potential to survive under varied abiotic stresses. Maximum utilization of this crop can be acheived throgh understanding its genetics, molecular and climate resilient properties.

The arid tropics of Asia and Africa are dominated by the abiotic stresses like drought and salinity, which were the key players affecting the yield in this climate change era. Drought tolerance is attributed by osmotic adjustment which maintains the turgor pressure, by accumulation of osmolytes and through production of antioxidants, ROS scavenging enzymes *viz.*, catalases, peroxidases, super oxide dismutase, ascorbate peroxidases. Increased root length is one of the parameters which attributes tolerance by capturing water from substrate during stress condition. Root length increased in tolerant genotype by plant memory which acts upon stress. Stress-dependent plant memory was activated and it redirected more carbohydrates to the plants which increases the root length. Apical meristem is responsible for shoot growth and is severely affected by drought through impairment of mitosis.

Cell elongation and cell division was affected and it impairs growth and yield-related traits which finally reflects on yield. Hence, there is an alteration in the source sink ration by reduced shoot length and increased root length under drought in comparison to control in little millet (Ajithkumar and Panneerselvam 2014). Osmoprotectants like proline and glycine betaine were increased under drought stress in little millet. Proline regulates drought tolerance by maintaining turgour pressure in cell, whereas glycine betaine regulates through stabilizing function of thylakoid membranes to suppress chlorophyll degradation. Superoxide dismutase which catalyses super oxide dismutation limits oxidative damage in little millet under drought stress was highly expressed. SOD automatically induces more production of peroxidases and catalases which prevents oxidative damage by breaking peroxide into water and oxygen. Hence these are some of the drought stress response determinants behind the drought tolerance in little millet. Metabolomic profiling is being used to find novel and stress-related low molecular weight compounds such as polyamines, peptides, sugars, phenolics and alkaloids. Studies on the metabolome profiling of small millets are limitted. Since all small millets are C4 in nature, cross application of research findings will give a leadto procedd with the research in any of the small millet complex. Three finger millet varieties were studied to reveal the metabolome profile regulated under stress. The study by Kim et al. (2013) identified 43 primary metabolites (19 amino acids, 17 organic acids, 8 sugars, 3 sugar alcohols and one amine) and 5 phenolic acids (*p*-coumaric, ferulic, *p*-hydroxybenzoic, salicylic and vanillic acids). This metabolomics approach has been used in little millet to dissect the metabolites involved in drought tolerance. Drought tolerant genotype Tarini was involved in the study by Dhawale *et al.* (2022). Drought stress significantly affected 25 metabolomic pathways, including galactose metabolism, fatty acid metabolism, starch and sucrose metabolism, beta oxidation of very long chain fatty acids, citric acid cycle, fructose and mannose metabolism, vitamin K metabolism, pentose phosphate pathway alpha-linoleic acid and linoleic acid metabolism, butyrate metabolism, homocysteine degradation, lactose degradation, glycerolipid metabolism, Warburg effect, malate-aspartate shuttle, glycerol phosphate shuttle, ketone body metabolism, glucose alanine cycle, gluconeogenesis, mitochondrial electron transport chain, Valine-leucine and isoleucine degradation, catecholamine biosynthesis, vitamin B6 metabolism, riboflavin metabolism, and lactose synthesis were significantly affected by water-deficit conditions. According to reports from various crops, millets are a good source of phenolics and antioxidants (Xiang et al. 2019; Guo et al. 2018; Ofosu et al. 2020). Salinity tolerance in little millet is attributed to increased SOD, CAT, POD and APX activity (Bhaskaran and Panneerselvam 2013). The first identified differentially expressed gene in foxtail millet during abiotic stress was salinity-induced phospholipid hydroperoxide glutathione peroxidase (PHGPX) (Sreenivasulu et al. 2004). Proteomic analysis of salt-stressed foxtail millet revealed differentially expressed proteins. Proteins expressed were found to be linked to proteins involved in the cellular pathways of lipid, nitrogen, carbohydrate, carotenoid, flavonoid and cell wall biosynthesis (Veeranagamallaiah et al. 2008). Pan et al. (2018) looked for proteins associated with drought stress in a drought-affected foxtail millet variety (Yugu 1). The majority of the 2500 differentially expressed proteins were found in chloroplasts.

Thus, photosynthesis-related proteins were overexpressed in water-stressed conditions. Among 2500 differentially expressed proteins, most of the proteins were localized to chloroplast. Thus proteins related to photosynthesis were highly expressed under water deficit conditions. Hence, multiomics approaches and properties reported in other millets can be applied and compared with little millet respectively to dissect its properties.

29.4 Population Genomics in Little Millet

Genomic information for little millet is scarce, and it must be expanded in order to carry out multifaceted crop improvement programs. Population genomics is one of the most effective approaches for helping scientists understand population structure, evolution history and domestication history through the use of SNPs or SSRs. It also aids in the identification of candidate genes for desirable traits, which can then be validated and used in marker-assisted breeding. Molecular markers reveal genetic diversity within populations and are widely used in genomics-assisted breeding. The evolution of next-generation sequencing has paved the way for the discovery of SNPs and SSRs in the available population. Using these advanced techniques, robust markers associated with economically significant traits can be designed, allowing marker assisted selection (MAS) to be used to accelerate crop improvement programs. SNPs are notable for their abundance throughout the genome and for being mostly biallelic in nature (Singh and Prasad 2017). RAPD markers were used to assess the molecular diversity of little millet landraces (Arunachalam et al. 2005). At the time, markers were insufficient to distinguish landraces. Later during in 2017, Ali *et al.* used switch grass EST-SSR markers to examine the genetic relationship between little millet accessions. A total of 779 primer pairs were designed from switch grass sequences, 48 of which were polymorphic with 160 alleles and an average of 3.3 alleles per locus. During dehydration, an expressed sequence tag (EST) library of foxtail millet identified 95 and 57 ESTs induced in roots and shoots, respectively. Distinct ESTs were expressed in both organs, and the majority of them were related to the protein degradation pathway (Zhang et al. 2007). To advance research and to study the crop, genome-wide marker dataset was generated using 190 little millet accession using GBS. After filtering 161–165 accessions were retained with 2245 SNPs. Seven putative sub populations were revealed through population genetic analysis. Little millet population structure was not correlated with existing core collection based on race as a centre of focus. Plotting 29 accessions based on geographic location distinctly differentiated population 4 and 5. Significant SNPs identified were validated with public phenotypic data. It revealed that flowering time and plant height were the most correlated traits with heritability of 0.209–0.807 (Johnson et al. 2019). These traits are reported to be under strong genetic control in other grass crops (Morris et al. 2012; Ma et al. 2016). SNPs generated represented the variability present in the existing little millet collection. These SNPs can be further used to study the genetics of various traits in little millet and can be used in selection of good breeding genotype. Desai et al. (2021) sequenced the transcriptome of three little millet samples. A total of 4443 genic-

SSR motifs were identified in 30,220 unigene sequences. Simple sequence repeats were found at the rate of 12.25% with one SSR locus per 10 kp as an average. Among different repeats, tri-nucleotide repeats were the most abundant one. Unigenes annotated were closely associated with cellular metabolism, cell communication and stress responses. Hence it could be linked with little millet's basic metabolism, growth and development. Especially, thiamine and purine metabolism pathway was highly enriched one through KEGG pathway analysis in little millet. Twelve genes were identified in thiamine metabolism and was highly expressed in vegetative stage and reproductive stage when compared to maturity stage. It implied that genes of thiamine biosynthesis was highly expressed in photosynthesizing tissues (Colinas and Fitzpatrick 2015). Thiamine biosynthesis was not still fully explored for its responsible genes. Thiamine being the important part of nutrients in human health, is vital to study the same to reap the benefits from little millet as well other crop. Fifty primers were randomly selected from identified SSR motifs and validated in 20 minor millet and 5 little millet genotypes. It was observed to have 48% polymorphism level with high tranferrability (70%). Hence, identified SSRs were can further used in MAS, QTL identification and in evolutionary genetics. In foxtail millet, 916 accessions were re-sequenced, resulting in the identification of 2.58 million SNPs. 0.8 million common SNPs were also used to create a haplotype map (Jia et al. 2013). Through genome-wide association studies, 512 different loci linked to 47 agronomic traits were identified, and marker-trait associations were established for several agronomic traits, including abiotic stress (Upadhyaya et al. 2015). Similarly, thousands of genome-wide SNPs in pearl millet and finger millet were identified using a genotyping-by-sequencing (GBS) approach to discover population diversity (Hu et al. 2015; Kumar et al. 2016; Gimode et al. 2016). Pearl millet F7 recombinant inbred lines (RILs) developed through hybridization between 863B X ICMB 841 were used to map QTLs associated with drought tolerance (Aparna et al. 2015). Four major QTLs controlling water use efficiency were identified, one of these was mapped on linkage group (LG) 6 and contributes to growth potential, transpiration rate and drought tolerance. Later, six water use-related QTLs were identified from pearl millet and it revealed 10-37 % phenotypic variation for water stress adaptation strategies (Tharanya et al. 2018).

29.5 Nutrient Genomics in Little Millet

Enhanced nutrient and antioxidant properties in small millets made it a potential source for mining genes governing nutritional traits like Protein, Fe and Zn and antioxidant compounds. Phenolics serve as dietary anti-oxidants by donating electrons or hydrogen. Its stable radical intermediates prevent oxidation, especially fatty acids and oils. Polyphenols are the potent phytochemicals used in food industries and in pharmaceutics. Besides this, diet fortification with foods rich in polyphenols impart anti-glycaemic, anti-diabetic and anti-mutagenic properties. It can be taken as a criterion in healthy food formulation. In this way, Pradeep and Guha (2011) found that steamed little millet phenolics contains ferulic acid (18.29 mg/100 g) and gallic acid (1.9 mg/100 g). DPPH reduction capacity and Fe

reducing power assay being the well-studied mechanism to detect anti-oxidant activity was directly correlated with total phenolics content in processed little millet. Thus, processed little millet can be utilized in pharmaceuticals to make use of the anti-oxidant properties in healthy diet formulation. TNPsu 25, TNPsu 23, TNPsu 22 and TNPsu 141 were the genotypes rich in iron, zinc and calcium identified by Manimozhi Selvi et al. (2015). More accumulation of these nutrients was attributed by translocation efficiency of genotype from source to sink. Little millet is a good source of macronutrients as well as dietary fibre (Patil et al. 2015). Little millet processing to RTE flakes, besides convenience, also reap the benefits of medium glyceamic index and trans-fat-free food with good shelf-life capacity. However, through long-term feeding intervention, it can be used in management of metabolic disorders. Chandel et al. (2014), identified a nutrient rich little millet also called "kutaki" through preliminary screening of micronutrient among different genotypes of little millet. Kutaki being rich in Fe, Zn and protein is consumed by tribals of Chattisgarh. Cereals are the major food source but it has very less availability of micronutrients. Plants typically have sophisticated mechanisms for maintaining metal ion homeostasis, which involves the uptake and distribution of metal ions to various tissues. Plant phenotype is, in general, a combination of genotype and environment (Kehr 2013). The environment is also a critical factor influencing the plan's potential for its yield and nutrients. Nutritional traits are more prone to environmental variation, especially through field heterogeneity. In such cases, plants need to cope with the environment by continuously sensing the change and it maintains the plant growth and metabolism through mineral ion-homoestasis. To trigger plant adaptive response, nutrient status information must flow from cell to cell and to other organs via a long-distance transport tube. Little millet one of the nutrient-rich crop screened for its nutrient traits and kutaki was identified as genotype rich in Fe, Zn and protein. Using next-generation technologies many homoestasis-related genes were explored in rice (Gupta et al. 2017). In rice, 39 metal homoeostasis-related genes were identified where four were well-characterized homologs. In soil iron will be in soluble form due to interaction with oxygen it will be available as ferric oxide as an insoluble form. The enzyme ferric chelate reductase in plants is responsible for iron uptake from soil. *AtFRO1*, *AtFRO2* were the genes responsible for iron uptake and was characterized in *Arabidopsis* under iron deficient condition. Narayanan et al. (2007) found the same gene involved with Fe and Zn uptake especially at grain-filling stage in rice. Since little millet lacks genomic information when compared to other millets, molecular network behind nutrient-rich little millet was identified through *FRO2* a rice ortholog using PCR and NGS techniques. Computational genomics revealed good correspondence in the context of sequence, structural and functional similarity. Hence, it is a ortholog. Further cloning and characterization of ion homoeostasis gene under nutrient deficient vs nutrient enriched conditions and to validate the same is in progress (Chandel et al. 2017). Little millet kernels are one among the protein-rich kernel category. Among various plant kernel proteins zeins, coixins and kafirins accumulate in the endosperm of the seed and it belongs to the prolamin family. It is composed of four types of polypeptides viz., α_, β_, γ_ and δ_zeins (Coleman et al. 1997). Hence, zein

was cloned and characterized in little millet by isolating samples at grain filling stage. *In silico* analysis found zeins from little millet as similar to alpha prolamins. This protein family consists of zeins from maize, coixins from Job's tears, alpha kafirins from sorghum. Threading analysis of little millet zein revealed that it forms anti-parallel alpha-helical hairpin with hydrophobic and hydrophilic surfaces opposite to each other which would involve in formation of protein storage assembly (Sivakumar et al. 2006).

29.6 Applicability for Major Cereals Through Gene Editing Approaches

Plant breeding was one of the best approaches for crop improvement to transfer desirable traits from donor to recipient through hybridization programmes. The use of modern biotechnological tools to transfer advantageous genes from different species, particularly from rice, wheat, maize and other cereal crops, was made possible by the elimination of the cross-genus barrier, minimal host genome rearrangement, targeted gene transfer and short duration (Singh et al. 2021). Among the various gene transfer techniques, one of the reliable approaches widely used in cereals and millets is *Agrobacterium tumefaciens*-mediated transformation (Sood et al. 2019). Under salinity and drought stress, the EcNAC67 transcription factor was overexpressed in finger millet. Rice has shown similar results (Rahman et al. 2016). The transgenic rice displayed profound root and shoot biomass with a higher revival percentage after the removal of abiotic stress. Most of the abiotic stress tolerance genes were present in millets, especially among nutricereals.

29.7 The Way Forward

Pangenomes is a rich resource to observe the genomic variation present within a species or genera where it includes more than one reference genome sequence. Such pangenomics need to be extended to sequenced crop, among small millets like foxtail millet and proso millet. In spite of the beneficial properties and adaptive traits present in little millet, lack of genomic information hinders its improvement. However genomics resources are available for chloroplast genome and transcriptome assembly of abiotic stress tolerance genes. Efforts need to be taken to sequence the crop for gene mining. In most explored crops like maize and rice pangenome data is available and it can be applied in little millet through synteny mapping approaches. Also chickpea, pigeonpea and qunioa once underutilized were possessed to have genome sequenced and it resolved the genetic basis behind agronomic and adaptive traits in these crops. Hence, little millet can be explored for its natural variation and its indigenous knowledge can be further explored at the genomic level. A common database specific to little millet can be created and problems prevailing in the crop, indigenous knowledge and research strategies can be updated in that database. So that repetition and wastage of resources can be

avoided for efficient utilization of resources to improve the crop. Sequencing of little millet will be a solution to advance the crop to the next level of research.

References

Ajithkumar IP, Panneerselvam R (2014) ROS scavenging system, osmotic maintenance, pigment and growth status of Panicum sumatrense roth under drought stress. Cell Biochem Biophys 68(3):587–595

Ali A, Choi YM, Hyun DY, Lee S, Kim JH, Oh S, Lee MC (2017) Development of EST-SSRs and assessment of genetic diversity in little millet (Panicum sumatrense) germplasm. Korean J Plant Resour 30(3):287–297

Amadou I, Gounga ME, Le GW (2013) Millets: nutritional composition, some health benefits and processing—a review. Emirates J Food Agric 25:501–508

Aparna K, Nepolean T, Srivastsava RK et al (2015) Quantitative trait loci associated with constitutive traits control water use in pearl millet [*Pennisetum glaucum* (L.) R. Br]. Plant Biol (Stuttg) 17:1073–1084

Arunachalam V, Rengalakshmi R, Raj K (2005) Ecological stability of genetic diversity among landraces of little millet (Panicum sumatrense) in south India. Genet Resour Crop Evol 52(1): 15–19

Bhaskaran J, Panneerselvam R (2013) Accelerated reactive oxygen scavenging system and membrane integrity of two Panicum species varying in salt tolerance. Cell Biochem Biophys 67(3): 885–892

Chandel G, Dubey M, Gupta S, Patil AH, Rao AR (2017) Identification and characterization of a grain micronutrient-related OsFRO2 rice gene ortholog from micronutrient-rich little millet (Panicum sumatrense). 3 Biotech 7(1):1–9

Chandel G, Meen AR, Dubey M, Kumari M (2014) Nutritionalproperties of minor millets: neglected cereals with potentials tocombat malnutrition. Curr Sci 107(7):1109–1111

Coleman CE, Clore AM, Ranch JP, Higgins R, Lopes MA, Larkins BA (1997) Expression of a mutant α-zein creates the floury 2 phenotype in transgenic maize. Proc Natl Acad Sci 94(13): 7094–7097

Colinas M, Fitzpatrick TB (2015) Natures balancing act: examining biosynthesis de novo, recycling and processing damaged vitamin B metabolites. Curr Opin Plant Biol 25:98–106

Desai H, Hamid R, Ghorbanzadeh Z, Bhut N, Padhiyar SM, Kheni J, Tomar RS (2021) Genic microsatellite marker characterization and development in little millet (Panicum sumatrense) using transcriptome sequencing. Sci Rep 11(1):1–14

Dhawale RN et al (2022) Metabolomic profiling of drought-tolerant little millet (Panicum sumatrense L.) genotype in response to drought stress. Pharm Innov 11:1754

Divya S, Geetha K, Parasuraman P (2021) Little millet-a dryland drought tolerant millet crop

Ganapathy KN (2017) Genetic improvement in little millet. In: Millets and sorghum: biology and genetic improvement. Wiley Blackwell, pp 170–183

Gimode D, Odeny DA, de Villiers EP et al (2016) Identification of SNP and SSR markers in finger millet using next generation sequencing technologies. PLoS One 11:e0159437

Guha M, Sreerama YN, Malleshi NG (2015) Influence of processing on nutraceuticals of little millet (Panicum sumatrense). In: Processing and impact on active components in food. Academic Press, pp 353–360

Guo X, Sha X, Rahman E, Wang Y, Ji B, Wu W, Zhou F (2018) Antioxidant capacity and amino acid profile of millet bran wine and the synergistic interaction between major polyphenols. J Food Sci Technol 55(3):1010–1020

Gupta S, Kumari M, Kumar H, Varadwaj PK (2017) Genome-wide analysis of miRNAs and TasiRNAs in Zea mays in response to phosphate deficiency. Funct Integr Genomics 17(2):335–351

Hu Z, Mbacke B, Perumal R et al (2015) Population genomics of pearl millet (*Pennisetum glaucum* (L.) R. Br.): comparative analysis of global accessions and Senegalese landraces. BMC Genomics 16:1048

Jia G, Huang X, Zhi H, Zhao Y, Zhao Q, Li W, Chai Y, Yang L, Liu K, Lu H, Zhu C (2013) A haplotype map of genomic variations and genome-wide association studies of agronomic traits in foxtail millet (Setaria italica). Nat Genet 45(8):957–961

Johnson M, Deshpande S, Vetriventhan M, Upadhyaya HD, Wallace JG (2019) Genome-wide population structure analyses of three minor millets: kodo millet, little millet, and proso millet. Plant Genome 12(3):190021

Kehr J (2013) Systemic regulation of mineral homeostasis by micro RNAs. Front Plant Sci 4:145

Kim JK, Park SY, Yeo Y, Cho HS, Kim YB, Bae H, Park CH, Lee JH, Park SU (2013) Metabolic profiling of millet (Paniciim miliaceum) using gas chromatography-time-of-flight mass spectrometry (GC-TOFMS) for quality assessment. Plant Omics 6(1):73–78

Kumar A, Sharma D, Tiwari A, Jaiswal JP, Singh NK, Sood S (2016) Genotyping-by-sequencing analysis for determining population structure of finger millet germplasm of diverse origins. Plant Genome 9:1–15

Kushwaha A, Singh A, Sirohi R, Tarafdar A (2019) Effect of hydrothermal treatment and milling parameters on milling and nutritional qualities of finger millet (Eleusine coracana). J Agric Eng 55(4):34–46

Ma X, Feng F, Wei H, Mei H, Xu K, Chen S et al (2016) Genome-wide association study for plant height and grain yield in rice under contrasting moisture regimes. Front Plant Sci 7. https://doi.org/10.3389/fpls.2016.01801

Manimozhi Selvi V, Nirmalakumari A, Senthil N (2015) Genetic diversity for zinc, calcium and iron content of selected little millet genotypes. J Nutr Food Sci 5(417):2

Morris GP, Ramu P, Deshpande SP, Hash CT, Shah T, Upadhyaya HD et al (2012) Population genomic and genome-wide association studies of agroclimatic traits in sorghum. Proc Natl Acad Sci U S A 110:453–458

Narayanan NN, Vasconcelos MW, Grusak MA (2007) Expressionprofiling of Oryza sativa metal homeostasis genes in differentrice cultivars using a cDNA macroarray. Plant Physiol Biochem 45(5):277–286

Ofosu FK, Elahi F, Daliri EBM, Chelliah R, Ham HJ, Kim JH, Han SI, Hur JH, Oh DH (2020) Phenolic profile, antioxidant, and antidiabetic potential exerted by millet grain varieties. Antioxidants 9(3):254

Pan J, Li Z, Wang Q, Garrell AK, Liu M, Guan Y, Zhou W, Liu W (2018) Comparative proteomic investigation of drought responses in foxtail millet. BMC Plant Biol 18(1):1–19

Paschapur AU, Joshi D, Mishra KK, Kant L, Kumar V, Kumar A (2021) Millets for life: a brief introduction. In: Millets and millet technology. Springer, Singapore, pp 1–32

Patil KB, Chimmad BV, Itagi S (2015) Glycemic index and quality evaluation of little millet (Panicum miliare) flakes with enhanced shelf life. J Food Sci Technol 52(9):6078–6082

Pradeep SR, Guha M (2011) Effect of processing methods on the nutraceutical and antioxidant properties of little millet (Panicum sumatrense) extracts. Food Chem 126(4):1643–1647

Rahman H, Ramanathan V, Nallathambi J, Duraialagaraja S, Muthurajan R (2016) Over-expression of a NAC 67 transcription factor from finger millet (Eleusine coracana L.) confers tolerance against salinity and drought stress in rice. BMC Biotechnol 16(1):7–20

Rai KN, Gowda CLL, Reddy BVS, Sehgal S (2008) Adaptation and potential uses of sorghum and pearl millet in alternative and health foods. Compr Rev Food Sci Food Saf 7(4):320–396

Singh RK, Prasad M (2017) Genome-wide association studies for improving agronomic traits in foxtail millet. In: The foxtail millet genome. Springer, Cham, pp 63–75

Singh RK, Muthamilarasan M, Prasad M (2021) Biotechnological approaches to dissect climate-resilient traits in millets and their application in crop improvement. J Biotechnol 327:64–73

Sivakumar S, Franco OL, Thayumanavan B, Murad AM, Manickam A, Mohan M, Mridula M (2006) Cloning and structural analysis of an Indian little millet (Panicum sumatrense) zein-like storage protein: implications for molecular assembly. Biochemistry (Moscow) 71(11): 1183–1191

Sood P, Singh RK, Prasad M (2019) Millets genetic engineering: the progress made and prospects for the future. Plant Cell Tissue Organ Cult 137(3):421–439

Sreenivasulu N, Miranda M, Prakash HS, Wobus U, Weschke W (2004) Transcriptome changes in foxtail millet genotypes at high salinity: identification and characterization of a PHGPX gene specifically upregulated by NaCl in a salt-tolerant line. J Plant Physiol 161:467–477

Tharanya M, Kholova J, Sivasakthi K, Seghal D, Hash CT, Raj B, Srivastava RK, Baddam R, Thirunalasundari T, Yadav R, Vadez V (2018) Quantitative trait loci (QTLs) for water use and crop production traits co-locate with major QTL for tolerance to water deficit in a fine-mapping population of pearl millet (Pennisetum glaucum LR Br.). Theor Appl Genet 131(7):1509–1529

Upadhyaya H, Vetriventhan M, Deshpande SP, Sivasubramani S, Wallace JG, Buckler ES, Hash CT, Ramu P (2015) Population genetics and structure of a global foxtail millet germplasm collection. Plant Genome 8:1–13

Veeranagamallaiah G, Jyothsnakumari G, Thippeswamy M, Reddy PCO, Surabhi GK, Sriranganayakulu G, Mahesh Y, Rajasekhar B, Madhurarekha C, Sudhakar C (2008) Proteomic analysis of salt stress responses in foxtail millet (Setaria italica L. cv. Prasad) seedlings. Plant Sci 175(5):631–641

Xiang J, Apea-Bah FB, Ndolo VU, Katundu MC, Beta T (2019) Profile of phenolic compounds and antioxidant activity of finger millet varieties. Food Chem 275:361–368

Zhang J, Liu T, Fu J et al (2007) Construction and application of EST library from *Setaria italica* in response to dehydration stress. Genomics 90:121–131

Breeding Kodo Millet for Biotic and Abiotic Stress Tolerance

30

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Abstract

Kodo millet (*Paspalum scrobiculatum* L.) is grown both as food and fodder crop across the world and specifically in the arid regions of tropical, sub-tropical and temperate regions. Their grain contains a variety of high-quality proteins, has high anti-oxidant activity and is also rich in fibre. Hence, these are preferred for diabetic patients. It is popular due to its excellent nutritional and climate-resilient features. Despite the numerous benefits of millets to humans, their productivity is limited due to multiple environmental constraints and impending climate change. The yield of these crops has been seriously jeopardized by the combined effects of heat and drought stress. Drought stress in millets affects yield at the seedling stage, while terminal drought has a great impact during the reproductive stage. Apart from these two, waterlogging and salinity stress cause many adverse effects on the growth and development of kodo millets. To combat the effects of multiple stresses that are associated with climate change, as well as to improve the millet production, it is necessary to develop high-yielding and stress-tolerant millet varieties. Several possible approaches are there to enhance the tolerance level

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of millets to different stress conditions as well as to boost the productivity. For this, there is a need for whole genome sequencing of all millets, including kodo millet as it will speed up genomic selection of superior millets through gene tagging, QTL mapping, marker-assisted selection, etc. Methods like PGPRs and CRISPR/Cas9 can be used to reduce the ill effects of biotic and abiotic stresses as well as to improve the productivity of millets.

Keywords

Millets · Drought · Stress · Climate · Marker-assisted selection

30.1 Introduction

One of the main cereal crops in the developing world is millet, which is used to feed both people and livestock in the arid and semi-arid tropical regions of Asia and Africa (Tadele 2016). Although millets are the sixth-highest-producing grain in the world after rice, wheat, maize, barley and sorghum, they are typically viewed as a poor man's crop and are only used for sustenance (Dwivedi et al. 2012; Lata et al. 2013). Six types of millet, including foxtail millet, finger millet, little millet, kodo millet, barnyard millet and proso millet, are included in the small millet group. Kodo millet (*Paspalum scrobiculatum L.*), one of the six crops, is a native of India (Sharma and Sharma 2021). Being a C4 plant, it is gaining interest since it adapts well to shifting agro-climatic conditions, as most of India's arable lands (69%) are dry and arid. Kodo millet (*P. scrobiculatum* L.), one of several small millet species, is primarily grown in arid, low-rainfall areas with poor soil fertility (Sharma and Sharma 2021). This crop is believed to have been domesticated in India (around 3000 years ago), and it is now grown there as well as in West Africa, Thailand, Indonesia, the Philippines and Vietnam (Upadhyaya et al. 2016). with Gujarat producing 0.07 lakh tonnes/year, Chhattisgarh 0.17 lakh tonnes/year, Uttar Pradesh 0.07 lakh tonnes/year, Madhya Pradesh 0.5 lakh tonnes/year and Tamil Nadu 0.12 lakh tonnes/year (Deshpande et al. 2015). India is the country that produces the majority of the kodo millet, with Gujarat producing 0.07 lakh tonnes/year, Chhattisgarh 0.17 lakh tonnes/year, Uttar Pradesh 0.07 lakh tonnes/year, Madhya Pradesh 0.5 lakh tonnes/year and Tamil Nadu 0.12 lakh tonnes/year (Deshpande et al. 2015). Kodo millet is rich in calcium (27 mg/100 mg grain), fibre (9%), carbs (66 g/100 g) and proteins (11%). The crop is suitable for food and feed due to its higher lecithin content, which encourages simple digestion (Ganapathy 2017). Kodo millet grains are also ideal for long-term storage due to their superior seed lifespan (Muthamilarasan and Prasad 2021).

Due to the predominance of major cereal crops like rice and wheat, the cultivated area of kodo millet has been declining since the green revolution. Small millets continue to contribute to the regional food security of the dry and marginal regions, where major cereal crops fail to produce, thus an increased effort to enhance the acreage of these crops is crucial. Since millets are more nutritious than the other

cereals, there is currently a push to grow them. When compared to other millets, Kodo millet has reportedly been shown to have a greater capacity to quench free radicals (Hegde and Chandra 2005). Additionally, it offers affordable protein, vitamins and minerals in the form of wholesome meals (Yadava and Jain 2006). Demand for these nutritional cereals, which are naturally anti-diabetic and anti-oxidant, is increased by consumers' growing health consciousness (Chandrasekara and Shahidi 2010). Therefore, to increase the production of this crop on a viable scale, technological intervention is required. The average yield for most crops has decreased by more than 50% as a result of several abiotic stresses that climate change has caused in plants and which are the primary causes of crop loss worldwide.

30.2 Origin, Taxonomy and Distribution

Kodo millet was domesticated around 3000 years ago in the Indian subcontinent (De Wet et al. 1983). It is reported as incompletely domesticated 'pseudo-cultivated' by some authors (De Wet 1992; Blench 1997). It belongs to the genus 'paspalum' and comprises approximately 400 species (Chase 1929). The chromosome number of *Paspalum scrobiculatum* is $2n = 2\times = 40$ (Hiremath and Dandin 1975). The main centre of origin of the 'paspalum' genus is South America (Chase 1929). It is cultivated throughout India, especially in the moist places in tropical and subtropical areas of the Old World. Based on panicle morphology, Kodo millet has been divided into three races, viz., *regularis*, *irregularis* and *variabilis.* The race '*regularis*' is the most common one in which there are two races of spikelets on a side of the rachis, '*irregularis*' has two-four rows of spikelets on the side of the rachis, and '*variabilis*' has two rows of irregular spikelets in the lower part and regular spikelets in the upper rows (Figs. 30.1 and 30.2).

30.3 Techniques of Germplasm Improvement in Kodo Millet

30.3.1 Conventional Breeding Method for Kodo Millet

There are a few breeding methods that are adopted in millets, like mass selection, pedigree selection, pureline selection, backcross breeding, selection from landraces and hybridization. APK 1, GK 2 and KMV 20 are the varieties of kodo millet that were developed by germplasm introduction. The germplasms can be introduced from NBPGR, New Delhi; NAGS, Bangalore; and ICRISAT, Hyderabad to any part of the country. PRL 1 was the first variety of the kodo millet that was released for the Tamil Nadu region in the year 1942, T 2 variety (1949), Niwas 1 (1971) (Yadava and Jain 2006). Pureline method is also used for the improvement of kodo millet against biotic and abiotic stresses. Breeding for shoot fly and head smut resistance (Yadava and Jain 1996; Verma 1989) has been developed in kodo millet. Thirty-three varieties have been developed in kodo millet through pureline selection. There is still a lot of scope for the improvement of biotic and abiotic stress resistance in kodo

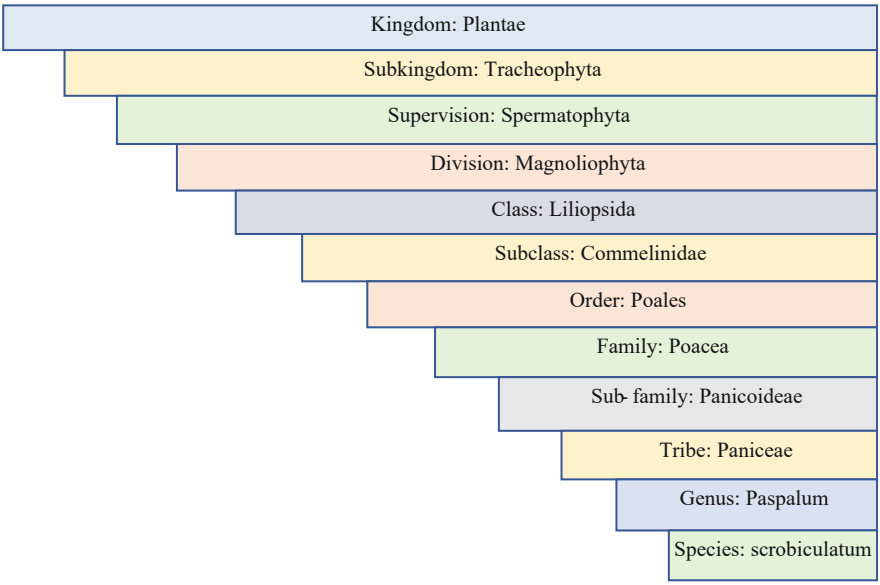


Fig. 30.1 Taxonomic hierarchy of kodo millet

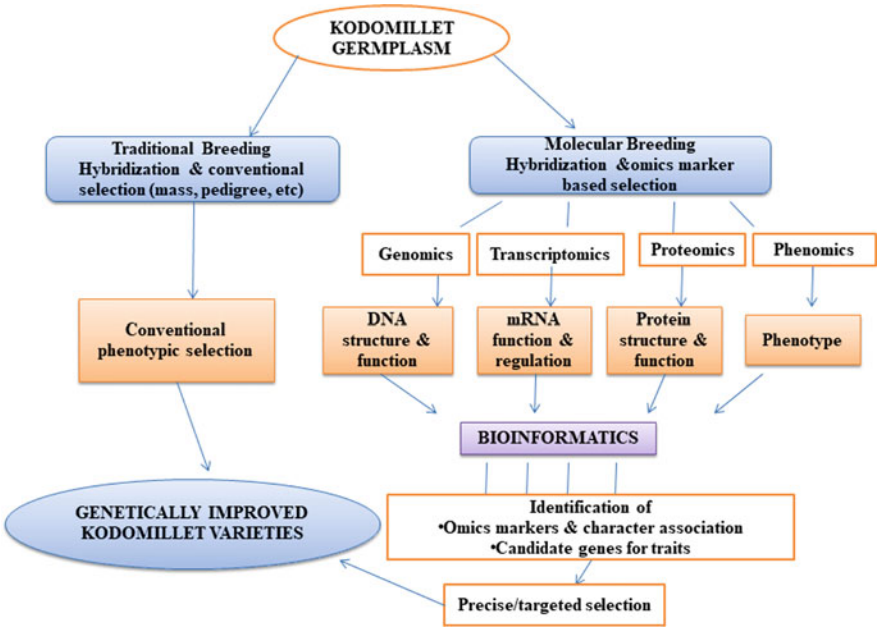


Fig. 30.2 Different methods of germplasm improvement in kodo millet

millet through pureline selection method. There are limited funds that are sanctioned for millet improvement, especially kodo millets. Due to which, the utilization of germplasms is very limited for these crops. A more economical approach is to develop core collections in case of these crops. The core collections of kodo millet have been developed by ICRISAT for evaluation of various biotic and abiotic stress tolerance. Out of all the methods, hybridization followed by selection is the most widely used method for millet improvement. In small millets, emasculation and hybridization are already reported (Li et al. 1935; Siles et al. 2001). The behaviour of anthesis and floral morphology of kodo millet makes it even more difficult for hybridization. Due to this reason, there is no reports of interspecific hybridization in kodo millet. Hand emasculation is an effective technique to carry out emasculation (Verma 1989; Yadava 1997). Another method of hybridization for kodo millet improvement is the contact method, which is the modification of finger millet contact hybridization method (Ayyangar and Warian Achuta 1934). The method has a characteristic of increasing the amount of cross-pollination by tying the panicles of desirable plants with the other plant on which the hybridization is to be performed. For hybrid seed production, male sterile lines are reported for foxtail as well as finger millets (Gupta et al. 1997). But in the case of kodo millets, no male sterile lines are reported. The physical mutagen (gamma-irradiation) was used to produce variations in kodo millet (Mishra et al. 1985). The effective gamma radiation dose is 25 Kr in case of kodo millets (Yadava 1997). Kodo millet 3 has been developed using the mutation-breeding programme in India. The effectiveness and efficiency of a mutagen are important to get a high number of useful mutations. EMS at 4% concentration is very effective to produce viable mutants in kodo millet (Jency et al. 2016). The millet is very responsive toward chemical as well as physical mutagens.

30.3.2 Genetic Transformation in Kodo Millets

The development of resistance to biotic and abiotic stress using biotechnological techniques has not been considered much. Agrobacterium-based transformation is the most studied and utilized method for millet transformation. This study was initiated in finger millet (Gupta et al. 2001) and foxtail millet (Liu et al. 2005). None of the complete genetic transformation work has been reported in kodo millet. Apart from kodo millet, few more millets like proso millet and little millet have not been studied for genetic transformation. For initiating the transgenic work in kodo millet, in vitro regeneration protocols can be used. Regeneration in kodo millet was started using somatic embryogenesis (Bovo and Mroginski 1989), from immature inflorescences (Nayak and Sen 1989; Vikrant and Rashid 2001) and from both immature and mature embryos (Vikrant and Rashid 2002). Somatic embryogenesis with 2,4-D, Picloram and Kinetin was reported by Kaur and Kothari (2003). Millets are not as responsive as cereals to the transformation techniques. No such variety has been reported using this technique.

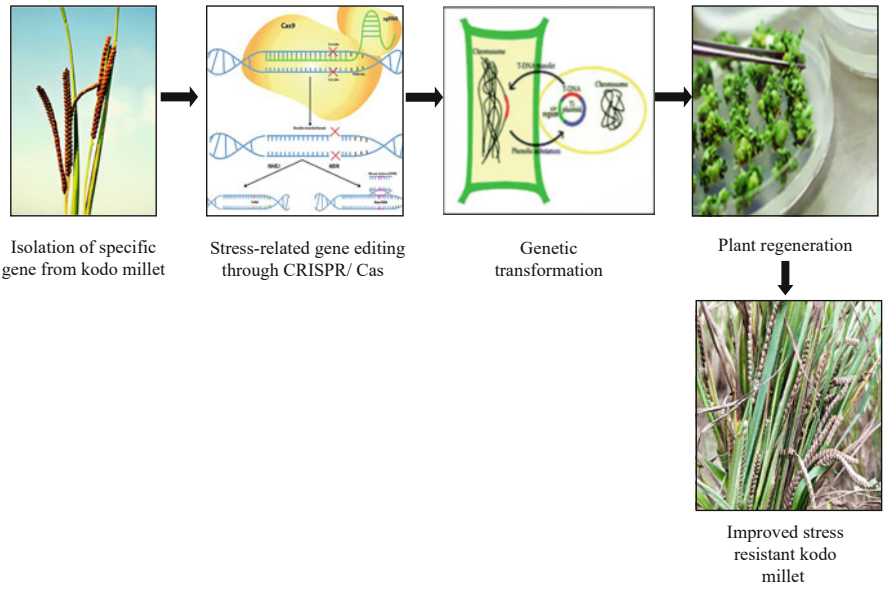
The transformation protocols can be optimized and utilized for kodo millet genetic transformation. This can be done using the earlier studies on finger millet

and foxtail millet, which also belong to the millets group. In finger millet, the transgenic was developed for leaf blast disease (Ignacimuthu and Ceasar 2012), salinity and drought tolerance (Hema et al. 2014), zinc improvement (Ramegowda et al. 2013) and herbicide resistance (Bayer et al. 2014). Similarly, in foxtail millet, this technique was used for developing salinity and drought tolerance (Li et al. 2014) and phosphorous transport (Ceasar et al. 2017).

30.3.3 CRISPR/Cas9 to Improve Stress Resistance in Kodo Millets

Genome editing is one of the most efficient technologies that offer precise genetic modifications. The nucleases like SSN (Site-Specific Nucleases) create double-stranded break in the DNA strand. HDR (Homology-Directed Repair) and NHEJ (Non-Homologous End Joining) are the techniques by which the breaks are repaired (Jinek et al. 2012). CRIPR/Cas 9 is utilized for improving different biotic and abiotic stress tolerance as well as yield-attributing traits in almost 20 crop species (Ricroch et al. 2017). For any biotic and abiotic stress tolerance, structural gene plays an important role by encoding important proteins (Zafar et al. 2020). The regulatory genes as well as cis-regulatory sequences indirectly affect the expression of these genes. The utilization of CRISPR/Cas is limited to a few crop species only. The improvement of millets through CRISPR/Cas 9 is attracting the scientists these days due to the increasing popularity of millet crops.

Some of the genes from the related species can be used for CRISPR/Cas 9 gene editing in case of millets in general, like from maize, *NF-YB* gene (Li et al. 1992); rice, *OsDIS1*, *OMTN 2–6*, *OsiSAP7* (Ning et al. 2011; Sharma et al. 2015; Fang et al. 2014); wheat, *TaWRKY146*, *WRKY* (Satapathy et al. 2018; Ma et al. 2017) and finger millet, threonine dehydratase (Hittalmani et al. 2017) for drought tolerance. Similarly, for heat tolerance, the genes from crops like wheat, *TaB2*, *TaGASR1*, *TaHsfA6f*, *TamiR159*, *TaFER-5B*, *TaMBF1c*, *TaOEP16-2-5B* (Wang et al. 2012; Xue et al. 2015; Qin et al. 2015; Zang et al. 2017; Singh and Khurana 2016) and rice, *hsp101*, *ZFP*, *SBPase*, *OsWRKY11*, *OsGSK1*, *Sp17*, *OsHsfA2e*, *FAD7*, *sHSP17.7* (Katiyar-Agarwal et al. 2003; Wei et al. 2013; Koh et al. 2007; Yokotani et al. 2008; Qi et al. 2011; Murakami et al. 2004; Sohn and Back 2007; Feng et al. 2007; Yamanouchi et al. 2002) can be utilized for editing genes in millets.



30.4 Impact of Abiotic Stress in Kodo Millet

Global food security is severely hampered by abiotic stresses, which not only reduce productivity but also lower the quality of collected produce (Wang and Frei 2011). The lowest vulnerability to environmental pressures, highest adaptability capacity to diverse ecological circumstances, highest tolerance to abiotic stresses and lowest input needs make kodo millet the most climate-resilient of all the millets (Bandyopadhyay et al. 2017).

30.4.1 Drought Stress

Although millets often adapt to abiotic stresses (salinity, drought, waterlogging, heat and cold) better than cereals, further research is needed to develop millets more robust to the increasing impacts of climate change and related environmental stresses (Muthamilarasan and Prasad 2021; Durairaj et al. 2019). Kodo millet is grown in agroecologies that are regularly subjected to the vagaries of nature, such as unpredictable heat stress, rainfall and moisture stress; hence drought tolerance is a crucial component of maintaining cultivar production levels. Following a brief period of drought, the cultivars should be able to make use of the moisture present and produce sufficient growth and grain output when precipitation is received throughout the crop season. Kodo millet may be planted in a range of soil types, from gravel to clay, and is known to be naturally drought-tolerant (De Wet et al. 1983; M'Ribu and Hilu

1996). It is now grown as a significant food crop on a global scale, which helps ensure regional food security, especially for dry and marginal soils where main cereal crops typically fail to grow (Sao et al. 2017).

Kodo millet is primarily a rain-fed crop, and as a result of the monsoon's failure, it is severely impacted by drought. Dehydration tolerance is an essential attribute that results immediately from drought, salt and cold stress (Hadebe et al. 2017). In order to deal with the water deficit conditions brought on by dehydration stress, plants frequently employ dehydration avoidance and tolerance mechanisms. Osmotic adjustment is typically the crop's first response to drought stress. The buildup of compatible solutes in the cytoplasm is thought to contribute to stress tolerance (Hare et al. 1998). Plants produce and accumulate suitable osmolytes, including proline and glycine betaine (GB), which are involved in osmotic adjustment, to counteract abiotic stress. This increases the osmotic potential of the cells (Kavikishore et al. 2005). The buildup of free amino acids under stress suggests that they may be involved in osmotic adjustment (Yadav et al. 2005). Plants naturally accumulate GB to thrive under stress conditions. Enzyme choline monooxygenase (CMO) first converts the Choline into betaine aldehyde and then glycine betaine is produced in the chloroplast stroma by the NAD-dependent enzyme betaine aldehyde dehydrogenase (BADH), whose activity rises in response to stress (Arakawa et al. 1990). Osmoticum can be maintained by GB if the plant's basic metabolism can support a high rate of synthesis of these substances to aid in osmotic adjustment for tolerance to drought stress. The crop may use many strategies to thrive in areas that are prone to drought. These defence mechanisms include drought avoidance, which is the capacity of the plant to maintain water balance in tissues and to prevent deficiency of water under stress conditions; drought tolerance, which is the capacity of the plant to produce biomass while withstanding reduced water potential; drought escape, which refers to a condition in which plants mature before experiencing drought stress and finally the drought recovery, which is a situation where plants produce some amount of yield while recovering from intermittent drought effects.

The total sugar content of *P. scrobiculatum* plants significantly increases as a result of drought stress. Together with other macromolecules like late embryogenesis abundant proteins (LEA Proteins), disaccharide and trehalose, which accumulate under drought stress and play a role in flower and embryo development as well as the control of carbon metabolism and photosynthesis, sugar is likely to stabilize membranes and prevent membrane fusion (Phillips et al. 2002; Iturriaga et al. 2009). The important strategies that plants adopt during dehydration are modifications to the root system and stomata closure, which are controlled by a number of signalling pathways and hormone-dependent gene expressions (Uga et al. 2013; Urano et al. 2016).

The transcriptomic approach per se enables thorough investigation and quantification of changes caused by abiotic stresses at the whole organism level. Rice's root architecture and responses to osmotic stress were previously discovered to be significantly regulated by the *WRKY28* and *WRKY76* (An et al. 2008). Differential expression of these genes during the early stages of kodo millet revealed that they may have an impact on the architecture of the root system under conditions of

dehydration stress. The manifestation of dehydration stress induces ABA-mediated stomatal closure to reduce the loss of water, and this is followed by a decrease in photosynthetic efficiency (Claeys and Inze 2013). The generation of ROS, which causes the breakdown of proteins, lipids and DNA, may be responsible for these dehydration stress reactions (Vessal et al. 2020). Transcriptional factors are essential for controlling signalling pathways. Transcripts encoding *MYB96*, *ERF113*, *MYB3R-2*, *bZIP12*, *ABI5 (bZIP)*, *SRM1 (MYB)*, *bZIP46* and *NAC48* were discovered, whose expression altered changes in response to dehydration stress in kodo millet. These TFs are known to regulate a number of physiological processes in a number of crops under dehydration stress, including antioxidant enzyme activity, stomatal closure, ABA biosynthesis and signalling, cuticular wax biosynthesis, pollen maturation, membrane modification, seed germination, phosphosulfate accumulation, phosphoadenosine and root growth (Liu et al. 2011; Seo et al. 2011; Wang et al. 2015; Hossain et al. 2010; Yang et al. 2011; Zou et al. 2008; Lee et al. 2017).

30.4.2 Salinity Stress

One of the most serious environmental factors limiting crop plant productivity is salt stress. Salinity has a significant impact on most areas of plant physiology, growth and development (Borsani et al. 2003). The excessive production of ROS, including the superoxide anion (O^{2-}), H_2O_2 and hydroxyl radicals are the significant effects of salinity stress in plants. Superoxide dismutase, peroxidase, polyphenol oxidase and catalase are oxygen radical detoxification enzymes that plants have in order to live in stressful environments (Jaleel et al. 2007). A method to improve plants' tolerance to salt may be provided through antioxidant mechanisms. ROS are lethal, highly reactive and capable of causing mutations (Halliwell 1997). Thus, oxidative stress is caused by a one-sided relationship between ROS and antioxidant defences. Oxidative stress causes deadly lipid peroxidation, DNA damage, enzyme inhibition, protein oxidation and the initiation of programmed cell death (PCD) or apoptosis in cells (Mishra et al. 2011; Malik et al. 2014). High soil salinity drastically lowers seed germination and frequently results in osmotic toxicity, which may reduce the germination rate. This decrease is brought on by osmotic pressure, which prevents water from absorbing. Thus, there is a decrease in cell division and differentiation as well as a shortening of the plumule and radical length. The buildup of Na^+ and Cl^- leads to an imbalance in the intake of nutrients and toxicity. Some metabolic functions during germination are inhibited by salt stress and low moisture content (Younis et al. 1991). The highest level of heavy metal tolerance (copper and zinc) was reported in kodo millet, followed by proso millet, during the seedling stage (Arora and Katewa 1999). Moreover, there are no prior reports found on how kodo millet responds to salt stress during germination. Therefore, in order to resist salinity stress, it is necessary to find genotypes that are salt tolerant. As proline builds up in the body, it protects certain enzymes by functioning as an osmoregulator (Sreenivasulu et al. 1999; Taffouo et al. 2009), leading to salinity stress tolerance in the genotypes that have been discovered. Identification of tolerant genotypes will

be aided by analysis of the proline, peroxidase, polyphenol oxidase and catalase activities.

30.5 Lodging in Kodo Millet

Lodging is the irreversible bending of the stem in an upright position, which is one of the major issues in millets, causing significant losses in grain yield. A number of factors, including wind, irrigation water, rainfall or a combination of them, can cause lodging. The stem and roots are the two primary targets of lodging; the literature refers to these targets as stem lodging and root lodging, respectively (Pinthus 1974; Piñera-Chavez et al. 2016). Root lodging differs from stem lodging in that the angle between the stem and soil varies as a result of wind pressure on the stem or crown bending/root disanchoring, whereas in stem lodging, the stems bend or break down towards the soil surface (Van Delden et al. 2010). Kodo millet is best suited for dryland farming because it needs the least amount of irrigation, crop protection, and fertilizing inputs. But it has been shown that practically all cultivated species lodge when they reach maturity. This is seen as a major constraint since it makes harvesting extremely difficult, both manually and by machine, as well as causing a financial loss in terms of quantity as well as grain quality. Thus, improving kodo millet for its non-lodging characteristic is an essential breeding objective in order to increase the importance of this crop. The traits "pushing resistance" (Terashima et al. 1992; Won et al. 1998), "stem diameter" and "culm weight" (Zuber et al. 1999) are implicated in culm strength and lodging resistance, according to the findings found in rice as a model crop. Additionally, reports claim that lignin and cellulose are involved in providing strength to culms, and lesser quantities of these substances make culms more fragile (Jones et al. 2001; Ma et al. 2002; Tanaka et al. 2003). Tilling practices, adjusting the date of seed sowing, increasing the intra-row space, or lowering the number of plants in a row are all examples of crop management practices that can minimize the problem of lodging (Piñera-Chavez et al. 2016; Cannarozzi et al. 2018; Van Delden et al. 2010). Since the lodging of the crop can occur prior to harvest, early maturity duration might also be considered (De Wet et al. 1983). A variety named 'JK 76' was identified as an early maturing variety in kodo millet (Tickle and Yadava 1992). The best way to overcome lodging is the through use of lodging-resistant cultivars along with good crop husbandry. Some cultivated landraces continue to initiate culms beyond the maturity of older shoots, maintaining the perennial nature of their wild ancestor (De Wet et al. 1983). If this regenerative feature can be cultivated through hybridization and breeding, it may help to reduce labour and fertilization costs. Gamma radiation or ethyl methane sulfonate (EMS) can generate a mutant named CO3, which is a non-lodging mutant in the kodo millet (Jency et al. 2020). Mutant (named second mutants or M2) was also created which exhibited greater lodging tolerance due to culm thickness and photosynthetic efficiency.

30.5.1 Waterlogging

In high-precipitation areas, waterlogging stress is the primary cause of low productivity. Under conditions of waterlogging, water seeps into the soil pores, causing hazardous chemicals to build up and preventing gas diffusion. This ultimately has an impact on photosynthesis, stomatal conductance and on roots (Linkemer et al. 1998). The kodo millet and other millets have two techniques to deal with this situation: one involves adventitious roots, and the other involves solubilized sugar and increased nitrogen reductase activity in the shoots (Cannarozzi et al. 2018; Kulkarni and Chavan 2014).

30.5.2 Heat Stress

One of the main factors affecting crop productivity and growth is temperature. Kodo millet is a thermosensitive crop. Pre-anthesis phenological events are exposed to current temperatures by late sowing in a rainfed farming system, which affects grain development and, eventually, yield (Nagarajan et al. 2008). The buildup of heat units above the threshold or base temperature is related to the phenological development of plants from sowing to maturity. It takes a specific amount of heat units to attain a specific phenophase. The most vulnerable biological functions to heat stress are photosynthesis and respiration, which have a significant impact on crop output (Ayele et al. 2001). The effects of high-temperature stress include decreased electron transport, impaired PS II performance and increased ROS buildup (Ray et al. 2019). Additionally, it desiccates the reproductive components, which can cause plant infertility, seed abortion, a decrease in the number of seeds and a shortening of the grain filling time (Asthir 2015). Upregulation of the signalling molecules, antioxidant system, heat-shock proteins, ion transporters, transcription factors and accumulation of osmoprotectants are some of the mechanisms of tolerance (Hatfield and Prueger 2015) (Fig. 30.3).

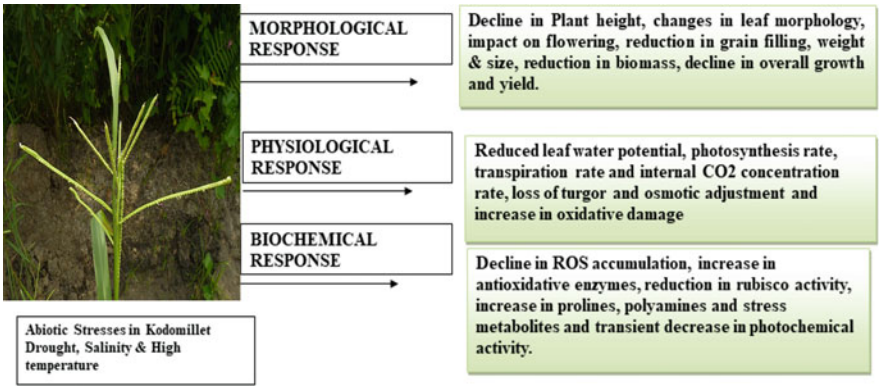


Fig. 30.3 Morpho-physiological and biochemical responses of kodo millet to abiotic stress

30.6 Biotic Factors

Kodo millet is very less hampered due to biotic stresses like diseases and pests. But there are some important biotic causes that affect crop yield drastically. Some of the important diseases that are reported in kodo millet are:

30.6.1 Head Smut of Kodo Millet

The disease was first observed in Australia. In India, it was reported in Bihar and Andhra Pradesh. It has become an endemic disease of kodo millet in India. The disease is caused by *Sorosporiumpaspali* H. Huge yield losses in kodo millet due to this disease were reported by Butler (1918), 30–40% yield loss was reported by Vishwanath (1992), 13–32% yield loss (Jain and Yadava 1997). GPLM-78, 96, 176, 322, 364, 621, 641, 679 and 720 (Jain 2005) and RK-31, 65-18, 87-9, 106, 162, ICK769, DPS-486, 516, 542, 672, 700, 727 (Ramappa et al. 2006) are the sources of resistance for head smut in kodo millet. They are used for gene introgression into the elite cultivars using different breeding programmes.

30.6.2 Ergot of Kodo Millet

The disease was identified in Burma in kodo millet crop (Butler and Bisby 1931). In India, in the year 1950, this was first reported from Kodaikanal, Assam and Gwalior (Ramakrishnan and Sundaram 1950). The disease occurs in both cultivated as well as wild forms of kodo millet. It is caused by *Claviceps paspali* S. The matured sclerotia cause paralysis and even the death of animals. The sweet and sticky liquid comes out from the infected flowers hence, it is named honeydew or sugary disease. After the release, these liquids are hardened into brown-coloured substances. With maturity, ergot is formed in the kernels of the panicle. The development of varieties resistant to ergot is an important objective of breeding.

30.6.3 Udbatta Disease

The disease was first identified in paddy as a pandemic disease. It is caused by *Ephelis oryzae* S. *Balansia oryzaesativae* H. is the perfect stage of this pathogen. It occurs in kodo millet in addition to Pennisetum, little millet and *Cyanodon dactylon.* In 2008, it was first reported in an All India Coordinated Research Project on small millets, GKVK farm, Bangaluru, Karnataka. It was spread like a sporadic disease in kodo millet. The highest incidence of this disease was reported in RBK 155 variety of kodo millet (Nagaraja et al. 2010). The disease is named so because the panicle looks like agarbatti and is thus named as Udbatta. The symptoms of this disease are visible in the panicle initiation stage. Breeding for the development of resistant varieties is the best measure to control this disease.

30.6.4 Sheath Rot

The disease is widespread in the state of Tamil Nadu, especially in the Vrundhachalam area. It is caused by *Sarocladiumoryzae* S. In the areas where kodo millet is grown as rabi or summer crop after paddy, the infected mycelium is carried by the rice crop residue which serves as a primary disease inoculum. This disease affects the emergence of panicle and leads to a reduction in grain yield. The topmost leaf sheath which encloses the young panicle shows the symptoms in the form of lesions. The grains are finally discolored and this affects the seed quality.

30.6.5 Aspergillus Fungi

Due to its detrimental effects on both human and animal health as well as its relevance in international trade, aflatoxin-contaminated food and feed is of great concern. Aflatoxicosis is a health risk that can result from dietary exposure to aflatoxins, as stated by the World Health Organization. Depending on the type, duration and amount of exposure, aspergillus species produce aflatoxins, which are secondary metabolites that hinder development, induce different malignancies and even result in death (Thomas et al. 2011; WHO 2000; Williams et al. 2004; Waliyar et al. 2015). Of the four aflatoxins viz., B1, B2, G1 and G2, aflatoxin B1 (AFB1) is the most potent carcinogen (Waliyar et al. 2016; Ajani et al. 2014; Boutrif and Canet 1998). Numerous assessments indicate that AFB1 is a widespread problem with food safety for consumers along the whole food delivery chain. According to the report by Food and Agricultural Organization (FAO), around 25% of the world's food crops are found to be contaminated with mycotoxins (Rao and Husain 1985). Aflatoxin poisoning has a significantly adverse effect on rural residents' quality of life, and mycotoxicosis-related economic losses are common in many nations. AFB1's significance in food, feed and trade-related issues is indicated by the regulatory standards for AFB1 limits that are implemented in more than 100 nations. In humid environments or if the crop is soaked during different conditions, such as pre-harvesting, harvesting and post-harvesting, Kodo millet grains are commonly infected with *Aspergillus tamari* Kita. The association of the mycotoxin, cyclopiazonic acid (CPA), with kodo millet seeds causing 'kodua poisoning' was first identified during the mid-1980s. Cyclopiazonic acid was first isolated from *Penicillium cyclopium* and later reported from *A. oryzae*, *Aspergillus versicolor*, *A. tamari*, *A. flavus* and *Penicillium camembertii*. Natural occurrence of this toxin was reported from peanuts and cheese and now we report it from Kodo millet. CPA was isolated and identified from a sample of kodo millet seed that caused symptoms of 'kodua poisoning' is a toxic syndrome in cattle and humans often encountered in the areas where *Paspalum* species are grown. Cattle poisoning is characterised by signs of anxiety, loss of coordination in the muscles, stumbling stride, depression and spasms. The cattle often recover after a few days; however, reports of poisoning-related deaths in cattle have also been reported. Sleepiness, tremors and giddiness are symptoms of kodo poisoning in humans caused by unintentional ingestion of

contaminated grain. The symptoms last for 13 days followed by recovery. Hence, potential target for breeding may be resistance to the fungi *Aspergillus flavus* and *A. tamari* which produce cyclopiazonic acid.

30.6.6 Shoot Fly

Shoot fly occupies the topmost insect which infects kodo millet. *Atherigona simplex* reduces the yield significantly by up to 39–49% (Nagesh Chandra and Musthak Ali 1983). Some shoot fly-resistant cultivars are JK13, RPS 515, 584, 810, 834, 842, 846, 871, 872, 938, 974, IQS 147-1, etc.

30.6.7 Genomic-Assisted Breeding

A variety of crop plants have been effectively improved using traditional breeding techniques using germplasm resources, which are a rich supply of beneficial genes. Characterization of trait-specific markers is the primary requirement to identify genotypes differing for desirable target trait. Based on gene-specific pair of primer, few sets of DNA-based markers have been created (Kushwaha et al. 2014), random amplified polymorphic DNA (RAPD) markers and semi-targeted polymerase chain reaction amplification (Yadav et al. 2016). *P. scrobiculatum's* genetic diversity was examined by Dhagat's in the year 1978 using 90 domestic and 6 alien germplasm accessions and found that there was minimal correlation between geographic region and the genetic relationships of the 96 accessions used, which could be split into four categories.

For a few *P. scrobiculatum* germplasm collections, a diversity analysis of morphological traits was carried out. A key tactic to improve the use of different germplasm with agronomically advantageous features in applied breeding is the creation of a core collection (Upadhyaya et al. 2014). 20 morpho-agronomic traits were assessed in a collection of 656 accessions of kodo millet from India. 75 accessions (about 11%) were chosen as the core subset from the germplasm collection kept at the ICRISAT genebank in Patancheru, Hyderabad, India. The development of tolerant lines for both abiotic and biotic conditions uses these core collections as essential resources for allele mining investigations for the acquisition of agronomic studies (Upadhyaya et al. 2014). They are the best genetic resources to utilize as novel sources of diversity for crop improvement.

The development of omics studies and high-throughput sequencing technology has made it clear that stress regulation is a very complex and interdependent process that is essential for a plant's survival under suboptimal circumstances. Stress tolerance has been defined as involving a network of regulatory and signalling molecules that may work in concert or in opposition to one another. With its highly adaptive traits, Kodo millet is a treasure trove of crucial genes and regulatory proteins that can be used to create crops that are stress-resistant.

30.6.8 Genomics of Kodo Millet

Direct access to the coding and non-coding portions of the genome that control growth, development and reaction to environmental cues is made possible by genome sequencing. The sequenced data also makes it easier to create genome-scale markers that help with understanding the diversity, structure and evolution as well as mapping sequence variation linked to desirable traits and creating molecular tools for crop improvement using genomics. Genotyping by sequencing (GBS) was used by the ICRISAT and Cornell University to genotype a variety of Kodo millet, identify genome-wide single nucleotide polymorphisms and evaluatepopulation structure and diversity (Johnson et al. 2019; Upadhyaya et al. 2015; Wallace et al. 2015). Unfortunately, Kodo millet's genome does not appear to have any genetic or molecular maps (Dwivedi et al. 2012). This is probably because of the issue of recurrent cross-hybridization with its wild relatives. Kodo millet has a limited number of molecular markers, yet these markers have been used to characterize diversity and phylogeny (M'Ribu and Hilu 1996). Using ESTs from kodo, there has been some preliminary work on predicting the miRNA target site (Babu et al. 2013). Comparing genomes from different species can highlight the parallels and divergences in genome organization (comparative genomics). Such research may be helpful in anticipating important genes involved in the resistance to abiotic stress and can shed light on the evolutionary relationships between different species. In fact, even distantly related species of plants have been shown to share a great deal of similarities in their genomes (Guyot et al. 2012). The number of conserved sites can be determined by examining orthologous sequences in the aligned genomes. With the development of the 'genome zipper' idea, this basically compares the completely sequenced and annotated genomes with diverse sources of data originating from less well-studied species, comparative genomics aids in finding a virtual gene order in a partially sequenced genome (Mayer et al. 2011). Functional and structural genomics work together to properly characterize a genome. Genome-wide expression profiling can be used to find candidate transcription factors (TFs) genes for qualities like stress tolerance. For the production of transgenic crops with desired features, additional inactivation or over-expression studies of these stress-responsive TFs genes can be conducted.

30.6.9 Transcriptomics in Kodo Millet

The study of the transcriptome, or the entire collection of messenger RNAs (mRNAs), in a cell or tissue of an organism, is known as transcriptomics (Rodrigues et al. 2014). It entails in-depth evaluation and quantification of transcript alterations across the entire genome (Lata 2015).

30.6.10 Proteomics

The term "proteome" refers to the total collection of proteins expressed in a single organ, tissue, or cell, or in the body as a whole. In order to comprehend the underlying regulatory mechanism of the expressed proteins, "proteomics" refers to a thorough structural and functional examination of the "proteome" (Muthamilarasan and Prasad 2021). Although study at the proteome level is required to understand the functional gene expression profiles, the transcriptome approach is effective for identifying the genes related to tolerance to abiotic stress. Additionally, the research to date shows that changes in gene expression levels are not always associated with changes in protein levels (Gygi et al. 1999; Kosova et al. 2013). The main reason is that cells contain mechanisms for post-transcriptional and post-translational control. In order to separate and identify proteins, proteomics analysis is performed utilising (1) 2-DE or paired gel-free shotgun liquid chromatography-tandem mass spectrometry (LC-MS/MS) technologies. Unravelling the functions of proteins and their networks of metabolic and signalling pathways in plants can be done (2) through protein mapping and characterization of post-translational modifications and protein-protein interactions, (3) using bioinformatics methods and the use of databases for model and non-model plant species. Due to the size of its genome, the lack of repetitive DNA, the length of the crop and its propensity for inbreeding, foxtail millet is regarded as a model crop for abiotic stress tolerance studies and performs better under those conditions. After treating foxtail millet seedlings to drought stress, TMT and LC-MS/MS-based proteomic techniques were used (Pan et al. 2018) to quantify the differentially expressed proteins (DEPs). There are no other proteomics studies on the reaction of minor millet to salinity stress. Additionally, no efforts have been undertaken to investigate the kodo millet's proteome responses to abiotic stress. As a result, there is a huge gap in the literature because no proteins in these millets have been linked to abiotic stress tolerance mechanisms.

References

Ajani J, Chakravarthy DVS, Tanuja P, Vali Pasha K (2014) Aflatoxins. Indian J Adv Chem Sci 3: 49–60

An SH, Sohn KH, Choi HW, Hwang IS, Lee SC, Hwang BK (2008) Pepper pectin methylesterase inhibitor protein CaPMEI1 is required for antifungal activity, basal disease resistance and abiotic stress tolerance. Planta 228:61–78

Arakawa K, Katayama M, Takabe T (1990) Levels of betaine and betaine aldehyde dehydrogenase activity in the green leaves and etiolated leaves and roots of barley. Plant Cell Physiol 31(6): 797–803

Arora A, Katewa SS (1999) Germination as a screening index of heavy metal tolerance in three ethno food grasses. J Environ Biol 20:7–14

Asthir B (2015) Mechanisms of heat tolerance in crop plants. Biol Plant 59:620–628

Ayele M, Blum A, Nguyen HT (2001) Diversity for osmotic adjustment and root depth in TEF [*Eragrostis* tef (Zucc) Trotter]. Euphytica 121:237–249

Ayyangar GNR, Warian Achuta V (1934) Anthers and pollination in ragi. Indian J Agric Sci 4:386

Babu RN, Jyothi MN, Sharadamma N, Sahu S, Rai DV, Devaraj VR (2013) Computational identification of conserved micro RNAs from kodo millet (*Paspalum scrobiculatum*). Afr Crop Sci J21:75–83

Bandyopadhyay T, Muthamilarasan M, Prasad M (2017) Millets for next generation climate smart agriculture. Front Plant Sci 8:1266

Bayer GY, Yemets AI, Blume YB (2014) Obtaining the transgenic lines of finger millet *Eleusine coracana* (L.) with dinitroaniline resistance. Cytol Genet 48:139–144

Blench R (1997) Neglected species, livelihoods, and biodiversity in difficult areas: how should the public sector respond? Nat Resour Perspect 23:1–10

Borsani O, Valpuesta V, Botella J (2003) Developing salt tolerant plants in a new century: a molecular biology approach. Plant Cell Tissue Org Cult 73:101–115

Boutrif E, Canet C (1998) Mycotoxin prevention and control: FAO programmes. Revue de Medecine Veterinaire 149:681–694

Bovo OA, Mroginski LA (1989) Somatic embryogenesis and plant regeneration from cultured mature and immature embryos of *Paspalum notatum* (Gramineae). Plant Sci 65:217–223

Butler EJ, Bisby GR (1931) Fungi of India. Sci. Monogr. XVIII, Calcutta

Butler EJ (1918) Fungi and diseases in plants, Thaker Spinck and Co., Calcutta, pp 547

Cannarozzi G, Weichert A, Schnell M, Ruiz C, Bossard S, Blösch R, Plaza-Wüthrich S, Chanyalew S, Assefa K, Tadele Z (2018) Waterlogging affects plant morphology and the expression of key genes in tef (*Eragrostis* tef). Plant Direct 2:e00056

Ceasar SA, Baker A, Ignacimuthu S (2017) Functional characterization of the PHT1 family transporters of foxtail millet with development of a novel agrobacterium-mediated transformation procedure. Sci Rep 7:1–16

Chandrasekara A, Shahidi F (2010) Content of insoluble bound phenolics in millets and their contribution to antioxidant capacity. J Agric Food Chem 58:6706–6714

Chase A (1929) The North American species of *Paspalum* (contributions from the United States National Herbarium), vol 28. U.S. Govt. Print. Off., Washington, DC, pp 1–310

Claeys H, Inze D (2013) The agony of choice: how plants balance growth and survival under water-limiting conditions. Plant Physiol 162:1768–1779

De Wet JMJ (1992) The three phases of cereal domestication. In: Chapman GP (ed) Grass evolution and domestication. Cambridge University Press, Cambridge, pp 176–191

De Wet JMJ, Rao KEP, Mengesha MH, Brink DE (1983) Diversity in kodo millet, *Paspalum scrobiculatum*. Econ Bot 37:159–163

Deshpande SS, Mohapatra D, Tripathi MK, Sadvatha RH (2015) Kodo millet-nutritional value and utilization in Indian foods. J Grain Process Storage 2:16–23

Dhagat NK (1978) Variability in inter-relationship and genetic divergence, in Paspalum scrobiculatum L. PhD Thesis. Jabalpur, Madhya Pradesh, India: JNKVV

Durairaj M, Gurumurthy G, Nachimuthu V, Muniappan K, Balasubramanian S (2019) Dehulled small millets: the promising nutricereals for improving the nutrition of children. Matern Child Nutr 15:e12791

Dwivedi S, Upadhyaya H, Senthilvel S, Hash C, Fukunaga K, Diao X, Santra D, Baltensperger D, Prasad M (2012) Millets: genetic and genomic resources. In: Janick J (ed) Plant breeding reviews, vol 35. John Wiley & Sons, Hoboken, pp 247–375

Fang Y, Xie K, Xiong L (2014) Conserved miR164-targeted NAC genes negatively regulate drought resistance in rice. J Exp Bot 65:2119–2135

Feng L, Wang K, Li Y, Tan Y, Kong J, Li H, Li Y, Zhu Y (2007) Overexpression of SBPase enhances photosynthesis against high temperature stress in transgenic rice plants. Plant Cell Rep 26:1635–1646

Ganapathy KN (2017) Improvement in finger millet: status and future prospects. In: Patil JV (ed) Millets and sorghum: biology and genetic improvement. John Wiley & Sons, Chichester, pp 87–111

Gupta SC, Muza FR, Andrews DJ (1997) Registration of INFM 95001 finger millet genetic male-sterile line. Crop Sci 37:1409

Gupta P, Raghuvanshi S, Tyagi AK (2001) Assessment of the efficiency of various gene promoters via biolistics in leaf and regenerating seed callus of millets, *Elusine coracana* and *Echinochloacrusgalli*. Plant Biotechnol 18:275–282

Guyot R, Lefebvre-Pautigny F, Tranchant-Dubreuil C, Rigoreau M, Hamon P, Leroy T, Hamon S, Poncet V, Crouzillat D, de Kochko A (2012) Ancestral synteny shared between distantly-related plant species from the asterid (*Coffea canephora* and *Solanum* sp.) and rosid (*Vitis vinifera*) clades. BMC Genomics 13:103

Gygi SP, Rochon Y, Franza BR, Aebersold R (1999) Correlation between protein and Mrna abundance in yeast. Mol Cell Biol 19(3):1720–1730

Hadebe S, Modi A, Mabhaudhi T (2017) Drought tolerance and water use of cereal crops: a focus on sorghum as a food security crop in sub-Saharan Africa. J Agron Crop Sci 203:177–191

Halliwell B (1997) Antioxidants and human disease: a general introduction. Nutr Rev 55(1):S44–S49. https://doi.org/10.1111/j.1753-4887.1997.tb06100.x

Hare PD, Cress WA, Van Staden J (1998) Dissecting roles of osmolyte accumulation during stress. Plant Cell Environ 21:535–553

Hatfield JL, Prueger JH (2015) Temperature extremes: effect on plant growth and development. Weather Clim Extremes 10:4–10

Hegde PS, Chandra TS (2005) ESR spectroscopic study reveals higher free radical quenching potential in kodo millet (*Paspalum scrobiculatum*) compared to other millets. Food Chem 92: 177–182

Hema R, Vemanna RS, Sreeramulu S, Reddy CP, Senthil-kumar M, Udayakumar M (2014) Stable expression of *mtlD* gene imparts multiple stress tolerance in finger millet. PLoS One 9:e99110

Hiremath SC, Dandin SB (1975) Cytology of *Paspalum scrobiculatum* Linn. Curr Sci 44:20–21

Hittalmani S, Mahesh H, Shirke MD, Biradar H, Uday G, Aruna Y, Lohithaswa H, Mohanrao AJB (2017) Genome and transcriptome sequence of finger millet (*Eleusine coracana* (L.) Gaertn.) provides insights into drought tolerance and nutraceutical properties. BMC Genomics 18(1):465

Hossain MA, Lee Y, Cho JI, Ahn CH, Lee SK, Jeon JS, Kang H, Lee CH, An G, Park PB (2010) The *bZIP* transcription factor *OsABF1* is an ABA responsive element binding factor that enhances abiotic stress signaling in rice. Plant Mol Biol 72:557–566

Ignacimuthu S, Ceasar SA (2012) Development of transgenic finger millet (*Eleusine coracana* (L.) Gaertn.) resistant to leaf blast disease. J Biosci 37:135–147

Iturriaga G, Suarez R, Nova-Franco B (2009) Trehalose metabolism: from osmoprotection to signaling. Int J Mol Sci 10(9):3793–3810

Jain AK (2005) Stable sources of resistance for head smut in kodo millet. Indian Phytopathol 58: 117

Jain AK, Yadava HS (1997) Recent approaches in disease management of small millets. Proc Nat Semi on Small Millets – Current Research Trends and Future Priorities as Food, Feed and in processing for Value Addition, held at TNAU, Coimbatore (T.N.) from 23 – 24 April, 1997, pp 31–33

Jaleel CA, Gopi R, Sankar B, Manivannan P, Kumar AK, Sridharan R, Panneerselvam R (2007) Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in Catharanthus roseus seedlings under salt stress. S Afr J Bot 73:190–195

Jency JP, Ravikesavan R, Sumathi P, Raveendran M (2016) Determination of lethal dose and effect of physical mutagen on germination percentage and seedling parameters in kodo millet variety CO3. Electron J Plant Breed 7(4):1122–1126. https://doi.org/10.5958/0975-928X.2016.00155.1

Jency JP, Rajasekaran R, Singh RK, Muthurajan R, Prabhakaran J, Mehanathan M, Prasad M, Ganesan J (2020) Induced mutagenesis enhances lodging resistance and photosynthetic efficiency of kodomillet (*Paspalum scrobiculatum*). Agronomy 10:227

Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E (2012) A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. Science 337:816–821

Johnson M, Deshpande S, Vetriventhan M, Upadhyaya HD, Wallace JG (2019) Genome-wide population structure analyses of three minor millets: kodo millet, little millet, and proso millet. Plant Genome 12:190021

Jones L, Ennos AR, Turner SR (2001) Cloning and characterization of irregular xylem4 (irx4): a severely lignin-deficient mutant of Arabidopsis. Plant J 26:205–216

Katiyar-Agarwal S, Agarwal M, Grover A (2003) Heat-tolerant basmati rice engineered by over-expression of *hsp101*. Plant Mol Biol 51:677–686

Kaur P, Kothari SL (2003) Embryogenic callus induction and efficient plant regeneration from root cultures of kodo millet. Phytomorphology 53:49–56

Kavikishore PB, Sangam S, Amrutha RN, Srilaxmi P, Naidu KR, Rao KRSS, Reddy KJ, Theriappan P, Sreenivasulu N (2005) Regulation of proline biosynthesis, degradation uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. Curr Sci 88:424–438

Koh S, Lee SC, Kim MK, Koh JH, Lee S, An G, Choe S, Kim SR (2007) T-DNA tagged knockout mutation of rice OsGSK1, an orthologue of Arabidopsis BIN2, with enhanced tolerance to various abiotic stresses. Plant Mol Biol 65:453–466

Kosova K, Prail IT, Vitamvas P (2013) Protein contribution to plant salinity response and tolerance acquisition. Int J Mol Sci 14(4):6757–6789

Kulkarni S, Chavan P (2014) Study of effect of waterlogging on root anatomy of ragi and rice. Am J Plant Physiol 9:46–51

Kushwaha H, Jillo KW, Singh VK, Kumar A, Yadav D (2014) Assessment of genetic diversity among cereals and millets based on PCR amplification using Dof (DNA binding with one finger) transcription factor gene-specific primers. Plant Syst Evol 301:833–840

Lata C (2015) Advances in omics for enhancing abiotic stress tolerance in millets. Proc Indian Natl Sci Acad 81:397–417

Lata C, Gupta S, Prasad M (2013) Foxtail millet: a model crop for genetic and genomic studies in bioenergy grasses. Crit Rev Biotechnol 33:328–343

Lee DK, Chung PJ, Jeong JS, Jang G, Bang SW, Jung H, Kim YS, Ha SH, Choi YD, Kim JK (2017) The rice *OsNAC6* transcription factor orchestrates multiple molecular mechanisms involving root structural adaptions and nicotianamine biosynthesis for drought tolerance. Plant Biotechnol J 15:754–764

Li HW, Meng CJ, Liu TN (1935) Problems in the breeding of millet (*Setariaitalic* L.) Beauv. J Am Soc Agron 27:963–970

Li XY, Mantovani R, Hooft van Huijsduijnen R, Andre I, Benoist C, Mathis D (1992) Evolutionary variation of the CCAAT binding transcription factor NF-Y. Nucleic Acids Res 20:1087–1091

Li C, Yue J, Wu X, Xu C, Yu J (2014) An ABA-responsive DRE-binding protein gene from *Setariaitalica*, *SiARDP*, the target gene of SiAREB, plays a critical role under drought stress. J Exp Bot 65:5415–5427

Linkemer G, Board JE, Musgrave ME (1998) Waterlogging effects on growth and yield components in late-planted soybean. Crop Sci 38:1576–1584

Liu Y, Yu J, Zhao Q, Zhu D, Ao G (2005) Genetic transformation of millet (*Setariaitalica*) by Agrobacterium-mediated. J Agric Biotechnol 13:32–37

Liu YC, Wu YR, Huang XH, Sun J, Xie Q (2011) AtPUB19, a U-box E3 ubiquitin ligase, negatively regulates abscisic acid and drought responses in *Arabidopsis thaliana*. Mol Plant 4:938–946

M'Ribu HK, Hilu KW (1996) Application of random amplified polymorphic DNA to study genetic diversity in *Paspalum scrobiculatum* L. (Kodo millet, Poaceae). Genet Resour Crop Evol 43: 203–210

Ma QH, Xu Y, Lin ZB, He P (2002) Cloning of cDNA encoding COMT from wheat which is differentially expressed in lodging-sensitive and -resistant cultivars. J Exp Bot 53:2281–2282

Ma J, Gao X, Liu Q, Shao Y, Zhang D, Jiang L, Li C (2017) Overexpression of *TaWRKY146* increases drought tolerance through inducing stomatal closure in Arabidopsis thaliana. Front Plant Sci 8:2036

Malik B, Pirzadah TB, Tahir I, Rehman RU, Hakeem KR, Abdin MZ (2014) Plant signaling: response to reactive oxygen species. In: Hakeem K, Rehman R, Tahir I (eds) Plant signaling: understanding the molecular crosstalk. Springer, New Delhi, pp 1–38

Mayer KFX, Martis M, Hedley PE, Simková H, Liu H, Morris JA, Steuernagel B, Taudien S, Roessner S, Gundlach H, Kubaláková M, Suchánková P, Murat F, Felder M, Nussbaumer T, Graner A, Salse J, Endo T, Sakai H, Tanaka T, Stein N (2011) Unlocking the barley genome by chromosomal and comparative genomics. Plant Cell 23:1249–1263. https://doi.org/10.1105/tpc.110.082537

Mishra PC, Singh CB, Sahi BG (1985) Study of induced variability in *Paspalum*. JNKVV Res J 9: 30–31

Mishra S, Jha AB, Dubey RS (2011) Arsenite treatment induces oxidative stress, upregulates antioxidant system, and causes phytochelatin synthesis in rice seedlings. Protoplasma 248(3): 565–577. https://doi.org/10.1007/s00709-010-0210-0

Murakami T, Matsuba S, Funatsuki H, Kawaguchi K, Saruyama H, Tanida M, Sato Y (2004) Over-expression of a small heat shock protein, sHSP17.7, confers both heat tolerance and UV-B resistance to rice plants. Mol Breed 13:165–175

Muthamilarasan M, Prasad M (2021) Small millets for enduring food security amidst pandemics. Trends Plant Sci 26:33–40

Nagaraja A, Anjaneya Reddy B, Govindappa MR (2010) Occurrence of Udabatta disease on Kodo millet (*Paspalum scrobiculatum* L.): a new report from South India. J Mycopathol Res 48:163–164

Nagarajan S, Anand A, Chaudhary HB (2008) Response of spring wheat (*Triticum aestivum*L.) genotypes under changing environment during grain filling period. Indian J Agric Sci 78:177–179

Nagesh Chandra BK, Musthak Ali TM (1983) Losses due to shoot fly on minor millets. MILWAI Newsl 2:16

Nayak P, Sen SK (1989) Plant regeneration through somatic embryogenesis from suspension cultures of a minor millet, *Paspalum scrobiculatum* L. Plant Cell Rep 8:296–299

Ning Y, Jantasuriyarat C, Zhao Q, Zhang H, Chen S, Liu J, Liu L, Tang S, Park CH, Wang X (2011) The SINA E3 ligase *OsDIS1* negatively regulates drought response in rice. Plant Physiol 157: 242–255

Pan J, Li Z, Wang Q, Garrell AK, Liu M, Guan Y, Zhou W, Liu W (2018) Comparative proteomic investigation of drought responses in foxtail millet. BMC Plant Biol 18(1):315

Phillips RL, Zak DR, Holmes WE, White DC (2002) Microbial community composition and function beneath temperate trees exposed to elevated atmospheric carbon dioxide and ozone. Oecologia 131(2):236–244

Piñera-Chavez F, Berry P, Foulkes M, Jesson M, Reynolds M (2016) Avoiding lodging in irrigated spring wheat. I. Stem and root structural requirements. Field Crop Res 196:325–336

Pinthus MJ (1974) Lodging in wheat, barley and oats: the phenomenon, its causes and preventive measures. In: Advances in agronomy, vol 25. Elsevier, Amsterdam, pp 209–263

Qi Y, Wang H, Zou Y, Liu C, Liu Y, Wang Y, Zhang W (2011) Over-expression of mitochondrial heat shock protein 70 suppresses programmed cell death in rice. FEBS Lett 585:231–239

Qin D, Wang F, Geng X, Zhang L, Yao Y, Ni Z, Peng H, Sun Q (2015) Overexpression of heat stress-responsive *TaMBF1c*, a wheat (*Triticum aestivum* L.) multiprotein bridging factor, confers heat tolerance in both yeast and rice. Plant Mol Biol 87:31–45

Ramakrishnan TS, Sundaram NV (1950) Ergot on two grasses from South India. Sci Cult 16:214

Ramappa HK, Ravishankar CR, Prakash P (2006) Reaction of kodo millet and barnyard millet entries to smut disease. In: Krishnegowda KT, Seetharama N, Khairwal IS et al (eds) Future policy options in India, Small millets, proceedings of the third national seminar on millets research and development, Mar 11–12, 2004, vol III. All India Coordinated Pearl Millet Improvement Project, Agricultural Research Station, Mandor, Jodhpur, Rajasthan, pp 73–74

Ramegowda Y, Venkategowda R, Jagadish P, Govind G, Hanumanthareddy RR, Makarla U (2013) Expression of a rice Zn transporter, *OsZIP1*, increases Zn concentration in tobacco and finger millet transgenic plants. Plant Biotechnol Rep 7:309–319

Rao BL, Husain A (1985) Presence of cyclopiazonic acid in kodo millet (*Paspalum scrobiculatum*) causing 'kodua poisoning' in man and its production by associated fungi. Mycopathologia 89(3):177–180

Ray DK, West PC, Clark M, Gerber JS, Prishchepov AV, Chatterjee S (2019) Climate change has likely already affected global food production. PLoS One 14:e0217148

Ricroch A, Clairand P, Harwood W (2017) Use of CRISPR systems in plant genome editing: toward new opportunities in agriculture. Emerg Top Life Sci 1:169–182

Rodrigues CM, Mafra VS, Machado MA (2014) Transcriptomics. In: Borém A, Fritsche-Neto R (eds) Omics in plant breeding. Wiley, New York, pp 33–57

Sao A, Singh P, Kumar P, Panigrahi P (2017) Determination of selection criteria for grain yield in climate resilient small millet crop kodo millet (*Paspalum scrobiculatum* L.). Bioscan 12(2): 1143–1146

Satapathy L, Kumar D, Kumar M, Mukhopadhyay K (2018) Functional and DNA–protein binding studies of *WRKY* transcription factors and their expression analysis in response to biotic and abiotic stress in wheat (*Triticum aestivum* L.). 3 Biotech 8(1):40

Seo PJ, Lee SB, Suh MC, Park MJ, Go YS, Park CM (2011) The *MYB96* transcription factor regulates cuticular wax biosynthesis under drought conditions in Arabidopsis. Plant Cell 23: 1138–1152

Sharma S, Sharma N (2021) Preparation of probiotic enriched functional beverage of Kodo millet (*Paspalum scrobiculatum*) a nutritionally enriched absolute new product for commercialization. J Pharmacogn Phytochem 10:752–758

Sharma G, Giri J, Tyagi AK (2015) Rice *OsiSAP7* negatively regulates ABA stress signalling and imparts sensitivity to water-deficit stress in Arabidopsis. Plant Sci 237:80–92

Siles MM, Baltensperger DD, Nelson LA (2001) Technique for artificial hybridization of foxtail millet [Setariaitalica (L.) Beauv.]. Crop Sci 41:1408–1412

Singh A, Khurana P (2016) Molecular and functional characterization of a wheat B2 protein imparting adverse temperature tolerance and influencing plant growth. Front Plant Sci 7:642

Sohn S, Back K (2007) Transgenic rice tolerant to high temperature with elevated contents of dienoic fatty acids. Biol Plant 51:340–342

Sreenivasulu NS, Ramanjulu K, Ramachandrakini PHS, Shekar-Shetty H, Savithri HS, Sudhakar C (1999) Total peroxidase activity and peroxidase isoforms as modified by salt stress in two cultivars of foxtail millet differential salt tolerance. Plant Sci 141:1–9

Tadele Z (2016) Drought adaptation in millets. In: Shanker AK, Shanker C (eds) Abiotic and biotic stress in plants—recent advances and future perspectives. IntechOpen, London

Taffouo VD, Keuamou J, Marie L, Ngalangue T, Alain Nandjou B, Akoa A (2009) Effects of salinity stress on growth ions partitioning and yield of some cowpea (*Vigna unguiculata* L. Walp) cultivars. Int J Bot 1:1–9

Tanaka K, Murata K, Yamazaki M, Onosato K, Miyao A, Hirochika H (2003) Three distinct rice cellulose synthase catalytic subunit genes required for cellulose synthesis in the secondary wall. Plant Physiol 133:73–83

Terashima K, Akita S, Sakai N (1992) Eco-physiological characteristics related with lodging tolerance of rice in direct sowing cultivation. Jpn J Crop Sci 61:380–387

Thomas WK, Bill DR, Gerald NW, John DG (2011) Aflatoxin: a 50 year odyssey of mechanistic and translational toxicology. Toxicol Sci 24:174–182

Tickle AN, Yadava HS (1992) Stability assessment of grain yield in kodo millet. Asv. Plant Sci 6(I, Suppl):34–38

Uga Y, Sugimoto K, Ogawa S, Rane J, Ishitani M, Hara N, Kitomi Y, Inukai Y, Ono K, Kanno N, Inoue H, Takehisa H, Motoyama R, Nagamura Y, Wu J, Matsumoto T, Takai T, Okuno K, Yano M (2013) Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. Nat Genet 45:1097–1102

Upadhyaya HD, Dwivedi SL, Singh SK, Singha S, Vetriventhana M, Sharma S (2014) Forming core collections in barnyard, kodo, and little millets using morpho-agronomic descriptors. Crop Sci 54:2673–2682. https://doi.org/10.2135/cropsci2014.03.0221

Upadhyaya HD, Vetriventhan M, Deshpande SP, Sivasubramani S, Wallace JG, Buckler ES, Hash CT, Ramu P (2015) Population genetics and structure of a global foxtail millet germplasm collection. Plant Genome 8(3):1–13

Upadhyaya HD, Vetriventhan M, Dwivedi SL, Pattanashetti SK, Singh SK (2016) Proso, barnyard, little, and kodo millets. In: Genetic and genomic resources for grain cereals improvement. Elsevier, pp 321–343

Urano K, Maruyama K, Jikumaru Y, Kamiya Y, Yamaguchi-Shinozaki K, Shinozaki K (2016) Analysis of plant hormone profiles in response to moderate dehydration stress. Plant J 90:17–36

Van Delden S, Vos J, Ennos A, Stomph T (2010) Analysing lodging of the panicle bearing cereal teff (*Eragrostis* tef). New Phytol 186:696–707

Verma SNP (1989) Researches on small millets at JNKVV. College of Agriculture, JNKVV, Rewa, Madhya Pradesh, p 126

Vessal S, Arefian M, Siddique KHM (2020) Proteomic responses to progressive dehydration stress in leaves of chickpea seedlings. BMC Genomics 21:523

Vikrant, Rashid A (2001) Direct as well as indirect somatic embryogenesis from immature (unemerged) inflorescences of a minor millet *Paspalum scrobiculatum* L. Euphytica 120:167–172

Vikrant, Rashid A (2002) Somatic embryogenesis from immature and mature embryos of a minor millet *Paspalum scrobiculatum* L. Plant Cell Tissue Organ Cult 69:71–77

Vishwanath S (1992) Management of biotic factors (diseases). In 6th Annual Small Millets Workshop at B.A.U., Ranchi – Kanke (Bihar) from 30th April to 2nd May, 1992

Waliyar F, Osiru M, Ntare BR, Kumar KVK, Sudini H, Traore A, Diarra B (2015) Post-harvest management of aflatoxin contamination in groundnut. World Mycotoxin J 8(2):245–252

Waliyar F, Kumar KVK, Diallo M, Traore A, Mangala UN, Upadhyaya HD, Sunidhi H (2016) Resistance to pre-harvest aflatoxin contamination in ICRISAT's groundnut mini core collection. Eur J Plant Pathol 145:901–913

Wallace JG, Upadhyaya HD, Vetriventhan M, Buckler ES, Hash CT, Ramu P (2015) The genetic makeup of a global barnyard millet germplasm collection. Plant Genome 8(1):1–7

Wang Y, Frei M (2011) Stressed food—the impact of abiotic environmental stresses on crop quality. Agric Ecosyst Environ 141(3–4):271–286

Wang Y, Sun F, Cao H, Peng H, Ni Z, Sun Q, Yao Y (2012) TamiR159 directed wheat *TaGAMYB* cleavage and its involvement in anther development and heat response. PLoS One 7:e48445

Wang T, Tohge T, Ivakov A, Mueller-Roeber B, Fernie AR, Mutwil M, Schippers JH, Persson S (2015) Salt-related MYB1 coordinates abscisic acid biosynthesis and signaling during salt stress in Arabidopsis. Plant Physiol 169:1027–1041

Wei H, Liu J, Wang Y, Huang N, Zhang X, Wang L, Zhang J, Tu J, Zhong X (2013) A dominant major locus in chromosome 9 of rice (*Oryza sativa* L.) confers tolerance to 48 C high temperature at seedling stage. J Hered 104:287–294

WHO (2000) Hazardous chemicals in humans and environmental health: International Programme on Chemical Safety. World Health Organization, Geneva, pp 7–9

Williams JH, Phillips TD, Jolly PE, Stiles JK, Jolly CM, Agarwal D (2004) Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences and interventions. Am J Clin Nutr 80:1106–1122

Won JG, Hirahara Y, Yoshida T, Imabayashi S (1998) Selection of rice lines using SPGP seedling method for direct seeding. Plant Prod Sci 1:280–285

Xue GP, Drenth J, McIntyre CL (2015) *TaHsfA6f* is a transcriptional activator that regulates a suite of heat stress protection genes in wheat (*Triticum aestivum* L.) including previously unknown Hsf targets. J Exp Bot 66:1025–1039

Yadav SK, Lakshmi NJ, Maheswari M, Vanaja M, Venkateswaarlu B (2005) Influence of water deficit at vegetative, anthesis and grain filling stages on water relation and grain yield in Sorghum. Indian J Plant Physiol 10:20–24

Yadav Y, Lavanya GR, Pandey S, Verma M, Ram C, Arya L (2016) Neutral and functional marker based genetic diversity in kodo millet (*Paspalum scrobiculatum* L.). Acta Physiol Plant 38(3): 1–12. https://doi.org/10.1007/s11738-016-2090-1

Yadava HS (1997) Gamma rays induced variation in kodo millet. Adv Plant Sci 9 (II Suppl):165–169

Yadava HS, Jain AK (1996) An overview of small millets research at Rewa. In: AICRP on small millets. ZARS, JNKVV, Rewa, Madhya Pradesh

Yadava HS, Jain AK (2006) Advances in kodomillet research. Directorate of Information and Publications of Agriculture, Indian Council of Agricultural Research

Yamanouchi U, Yano M, Lin H, Ashikari M, Yamada K (2002) A rice spotted leaf gene, Spl7, encodes a heat stress transcription factor protein. Proc Natl Acad Sci U S A 99:7530–7535

Yang X, Yang YN, Xue LJ, Zou MJ, Liu JY, Chen F, Xue HW (2011) Rice ABI5-Like1 regulates abscisic acid and auxin responses by affecting the expression of ABRE-containing genes. Plant Physiol 156:1397–1409

Yokotani N, Ichikawa T, Kondou Y, Matsui M, Hirochika H, Iwabuchi M, Oda K (2008) Expression of rice heat stress transcription factor *OsHsfA2e* enhances tolerance to environmental stresses in transgenic Arabidopsis. Planta 227:957–967

Younis SA, Shahatha HA, Hagop PG, Al-Rawi FI (1991) Effect of salinity on the viability of rice seeds. In: Plant growth, drought and salinity in the arab region. The Egyptian Botanical Society, pp 235–244

Zafar SA, Zaidi SS-E-A, Gaba Y, Singla-Pareek SL, Dhankher OP, Li X, Mansoor S, Pareek A (2020) Engineering abiotic stress tolerance via CRISPR/Cas-mediated genome editing. J Exp Bot 71:470–479

Zang X, Geng X, Liu K, Wang F, Liu Z, Zhang L, Zhao Y, Tian X, Hu Z, Yao Y (2017) Ectopic expression of TaOEP16-2-5B, a wheat plastid outer envelope protein gene, enhances heat and drought stress tolerance in transgenic Arabidopsis plants. Plant Sci 258:1–11

Zou M, Guan Y, Ren H, Zhang F, Chen F (2008) A bZIP transcription factor, OsABI5, is involved in rice fertility and stress tolerance. Plant Mol Biol 66:675–683

Zuber U, Winzeler H, Messmer MM, Keller M, Keller B, Schmid JE, Stamp P (1999) Morphological traits associated with lodging resistance of spring wheat (*Triticum aestivum* L.). J Agron Crop Sci 182:17–24

Botanical Description, Brief History of Browntop Millet and Its Spectacular Adaptations as a Hardy Food and Feed Crop

31

Srijan Ambati, Hirdayesh Anuragi, K. Rajendra Prasad, B. Vidhyadhar, and Balram Marathi

Abstract

Browntop millet is a hardy food and feed crop that tolerates flooding, dry spells, as well as shade. The origin though believed to be from Southeast Asia, there are archaeological evidences that strongly pins it down to the Indian subcontinent. The crop is also found in China, Australia, Arabia, and Africa. The crop was introduced to the USA in the early nineteenth century, especially to feed game birds. The crop is gaining popularity in recent times, especially in the cuisine of the southern states of India, owing to its high dietary fiber content to address the obesity problems. This miracle crop has great potential in adapting to the agro-forestry systems due to its shade tolerance feature. However, the genetic improvement efforts towards this crop were found to be very poor. Owing to the current era of omics, there is a great need for studying the crop at a molecular level to identify new genes conferring hardiness for its wide adaptive potential. This is necessary to increase the genetic base of the existing gene pools. This chapter focuses on the botany of browntop millet, origin, distribution, habitat, adaption, crop diversity, and nutritional aspects of the crop.

Keywords

Browntop millet · Habitat · Shade tolerance · Nutrition and improvement

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31.1 Introduction

Brachiaria ramosa (L.) Stapf. is an introduced annual grass that originated in Southeast Asia. It is grown in Africa, Arabia, China, and Australia (Clayton et al. 2006). The origins of browntop millet may be traced all the way back to the New Stone Age in the southern regions of the Indian subcontinent. Dry regions of Karnataka and Andhra Pradesh at lower altitudes of South India, witness more cultivation than other regions of the globe. A shorter growing and harvesting period than other millets like pearl millet, browntop millet takes only about 90 days to complete its life cycle (*Pennisetum glauccum*). It is often cultivated as a monoculture rather than in a field with other crops. To reduce grain loss from panicle cracking, early morning harvesting is ideal. Grain shattering is less severe than in the wild forms, but it is still present. Most of the crop is harvested from the ground up, then threshed, dehusked, and processed. Since its grains are semi-shattering, drying them alone is often enough to dislodge them, negating the need to thresh the grain. However, dehusking is still necessary, as is the case with other millets. Straw and chaff are often utilized for animal feed, but the grain is saved for humans and is supposedly more delicious than rice. Flat pancakes (chapati, dosa) are often made using flour made from browntop millet or polished and boiled to make thinner porridge or pudding (payasam). Certain foods of this kind find a place in religious rites, and that may be a factor in why they are still cultivated (Kimata et al. 2000).

Browntop millet grain and spikelets may be easily confused with foxtail millet owing to their similar appearance. Granules are quite similar to *Setaria's*, despite the panicle being less dense and bristly. A lengthy embryo, around two-thirds to three-quarters of grain length, is characteristic of ovoid to spherical grains. They are often shorter and wider than foxtail millet. Well-preserved grains may be identified by their undulating surface (Fuller et al. 2004), which is reminiscent of foxtail millet but has its own unique characteristics. Similarly to *Setaria* spp., the husk has a beaded and rugose pattern, although it is coarser compared to *Setaria italica* and finer than *Setaria verticillata*. The domestication of foxtail millet began in northern China. Because it could be confused with native browntop millet, it is still unknown when and how it entered South Asia. For the purpose of differentiating between these two tiny millet species, geometric morphometrics (GM) provides an alternative to conventional archaeobotanical techniques. Using the identical samples before and after charring, photographs of ancient and contemporary caryopses were taken using a Leica EZ4D stereoscope. The pictures were scaled using the TPSdig program, and the landmark configuration created for their research was manually applied (Rohlf 2013). The landmark configuration concentrated on the form of the embryo, which was less influenced by charring, in order to overcome the morphological bias on the overall grain shape produced by carbonization.

It has been shown that a geometric morphometric technique may be used to differentiate between foxtail millet and browntop millet embryos before and after charring. Charring, on the other hand, has a tendency to even out the dimensional discrepancies between the two classes. This highlights the need for a geometric

morphometric method to analyze small shape changes, which is especially important when working with archaeobotanical assemblages (García-Granero et al. 2016).

Since browntop millet often grows as a mimic weed in fields of *Panicum sumatrense*, it has earned the local term "illegitimate spouse of little millet" in numerous regions of India (Sakamoto 1987).

31.2 Botanical Description (Morphology)

Browntop millet is an annual grass tufted loosely with culms which may be either erect or prostrate along the surface, with height ranging from 37.9 to 59.0 cm, along the stem nodes are present which are minute to slightly hairy. Leaf blades are lanceolate and narrow, with length 3.1–15.7 cm and width 0.2–1.5 mm wide, thickened margins and moderately wavy, apex is acuminate, leaf sheaths may be glabrous and/or pubescent, short ligule, ciliate, 1–2 mm long. Inflorescence is raceme that have bristles bearing the spikelets. Spikes/florets are greenish and 2.8–10.2 cm long, borne along a central axis, subtended by the bristles. Bristles are light greenish in color and 0.6–1.2 cm long. Minute hairs are seen at rachilla, rachis is triquetrous and hispid (Shaheen et al. 2013). Browntop millet has a compact panicle or a loose one, and its spikelets may either exhibit dehiscence or be non-shattering. However, the domestic variants usually behave like other cultivated grain types with a compact and slightly indehiscent nature (Kimata et al. 2000). Spikelets are elliptic in shape and 2–3.5 mm long, little hairy, glabrous, and acute. Lower glume is ovate, acute or obtuse apex, 5-nerved while upper glume is ovate, acute apex, 5–7 nerved. Lemma is ovate with smooth surface, involute margins, acute, papery lemma, 5–7 nerved, 0.3 mm long and 0.1 mm wide, while palea is hard, generally 0.2 mm long and 0.1 mm wide. Anthers are three. Roots are adventitious (fibrous).

31.2.1 Palynology

The pollen is circular in the equatorial view, while it is circular, semi-circular or tubular in the polar view. Polar diameter is 32.5 μm (25–40 μm) and equatorial diameter is 30 μm (25–30 μm). *P*/*E* ratio is 1.08. Exine thickness is 0.85 μm (0.7–1 μm) and intine thickness is 1.15 (1.0–1.3). The position of pore is proximal and pollen is monoporate. Tectum is scabrate. Pore is endoporus. Percentage of pollen fertility in this species is 61.42% (Shaheen et al. 2013).

31.2.2 Chromosome Number

Basappa et al. (1987) collected specimens of *Brachiaria ramosa* (Linn.) Stapf from 23 different populations. Multiple morpho- and cyto-types of *Brachiaria ramosa* sensu Stapf are documented. It is believed that Stapf's (1919) larger perspective on

Table 31.1 Previous reports of chromosome numbers for *Brachiaria* species (adopted from Basappa et al. 1987)

Species	Chromosome number		Authors
	2*n*	*n*	
Brachiara ramosa	36	–	Moffett and Hurcombe (1949), Mulay and Leelamma (1956)
	32	–	Patil and Ghosh (1962)
	–	16	Mehra et al. (1968)
	–	21	Malik and Tripathi (1970)
	–	18	Gupta (1971), Saxena and Gupta (1972)
	72	–	Narayan and Muniyamma (1972)
	28	–	Sinha and Jha (1972)
	–	7	Mehra and Chowdhury (1974)
	–	16, 18	Mehra and Sharma (1975)
	–	16, 36	Christopher and Abraham (1976)
	–	16, 18	Sharma and Sharma (1979), Sharma and Kour (1980)
	32, 36	–	Basappa and Muniyamma (1981)
B. ramosa var. *pubescens*	32	–	Basappa and Muniyamma (1983)

Table 31.2 Base numbers and ploidy levels in *Brachiaria ramosa*

	Base number	Ploidy
B. ramosa var. *ramosa*	$x = 8$	$4x = 32$
B. ramosa var. *pubescens*	$x = 8$	$4x = 32$

this species contributed to the complexity of the issue. Two chromosomal races, with respective chromosome counts of 32 and 36 and respective chromosome bases of 8 and 9, have been identified in this species. Basappa and Muniyamma (1983) provide a circumscription of *B. ramosa* that eliminates the cytotype $2n = 36$ (which is treated as a distinct species, *B. stapfiana* Basappa and Muniyamma). Basappa and Muniyamma recognize two variants of this species: var. ramosa and var. pubescens. In all 23 studied groups, both types were found to contain $2n = 32$ chromosomes. Without access to voucher specimens, it is impossible to comment on reports that differ from $2n = 32$ and 36, such as those with $n = 7$ (Mehra and Chowdhury 1974), $n = 21$ (Malik and Tripathi 1970), and $n = 36$ (Christopher and Abraham 1976). Plant of *B. subquadripara* used for $2n = 72$ report (Narayan and Muniyamma 1972) (Tables 31.1 and 31.2).

31.3 Domestication

It is likely that browntop millet was first domesticated in the Deccan region of South India, and from there it spread to other regions of India throughout the prehistoric period. Charred grains that have been found to be of the "*B. ramosa* type" having been retrieved from the entirety of the early New Stone Age South Indian sites that have undergone systematic archaeobotanical studies. Browntop millet is rather widespread and common in these areas. Little archaeobotanic work has been done on initial New Stone Age or preceramic period (Mesolithic) sites, making it difficult to determine when this crop was domesticated; but even so, the finding demonstrates that this crop, in conjunction with other crops of South India (i.e., horsegram, mung bean, and bristly foxtail), developed from indigenous wild populations all around early 3000 BCE (Fuller 2006).

It was around this time that the southern, Stone Age Indian ash-mound civilization, which practiced both nomadic grazing of cattle and intensive, small-scale crop cultivation, began to include indigenous millets and legumes into their agropastoral system. The cultivation of browntop millet began in the Deccan and continued all the way down to Tamil Nadu in the south (Cooke et al. 2005) and up to Gujarat in the north till the end of the second millennium BCE. It has also been discovered in traces on the eastern side of Orissa (Odisha) and at several sites in the Ganges plains, which date to the copper age (late second–early first millennium BC) (Harvey 2006).

The amount of grains found, however, does not always point to agriculture and may instead represent wild plants. Although browntop millet was still sown in the Paithan province in Maharashtra as recently as the seventh century of the current era, its popularity has waned over time. Replacement by more productive millets such as jowar and finger millet in Africa and foxtail millet elsewhere likely led to its steady decline in popularity. The browntop millet that still exists today is a relic cultivar, yet it nonetheless serves a vital ceremonial purpose.

31.3.1 Nutritional Composition

Browntop millet is said to be gluten-free, essential nutrients rich, a good source of iron, zinc, and fiber and supply 338 kcal of energy with 100 g of browntop millet. The mineral composition constitutes calcium (28 mg), iron (7.72 mg), phosphorus (276 mg), potassium (60 mg), magnesium (94.5 mg), manganese (1.99 mg), sodium (7.6 mg), zinc (2.5 mg), copper (1.23 mg), and a good natural fiber source (8.5%) because of which it serves as the best choice to cope with lifestyle diseases. Incidence level of cardiovascular diseases, diabetes, and duodenal ulcer is reported at lower circuit among people who are regularly consuming millets (Kishore et al. 2021).

31.4 Adaptations as Hardy Food and Feed Crop

Brown top millet has a high tolerance for dry conditions and thrives in semiarid environments. It does best with a yearly precipitation of 75–150 cm at even elevations of 2000–2500 m as well (Roecklein and Leung 1987). Browntop millet is often considered an insurance crop in areas where it grows as a weed in millet fields. It is seen growing in poor and marginal soils in southern India. It is popular as a short-duration, shade-tolerant crop. It thrives well in full sun and can tolerate up to 50% shade (Mitchell and Tomlinson 1989). There are also evidences that it can be grown even under the shade of a tamarind tree. Whatever be the cropping system, it can fit into it. It is a potential cover crop as it can easily spread its roots wherever it comes in contact with the ground, enabling it to cover the ground like a carpet and hold the soil firmly, thereby preventing soil erosion also. It is generally observed to be growing in areca nut and coconut plantations for prevention of rodent damage owing to its sharp leaf edges, thus acting as a nurse crop. It can also suppress root-knot nematode in the soil. It also features another important character, i.e., phytoremediation. It is capable of accumulating zinc and lead in shoot and root tissues in considerable amounts in the contaminated soils (Sujata et al. 2018).

31.5 Necessity for Crop Improvement: Traits to Be Focused

In general, crop improvement is targeted at yield per se, primarily followed by its adaptability to wider environmental conditions or locations. In nature, G × E interactions exert immense pressure on yield per se across locations. So, breeding high-yielding and wider adaptable genotypes shall be the primary focus. Further, it is necessary to understand the negative direct or indirect traits affecting yield per se that demand a breeder's attention.

It was observed that all the small millets are prone to lodging owing to the softness of their stalks, crop management, and environmental factors (Upadhyaya et al. 2015; Santra et al. 2019; Vetriventhan et al. 2020). So far, exact figures on yield losses due to lodging have not been worked out. However, a 50% loss in yields was recorded with cereals like wheat and rice (Tian et al. 2018). As lodging character has a genetic basis and its expression is environmentally influenced, this problem can be solved with development of lodging-resistant types.

Shattering is another important trait on which breeders need to focus. Grain shattering is resulting in significant yield losses in smaller millets (Vetriventhan et al. 2020). Therefore, breeding shattering-resistant types is important in fixing yield losses.

Special focus on breeding traits to increase the extent of cultivation as well as consumption of browntop millet are development of machine harvestable cultivars, improving the nutritive value of grain, developing cultivars suitable to make value-added products such as rice, flour, vermicelli, baked foods, snacks, instant foods like noodles, short-duration genotypes for inter-cropping, and genotypes amenable for rice-fallows.

31.6 Conclusions

Browntop millet is an extraordinary species, hardy and stubborn to adapt to changing climatic scenarios. Its composition gives hope of using it as a Nutri-cereal to address modern lifestyle health problems such as obesity, diabetes, etc. Further, the genomic resources are yet unexplored in this crop. Once explored, it may also cater to the genetic needs of other crops for addressing various challenges which however demands sequencing, annotation and Omics approaches application.

References

Basappa GP, Muniyamma M (1981) IOPB chromosome number reports. LXXII. Taxon 30:694–708

Basappa GP, Muniyamma M (1983) New taxa in Brachiaria Griseb (Poaceae). Proc Indian Natl Sci Acad B 49:377–384

Basappa GP, Muniyamma M, Chinnappa CC (1987) An investigation of chromosome numbers in the genus *Brachiaria* (Poaceae: Paniceae) in relation to morphology and taxonomy. Can J Bot 65(11):2297–2309. https://doi.org/10.1139/b87-313

Christopher J, Abraham A (1976) Cytology and phylogeny of South Indian grasses. LU. Subfam. Panicoideae, Tribe-I, Paniceae. Cytologia 41:621–637

Clayton WD, Vorontsova MS, Harman KT, Williamson H (2006) Grass base–the online world grass flora. Royal Botanic Gardens, London

Cooke M, Fuller DQ, Rajan K (2005) Early historic agriculture in southern Tamil Nadu: archaeobotanical research at Mangudi, Kodumanal and Perur. In: Franke-Vogt U, Weisshaar J (eds) South Asian archaeology 2003: proceedings of the European Association for South Asian Archaeology Conference, Bonn, Germany, 7th–11th Jul 2003. Linden Soft, Aachen, pp 329–334

Fuller DQ (2006) Agricultural origins and frontiers in South Asia: a working synthesis. J World Prehist 20:1–86

Fuller DQ, Korisettar R, Venkatasubbaiah PC, Jones MK (2004) Early plant domestications in southern India: some preliminary archaeobotanical results. Veg Hist Archaeobotany 13:115–129

García-Granero JJ, Arias-Martorell J, Madella M et al (2016) Geometric morphometric analysis of *Setariaitalica* (L.) P. Beauv. (foxtail millet) and *Brachiaria ramosa* (L.) Stapf. (browntop millet) and its implications for understanding the biogeography of small millets. Veg Hist Archaeobotany 25:303–310. https://doi.org/10.1007/s00334-015-0541-z

Gupta BK (1971) Cytological investigation in some north Indian grasses. Genet Iber 23:183–198

Harvey EL (2006) Early agricultural communities in northern and eastern India: an archaeobotanical investigation. Unpublished PhD dissertation. University College London

Kimata M, Ashok EG, Seetharam A (2000) Domestication, cultivation and utilization of two small millets, Brachiaria ramosa and Setariaglauca (Poaceae) in South India. Econ Bot 54(2):217–227

Kishore AS, Rekha KB, Hussain SA, Madhavi A (2021) Quality enhancement of nutri-cereal BTM through agronomic practices. Curr Sci 120:468–470

Malik CP, Tripathi RC (1970) IOPB chromosome number reports. XXVII. Taxon 19:437–442

Mehra PN, Chowdhury JB (1974) IOPB chromosome number reports. XLVI. Taxon 23:801–812

Mehra PN, Sharma ML (1975) Cytological studies in some central and eastern Himalayan grasses. 11. The Paniceae. Cytologia 40:75–89

Mehra PN, Khosla PK, Kohli BL, Koonar JS (1968) Cytological studies in the north Indian grasses. Part I. Res Bull Panjab Univ Sci 19:157–230

Mitchell WA, Tomlinson WH Jr (1989) Browntop millet (Panicumramosum): section 7.1.5, US Army Corps of Engineers Wildlife Resources Management Manual, Technical Report EL-89-12. US Army Engineer Waterways Experiment Station, Vicksburg, MA

Moffett AA, Hurcombe R (1949) Chromosome numbers of south African grasses. Heridity 3:369–373

Mulay BN, Leelamma PJ (1956) Chromosome numbers of some desert grasses of Rajasthan. Proc Indian Acad Sci 55:65–69

Narayan K, Muniyamma M (1972) IOPB chromosome number reports. XLIV. Taxon 6:679–684

Patil RP, Ghosh K (1962) Cytological observations on some grasses from Allahabad and Kotwa in Uttar Pradesh. In: Proc Indian Sci Congr Part 3. pp 330–331

Roecklein JC, Leung P (1987) A profile of economic plants. Transaction Publishers, New Brunswick

Rohlf FJ (2013) TpsDIG v2.17. Ecology and evolution. http://life.bio.sunysb.edu/morph/index.html

Sakamoto S (ed) (1987) A preliminary repost of the studies on millet cultivation and its agro-pastoral culture complex in the Indian subcontinent (1985). Kyoto University Research Team for the Studies on Millet Cultivation and Its Agro-pastoral Culture Complex in the Indian Subcontinent, Kyoto

Santra DK, Khound R, Das S (2019) Proso millet (Panicum miliaceum L.) breeding: progress, challenges and opportunities. In: Al-Khayri J, Jain SM, Johnson DV (eds) Advances in plant breeding strategies: cereals. Springer, Cham, pp 223–257

Saxena BK, Gupta BK (1972) Chromosome numbers of some grasses of Dehra Dun. Bull Bot Surv India 11:443–444

Shaheen S, Haroon N, Shinwari ZK, Tareen RB, Shinwari MI, Samiullah T (2013) Systematic identification of genus Brachiaria on the basis of vegetative and floral morpho-palynological markers (LM & SEM). Pak J Bot 4(5):143–149

Sharma ML, Kour S (1980) IOPB chromosome number reports. LXIX. Taxon 29:703–730

Sharma ML, Sharma K (1979) Cytological studies in the north Indian grasses. Cytologia 44:861–872

Sinha RRP, Jha RP (1972) Cytological studies in some grasses of Bihar. Proc Indian Sci Congr 59: 352–353

Stapf O (1919) Brachiaria ramosa (Linn.) Stapf. In: Prain D (ed) Flora of tropical Africa, part 3, vol 9. Lovell Reeve and Co., London, p 542

Sujata B, Prabhu CG, Nandini C, Prabhakar, Thippeswamy V (2018) Browntop millet—a review. Agric Res Technol 14(5):555937. https://doi.org/10.19080/ARTOAJ.2018.14.555937

Tian B, Luan S, Zhang L, Liu Y, Zhang L, Li H (2018) Penalties in yield and yield associated traits caused by stem lodging at different developmental stages in summer and spring foxtail millet cultivars. Field Crop Res 217:104–112

Upadhyaya HD, Vetriventhan M, Dwivedi SL, Pattanashetti SK, Singh SK (2015) Proso, barnyard, little and kodo millets. In: Singh M, Upadhyaya HD (eds) Genetic and genomic resources for grain cereals improvement. Academic Press, Oxford, pp 321–343

Vetriventhan M, Azevedo VC, Upadhyaya H, Nirmalakumari A, Kane-Potaka J, Anitha S et al (2020) Genetic and genomic resources, and breeding for accelerating improvement of small millets: current status and future interventions. Nucleus 63:217–239. https://doi.org/10.1007/s13237-020-00322-3

Breeding Brown Top Millet (*Brachiaria ramose*) for Biotic and Abiotic Stress Resistance

32

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Abstract

Millets, resilient plants suitable for growth in various tropical and subtropical environments, outperform grains in terms of nutrition. They are not only more cost-effective than cereals but also boast higher levels of protein, vitamins and fibre. Southeast origin annual grass Brown top millet (*Brachiaria ramose*) is grown in Africa, Arabia, China and Australia. Distinguishing itself from other minor millets, it possesses unique qualities such as the shortest cultivation period, the ability to tolerate shade and the capacity to suppress root-knot nematode populations. It is gluten-free, has low GI and so its consumption reduces cholesterol. It is rich in fibre, iron, calcium, magnesium and many minerals and is gluten-free. It is grown mostly in southern India and also grown in some parts of the USA as a fodder crop and bird feed. In comparison, millet production and productivity is lower than that of cereals and has further hindered its yield potential due to biotic and abiotic stress. Biotic stress primarily leads to a greater reduction in yield capacity. Diseases mainly include leaf blight, blast and downy mildew. The insect pests recorded are shoot flies *A. oryzae*, *A. pulla* and *A. punctata*; caseworm *Paraponyx stagnalis*; and red hairy caterpillars *A. albistriga* and *A. moorei*. Apart from the disease and pest, many weeds and

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abiotic factors also hinder the yield potential of the crop. The need to focus more research and development on millets is underlined by growing consumer interest in healthy nutrition, together with the climate-resilient properties of small millets. Except for finger millet and foxtail millet, other small millets like brown top millet have received minimal research attention towards development genetic and genomic resources and breeding for biotic and abiotic stress tolerance. Available germplasm diversity along with advances in phenotyping and genomics technologies could be utilized in improvement of brown top millet.

Keywords

Browntop millet · Biotic stress · Abiotic stress · Resistance · Improvement

32.1 Introduction

Today's major global challenges including increasing population and changing climatic conditions make it necessary to explore newer indigenous plant resources that are stress-tolerant such as millets to ensure food security. Millets belonging to the Gramineae family are small-seeded edible cereals, grown under tropical and arid climates (Maharajan et al. 2019). Besides having additional high protein, vitamin and fibre content millets are nutritionally superior and also less expensive. Many unexplored and underexploited indigenous plant species like brown top millet are neglected by the current mono-crop-based agriculture system. Southeast origin annual grass Brown top millet (*Brachiaria ramose*) is grown in Africa, Arabia, China and Australia. The shortest growing season, shade tolerance and root-knot nematode population suppression make it distinct from other small millets. Recognizing brown top millet grains and spikelets can pose a challenge due to their resemblance to *Setaria italica* (shown in Fig. 32.1). While the panicle can be differentiated from Setaria by its looser, non-bristly nature, the actual grains share a strong likeness. These grains are roughly oval to circular in shape and contain a lengthy embryo, comprising around two-thirds to three-fourths of the grain's length. It has low Glycaemic Index (GI) so its consumption reduces cholesterol. It is gluten-free and rich in fibre, iron, calcium, magnesium and many minerals. It is grown mostly in southern India and also grown in some parts of the USA as a fodder crop and bird feed. The plant is well liked and cultivated in rainfed regions within the districts of Tumakuru, Chitradurga and Chikkaballapura in the state of Karnataka, as well as in the Ananthapur district of Andhra Pradesh. This millet seed is grown in a variety of soils and climates. Like other millets, it is a hardy crop and well suited for dry land. Compared to other warm-season forage grasses, brown top millet is relatively low yielding and often used as a catch crop, cover crop or nurse crop (Sheahan 2014).

The mineral content includes 28 mg of calcium, 7.72 mg of iron, 276 mg of phosphorus, 60 mg of potassium, 94.5 mg of magnesium, 1.99 mg of manganese, 7.60 mg of sodium, 2.5 mg of zinc and 1.23 mg of copper. It also contains a

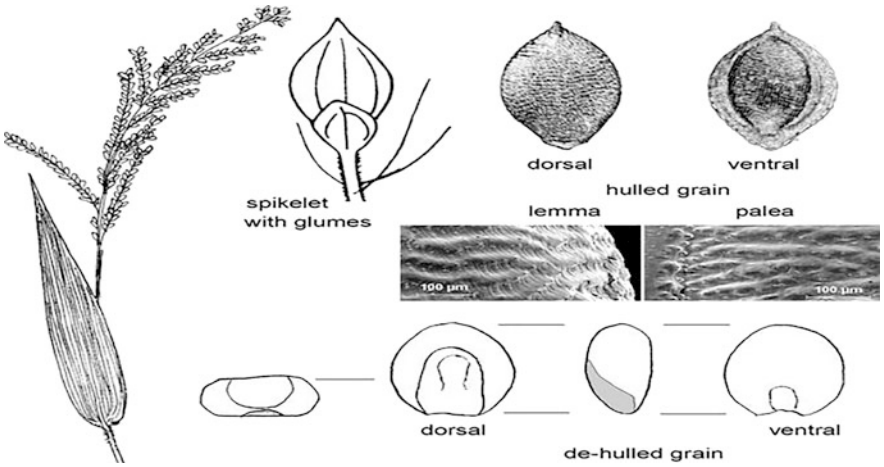

Fig. 32.1 Illustrations depicting the panicles, spikelets and grains with and without husks of brown top millet are presented, showcasing the textured husk designs on the lemma and palea. Scanning electron microscope (SEM) images of these lemma and palea patterns are included as insets. (Courtesy: Eleanor Kingwell-Banham and Dorian Q. Fuller, Institute of Archaeology, University College London, London, UK; Source: https://www.researchgate.net/publication/286351352_Brown_Top_Millet_Origins_and_Development)

substantial amount of natural fibre (8.5%), making it a valuable choice for managing lifestyle-related ailments (Roopa 2015). People who consume millets regularly tend to experience fewer instances of cardiovascular diseases, duodenal ulcers and hyperglycemia (diabetes). Although agronomists have amassed a considerable amount of information about optimal farming practices for various millet crops, there is a scarcity of information specifically regarding the agricultural methods for browntop millet.

Despite possessing climate-resilient and nutrient-rich properties, it also faces multiple constraints that can influence its production. Among biotic constraints, diseases mainly include leaf blight, bacterial streak, blast, downy mildew, head mold, rust, head smut and kernel smut. The insect pests recorded are shoot flies *A. oryzae*, *A. pulla* and *A. punctata*; caseworm *Paraponyx stagnalis*; and red hairy caterpillars *A. albistriga* and *A. moorei*. Apart from disease and pest, many weeds also hinder the yield potential of the crop.

Brown top millet cultivation is subjected to abiotic stresses associated with soil and climate that reduce output and productivity. Inadequate soil moisture and rainfall, along with heat and light-related stress, atmospheric drought (arid conditions), poor soil fertility and elevated soil salinity constitute several factors that significantly impede its cultivation. Despite being a tough crop, it is frequently farmed in arid environments and on sparsely fertile areas, making it more vulnerable to abiotic stress.

32.2 Biotic Stresses

Brown top millet is able to thrive in a variety of climates and is less vulnerable to severe biotic and abiotic challenges. However, a few diseases and insect pests are significantly reducing yields, thus it is crucial to breed for cultivars that are disease- and pest-resistant. The major obstacles to agricultural output are biotic pressures arising from living entities like fungi, bacteria, mycoplasma, nematodes, insects, avian species, unwanted plants and parasitic vegetation. Factors like weather, cropping systems, cultivation techniques, crop types, crop varieties and levels of crop resistance all affect how intense the biotic stress is felt. The crop is typically prone to these stresses due to hot, humid weather, intense farming with plenty of inputs and inadequate crop management techniques. Effect of biotic stresses in brown top millet is less compared to other crops as it is mostly grown in dry climates.

32.2.1 Pathogens

32.2.1.1 Leaf Blight

Among the diseases, most of the researches are concentrated on leaf blight disease, while in other diseases reports are scanty. Misra and Prakash (1972) reported that *Helminthosporium setariae* caused leaf spot on brown top millet in India, but they did not provide any morphological or molecular evidence of identification. Through the examination of both physical traits and genetic characteristics, the causative agent of leaf blight disease in brown top millet was determined to be *B. setariae* (Shoemaker), a member of the Pleosporaceae family. This finding, reported by Ramesh et al. in 2021, marks a novel occurrence of this pathogen on this host in India. The confirmation of the pathogen's identity as *B. setariae* was established through additional sequencing and a combined analysis of specific genes, including the ITS (internal transcribed spacer of rDNA), GAPDH (glyceraldehyde 3-phosphate dehydrogenase) and LSU (large subunit).

In the early stages of the disease, small brown spots with a yellow halo emerge on both sides of the leaves. As the infection advances, these spots enlarge and merge, leading to a blighted aspect (depicted in Fig. 32.2). The symptoms closely resembled those of maize leaf spot induced by *B. setariae*, as observed by Xiao et al. (2019).

Utilizing agronomic practices like managing crop residues and applying fungicide (2.5 g of mancozeb per litre of water) when the disease manifests can enhance tolerance. The research indicated that *B. setariae* exhibited a strong affinity for brown top millet exclusively. This might be attributed to alterations in the pathogen's genome over time, including processes like hybridization, horizontal gene transfer, point mutation, partial or complete gene deletion, as well as changes in nucleotides and/or amino acids, ultimately leading to the transition to a new host (Ramesh et al. 2021).

The production of brown top millet is being threatened by the destructive disease leaf blight. Due to its ability to rapidly break host resistance, the high degree of variability in *Bipolaris* spp. continues to pose a challenge for researchers. Although

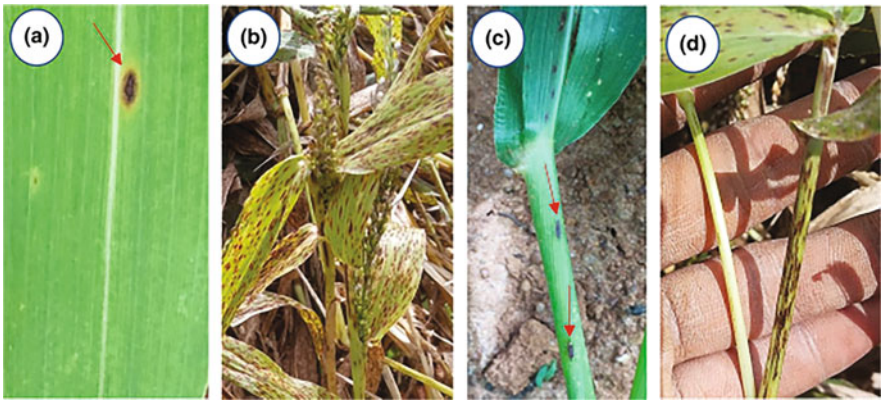

Fig. 32.2 Symptoms of the disease on brown top millet triggered by *B. setariae* include: (**a**) Tiny brown marks encircled by a yellow ring on the leaves (**b**) Numerous lesions present on nearly all leaves (**c**) Initial signs manifesting on the petioles of the leaves.(**d**) Intense symptoms observed on the stems of the plants (Courtesy Ramesh et al. 2021)

there are attempts to take advantage of host resistance to *Bipolaris* in crops including wheat, barley and maize, the majority of the resistance is polygenic and quantitative in nature. There is currently no brown top millet cultivar known to give a considerable level of resistance to the leaf blight disease. As a result, research into the genetics of resistance as well as the identification of new resistance sources must be undertaken. It should also be a top focus to unravel the host-pathogen interaction and create resilient, long-lasting variants using CRISPR-Cas and transgenic technology.

32.2.1.2 Blast

Among the fungal diseases, *Pyricularia grisea* (Cke.) causing blast in Australia, India and USSR is severe during wet conditions. Numerous little brown flecks that grow into brown spindle-shaped dots on the leaves are its key characteristic. Later patches become larger and merge to form a blasted appearance. They also have ash-grey centres with brown edges. Spots are 0.5–1.5 mm wide and 3–8 mm long.

At some locations, culm nodes can lodge and turn black. At the base of the panicles, brown to black patches enlarge and frequently girdle the neck beneath the panicles. Shrunken and covered in mycelium, conidiophores and conidia, the diseased neck becomes a sign of the condition. In damp conditions, nodes and necks develop an abundance of conidia and conidiophores. Panicles fail to appear when the neck blast is severe. Spikelet develops an infection and turns black. Early neck infection prevents the grains from filling and keeps the panicles upright. Later infection causes panicles that are just partly full to lodge.

Numerous crops, including rice, wheat, finger millet, foxtail millet, pearl millet and several grasses are infected by *Pyricularia grisea*. The pathogen population that infects rice or any other host does not infect pearl millet and vice versa because of the pathogen's extremely specific host range. This fungus has been found to have a high

degree of pathogenic variation in rice, finger millet, foxtail millet, wheat and numerous weed hosts (Prabhu et al. 1992; Takan et al. 2012). Operation of mechanisms like sexual recombination, heterokaryosis and parasexual recombination in this pathogen help it in frequent race changes. A significant obstacle to the establishment of long-lasting resistance to this disease in other crops is the recurrent race formation (Suh et al. 2009). Blast disease can be prevented by using management techniques like wide plant spacing and controlling the amount of nitrogenous fertiliser. A wide range of chemicals are frequently employed to manage blast in the absence of resistant types.

32.2.1.3 Downy Mildew

This condition is referred to as "green ear disease" due to the influence of *Sclerospora graminicola* (Sacc.). The symptoms closely resemble those induced by *Sclerophthora macrospora* (Sacc.). It's commonly found in regions where millet is cultivated, with a notable prevalence of downy mildew in Africa and India.

Sclerospora graminicola (Sacc.) has the ability to endure for multiple years in the soil as oospores. The fungus is spread through oospores present in seeds and by any means that can transport soil or residue contaminated with oospores. Oospores typically sprout and generate one or more germ tubes that directly infiltrate the growing tissue of young plants. Sporangia (also known as conidia) form at the tips of conidiophores that emerge through stomatal openings and are carried by the wind to other host plants. These sporangia then produce four zoospores, which encyst and later form a germ tube capable of infecting the meristematic tissue. Eventually, oospores are produced within the infected tissue. The disease exhibits its most severe effects when the environmental conditions are moist. Plants are dwarfed with excessive tillering from the crown and development of axillary buds along the culm. Flower parts develop leaflike structures with no kernel development. A downy greyish growth, which is the conidia and conidiophores, develop on infected tissue during wet or humid weather. As plants approach maturity, leaves become brown, necrotic and split or shred.

There is an absence of an organized program for developing resistance in brown top millet. While using protective fungicides on seeds and adopting effective fertilizer management practices can decrease disease occurrence, the identification of sources with resistance remains to be accomplished for a meaningful approach to disease management.

32.2.1.4 Insects

Brown top millet may become infested with armyworms and grasshoppers (Baker 1996) and is susceptible to mungbean yellow mosaic bigeminy virus (Brunt et al. 1996). Other insect pests recorded are shoot flies *Atherigona oryzae*, *A. pulla* and *A. punctate*: caseworm *Paraponyx stagnalis*; and red hairy caterpillars *Amsacta albistriga* and *A. moorei*. Shoot fly causes considerable damage to the seedlings and caterpillars are the occasional leaf-feeding insects that feed on leaves and cause defoliation. However, their damaging potential, supported by actual yield loss data are lacking.

Host-plant resistance is an effective, economical and environmentally friendly approach to managing insect pests. Plants that possess resistance could exhibit either one or a blend of the three mechanisms (non-preference, antibiosis and tolerance as elucidated by Painter (1951)), which collectively determine the extent of insect resistance. Each form of resistance mechanism functions during various phases of the interaction between insects and plants, aided by physical or chemical traits of the plant that can be considered as elements of resistance. As there haven't been comprehensive investigations in brown top millet, species that are resistant or tolerant are yet to be identified. The mechanism behind resistance and the sources of resistance will be discerned in forthcoming studies.

32.2.1.5 Weeds

Agriculture across the world is hampered by weed issues. They are a significant obstacle to boosting productivity, particularly during the rainy season when the weather is favourable for their growth. As the crop is grown in rainfed environment the major limiting elements are soil moisture and nutrients. Brown top millet faces competition from weeds for light, soil moisture and nutrients, which lowers the amount of grain produced.

The decrease in grain yield is influenced by the type and intensity of weeds, the length of time they infest and the prevailing environmental circumstances. Brown top millet is a prolific seed producer making it competent with the weeds. The viable seeds of a current planting will continue to reproduce until a killing frost, inundation, or other factors cause its seasonal demise (Mitchell and Tomlinson 1989).

32.2.2 Other Biotic Constraints

Currently, millet growers are thought to be at risk from avian damage to grains. Small-grain crops are typically more vulnerable to severe bird damage than large-grain crops. The degree of damage varies with the crop and the growing environment, and in isolated and unprotected situations, the loss could reach 100%. Although it is a widespread issue in many nations, it is especially awful in several African nations. In eastern and southern Africa, Quelea and other birds are thought to reduce crop productivity by roughly 1.6 million tonnes annually (Wortmann et al. 2009).

32.3 Abiotic Stress

Cultivation of brown top millet in marginal areas with poor irrigation and management practices makes it more vulnerable to abiotic stresses. In comparison to biotic stress resistance, abiotic stress resistance is physiologically more complex by nature, often subject to significant environmental impacts, and has received less research.

The presence or absence of different pressures may affect how a plant responds to a stress. For instance, poor soil fertility or a compacted soil structure may hinder root

development and the plant's ability to exhibit drought resistance. The ability to withstand abiotic stress is typically controlled by polygenic inheritance and may be affected by a number of interrelated ways. Abiotic stress resistance is very challenging to research, both physiologically and genetically, because of these and other reasons.

32.3.1 Drought

In rainfed habitats, brown top millet productivity is severely constrained by drought. The amount and distribution of rainfall in regions where the crop is grown by rainfed irrigation strongly influences production. Drought or moisture stress can develop during the seedling stage, vegetative stage, or grain filling. In many African countries, millet production is thought to be one of the most vulnerable to drought and water stress (Matanyaire 1996; Gebretsadik et al. 2014). In Ethiopia, soil-water deficits are frequent throughout the stages of millet crop establishment and grain fill, while shortfalls at the midseason are typical in Kenya and Uganda. In India throughout the rainy and postrainy seasons, millet production is low due to frequent dry spells of different lengths, intensities and timing (Patil 2007).

32.3.2 Soil Fertility and Others

Millet is primarily planted in sandy soils with poor nutrient status and limited water-holding capacity, which has a negative impact on its productivity. Most of the time, the soils have low levels of organic carbon and are lacking in nitrogen and phosphorus. Crop rotation and fertiliser application are rarely practised by farmers. The production potential of a crop in the semiarid tropics is severely constrained by these stresses and drought. Another significant barrier that affects millet productivity is soil salinity. However, this issue is not as widespread as soil fertility and drought are. Salinity issues are made worse by inadequate drainage systems and poor irrigation water quality.

Many beneficial genes, especially those that are tolerant to biotic and abiotic stress, can be found in wild and exotic accessions. With the use of molecular markers, backcrossing programmes may now introduce these helpful genes with the least amount of linkage drag. Research works need to progress on the biotic and abiotic challenges of economic importance in producing places around the world in order to sustainably produce improved cultivars.

32.4 Conclusion

Despite all the advantages brown top millet provides cultivation is still restricted to a few locations. It is a less popular crop that is in danger of being extinct. Food security, malnutrition and climate change could all be addressed while safeguarding

the livelihoods of farmers through the preservation, popularisation and development of suitable and simple processing equipment for the millet.

Employing landrace selection offers an avenue to enhance essential traits linked to domestication in brown top millet, encompassing characteristics like seed type that doesn't shatter and resistance to lodging. This approach also facilitates the cultivation of resilience against both living organism-related and environmental challenges. However, the utilization of genome editing and alternative methods for crop enhancement faces obstacles due to the absence of standardized genetic transformation procedures, tissue culture methods, well-annotated genome sequences, and other pertinent resources and techniques. To establish effective integrated production systems, incorporating intercropping, a comprehensive breeding strategy is recommended, focusing on domestication traits alongside factors like processability, ease of harvesting, plant structure and phenology.

References

Baker RD (1996) Millet production. NSMU cooperative extension guide # A-414. Lubock.tamu.edu/files/2011/10/Millet-Production.pdf

Brunt AA, Crabtree K, Dallwitz MJ, Gibbs AJ, Watson L, Zurcher EJ (1996) Plant viruses online: descriptions and lists from the VIDE database. http://biology.anu.edu.au/Groups/MES/vide

Gebretsadik R, Shimelis H, Laing MD, Tongoona P, Mandefro N (2014) A diagnostic appraisal of the sorghum farming system and breeding priorities in Striga infested agro-ecologies of Ethiopia. Agric Syst 123:54–61

Maharajan T, Ceasar SA, Krishna TPA, Ignacimuthu S (2019) Phosphate supply influenced the growth, yield and expression of *PHT1* family phosphate transporters in seven millets. Planta 250:1433–1448

Matanyaire CM (1996) Pearl millet production system(s) in the communal areas of northern Namibia: priority research foci arising from a diagnostic study. In: Leuschner K, Manthe CS (eds) Drought-tolerant crops for southern Africa: proceedings of the SADC/ICRISAT regional sorghum and pearl millet workshop, 2529 July 1994, Gaborone, Botswana. ICRISAT, Patancheru, Andhra Pradesh, pp 43–58

Misra AP, Prakash O (1972) *Helminthosporium* species occurring on *graminaceous* hosts in India. Indian J Mycol Plant Pathol 2:95–97

Mitchell WA, Tomlinson WH (1989) Browntop millet *(Panicum ramosum)*: section 7.1. 5. US Army Corps of Engineers Wildlife Resources Management Manual

Painter RH (1951) Insect resistance in crop plants. University Press of Kansas, Lawrence

Patil SL (2007) Performance of sorghum varieties and hybrids during post-rainy season under drought situations in Vertisols in Bellary, India. J SAT Agric Res 5(1):1–3

Prabhu AS, Filippi MC, Castro N (1992) Pathogenic variation among isolates of *Pyricularia oryzae* affecting rice, wheat, and grasses in Brazil. Int J Pest Manag 38(4):367–371

Ramesh GV, Palanna KB, Vinaykumar HD, Kumar A, Koti PS, Mahesha HS, Nagaraja TE, Tonapi VA, Jeevan B (2021) Occurrence and characterization of *Bipolaris setariae* associated with leaf blight of browntop millet (*Brachiaria ramosa*) in India. J Phytopathol:1–10. https://doi.org/10.1111/jph.1303

Roopa OM (2015) M.Sc. thesis. University of Agricultural Sciences, Bengaluru

Sheahan CM (2014) Plant guide for browntop millet (*Urochloaramosa*). USDA-Natural Resources Conservation Service, Cape May Plant Materials Center, Cape May

Suh JP, Roh JH, Cho YC, Han SS, Kim YG, Jena KK (2009) The *Pi40* gene for durable resistance to rice blast and molecular analysis of *Pi40*-advanced backcross breeding lines. Phytopathology 99:243–250

Takan JP, Chipili J, Muthumeenakshi S et al (2012) *Magnaporthe oryzae* populations adapted to finger millet and rice exhibit distinctive patterns of genetic diversity, sexuality and host interaction. Mol Biol Biotechnol 50:145–158. https://doi.org/10.1007/s12033-011-9429-z

Wortmann CS, Mamo M, Mburu C, Letayo E, Abebe G, Kayuki KC et al (2009) Atlas of sorghum production in eastern and southern Africa. University of Nebraska, Lincoln, NE

Xiao SQ, Zhang D, Zhao JM, Yuan MY, Wang JH, Xu RD, Li GF, Xue CS (2019) First report of leaf spot of maize (*Zea mays*) caused by *Bipolarissetariae* in China. Plant Dis 104:582